Gastrointestinal Handling of Water-Soluble Vitamins

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ABSTRACT

Nine compounds are classified as water-soluble vitamins, eight B vitamins and one vitamin C. The vitamins are mandatory for the function of numerous enzymes and lack of one or more of the vitamins may lead to severe medical conditions. All the vitamins are supplied by food in microgram to milligram quantities and in addition some of the vitamins are synthesized by the intestinal microbiota. In the gastrointestinal tract, the vitamins are liberated from binding proteins and for some of the vitamins modified prior to absorption. Due to their solubility in water, they all require specific carriers to be absorbed. Our current knowledge concerning each of the vitamins differs in depth and focus and is influenced by the prevalence of conditions and diseases related to lack of the individual vitamin. Because of that we have chosen to cover slightly different aspects for the individual vitamins. For each of the vitamins, we summarize the physiological role, the steps involved in the absorption, and the factors influencing the absorption. In addition, for some of the vitamins, the molecular base for absorption is described in details, while for others new aspects of relevance for human deficiency are included. © 2018 American Physiological Society. *Compr Physiol* 8:1291-1311, 2018.

Didactic Synopsis

Major teaching points

- Water-soluble vitamins cover eight B vitamins and vitamin C.
- The vitamins are supplied with food in quantities of microgram to milligram, and some are synthesized also by gut microbiota.
- The intestinal uptake requests concerted action by several cellular transporters.
- The vitamins function as coenzymes or antioxidants for numerous intracellular metabolic reactions.
- Lack of one or the other of the vitamins may lead to severe disease.

General Introduction

The water-soluble vitamins are fascinating low molecular weight substances of importance for human and animal health. Most of them evolved billions of years ago, in the pre-DNA/RNA era (131) but were not discovered and characterized until the twentieth century.

For some of the vitamins the discovery is based on unexplained human conditions as exemplified for vitamin B1. Dutch soldiers in action in Indonesia in the 1890s developed a severe affliction that was assumed to be an infection at the time. To isolate the bug, groups of hens were infected with the soldier's blood; like the soldiers, the hens were fed polished rice. When changed to unpolished rice, they recovered—independent of the blood injections. This led Eijkman to speculate that the rice coating contained a substance of importance for human and animal health. Eventually the substance was isolated and named vitamin B1 or—later—based on its chemical structure thiamin (for a historical account see (182)).

The term vitamin was introduced around 1900 as a combination of the words vital (important for life) and amine (a substance containing nitrogen). Vitamins are organic substances needed in quantities that are small, but more than can be supplied by endogenous synthesis. Thus, vitamins must be obtained from exogenous sources (diet and gut microbiota) and consequently taken up in the gastrointestinal tract.

Vitamin B1 (thiamin) and vitamin C (ascorbic acid) were isolated in 1926. Relatively soon thereafter structures were clarified and modern naming followed (named as their structure was clarified) riboflavin (B2), pyridoxine (B6), niacin (B3), biotin (B7), pantothenic acid (B5), and folate (B9). Vitamin B12 (cobalamin) was finally isolated and named in 1948. Some initially identified B vitamins proved not to be well-defined substances and because of that there are

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missing numbers in the B-vitamin series. For example, we have B12 though altogether we have only eight B vitamins (for an extensive historical account on the vitamins see (33)).

In our continuous presentation of the vitamins, we will use the abbreviations indicated in Table 1.

Occurrence, recommended daily allowance (RDA), and turnover

Most of the water-soluble vitamins are present in a wide variety of food. An exception is vitamin B12, which is present only in food of animal origin (Table 2). The recommended daily allowance ranges from a few micrograms (vitamin B12) to more than 50 mg (vitamin C). The values as recommended by the Food and Nutrition Board/USA are presented in Table 2 (41). Only values for adults are provided. As a rule of thumb, children need a little less; in contrast, pregnant and lactating women needs up to 40% more. To ensure a sufficient supply of each vitamin, vitamin fortification has increased over the years. For example, fortification with vitamin C (juices and other soft drinks and folate (cereals) is widespread), http://www.ffinetwork.org/global_progress.

In food, the vitamins are usually bound to proteins; they are released from proteins by the action of digestive enzymes. The released vitamins are then absorbed by a specific carrier-mediated process that has a limited capacity (Fig. 1). This carrier-mediated transport can be circumvented by intake of high doses of the vitamins. Around 1% of luminal vitamin is expected to cross the intestine by passive absorption.

Until recently vitamins were considered only to be absorbed in the small intestine, but now also synthesis by the microbiota and uptake in colon is judged of importance for many of the vitamins, as summarized in Figure 2.

The turnover rates for the vitamins are relatively fast. Because of that signs and symptoms of deficiency may occur within months. An exception is vitamin B12, which has a very slow turnover rate, thus delaying the development of symptoms up to years of insufficient intake/uptake.

Function and common causes of lack

Vitamins function as coenzymes for many different enzymes and as antioxidants (Table 3). Vitamin deficiency and suboptimal levels are common in underdeveloped countries and amongst individuals not receiving an adequate diet. In addition to nutritional deprivation, chronic diseases such as alcoholism and conditions affecting the intestine will often lead to an insufficient body supply. In rare cases, genetic defects in any step of the digestion/transport of the vitamin may lead to a functional deficiency (see the individual vitamins).

Ascorbate (Vitamin C)

Two forms of vitamin C exist in the diet: a reduced form (ascorbic acid) and an oxidized form (dehydro-L-ascorbic

Table 1 List of Abbreviations

AA: Amino acids
ATP: Adenosine triphosphate
B12: Cobalamin, vitamin B12
BBMV: Brush border membrane vesicles
BLM: Basolateral membrane
Bp: Base pairs
cKO: Conditioned knockout
CN-B12: Cyano-B12
Cnn: Cysteine, nn: indicates amino acid number
DHAA: Dehydro-L-ascorbic acid
DYNLRB1: Dynein light-chain road block-1
EnnQ: Glutamine (Q) instead of glutamic acid (E) in position nn
FAD: Flavin adenine dinucleotide
FMN: Flavin mononucleotide
Gnn: Glycine, nn: indicates the amino acid number
GC-box: A pattern of nucleotides that binds promotion factors
GKLF: Gut-enriched Kruppel-like factor/cis element
h: used to indicate human origin
HFMS: Hereditary Folate Malabsorption Syndrome
HO-B12: Hydroxo/aquo-B12
KnnE: Glutamic acid (E) instead of Lysine(K) in position nn
miRNA: MicroRNA
NAD: Nicotinamide adenine dinucleotide
NADP: Nicotinamide adenine dinucleotide phosphate
Nnn: Asparagine, nn: indicates the amino acid number
PCFT: Proton-coupled folate transporter
RFC: Reduced folate carrier
RFVT: Riboflavin transporter
SLC5A6: The gene coding for SMVTSLC19A1: The gene coding for RFC
SLC19A2/3: Genes coding for THTR-1 and THTR-2
SLC23A: The gene coding for SVCT
SLC44A4: The gene coding for TPPT
SLC46A1: Gene coding for PCTF
SLC52A: The gene coding for RFVT
SMVT: Sodium-dependent multivitamin transporter
Sp1: GC-box binding factor/cis element
SVCT: Sodium-dependent vitamin C transporter
THTR: Thiamin transporter
TMD: Transmembrane domain
TTP: Thiamin pyrophosphate/triphosphate
TPPT: Thiamin pyrophosphate transporter

Table 2 Daily Requirement and Contribution from Food Items

Vitamin	Recommended daily allowance (RDA) Men (Women)[a]	The numbers indicate % supplied by a Western diet[b]				
		Bread, cereals	Fruit, vegetables	Meat, poultry, fish	Dairy, eggs	Others[c]
Ascorbate (Vitamin C) (mg)	90 (75)	<0.5	72	7	3	20
Biotin (Vitamin B7) (μg)	30 (30)	X	X	X	X	X
Cobalamin (Vitamin B12) (μg)	2.4 (2.4)	<0.5	<0.5	55	38	8
Folate (Vitamin B9) (μg)	400 (400)	22	44	9	17	9
Niacin (Vitamin B3) (mg)	16 (14)	11	15	37	21	18
Pantothenic acid (Vitamin B5) (mg)	5 (5)	X	X	X	X	X
Pyridoxine (Vitamin B6) (mg)	1.3 (1.3)	16	32	28	15	8
Riboflavin (Vitamin B2) (mg)	1.3 (1.1)	11	9	19	49	14
Thiamin (Vitamin B1) (mg)	1.2 (1.1)	32	16	35	14	4

Note. X indicates that the vitamin is present, but it is not possible to indicate exact contribution.
[a]From (41).
[b]Based on "Danskernes kostvaner" 2003 to 2008, ISBN: 978-87-92158-67-3.
[c]Others: Fat, sugar, sweets, drinks, etc.

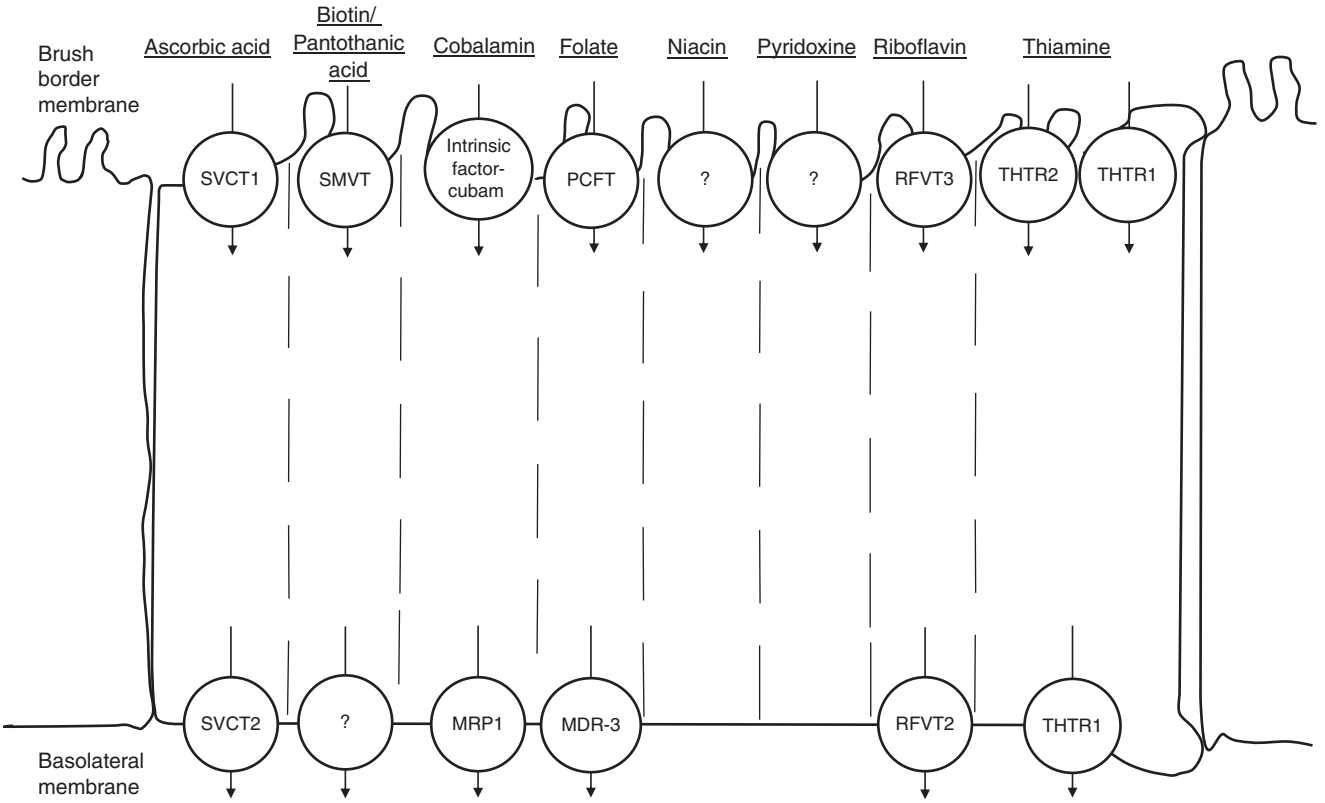


Figure 1 Absorption of water-soluble vitamins in the small intestine. The figure depicts key proteins involved in the uptake of vitamins supplied with food or recycled with bile and absorbed in the small intestine. The question mark indicates that the molecular identity of the system involved has not been identified yet.

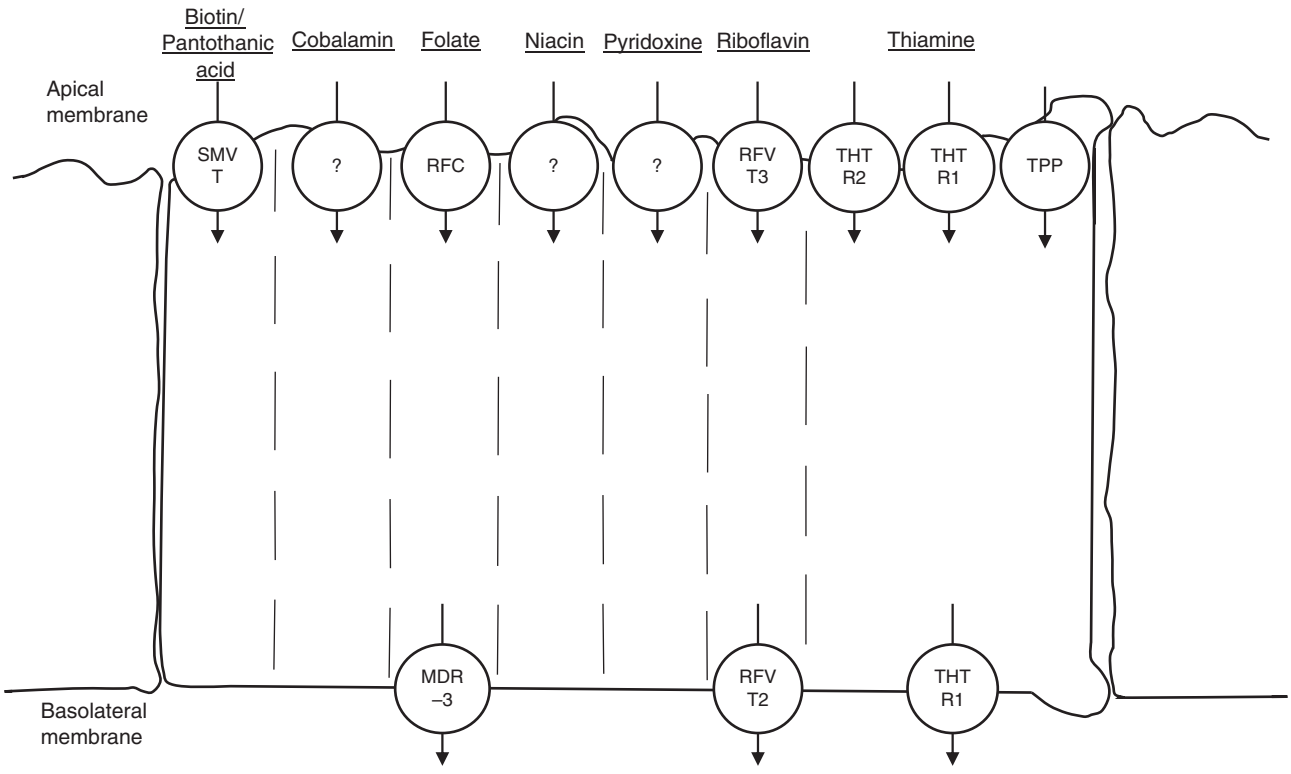


Figure 2 Absorption of water-soluble vitamins in the large intestine. The figure depicts key proteins involved in the uptake of vitamins synthesized by the gut microbiota and absorbed in the large intestine. The question mark indicates that the molecular identity of the system involved has not been identified yet.

acid; DHAA). Ascorbic acid acts as a cofactor in reactions related to normal iron and copper metabolism, synthesis of collagen, and metabolism of carnitine and tyrosine; it also acts as free-radical scavenger and plays a role in normal immune function. Most mammals generate vitamin C from D-glucose endogenously, but humans (as well as other primates and the guinea pigs) cannot do so because they lack the needed enzyme, that is, L-gulonolactone oxidase. Thus, they need to obtain the vitamin from exogenous sources. Deficiency of vitamin C leads to a variety of clinical abnormalities including scurvy, poor wound healing, vasomotor instability, and connective tissue disorders.

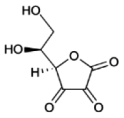
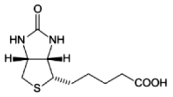
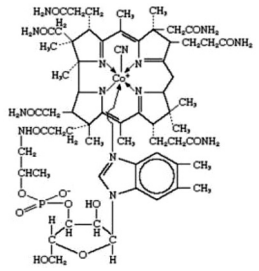
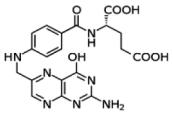
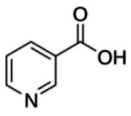
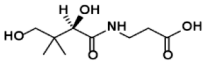
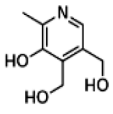
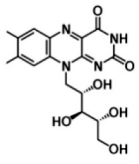
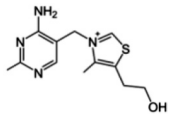
Absorption of dietary vitamin C

The diet is the main source of vitamin C for humans as little is generated by the intestinal microbiota (258). Uptake of ascorbic acid in the small intestine occurs via specific sodium-dependent carrier-mediated process (181, 217). Uptake of DHAA occurs via a sodium-independent carrier-mediated process that is competitively inhibited by sugar (due to structural similarities) (18, 251). DHAA is converted inside the enterocyte to ascorbic acid by the action of the enzyme DHAA-reductase (18, 32, 211).

The human intestine expresses two transporters for ascorbic acid: the sodium-dependent vitamin C transporter-1 (SVCT-1; a 598 AA protein; product of the *SLC23A1* gene) and the sodium-dependent vitamin C transporter-2 (SVCT-2; a 650 AA protein product of the *SLC23A2* gene) (Fig. 1) (247, 253). SVCT-1 and SVCT-2 share significant sequence similarity with one another. In the intestine, SVCT-1 is expressed at a higher level than SVCT-2 and both carriers transport ascorbic acid via a specific, electrogenic, and sodium-dependent process. While the affinity of SVCT-2 for ascorbic acid appears to be higher than that of SVCT-1 (114), neither of them transports DHAA. As to the system(s) involved in the intestinal absorption of DHAA, both glucose transporters GLUT2 and GLUT8 appear to be involved (Fig. 1) (36, 114).

Knowledge about cell biology and structure-activity relationship of the SVCT-1 protein has also been forthcoming. Thus, expression of the hSVCT-1 protein at the apical membrane domain appears to be determined by a sequence (amino acids 563-572) in the C-terminal tail of the polypeptide (226). Intracellular trafficking of the SVCT-1 protein appears to involve heterogeneous population of intracellular vesicles, the mobility of which depends on temperature and on intact microtubule network (226). With regards to SVCT-2, this transporter is expressed at the basolateral membrane (BLM) domain of enterocytes (23), and expression is dictated by a basolateral targeting sequence (26). Other investigations have reported that the hSVCT1 has an accessory protein: the enzyme glyoxalate reductase/hydroxypyruvate reductase (hGR/HPR), and that their interaction has functional

Table 3 Structure and Function of Water-Soluble Vitamins

Vitamin	Structure	Active forms	Function
Ascorbic acid		Ascorbic acid	– Antioxidant – Iron and copper metabolism – Collagen synthesis – Carnitine and tyrosine metabolism – Immune function
Biotin		Biotin	– Metabolism of fatty acids, carbohydrates, and amino acids – Energy metabolism – Regulation of cellular oxidative stress – Gene expression – Immune function – Maintenance of normal intestinal homeostasis
cobalamin		Upper ligand (replacing the cyanide (CN) group: – Methyl-5′-deoxyadenosyl-	– Methyl transfer – Metabolism of odd chain fatty acids, branched amino acids, and cholesterol
Folate		Many derivatives, e.g., tetrahydrofolate (THF) N^5-methyl-THF, N^{10}-formyl-THF	– DNA synthesis – Metabolism of amino acids
Niacin		Niacin-adenine-dinucleotid (NAD), Niacin-adenine-dinucleotid-phosphate (NADP)	– Glycoysis – Maintenance of cellular redox state – Maintenance of normal intestinal homeostasis
Pantothenic acid		Part of coenzyme A	– Metabolism of carbohydrates, lipid and protein
Pyridoxine		Pyridoxin, pyridoxal pyridoxamin	– Metabolism of carbohydrates, lipids and proteins – Production of neurotransmitters
Riboflavin		Flavinmononucleotide (FMN), flavinadenindinucleotid (FAD)	– Oxidation-reduction – Protein foldings, energy metabolism, antioxidant, anti-inflammatory
Thiamin		Thiamin pyrophosphate (TPP)	– Energy metabolism – ATP production – Reduction of cellular oxidative stress

consequence (233). Finally, a role for the histidine residue at position 51 of the hSVCT-1 protein and the histidine residue at position 109 of the hSVCT-2 protein in the function of these transporters have been reported (250); also, both of the putative N-glycosylation sites of the hSVCT-1 polypeptide (located at positions 138 and 144) and of the hSVCT-2 polypeptide (located at positions 188 and 196) appear to be glycosylated and are important for function (229).

As to regulation of the intestinal ascorbic acid uptake process, important knowledge regarding transcriptional regulation of the *SLC23A1* and *SLC23A2* genes has been reported. Thus, the promoter of the *SLC23A1* gene has been cloned and characterized, its minimal region required for basal activity has been identified to be within the 135-bp region upstream of the transcriptional start site, and a role for the hepatic nuclear factor 1 (HNF-1) in promoter activity has been reported (128). With regards to the *SLC23A2* gene, again its promoter has been cloned and characterized, the minimal region identified, and a role for Krupp-like factor (KLF)/Sp1 in promoter activity has been reported (172).

Other investigations have shown that the intestinal ascorbic acid uptake process is adaptively regulated by extracellular substrate level (95, 120). The process also undergoes differentiation-dependent regulation that involve changes in the expression of hSVCT-1 (but not the hSVCT-2) (126). Finally, both hSVCT-1 and hSVCT-2 appears to be under the regulation of an intracellular protein kinase C (PKC)-mediated pathway (115).

Biotin (Vitamin B7)

Biotin acts as a cofactor for multiple carboxylases involved in fatty acid, glucose and amino acid metabolism. Recent studies have suggested new roles for biotin in energy metabolism (i.e., ATP production), regulation of cellular oxidative stress (121), and gene expression (179). Furthermore, a role for biotin in normal immune functions (2,103-105), in maintenance of normal integrity/homeostasis of gut mucosa (63, 183), and in the colonization/invasiveness of certain enteropathogenic bacteria (261) have been reported. Biotin deficiency/suboptimal levels have been reported in different conditions including chronic alcoholism (21), inflammatory bowel disease (IBD) (55), the inborn errors leading to multiple carboxylase deficiency (244), and biotin-responsive basal ganglia disease (98), and patients on long-term anticonvulsant therapy (100), or long-term parenteral nutrition (130). Overt severe biotin deficiency leads to dermatological abnormalities, neurological disorders, and growth retardation.

Absorption of dietary and microbiota-generated biotin

Biotin is available to the host from two exogenous sources: the diet and the gut microbiota (258). In the diet, biotin exists in both the free and protein-bound forms; the latter is enzymatically hydrolyzed (in the gut lumen) first to biocytin (biotinyl-L-lysine) and biotin bound to oligo peptides via the action of gastrointestinal proteases and peptidases. Free biotin is released via the action of the enzyme biotinidase that hydrolyzes the amide bond between the carboxyl group of biotin and the epsilon amino group of lysine (208, 257). Because the intestine absorbs biocytin (biotinyl-L-lysine) or biotin-short peptides poorly (208), mutations in the enzyme biotinidase (as in patients with the autosomal recessive disorder "biotinidase deficiency") leads to biotin deficiency/suboptimal levels and is associated clinical (neurological and cutaneous) abnormalities; likely the result of multiple carboxylase deficiencies (19, 208, 257).

Absorption of free biotin (a negatively charged molecule) in the small intestine occurs via an efficient sodium-dependent carrier-mediated process that takes place mainly in the proximal small intestine (196, 202). This sodium-dependent event reflects the function of the uptake system at the apical membrane of the polarized enterocytes as shown by functional [e.g., purified intestinal brush border membrane vesicles (BBMV)] as well as immunological, and live-cell confocal imaging studies (Fig. 1) (196, 202, 203, 205). A unique feature of this system is that it also transports vitamin B5 (pantothenic acid) and the antioxidant lipoate (187, 199). For this reason, the system has been named the "sodium-dependent multivitamin transporter," or "SMVT." Biotin exits the polarized absorptive cells via a sodium-independent carrier-mediated process (203).

In the large pool of microbiota-generated biotin, a considerable portion exists in the free form, and hence available for absorption (258). Indeed, human/mammalian large intestine is capable of absorbing free biotin via the SMVT system (Fig. 2) (24, 199).

The SMVT system is product of the *SLC5A6* gene. In the intestine, SMVT is exclusively expressed at the apical membrane of the polarized intestinal absorptive cells as shown by functional, immunological, and confocal imaging studies (Fig. 1) (190). The SMVT system appears to be the only biotin uptake system that operates in the mammalian gut as shown by *in vitro* gene-specific silencing (siRNA) approach (12), and *in vivo* conditional (intestinal-specific) SMVT-knockout (cKO) mouse investigations (63).

Aspects of the SMVT cell biology and structure-function relationship have been delineated. The structural component that determines apical membrane targeting of the hSMVT in absorptive epithelial is located in to the C-terminal tail of the polypeptide (225). Both distinct trafficking vesicles and the microtubule network appear to be involved in intracellular trafficking of the hSMVT (225). Furthermore, PDZD11, an accessory protein containing the PDZ domain has been identified as an interacting partner with SMVT in gut epithelia; this interaction (which occurs at the PDZ binding domain located at the cytoplasmic tail of the SMVT polypeptide) has functional consequences for vitamin transport (142). Moreover, C294 of SMVT is important for function (66), and protein glycosylation at N138 and N489 is important for function (67).

Important knowledge regarding transcriptional regulation of the *SLC5A6* gene and resulting regulation of the intestinal biotin uptake has been reported. The 5′ regulatory region of the *SLC5A6* gene has been cloned and characterized; that region contains two promoters (39); activity of these promoters appears to be regulated by the nuclear factors Kruppel-like factor 4 (KLF-4) and activator protein (AP-2) (39,174). From a whole organism standpoint the intestinal biotin absorption is adaptively regulated by biotin availability (171,195). A significant upregulation in intestinal carrier-mediated biotin uptake was observed in biotin deficiency, and a significant downregulation occurs following oversupplementation. This adaptive regulation appeared to be mediated at the level of transcription of the *SLC5A6* gene (171). The biotin level-responsive region in the *SLC5A6* promoter was identified to be within a 103-bp region sequence and contains gut-enriched Kruppel-like factor (GKLF) cis-elements that confer the upregulatory response to extracellular biotin level (171). The intestinal biotin uptake process also undergoes developmental regulation during early life and this regulation also occurs via transcriptional mechanisms involving the *SLC5A6* gene (143, 204). A role for protein kinase C (PKC) in regulating intestinal biotin uptake has also been reported and appears to occur at T286 of the hSMVT (67). Also, a protein casein kinase-2 (CK-2) -mediated pathway appears to exert a regulatory effect on SMVT via regulating its level of expression at the cell surface and that this effect involves T78 of the SMVT (109).

Factors influencing the uptake of biotin

Chronic alcohol consumption is associated with suboptimal levels of biotin (21). At least in part this appears to be mediated via inhibition in intestinal (and colonic) biotin uptake and involves transcriptional mechanisms affecting the *SLC5A6* gene (239). Other studies have shown that intestinal infection with *Salmonella enterica* serovar Typhimurium (*S. typhimurium*) leads to a significant reduction in intestinal biotin uptake (64). However, the latter effect appears to be indirect, involves proinflammatory cytokines, and is mediated via NF-κB signaling pathway (64). Similarly, exposure of colonic epithelial cells to the bacterial endotoxin lipopolysaccharide (LPS) (a major component of the outer membrane of gram-negative bacteria) leads to a significant inhibition in biotin uptake via interference with the portion of the SMVT protein exposed at the cell surface; the latter appears to be mediated via a protein casein kinase (CK-2) -mediated pathway (109). Finally, intestinal biotin uptake process appears to be sensitive to the effect of the anticonvulsant drugs carbamazepine and primidone (206).

Cobalamin (Vitamin B12)

The discovery of vitamin B12 (B12) and its almost magic effect is related to the disease pernicious anemia. Back in time patients suffering from this condition presented with severe megaloblastic anemia and often devastating neurological symptoms. Searching for a suitable treatment of the deadly disease finally led to the isolation of B12 in 1948, and to a pathophysiological explanation for the condition. The patients had lost the ability for gastrointestinal absorption of the vitamin; but if treated with pharmacological doses of B12 in time—and for life—all symptoms could be prevented [for a historical review see (25)]. The pathophysiological explanation for the disease has turned out to be an autoimmune-induced destruction of the parietal cells that produce intrinsic factor, a protein needed for the gastrointestinal uptake of B12. Details are described in the following sections.

The chemical name for B12 is cobalamin, which in turn reflects its structure. The core of the molecule is a corrin ring surrounding a central cobalt atom. Attached to the cobalt atom is a lower ligand common to all forms of B12, and an upper ligand, specific for the various forms of the vitamin, Table 3 [for details on chemistry see (160)]. Two forms have coenzyme functions, the so-called coenzyme-B12's, methyl-B12, and 5′-deoxyadenosyl-B12. Methyl-B12 is coenzyme for the cytoplasmic methionine synthetase (5-methyltetrahydropteroyl-L-glutamate: L-homocysteine-S-methyltransferase; EC 2.1.1.13). The enzyme converts homocysteine to methionine which in turn leads to the formation of the methyl donor S-adenosylmethionine. In addition, the enzyme is essential for a normal DNA synthesis, where also folate is at play (see the section on folate). The B12-form 5′-deoxyadenosyl-B12 is coenzyme for the mitochondrial methylmalonyl-CoA mutase (methylmalonyl-CoA CoAcarbonylmutase; EC 5.4.99.2) that plays a role for the catabolism of odd chain fatty acids, branched amino acids, and cholesterol. Coenzyme-B12's are converted to hydroxo/aquo-B12 (HO-B12) upon exposure to light, and because of that HO-B12 accounts for the major part of endogenous B12 in food. The synthetic form of the vitamin, cyano-B12 (CN-B12), is widely employed in vitamin pills [for reviews see (31, 157)].

In humans, the total amount of B12 is in the magnitude of 2 to 3 mg. This figure is derived from old studies employing two different approaches, isotope dilution or direct measurement [for review see (42)]. The principle of the isotope dilution method is to administer labeled B12, wait until it is assumed that an equilibrium has been reached between administered and endogenous B12 and then calculating the total amount of endogenous B12 from a measure of label and endogenous B12 in a representative biopsy, for example, from the liver (1). Though several concerns can be raised against this method the results fit reasonably well with estimates based on *post mortem* measures of the B12 content in tissues (167).

The absorptive capacity of B12 is limited. Only around 1 μg is absorbed from a single dose of the vitamin, and following this there is a lag of 4 to 6 h before a new dose can be absorbed with the same efficiency [for review, see (31)]. This in turn implies that the maximal capacity for active B12 uptake is in the magnitude of 4 to 6 μg/24 h. When exposed to pharmacological amounts of the vitamin 1% of the dose will be absorbed by passive absorption (27).

The turnover rate of B12 in healthy humans is exceptionally slow, with a daily loss of no more than around 0.13% [for review see (42)] but somewhat faster in individuals unable to absorb the vitamin, because they do not benefit from B12 recycled with bile (5).

Part of the daily loss is possibly explained by degradation of B12 to inactive corrinoids, also referred to as B12-analogues. B12-analogous are present in human stool, bile, and blood, but it is still argued to which extent they are derived from metabolism of B12 and to which extent they are produced by microorganisms in the gut (4, 48, 84). Cobinamide has been suggested as a predominant analogue (84), but final proofs as to the nature of the analogues are pending.

Absorption of dietary vitamin B12

Vitamin B12 is requested in the lowest quantity of all vitamins. The daily requirement is 2.4 μg for adults, 2.6 μg during pregnancy, and 2.8 μg during lactation (41). The vitamin is exclusively produced in microorganisms, and is supplied to humans through food of animal origin. Because of that it is often supplied in insufficient amounts in a vegetarian diet [for reviews see (68, 158)].

In food B12 is present either as the coenzyme forms or as HO-B12, and most is protein bound. Its gastrointestinal uptake depends on a coordinated action between enzymes capable of liberating the vitamin from food items, soluble B12 binding proteins, and receptor molecules [for review see (154)].

Three classes of soluble B12 binding proteins have been described, each of them present in the gastrointestinal tract of either humans or other animals. The three proteins, transcobalamin, intrinsic factor, and haptocorrin are phylogenetically related with transcobalamin (gene: TCN2) as the oldest, then follows intrinsic factor (gene: GIF) and finally haptocorrin (gene: TCN1) that most likely is evolved from duplication of the intrinsic factor gene (74, 94). The proteins have been purified from natural sources (152) and after *in vitro* cloning from man as well as other species (50, 52, 93). They are structurally comparable (59, 125, 259) and all consists of two parts that is sandwiched around the B12 molecule [for review see (54)]. Intrinsic factor is always expressed in relation to the gastrointestinal tract. Interestingly the expression of transcobalamin and haptocorrin varies across species; some species secrete transcobalamin in saliva and milk, while others, including man, secrete haptocorrin (53, 75, 91, 153).

The function of haptocorrin is unsettled. In humans and many animal species it is present in most extracellular fluids, but curiously it is not expressed at all in rodents (74, 91). Haptocorrin is characterized by its ability to recognize both active forms of B12 and B12-analogues (243). In the human gastrointestinal tract, it is synthesized by the salivary glands and released into saliva (153), with an estimated daily output of more than 15 nmol (corresponding to a binding capacity of more than 20 μg B12). It is also released from the gallbladder together with B12 (78). In addition, human milk contains an increasingly high concentration during the lactation period with an output of more than 100 nmol/24 h at lactation month nine (75). Other species like dog and hog synthesize the protein in the stomach (86, 123).

In the gastrointestinal tract haptocorrin may play a role for the protection of B12 during its journey through the acidic and pepsin attacking gastric juice. Haptocorrin and its binding of B12 are resistant to both low pH and pepsin (3). In the small intestine haptocorrin is degraded, notably by trypsin and chymotrypsin and the attached B12 is liberated (3).

Intrinsic factor is mandatory for a normal intestinal uptake of B12. It is a glycoprotein, and the carbohydrate moiety is considered of importance for its unique stability toward enzymatic attacks on its journey from the upper gastrointestinal tract and until it is recognized by specific receptors in the distal part of the small intestine. Intrinsic factor consists of one amino acid chain (417 amino acids) with N-terminal α-subunit united by a single amino acid strand to a C-terminal β-subunit. Disruption of the uniting strand has no apparent influence on binding to B12 or to the receptor [for review see (54)].

The daily output of intrinsic factor is in the magnitude of 20 nmol, which is considerably higher than the few nanomol of B12 absorbed every day (31). Intrinsic factor binds B12 independent of the upper ligand (e.g., HO-B12 or CN-B12) with a dissociation constant exceeding 10^{12} mol/L (51), but does not recognize B12-analogues (243). These features ensure that only active B12 forms are presented for absorption in the terminal ileum, that even very low concentrations of B12 is captured and that various B12 forms are absorbed to the same extent.

The production site for intrinsic factor shows some variation among species. In humans, intrinsic factor is mainly synthesized in the parietal cells of the stomach (85, 113). Dog intrinsic factor is produced in pancreatic duct cells and rodent intrinsic factor in gastric chief cells (215, 218).

Transcobalamin is best known as the B12 transport protein that ensures a receptor-mediated transport of B12 from the bloodstream and into all the cells of the body (165). In addition, the protein is present in saliva of rodents and in the milk of certain species including the cow (53, 91). Potentially the protein may have two functions in the gastrointestinal tract. Like haptocorrin it may cargo B12 until it is transferred to intrinsic factor in the small intestine. In addition, transcobalamin may promote an intrinsic factor independent uptake of B12. So far this has only been supported by *in vitro* studies (87).

B12 bound to intrinsic factor or possibly transcobalamin is internalized after binding to specific receptors present on the brush border membranes of the enterocytes. Cubam recognizes B12 in complex with intrinsic factor and megalin recognizes transcobalamin [for review see (154)].

The multifunctional receptor cubam is mandatory for a normal intestinal uptake of B12. Cubam is an abbreviation for cubilin and amnionless. Cubilin is a huge one amino acid

chain protein with no membrane spanning motif. Initial studies suggested cubilin to act in concert with megalin for uptake of intrinsic factor bound B12, but later the membrane spanning molecule, amnionless was proven to be the partner (61). Cubam has been identified in several tissues including the intestine and the proximal tubules of the kidney. In the intestine, cubam is located to the brush border membranes of the distal ileum, where its main function is recognition of intrinsic factor-B12. In the kidney, cubam is located to the luminal part of the proximal tubules and plays an important role for ensuring reabsorption of multiple proteins from the ultrafiltrate including vitamin D binding protein (Gc) transferrin and albumin [for review see (155)]. This dual function explains why patients—and dogs—with mutations in the receptor may display both B12 malabsorption and proteinuria (60, 242).

The N-terminal part of cubilin is responsible for recognizing intrinsic factor. The binding is calcium dependent, and the receptor recognizes only intrinsic factor saturated with B12 (7).

Amnionless provides membrane anchorage of cubilin and thereby endocytic capacity of the entire complex including intrinsic factor-B12 (61). Internalization is promoted via binding to the clathrin-associated sorting proteins disabled-2 (Dab2) and autosomal recessive hypercholesterolemia protein (ARH) (159). In addition, amnionless is essential for escorting cubilin to the surface of the cells (149).

Once internalized into the endosomes intrinsic factor-B12 dissociates from the receptor, and cubam recycles to the surface of the enterocyte. Intrinsic factor is degraded in the lysosome, a degradation that may involve cathepsin L (70). Export of B12 from the lysosomes and into the cytoplasm of the enterocytes request two membrane anchored transporters, LMBRD1, a nine-path protein, ABCD4 (38), and the ZNF143 transcriptional factor (161). The major part of the absorbed B12 is destined for the circulation and unlikely to be further modified in the enterocyte. For example, most of orally administered CN-B12 is recovered as such in the circulation (83).

The export of B12 from the enterocyte remains controversial. A persistent view has been that B12 is bound to transcobalamin within the enterocyte and that the complex is released into the circulation. This view has been supported by a high expression of mRNA coding for transcobalamin in the intestinal cells (164). The concept of the Schillings test (no longer available) conflicts with this view. The Schillings test is an absorption test that measure B12 excreted in the urine following an oral test dose of labeled B12 combined with an intramuscular dose of B12 that saturates all the circulating B12-binding capacity (212). If B12 was delivered to the circulation bound to transcobalamin no B12 would reach the urine. This argument let to the search for transporters capable of escorting B12 out of the enterocyte, and led to the identification of MRP1. MRP1 is a multifunctional ATP-dependent transporter localized on basolateral membranes of polarized cells and involved in export of endogenous molecules such as steroids and prostaglandins and several drugs [for review see (34)]. Both *in vitro* studies and studies on MRP1 knockout mice (16) strongly supports that MRP1 is involved in the transport of B12 across the intestinal basolateral membrane, but the results also underscore that other exporters are at play. Though the capacity for uptake of B12 is decreased in MRP1 knockout mice they are still capable of absorbing enough of the vitamin to prevent any symptoms of B12 deficiency (16).

The other B12-related receptor present in the gastrointestinal tract is megalin. This multifunctional receptor is widely distributed in the body. Like cubam, it participates in the reabsorption of proteins in the kidney [for review see (155)]. Its localization and function in the gastrointestinal tract remains controversial. It binds transcobalamin both saturated and unsaturated with B12 and *in vitro* studies have shown that megalin expressing Caco2 cells (a proxy for intestinal enterocytes) display a specific uptake of transcobalamin bound B12 (87). It has been speculated that this may represent a mechanism whereby B12 bound to transcobalamin in, for example, cow's milk (53) can be absorbed independent of the intrinsic factor pathway, but so far convincing evidence is lacking.

Over the year's researchers have attempted to use the intestinal uptake system for B12 to ensure uptake of other cargoes, such as orally administered peptides (e.g., insulin). The work has been driven by the observation that binding of the peptide-B12 conjugates to intrinsic factor decrease the susceptibility toward enzymatic digestion (20). An unsolved problem is that the B12 conjugates may well compete for the limited uptake capacity of B12, and thereby induce B12 deficiency. A study on mice highlights this concern (133).

Factors influencing the uptake of vitamin B12

The gastrointestinal uptake of B12 is vulnerable and influenced not only by the supply of B12 but also by many other factors. An insufficient uptake is common in areas with a low intake of animal products, but even in communities where the dietary supply of B12 is sufficient up to 20% of individuals above the age of 60 years is considered to have an impaired B12 status [for review see (72)].

The form of the vitamin supplied (e.g., CN-B12 in vitamin pills or HO-B12 in food items) is unlikely to influence the gastrointestinal uptake as recently confirmed in a study in rats. Curiously, the study showed that the liver accumulated high amounts of the absorbed HO-B12 while the kidney accumulated a relatively high amount of the absorbed CN-B12 (99).

Microbial consumption of B12 may influence the B12 available for gastrointestinal uptake as exemplified by older work on the fish tapeworm, *Diphyllobothrium latum* (156).

To some extent the source of food B12 will influence the uptake of B12 because the bioavailability varies. Dairy products have a high bioavailability of B12 while it is low in seafood and eggs, because these food sources are reach in B12 binding proteins (42, 112).

Age is an important factor. With age the capacity to degrade food-bound B12 decreases, and at the same time the

gastric function decreases typically followed by a decrease in the output of intrinsic factor (29, 96). A decreased capacity for degradation of B12 binding proteins is observed also in patients with an impaired pancreatic function, and thereby a decreased release of digestive enzymes [for review see 72, 77)].

The output of intrinsic factor strongly influences the B12 absorption. In healthy humans, the synthesis of intrinsic factor occurs in parallel with gastric acid stimulation (97, 176) and this in turn may suggest a poor uptake of B12 administered as a vitamin pill without simultaneous intake of food. Lack of intrinsic factor totally impairs active B12 absorption, as evident in patients with pernicious anemia.

Pernicious anemia occurs in about 0.1% of the population of the Western world. The condition is caused by an autoimmune-induced chronic atrophic gastritis with destruction of gastric parietal cells. The gastric H+/K+ATPase (proton pump) is the primary causative autoantigen [for review see (72)]. Molecular mimicry by *Helicobacter pylori* antigens has questioned a microbial trigger for the initiation of the autoimmune gastritis [for review see (108)].

An impaired release of intrinsic factor is related to numerous other conditions including any kind of gastric body atrophy, total gastrectomy, gastric bypass, or any drug affecting the output of gastric acid, notably proton pump inhibitors. Finally, mutations in the GIF gene coding for intrinsic factor represents a rare cause for an impaired production of intrinsic factor [for review, see (71)].

Factors affecting the absorption of B12 in the terminal ileum include intestinal diseases and specific alterations in the expression of the cubam receptor. Patients with Crohn's disease and ulcerative colitis, notably those undergoing resection of more than the 20 distal centimeters of ileum will have an impaired expression of cubam and thereby a decreased capacity for absorption of B12 (15).

A few studies show the expression of cubam to be regulated by hormones. Thyroidectomized rats show a 70% decrease in the expression of cubilin that can be reverted upon treatment with thyroxin (260). Placental lactogen peptide increase the capacity for B12 uptake as judged from studies in the mice (178). This has been interpreted to indicate an increased uptake of B12 during pregnancy, a theory not confirmed in human studies (73).

Mutations in both cubilin and amnionless have been reported in humans suffering from the Imerslud-Gräsbeck syndrome, and such mutations have underscored the function of the two parts of the receptor in relation both to the uptake of B12 and in relation to protein reabsorption in the kidney (245).

Today the capacity for absorption of B12 can be explored with the CobsSorb test, a test that measures the increase in circulating B12 following a test dose of the vitamin (76). The test cannot clarify the cause for malabsorption of the vitamin, but it can help identifying patients unable to absorb B12 and thereby in need for life-long treatment either with injections or with high-dose oral B12 [for review see (72)].

Folate (Vitamin B9)

Folate (a term that refers to derivatives of folic acid) is involved in the synthesis of pyrimidine and purine nucleotides, and in the metabolism of several amino acids (including homocysteine). Folate deficiency/suboptimal levels occur in a variety of conditions including chronic alcoholism, Hereditary folate malabsorption syndrome (HFMS; an autosomal-recessive disorder; 62), patients with inflammatory bowel disease celiac disease, tropical sprue, and those on long-term use of certain therapeutic agents (e.g., sulfasalazine, trimethoprim, pyrimethamine, diphenylhydantoin). Such deficiency leads to a variety of conditions including megaloblastic anemia and growth retardation; when it occurs in pregnancy, it could lead to neural tube defects in the developing embryo. In addition, it has been related to an increased risk of developing cardiovascular diseases.

Absorption of dietary and microbiota-generated folate

Folate in the diet exists in the free (i.e., monoglutamate) and polyglutamate forms. Folate polyglutamates are hydrolyzed in the small intestine to folate monoglutamates prior to absorption via the action of the enzyme folate hydrolase (30). Uptake of the generated folate monoglutamates occurs mainly in the proximal part of the small intestine and involves a specific, acidic pH- (but not Na-) dependent carrier-mediated process (Fig. 1) (reviewed in 202). The mechanism of folate exit out of the enterocytes across the BLM, also occurs via a specific carrier-mediated mechanism (43).

As to the folate generated by the gut microbiota, a considerable amount of this folate exists in the free (absorbable) monoglutamate forms (180). Absorption, again involves an efficient and specific carrier-mediated process (Fig. 2) (44, 101).

The normal human (mammalian) intestine expresses two folate uptake systems: the proton-coupled folate transporter (hPCFT; product of the *SLC46A1* gene; 89, 162, and 263), and the reduced folate carrier (hRFC; product of the *SLC19A1* gene; 255). The hPCFT protein (459 AA) plays a critical role in the uptake of dietary folate monoglutamates (89, 162, and references therein). Mutations in this transporter are the cause of HFMS (89, 162, and references therein). PCFT operates optimally at acidic pH 5.5 to 6.0 (with minimal activity at pH 7.0 and above) and cotransport folate$^-$ with H^+ via an electrogenic process (89, 162, 263, and references therein). The hPCFT protein is mainly expressed in the proximal small intestine (89, 162), and its expression is restricted to the apical membrane domain of the polarized enterocytes (Fig. 1) (230). As to the hRFC, this carrier functions as an anion exchanger and operates optimally at neutral/alkaline pH of 7.0 to 7.4 (89, 255). The hRFC protein (591 AA) is expressed along the intestinal tract and its expression is also restricted to the apical membrane domain of epithelial cells (254). Since this carrier operates at neutral/alkaline pHs (which is the pH at

the luminal surface of the distal small intestine and the large intestine; 190), it is reasonable to assume that this carrier plays a role in folate uptake in the distal gut (Fig. 2).

Knowledge about cell biology and structure-activity relationship of the PCFT and RFC proteins has also been forthcoming. Thus, cell surface expression of the hPCFT appears to be determined by a consensus beta-turn sequence separating predicted TM2 and TM3 (230); also, an intact microtubule network appears to be important for intracellular trafficking of the protein (230). With regards to the hRFC protein, the molecular determinants that dictate its targeting to cell membrane appear to reside within the hydrophobic backbone of the polypeptide (122, 228). Intracellular trafficking of the hRFC protein again involves trafficking vesicles whose mobility depends on the microtubule network (122). Other investigations have identified the dynein light-chain road block-1 (DYNLRB1) protein as an interacting partner with hRFC, and showed that this interaction has functional consequence (11).

With regards to structure-function relationship of the hPCFT and hRFC, a critical role for the conserved histidine residues located at positions 247 and 281 in the function of hPCFT has been reported (249). Also, clinical mutations found in the PCFT protein in patients with the HFMS (located at positions 65, 66, 113, 147, 318, 376, and 425; ref. 263 and references therein) were found to be dysfunctional due to early stop codon and a frame shift (both lead to absence of the hPCFT protein), defect in intracellular trafficking/membrane targeting of the protein, and/or protein instability (89, 263). A role for residues 161, 232, 299, and 304 in the function of the hPCFT protein has also been reported (89, 148). Finally, the hPCFT protein appears to form homo-oligomers, and that TMDs 2, 3, 4, and 6 are important for these formations (256); also, TMDs 1, 2, 7, and 11 of the hPCFT appear to form an extracellular gate in the inward-open confirmation (264). As to RFC, studies have shown a role for residues 29, 44, 45, 46, 48, 106, 107, 132, 133, 313, and 373 in the function of the transporter (89, 263, and references therein); also important is the intracellular loop between TMDs 6 and 7 (118). Other studies have reported that the RFC protein is N-glycosylated at position N58 (89). Finally, the membrane translocation pathway of hRFC appears to involve TMDs 1, 2, 4, 5, 7, 8, 10, and 11 (89).

As to regulation of the intestinal folate uptake process, important knowledge regarding transcriptional regulation of the *SLC46A1* and *SLC19A1* genes has been reported. Thus, the *SLC46A1* promoter has been cloned and characterized, its minimal region required for basal activity has been mapped to a region that is 157 bp upstream of the ATG site and contains putative GC-box sites as well as enhancer elements (YY1 and AP1), which appear to play a role in promoter activity (241). With regards to *SLC19A1*, again the promoter region of this gene has been cloned and characterized [shown to contain multiple alternative promoters that lead to the generation of distinct 5′-untranslated regions (UTRs) but with common hRFC open reading frame] (reviewed in 69, 255, 263)]. A role for nuclear factors SP, USF AP1, and C/EBP in regulating the expression of RFC in different tissues has been reported. Finally, methylation and chromatin structure also appear to play a role in regulating the function of the hRFC promoters (69, 255, 263).

Other investigations have shown that the intestinal folate uptake process is adaptively regulated by the prevailing level of the vitamin. Thus, folate deficiency was shown to lead to a specific and significant induction in intestinal folate uptake that is associated with an increase in the level of expression of RFC and PCFT mRNA and appears to involve transcription mechanism(s) (10, 163, 188, 221). The intestinal folate uptake process also appears to be developmentally regulated during early stages of life (14). In addition, the process undergoes differentiation-dependent regulation mediated via alteration in the transcription of the *SLC46A1* and *SLC19A1* genes (236). Finally, the intestinal folate uptake process appears to be under the regulation of an intracellular protein-tyrosine kinase (PTK) and a cAMP-mediated pathways (101, 194).

Factors influencing the uptake of folate

Chronic alcohol consumption is associated with folate suboptimal/deficient states. This appears to be, at least in part, mediated via inhibition in both the initial hydrolysis phase of dietary folate polyglutamates and the subsequent uptake of the generated folate monoglutamates (80, 124, 150, 252). Chronic use of certain drugs (e.g., sulfasalazine; 57) also interferes with normal intestinal folate uptake process. Finally, activity of the intestinal folate hydrolase is suppressed in diseases like celiac disease and tropical sprue (35, 79, 81).

Niacin (and Nicotinic Acid; Vitamin B3)

Niacin, a precursor for the coenzymes nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), is important for glycolysis and the pentose phosphate shunt. Deficiency/suboptimal levels of niacin occurs in chronic alcoholism and in patients with Hartnup's disease (which is caused by mutations in the gene that encodes the membrane transporter of tryptophan). Severe deficiency of niacin leads to pellagra.

Absorption of dietary and microbiota-generated niacin

There is an endogenous and exogenous source for niacin. The former is in reference to the niacin that is generated from tryptophan. The latter is in reference to the vitamin that exists in the diet and that is produced by the normal intestinal microbiota. Absorption of dietary niacin in the small intestine as well as the niacin that is generated by the microbiota occurs via an acidic pH- (but Na^+-) dependent, specific, and high-affinity carrier-mediated process (Figs. 1 and 2) (102, 135). Molecular identity of the transport system involved, however, is not known so far. The intestinal niacin uptake process

appears to be under the regulation of an intracellular protein-tyrosine kinase (PTK)-mediated pathway; it is also adaptively regulated by the prevailing substrate level (102).

Pantothenic Acid (Vitamin B5)

Pantothenic acid plays an important role in normal carbohydrates, fat, and protein metabolism via its role in the biosynthesis of coenzyme A and acyl carrier proteins. Since pantothenic acid is widely distributed in diet, spontaneous deficiency of the vitamin has not been reported in humans.

Absorption of dietary and microbiota-generated pantothenic acid

Like a number of other water-soluble vitamins, pantothenic acid is presented to the intestinal tract from two sources: the diet and the gut microbiota (258). In the diet, pantothenic acid exists mainly in the form of coenzyme A; this form is converted to free pantothenic acid prior to absorption (216). Absorption of free pantothenic acid in the small intestine and that generated by the gut microbiota in the large intestine occurs by a carrier-mediated process that involves the sodium-dependent multivitamin transport (SMVT) system, that is, its shares the same transport system with biotin (Figs. 1 and 2) (187,190,199). Little is known about how the intestinal/colonic pantothenic acid uptake process is regulated.

Pyridoxine (and Derivatives; Vitamin B6)

Pyridoxine (mainly in the form of pyridoxal 5′-phosphate, the most biologically active form of the vitamin) is a cofactor for enzymes involved in carbohydrate, lipid, and protein (including the amino acid homocysteine) metabolism, as well as in the production of neurotransmitters. Vitamin B6 deficiency/suboptimal levels occur in different conditions including chronic alcoholism, diabetes, and celiac and renal diseases; it also occurs in patients on long-term use of hydrazines (antidepressant) and penicillamine (antirheumatic drug). Such deficiency leads to a variety of clinical abnormalities including microcytic anemia, dermatitis/glossitis, and neurological disorders.

Absorption of dietary and microbiota-generated vitamin B6

Pyridoxine like other members of the vitamin B6 family, that is, pyridoxal and pyridoxamine, exist in the diet in the phosphorylated and nonphosphorylated forms, which are hydrolyzed to free forms prior to absorption (82). Absorption of the liberated free forms of vitamin B6 occurs via an efficient and specific carrier-mediated mechanism (Fig. 1) (198). This process is acidic pH- (but is sodium-) dependent and amiloride sensitive (198).

As to vitamin B6 generated by the gut microbiota, considerable amount of this vitamin exists in the free form, and thus, available for absorption (116). As to the mechanism involved in this uptake process, evidence has emerged in recent years to show that this occurs via an efficient and specific carrier-mediated mechanism (Fig. 2) (209). Nothing, however, is currently known about molecular identity of the transport system involved.

Factors influencing the uptake of vitamin B6

The intestinal vitamin B6 uptake process is adaptively regulated by the prevailing level of the vitamin in the surrounding media. Thus, cells maintained under low pyridoxine level takes in more vitamin than cells maintained in the presence of high levels. This induction appears to be transcriptionally mediated (209). Other studies have reported that the intestinal vitamin B6 uptake process is under the regulation of an intracellular protein-kinase A-mediated signaling pathway (198).

Riboflavin (Vitamin B2)

In its biologically active forms [i.e., flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD)], riboflavin plays important roles in oxidation-reduction reactions involving carbohydrate, lipid, amino acids, and processing of certain water-soluble vitamins. Other studies have shown that riboflavin plays a role in protein folding (248), energy metabolism (88), has antioxidant and anti-inflammatory properties (111, 117, 214), and is needed for normal immune function (127). Riboflavin deficiency/suboptimal levels occur in chronic alcoholism, diabetes mellitus, and inflammatory bowel disease; they also occur in Brown-Vialetto Van Laere and Fazio Londe syndromes [rare neurological disorders caused by mutations in membrane riboflavin transporter-2 and -3 (RFVT-2 and RFVT-3)] (22, 71). Such deficiency leads to a range of clinical abnormalities including degenerative changes of the nervous system, anemia, skin lesions, cataract, and growth retardation.

Absorption of dietary and microbiota-generated riboflavin

Riboflavin in the diet exists mainly in the forms of FMN and FAD and is bound to protein. Digestions begins by releasing these forms from the binding proteins (via the action of gastric acid and hydrolases), followed by hydrolysis (by intestinal phosphatases) to free riboflavin (37). Absorption of riboflavin then takes place via a specific and efficient, sodium-independent carrier-mediated process (Fig. 1) (129,189,192,193,246). Internalized riboflavin is then transported out of the enterocytes across the BLM via a specific carrier-mediated mechanism (199).

As to the riboflavin generated by the large intestinal microbiota, a considerable amount of this riboflavin exists in the free

form, and thus, available for absorption (92, 220). Indeed, the large intestine is capable of absorbing riboflavin and for this, it utilizes an efficient and specific carrier-mediated mechanism (Fig. 2) (200).

Three riboflavin vitamin transporters, RFVT-1, RFVT-2, and RFVT-3 (encoded by *SLC52A1*, *SLC52A2*, and *SLC52A3* genes, respectively) that share varied degrees of sequence similarity have been identified in human (mammals) tissues (262). All these transporters are expressed in the gut with expression of the RFVT-3 being significantly higher than RFVT-1 and RFVT-2 (238, 262); RFVT-3 also appears to be a more efficient transporter for the vitamin than the other two transporters (238). Furthermore, while expression of RFVT-3 is restricted to the apical membrane domain of the polarized absorptive epithelial cells, expression of RFVT-1 is mostly at the BLM domain of these cells while that of RFVT-2 is mainly expressed inside intracellular vesicles (238).

The RFVT-3 is the major contributor toward carrier-mediated riboflavin absorption in the gut. This was established in studies utilizing an *in vitro* gene-specific silencing (siRNA) approach (238), and an *in vivo* intestinal-specific (conditional) *SLC52A3* knockout (cKO) mice (224). In the latter study, all the RFVT-3 cKO mice developed riboflavin deficiency, demonstrating the importance of RFVT-3 in regulating the overall body homeostasis of the vitamin (224).

Knowledge about cell biology and structure-activity relationship of the hRFVT-3 has also been forthcoming in recent years. Thus, cell surface expression of the hRFVT-3 protein appears to be determined by a sequence in the C-terminal tail of the polypeptide, with a special role for the conserved cysteine residues located at positions 463 and 467 (235). The putative disulfide bridge between C463 and C386 also appears to be important for membrane targeting of the RFVT-3 protein (235). In addition, intracellular trafficking of the RFVT-3 protein appears to involve distinct vesicular structures, and that mobility of these vesicles depends on the microtubule network (235). Moreover, the recent identification of clinical mutations in *SLC52A3* in patients with the Brown-Vialetto-Van Laere syndrome has furthered our understanding of the structure-function and cell biology of hRFVT-3 (144). Thus, clinical mutants P28T, E36K, E71K, and R132W were all found to be functionally defective and that this is due to intracellular retention of the mutated hRFVT-3 (144).

As to regulation of the intestinal RF uptake process, important knowledge regarding transcriptional regulation of the *SLC52A1* and *SLC52A3* genes has also been reported in recent years. Thus, the S*LC52A1* promoter has been cloned and characterized, its minimal region required for basal activity has been identified (between −234 and −23), and a role for the cis-element Sp-1 has been shown (184). With regards to *SLC52A3*, again the promoter of this gene has been cloned and characterized *in vitro* and *in vivo*, its minimal promoter region required for basal activity was identified (between −199 and +8 bop), and a role for the Sp1 binding site (at position −74/−71 bop) in determining the activity of the *SLC52A3* promoter has been demonstrated (65). More recent investigations have shown that the RFVT3 is a target for post-transcriptional regulation by miRNAs (specifically miR-423-5p) in intestinal epithelial cells, and that this regulation has functional consequences on intestinal riboflavin uptake (110).

Other investigations have shown that the intestinal riboflavin uptake process is adaptively regulated by the prevailing level of the vitamin (191, 193). This adaptive regulation appears to be mediated via changes in the level of expression of the hRFVT-3 (and hRFVT-2; but not hRFVT-1) (231). Focusing on the predominant hRFVT-3, the adaptive regulation appears to be exerted at the level of *SLC52A3* transcription and involves the nuclear factor Sp1 (222); it also involved epigenetic mechanism(s) and changes in the level of expression of the hRFVT-3 protein at the cell surface (222).

The intestinal riboflavin uptake process also appears to be developmentally regulated during early stages of life and that this regulation is mediated via a decrease in the V_{max} and an increase in the apparent K_m of the riboflavin carrier-mediated uptake process. The molecular mechanism(s) that mediates this type of regulation in intestinal riboflavin uptake, however, is not known at present. In addition, the process was found to undergo differentiation-dependent regulation mediated via alteration in the transcription rate of the *SLC52A1* and *SLC52A3* genes (223). Finally, the intestinal riboflavin uptake process appears to be under the regulation of an intracellular protein-kinase A- and Ca^{2+}/calmodulin-mediated signaling pathways (192).

Factors influencing the uptake of riboflavin

Chronic alcohol exposure is associated with riboflavin suboptimal/deficient states. This appears to be, at least in part, mediated via inhibition in small intestinal and colonic riboflavin uptake and based on studies with animal models believed to be exerted at the level of transcription of the *slc52a1* and *slc52a3* genes (237). The Na^+/H^+ exchanger amiloride and the antipsychotic tricyclic phenothiazine agent chlorpromazine (the latter shares structural similarities with riboflavin) also cause inhibition in intestinal riboflavin uptake (246).

Thiamin (Vitamin B1)

Thiamin (mainly in its diphosphate form, i.e., thiamin pyrophosphate, TPP) acts as a cofactor for a number of enzymes that catalyze important metabolic reactions relate to energy metabolism and ATP production (reviewed in 188); the vitamin also plays a role in reducing cellular oxidative stress (28, 58). It is therefore not surprising that deficiency of this vitamin at the cellular level leads in impairment in energy metabolism/ATP production and to oxidative stress. Other forms of thiamin, like thiamin triphosphate (TTP), have also been reported as having biological activity (e.g., TTP regulates the function of membrane chloride channels in nerve cells; 17). Thiamin deficiency/suboptimal levels occur in chronic alcoholism, in patients with diabetes, celiac sprue,

renal diseases, those undergoing bariatric surgery, those with sepsis, following long-term use of furosemide, and in the elderly (reviewed in 188). Such deficiency leads to a range of clinical abnormalities including neurological and cardiovascular disorders.

Absorption of dietary and microbiota-generated thiamin

In the diet, thiamin exists mainly in the phosphorylated forms which are hydrolyzed to free thiamin (a monocationic compound at luminal pH) prior to absorption. This step is catalyzed by intestinal phosphatases (219) [the small intestine does not have an uptake system for TPP, i.e., it is unlike the large intestine which possess such an efficient uptake system; see succeeding text]. The proximal small intestine is the preferential site of absorption of the liberated free thiamin, and absorption occurs via a specific, pH (but not sodium) -dependent, electroneutral, and amiloride-sensitive carrier-mediated process (Fig. 1) (46, 177, 197). Internalized free thiamin is then transported out of the enterocytes across the BLM; this event is also specific and involves a carrier-mediated mechanism (45, 177).

As to the thiamin that is generated by the gut microbiota, this source of vitamin provides thiamin in both free and phosphorylated (mainly TPP) forms (8, 147, 258). Recent studies have shown that human colonocytes can absorb both forms of thiamin and that they do so via distinct and efficient carrier-mediated processes (Fig. 2) (134, 139, 201).

Thiamin transporter-1 and -2 (THTR-1 and THTR-2; products of the *SLC19A2 and SLC19A3* genes, respectively; 40, 47, 56, 106, 166) are both expressed in the small and large intestines (169, 175, 185). Both transporters appear to be involved in thiamine uptake as shown in studies utilizing gene-specific siRNA knockdown, and *SLC19A2* and *SLC19A3* knockout approaches (169, 185). The human (h)THTR-1 (a 497 AA protein) and hTHTR-2 (496 AA protein) share 48% identity with one another; they also share around 40% identity with the human-reduced folate carrier (hRFC) (47, 49). However, neither hTHTR-1 nor hTHTR-2 transports folate, and hRFC does not transport free thiamin (166). As to function, the hTHTR-1 appears to operate in the micromolar range, while the hTHTR-2 appears to operate in the nanomolar range of the vitamin (9). The hTHTR-1 is expressed at a higher level than hTHTR-2 in the human intestine (175, 185); it is also expressed at both the apical and the basolateral membrane domains of the polarized enterocytes, while expression of the hTHTR-2 is restricted to the apical membrane domain only (185, 227).

As to absorption of the microbiota-generated TPP in the large intestine, this occurs via a specific and efficient carrier-mediated process (139) that involves the thiamin pyrophosphate transporter (TPPT; product of the *SLC44A4* gene) (134). The hTPPT is expressed in the colon but not in the small intestine with expression being restricted to the apical membrane domain of the polarized colonocytes (134). This tissue-specific expression of the TPPT along the intestinal tract appears to be established by epigenetics as well as miRNA-mediated mechanisms (136).

Knowledge about cell biology and structure-activity relationship of the hTHTR-1 and hTHTR-2 has also been forthcoming in recent years. Thus, an essential role for the N-terminal and the backbone of the hTHTR-1 polypeptide in membrane targeting of the transporter has been demonstrated (227). Intracellular movement of the hTHTR-1 appears to involve trafficking vesicles whose mobility depends on the microtubule network (227). As to the hTHTR-2 protein, an essential role for the transmembrane backbone in membrane targeting has been reported (232); again, intracellular movement of hTHTR-2 to the cell surface involves trafficking vesicles that depends on intact microtubule network (232). Other investigations have identified a member of the human tetraspanin family of proteins, the hTspan-1, and the human transmembrane 4 superfamily 4 (hTM4SF4) as interacting partners with the hTHTR-1 and hTHTR-2, respectively, in human intestinal epithelial cells (141, 234). These accessary proteins were shown to influence the functionality and/or cell biology of these thiamin transporters.

Knowledge about structure-function relationship of the hTHTR-1 protein came mainly from clinical findings of mutations in the transporter in patients with the autosomal recessive disorder, thiamin responsive megaloblastic anemia (TRAM), a condition caused by mutation in the *SLC19A2* gene (107, 132, 153). These mutations were found to alter the functionality of hTHTR-1 via their effects on level of expression/stability, membrane targeting, and/or alteration in transport activity of the transporter. Experimentally, a role for the anionic amino acid residue located at position 138 of the hTHTR-1 polypeptide (the only conserved anionic residue in the TMDs of the protein) in the transport of the positively charged thiamin has been identified (13). With regards to the hTHTR-2 polypeptide, again data from clinical and experimental investigations have shown that the two clinical mutations (K44E and E320Q) identified in this transporter in patients with thiamin-responsive Wernicke's-like encephalopathy are important for function. Other studies have reported an important role for G23 and T422 in transport function of hTHTR-2 (231). Finally, insight into structure-function relationship of the colonic TPPT has also been forthcoming in recent years (145). The protein appears to be glycosylated at positions N69, N155, N197, N393, and N416; however, only glycosylation at N69, N155, and N393 appears to have functional importance (145).

As to regulation of the intestinal thiamin uptake process, important knowledge regarding transcriptional regulation of the *SLC19A1* and *SLC19A2* has also been gained. Thus, the *SLC19A2* promoter has been cloned, its minimal region required for basal activity has been identified (between −356 and −36), and a role for the nuclear factors GKLF, NF-1, and SP-1 was demonstrated (173, 175). Also, the human *SLC19A2* promoter appears to be a target for activation by the p53 tumor suppressor transcription factor (119). With

regards to *SLC19A3*, again the promoter of this gene has been cloned, its minimal promoter region required for basal activity was identified (between −77 and +59), and a role for the stimulating protein-1 (SP1)/guanosine cytidine box (GC-box) binding site (located at position −48/−45 bop) in promotor function has been reported (140). Similarly, the promoter of the *SLC44A4* gene has been cloned, the minimal region required for basal activity has been identified (between −178 and +88), and a role for ETS/ELF3 [E26 transformation-specific sequence (ETS) proteins], cAMP-responsive element (CRE), and SP1/GC-box sequence motifs in activity of the *SLC44A4* promoter has been demonstrated (137).

Other investigations have shown that the intestinal thiamin uptake process is adaptively regulated by the prevailing thiamin level (146, 168). This adaptive regulation appears to be mediated via a change in the level of expression of THTR-2 (but not THTR-1) and appears to be transcriptionally mediated (146, 168). The thiamin level-responsive region in the *SLC19A3* promoter appears to be located in a sequence between −77 and −29; also a role for the SP1/GC-box in mediating the effect of extracellular thiamin level on *SLC19A3* promoter activity has been demonstrated (146). Similarly, the colonic TPP uptake process appears to be adaptively regulated by extracellular TPP level. This regulation, however, appears to be of two types. After a short-term exposure, the adaptive regulation appears to be mediated via a change in the fraction of the protein that is expressed at the cell membrane (transcriptional regulation does not appear to be involved). After long-term exposure, however, the adaptive regulation appears to be mediated at the level of transcription of the *SLC44A4* gene (6).

The intestinal thiamin uptake process also appears to be developmentally regulated during early stages of life and that this type of regulation is also mediated at the level of transcription of the *SLC19A2* and *SLC19A3* genes (170). In addition, the process was found to undergo differentiation-dependent regulation that is transcriptionally mediated with the differentiation-responsive region being located between −356 to −275 bp in the case of the *SLC19A2* promoter, and between −77 and −13 bp in case of the *SLC19A3* promoter (138). Further, a role for the NF1 binding site (−348 to −345 bp) in the *SLC19A2* promoter and for the SP1/GC-box binding site (−48 to −45 bp) in the *SLC19A3* promoter in mediating the differentiation-dependent regulation has been reported (138). Finally, the intestinal uptake process of free thiamin and the colonic uptake process of TPP both appear to be under the regulation of an intracellular Ca^{2+}/calmodulin (CaM)-mediated pathway (139, 197, 201).

Factors influencing the uptake of thiamin

Chronic alcohol consumption impairs intestinal and colonic thiamin absorption process (90, 240), thus contributing to the development of thiamin deficiency. This impairment was associated with a significant reduction in the level of expression of THTR-1 (but not THTR-2) (240). Also, infection with the gram-negative enteropathogenic *Escherichia coli* (EPEC; a food-borne pathogen) significantly inhibit intestinal thiamin uptake (9). This inhibition appears to be associated with a decrease in cell surface expression of THTR-1 and THTR-2 proteins as well as with a decrease in level of expression of hTHTR-1 and hTHTR-2 mRNA and activity of their respective promoters. The EPEC structural components that mediate its effect on intestinal thiamin uptake appear to be products of the espF and espH genes (9). Finally, sepsis appears to be associated with a significant inhibition in intestinal thiamin uptake, and that the degree of inhibition correlates with the severity of sepsis and is associated with a significant decrease in the level of expression of THTR-1 and THTR-2 in the gut mucosa (210).

Concluding Remarks

Today many details concerning the gastrointestinal handling of water-soluble vitamins have been clarified, but more is needed. We know now that the uptake of vitamins is rate limited, but for many of the vitamins we do not know the maximal capacity for uptake, nor do we know all the factors that may influence the uptake, and thereby alter the amount of vitamin absorbed. Also, while we do know that many of the water-soluble vitamins are supplied both by diet and by the gut microbiota, we lack knowledge as to the relative contribution of the latter source toward overall body homeostasis of these vitamins and how this is altered in different conditions. Clarification of these issues should increase our knowledge and understanding of the physiology and pathophysiology of gastrointestinal handling of vitamins.

References

1. Adams JF, Tankel HI, MacEwan F. Estimation of the total body vitamin B12 in the live subject. *Clin Sci* 39: 107-112, 1970.
2. Agrawal S, Agrawal A, Said HM. Biotin deficiency enhances the inflammatory response of human dendritic cells. *Am J Physiol Cell Physiol* 311: C386-C391, 2016.
3. Allen RH, Seetharam B, Podell E, Alpers DH. Effect of proteolytic enzymes on the binding of cobalamin to R protein and intrinsic factor. In vitro evidence that a failure to partially degrade R protein is responsible for cobalamin malabsorption in pancreatic insufficiency. *J Clin Invest* 61: 47-54, 1978.
4. Allen RH, Stabler SP. Identification and quantitation of cobalamin and cobalamin analogues in human feces. *Am J Clin Nutr* 87: 1324-1335, 2006.
5. Amin S, Spinks T, Ranicar A, Short MD, Hoffbrand AV. Long-term clearance of [57Co]cyanocobalamin in vegans and pernicious anaemia. *Clin Sci (London)* 58: 101-103, 1980.
6. Anandam KY, Srinivasan P, Subramanian VS, Said HM. Molecular mechanisms involved in the adaptive regulation of colonic thiamin pyrophosphate (TPP) uptake process. *Am J Physiol Cell Physiol* 313(6): C655-C663, 2017.
7. Andersen CB, Madsen M, Storm T, Moestrup SK, Andersen GR. Structural basis for receptor recognition of vitamin-B(12)-intrinsic factor complexes. *Nature* 464: 445-448, 2010.
8. Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, Fernandes GR, Tap J, Bruls T, Batto JM, Bertalan M, Borruel N, Casellas F, Fernandez L, Gautier L, Hansen T, Hattori M, Hayashi T, Kleerebezem M, Kurokawa K, Leclerc M, Levenez F, Manichanh C, Nielsen HB, Nielsen T, Pons N, Poulain J, Qin J, Sicheritz-Ponten T, Tims S, Torrents D, Ugarte E, Zoetendal EG, Wang J, Guarner F, Pedersen O, de Vos WM, Brunak S, Doré J, Antolín M, Artiguenave F, Blottiere HM, Almeida M, Brechot C, Cara C, Chervaux C, Cultrone A,

Delrme C, Denariaz G, Dervyn R, Foerstner KU, Friss C, van de Guchte M, Guedon E, Haimet F. Enterotypes of the human gut microbiome. *Nature* 473: 174-180, 2011.
9. Ashokkumar B, Kumar JS, Hecht GA, Said HM. Enteropathogenic Escherichia coli inhibits intestinal vitamin B1 (thiamin) uptake: Studies with human-derived intestinal epithelial Caco-2 cells. *Am J Physiol* 297: G825-G833, 2009.
10. Ashokkumar B, Mohammed ZM, Vaziri ND, Said HM. Effect of folate oversupplementation on folate uptake by human intestinal and renal epithelial cells. *Am J Clin Nutr* 86: 159-166, 2007.
11. Ashokkumar B, Nabokina SM, Ma TY, Said HM. Identification of dynein light chain road block-1 as a novel interaction partner with the human reduced folate carrier. *Am J Physiol* 297: G480-G487, 2009.
12. Balamurugan K, Ortiz A, Said HM. Biotin uptake by human intestinal and liver epithelial cells: Role of the SMVT system. *Am J Physiol* 285: G73-G77, 2003.
13. Balamurugan K, Said HM. Functional role of specific amino acid residues in human thiamin transporter SLC19A2: Mutational analysis. *Am J Physiol* 283: G37-G43, 2002.
14. Balamurugan K, Said HM. Ontogenic regulation of folate transport across rat jejunal brush-border membrane. *Am J Physiol* 285: G1068-G1073, 2003.
15. Battat R, Kopylov U, Szilagyi A, Saxena A, Rosenblatt DS, Warner M, Bessissow T, Seidman E, Bitton A. Vitamin B12 deficiency in inflammatory bowel disease: Prevalence, risk factors, evaluation, and management. *Inflamm Bowel Dis* 20: 1120-1128, 2014.
16. Beedholm-Ebsen R, van de Wetering K, Hardlei T, Nexø E, Borst P, Moestrup SK. Identification of multidrug resistance protein 1 (MRP1/ABCC1) as a molecular gate for cellular export of cobalamin. *Blood* 115: 1632-1639, 2010.
17. Bettendorff L, Hennuy B, De Clerck A, Wins P. Chloride permeability of rat brain membrane vesicles correlates with thiamin triphosphate content. *Brain Res* 652: 157-160, 1994.
18. Bianchi J, Wilson FA, Rose RC. Dehydroascorbic acid and ascorbic acid transport in the guinea pig ileum. *Am J Physiol* 250: G461-G468, 1986.
19. Blanton SH, Pandya A, Landa BL, Javaheri R, Xia XJ, Nance WE, Pomponio RJ, Norrgard KJ, Swango KL, Demirkol M, Gyiden H, Coskun T, Tokatli A, Ozalp I, Wolf B. Fine mapping of the human biotinidase gene and haplotype analysis of five common mutations. *Intern J Hum Med Gen* 50: 102-111, 2000.
20. Bonaccorso RL, Chepurny OG, Becker-Pauly C, Holz GG, Doyle RP. Enhanced peptide stability against protease digestion induced by intrinsic factor binding of a vitamin B12 conjugate of exendin-4. *Mol Pharm* 12: 3502-3506, 2015.
21. Bonjour JP. Vitamins and alcoholism. V. Riboflavin, VI. Niacin, VII. Pantothenic acid, and VIII. Biotin. *Int J Vitam Nutr Res* 50: 425-440, 1980.
22. Bosch AM, Abeling NG, Ijlst L, Knoester H, Van der Pol WL, Stroomer AE, Wanders RJ, Visser G, Wijburg FA, Duran M, Waterham HR. Brown-Vialetto-Van Laere and Fazio Londe syndrome is associated with a riboflavin transporter defect mimicking mild MADD: A new inborn error of metabolism with potential treatment. *J Inherit Metab Dis* 34: 159-164, 2011.
23. Boyer JC, Campbell CE, Sigurdson WJ, Kuo S. Polarized localization of vitamin C transports, SVCT1 and SVCT2, in epithelial cells. *Biochem Biophys Res Commun* 334: 150-156, 2005.
24. Brown BB, Rosenberg JH. Biotin absorption by distal rat intestine. *J Nutr* 117: 2121-2126, 1987.
25. Bunn HF. Vitamin B12 and pernicious anemia—The dawn of molecular medicine. *N Engl J Med*, 370: 773-776, 2014.
26. Bürzl, M., Suzuki Y, Ackermann D, Miyazaki H, Maeda N, Clémençon B, Burrier R, Hediger M. The sodium-dependent ascorbic acid transporter family SLC23. *Mol Aspects Med* 34: 436-454, 2013.
27. Butler C, Vidal-Alaball J, Cannings-John R, McCaddon A, Hood K, Papaioannou A, Mcdowell I, Goringe A. Oral vitamin B12 versus instramuscular vitamin B12 for vitamin B12 deficiency: A systematic review of randomized controlled trails. *Fam Pract* 23: 279-285, 2006.
28. Calingasan NY, Gandy SE, Baker H, Sheu KF, Smith JD, Lamb BT, Gearhart JD, Buxbaum JD, Harper C, Selkoe DJ, Price DL, Sisodia SS, Gibson GE. Noval neuritic clusters with accumulations of amyloid precursor protein and amyloid precursor-like protein 2 immunoreactivity in brain regions damaged by thiamine deficiency. *Am J Pathol* 149: 1063-1071, 1996.
29. Carmel R. Malabsorption of food cobalamin. *Baillieres Clin Haematol* 8: 639-655, 1995.
30. Chandler CJ, Wang T, Halsted CH. Pteroylpolyglutamate hydrolase from human jejunal brush borders: Purification and characterization. *J Biol Chem* 261: 928-933, 1986.
31. Chanarin I. *The Megaloblastic Anaemias* (3rd ed). Boston: Blackwell Scientific Publications, 1990.
32. Choi JL, Rose RC. Regeneration of ascorbic acid by rat colon. *Proc Soc Exp Bio Med* 190: 369-374, 1989.
33. Combs GF, Jr., McClung JP. *The Vitamins. Fundamental Aspects in Nutrition and Health* (5th ed). London, United Kingdom: Academic Press, 2017.
34. Cole SP. Multidrug resistance protein 1 (MRP1, ABCC1), a "multitasking" ATP-binding cassette (ABC) transporter. *J Biol Chem* 289: 30880-30888, 2014.
35. Corcino JJ, Reisenauer AM, Halsted CH. Jejunal perfusion of simple and conjungated folates in tropical sprue. *J Clin Invest* 58: 298-305, 1976.
36. Corpe CP, Eck P, Wang J, Al-Hasani H, Levine M. Intestinal dehydroascorbic acid (DHA) trasport mediated by the facilitative sugar transporters, GLUT2 and GLUT8. *J Bio Chem* 288: 9092-9101, 2013.
37. Daniel H, Binninger E, Rehner G. Hydrolysis of FMN and FAD by alkaline phosphatase of the intestinal brush border membrane. *Int J Vitam Nutr Res* 53: 109-114, 1983.
38. Deme JC, Hancock MA, Xia X, Shintre CA, Plesa M, Kim JC, Carpenter EP, Rosenblatt DS, Coulton JW. Purification and interaction analyses of two human lysosomal vitamin B12 transporters: LMBD1 and ABCD4. *Mol Membr Biol* 31: 250-261, 2014.
39. Dey S, Subramanian VS, Chatterjee NS, Rubin SA, Said HM. Characterization of the 5′ regulatory region of the human sodium-dependent multivitamin transporter, hSMVT. *Biochim Biophys Acta* 1574: 187-192, 2002.
40. Diaz GA, Banikazemai M, Oishi K, Desnick RJ, Gelb BD. Mutations in a new gene encoding a thiamin transporter cause thiamin-responsive megaloblastic anaemia syndrome. *Nat Genet* 22: 309-312, 1999.
41. Dietary reference intakes (DRIs): Estimated average requirements. *Food and Nutrition Board, Institute of Medicine, National Academies*. https://www.ncbi.nlm.nih.gov/books/NBK56068/table/summary tables.t1/?report=objectonly
42. Doets EL, In't Veld PH, Szczecinska A, Dhonukshe-Rutten RA, Cavelaars AE, van't Veer P, Brzozowska A, de Groot LC. Systematic review on daily vitamin B12 losses and bioavailability for deriving recommendations on vitamin B12 intake with the factorial approach. *Ann Nutr Metab*, 62: 311-322, 2013.
43. Dudeja PK, Kode A, Alnounou M, Tyagi S, Torania S, Subramanian VS, Said HM. Mechanism of folate transport across the human colonic basolateral membrane. *Am J Physiol* 281: G54-G60, 2001.
44. Dudeja PK, Torania SA, Said HM. Evidence for the existence of an electroneutral, pH-dependent, DIDS-sensitive carrier-mediated folate uptake mechanism in the human colonic luminal membrane vesicles. *Am J Physiol* 272: G1408-G1415, 1997.
45. Dudeja PK, Tyagi S, Gill R, Said HM. Evidence for carrier-mediated mechanism for thiamin transport to human jejunal basolateral membrane vesicles. *Digest Dis Sci* 48: 109-115, 2003.
46. Dudeja PK, Tyagi S, Kavilaveettil RJ, Gill R, Said HM. Mechanism of thiamin uptake by human jejunal brush-border membrane vesicles. *Am J Physiol* 281: C786-C792, 2001.
47. Dutta B, Huang W, Molero M, Kekuda R, Leibach FH, Devoe LD, Ganapathy V, Prasad PD. Cloning of the human thiamin transporter, a member of the folate transporter family. *J Biol Chem* 45: 31925-31929, 1999.
48. el Kholty S, Gueant JL, Bressler L, Djalali M, Boissel P, Gerard P, Nicolas JP. Portal and biliary phases of enterohepatic circulation of corrinoids in humans. *Gastroenterology* 101: 1399-1408, 1991.
49. Eudy JD, Spiegelstein O, Baber RC, Wlodarczyk BJ, Talbot J, Finnell RH. Identification and characterization of the human and mouse SLC19A3 gene: A novel member of the reduced folate family of micronutrient transporter genes. *Mol Gen Metabol* 71: 581-590, 2000.
50. Fedosov SN, Berglund L, Nexo E, Petersen TE. Sequence, S-S bridges, and spectra of bovine transcobalamin expressed in Pichia pastoris. *J Biol Chem* 274: 26015-26020, 1999.
51. Fedosov SN, Grisson CB, Fedosova NU, Moestrup SK, Nexo E, Petersen TE. Application of a fluorescent cobalamin analogue for analysis of the binding kinetics. A study employing recombinant human transcobalamin and intrinsic factor. *FEBS J* 273: 442-453, 2006.
52. Fedosov SN, Laursen NB, Nexo E, Moestrup SK, Petersen TE, Jensen EO, Berglund L. Human intrinsic factor expressed in the plant Arabidopsis thaliana. *Eur J Biochem* 270: 3362336-3362337, 2003.
53. Fedosov SN, Petersen TE, Nexo E. Transcobalamin from cow milk: Isolation and physico-chemical properties. *Biochim Biophys Acta* 1292: 113-119, 1996.
54. Fedosov SN. Physiological and molecular aspects of cobalamin transport. *Subcell Biochem* 56: 347-367, 2012.
55. Fernandez-Banares F, Abad-Lacruz A, Xiol X, Gine JJ, Dolz C, Cabre E, Esteve M, Gonzalez-Huix F, Gassull MA. Vitamin status in patients with inflammatory bowel disease. *Am J Gastroenterol* 84: 744-748, 1989.
56. Fleming JC, Tartaglini E, Steinkamp MP, Schorderet DF, Cohen N, Neufeld EJ. The gene mutated in thiamine-response anaemia with diabetes and deafness (TRMA) encodes a functional thiamine transporter. *Nat Genet* 22: 305-308, 1999.

57. Franklin JL, Rosenberg IH. Impaired folic acid absorption in inflammatory bowel disease: Effects of salicylazosulfapyridine (azulfidine). *Gastroenterol* 64: 517-525, 1973.
58. Frederikse PH, Farnsworth P, Zigler JS. Thiamine deficiency in vivo produces fiber cell degeneration in mouse lenses. *Biochem Biophys Res Commun* 258: 703-707, 1999.
59. Furger E, Frei DC, Schibli R, Fischer E, Prota AE. Structural basis for universal corrinoid recognition by the cobalamin transport protein haptocorrin. *J Biol Chem* 288: 25466-25476, 2013.
60. Fyfe JC, Hemker SL, Venta PJ, Stebbing B, Giger U. Selective intestinal cobalamin malabsorption with proteinuria (Imerslund-Gräsbeck syndrome) in juvenile Beagles. *J Vet Intern Med* 28: 356-362, 2014.
61. Fyfe JC, Madsen M, Hojrup P, Christensen EI, Tanner SM, de la Chapell A, He Q, Moestrup SK. The functional cobalamin (vitamin B12)-intrinsic factor receptor is a novel complex of cubilin and amnionless. *Blood* 103: 1573-1590, 2004.
62. Geller J, Kronn D, Jayabose S, Sandoval C. Hereditary folate malabsorption: Family report and review of the literature. *Medicine* 81: 51-68, 2002.
63. Ghosal A, Jellbauer S, Kapadia R, Raffatellu M, Said HM. Salmonella infection inhibits intestinal biotin transport: Cellular and molecular mechanisms. *Am J Physiol* 309: G123-G131, 2015.
64. Ghosal A, Lambrecht N, Subramanya SB, Kapadia R, Said HM. Conditional knockout of the Slc5a6 gene in mouse intestine impairs biotin absorption. *Am J Physiol* 304: G64-G71, 2013.
65. Ghosal A, Sabui S, Said HM. Identification and characterization of the minimal 5′-regulatory region of the human riboflavin transporter-3 (*SLC52A3*) in intestinal epithelial cells. *Am J Physiol* 15: C189-C196, 2015.
66. Ghoshal A, Said HM. Cys(294) is essential for the function of the human sodium-dependent multivitamin transporter. *Biochim Biophys Acta* 1818: 97-102, 2012.
67. Ghosal A, Subramanian VS, Said HM. Role of the putative N-glycosylation and PKC-phosphorylation sites of the human sodium-dependent multivitamin transporter (hSMVT) in function and regulation. *Biochem Biophys Acta* 1808: 2073-2080, 2011.
68. Gille D, Schmid A. Vitamin B12 in meat and dairy products. *Nutr Rev* 73: 106-115, 2015.
69. Gong M, Cowan KH, Gudas J, Moscow JA. Isolation and characterization of genomic sequences involved in the regulation of the human reduced folate carrier gene (RFC1). *Gene* 233: 21-31, 1999.
70. Gordon MM, Howard T, Becich MJ, Alpers DH. Cathepsin L mediates intracellular ileal digestion of gastric intrinsic factor. *Am J Physiol* 268: G33-G40, 1995.
71. Green P, Wiseman M, Crow YJ, Houlden H, Riphagen S, Lin JP, Raymon FL, Childs AM, Sheridan E, Edwards S, Josifova DJ. Brown-Vialetto-Van Laere syndrome, a Ponto-Bulbar Palsy with deafness, is caused by mutations in C20orf54. *Am J Hum Genet* 86: 485-489, 2010.
72. Green R, Allen LH, Bjørke-Monsen AL, Brito A, Guéant JL, Miller JW, Molloy AM, Nexo E, Stabler S, Toh BH, Ueland PM, Yajnik C. Vitamin B12 deficiency. *Nat Rev Dis Primers* 3: 17040, 2017.
73. Greibe E, Andreasen BH, Lildballe DL, Morkbak AL, Hvas AM, Nexo E. Uptake of cobalamin and markers of cobalamin status: A longitudinal study of healthy pregnant women. *Clin Chem Lab Med* 49: 1877-1882, 2011.
74. Greibe E, Fedosov S, Sorensen BS, Hojrup P, Poulsen SS, Nexo E. A single rainbow trout cobalamin-binding protein stands in for three human binders. *J Biol Chem* 287: 33917-33925, 2012.
75. Greibe E, Lildballe DL, Streym S, Vestergaard P, Rejnmark L, Mosekilde L, Nexo E. Cobalamin and haptocorrin in human milk and cobalamin-related variables in mother and child: A 9-mo longitudinal study. *Am J Clin Nutr* 98: 389-395, 2013.
76. Greibe E, Mahalle N, Bhide V, Heegaard CW, Naik S, Nexo E. Increase in circulating holotranscobalamin after oral administration of cyanocobalamin or hydroxocobalamin in healthy adults with low and normal cobalamin status. *Eur J Nutr* 2017. doi: 10.1007/s00394-017-1590-0. [Epub ahead of print].
77. Guéant JL, Champigneulle B, Gaucher P, Nicolas JP. Malabsorption of vitamin B12 in pancreatic insufficiency of the adult and of the child. *Pancreas* 5: 559-567, 1990.
78. Gueant JL, Monin B, Biossel P, Gaucher P, Nicolas JP. Biliary excretion of cobalamin and cobalamin analogues in man. *Digestion* 30: 151-157, 1984.
79. Halsted CH, Reisenauer AM, Romero JJ, Cantor DS, Ruebner B. Jejunal perfusion of simple and conjugated folates in celiac sprue. *J Clin Invest* 59: 933-940, 1977.
80. Halsted CH, Reisenauer AM, Shane B, Tamura T. Availability of monoglutamyl and polglutamyl folates in normal subjects and in patients with celiac sprue. *Gut* 19: 886-891, 1978.
81. Halsted CH, Robles EA, Mezey E. Decreased jejunal uptake of labeled folic acid (3 H-PGA) in alcoholic patients: Roles of alcohol and nutrition. *N Engl J Med* 285: 701-706, 1971.
82. Hamm MW, Hehansho H, Henderson LM. Transport and metabolism of pyridoxamine and pyridoxamine phosphate in the small intestine. *J Nutr* 109: 1552-1559, 1979.
83. Hardlei TF, Mørkbak AL, Bor MV, Bailey LB, Hvas AM, Nexo E. Assessment of vitamin B(12) absorption based on the accumulation of orally administered cyanocobalamin on transcobalamin. *Clin Chem* 56: 432-436, 2010.
84. Hardlei TF, Obeid R, Herrmann W, Nexo E. Cobalamin analogues in humans: A study on maternal and cord blood. *PLoS One* 8: e61194, 2013.
85. Hewitt JE, Gordon MM, Taggart RT, Mohandas TK, Alpers DH. Human gastric intrinsic factor: Characterization of cDNA and genomic clones and localization to human chromosome 11. *Genomics* 10: 432-440, 1991.
86. Hewitt JE, Seetharam B, Leykam J, Alpers DH. *Isolation* and characterization of a cDNA encoding porcine gastric haptocorrin. *Eur J Biochem* 189: 125-130, 1990.
87. Hine B, Boggs I, Green R, Miller JW, Hovey RC, Humphrey R, Wheeler TT. Transcobalamin derived from bovine milk stimulates apical uptake of vitamin B12 into human intestinal epithelial cells. *J Cell Biochem* 115: 1948-1954, 2014.
88. Hino S, Sakamoto A, Nagaoka K, Anan K, Wang Y, Mimasu S, Umehara T, Yokoyama S, Kosai K, Nakao M. FAD-dependent lysine-specific demethylase-1 regulates cellular energy expenditure. *Nat Commun* 3: 1-12, 2012.
89. Hou Z, Matherly LH. Biology of the major facilitative folate transporters SLC19A1 and SLC46A1. *Curr Top Membr* 73: 175-204, 2014.
90. Hoyumpa AM. Mechanisms of thiamin deficiency in chronic alcoholism. *Am J Clin Nutr* 33: 2750-2761, 1980.
91. Hygum K, Lildballe DL, Greibe EH, Morkbak AL, Poulsen SS, Sorensen BS, Petersen TE, Nexo E. Mouse transcobalamin has features resembling both human transcobalamin and haptocorrin. *PLoS One* 6: e20638, 2011.
92. Iinuma S. Synthesis of riboflavin by intestinal bacteria. *J Vitam* 2: 6-13, 1995.
93. Johnston J, Yang-Feng T, Berliner N. Genomic structure and mapping of the chromosomal gene for transcobalamin I (TCN1): Comparison to human intrinsic factor. *Genomics* 12: 459-464, 1992.
94. Kalra S, Li N, Yammani RR, Seetharam S, Seetharam B. Cobalamin (vitamin B12) binding, phylogency, and synteny of human transcobalamin. *Arch Biochem Biophys* 431: 189-196, 2004.
95. Karasov WH, Darken BW, Bottum MC. Dietary regulation of intestinal ascorbate uptake in guinea pigs. *Am J Physiol* 260: G108-G118, 1991.
96. Kittang E, Aadland E, Schjønsby H, Røhss K. The effect of omeprazole on gastric acidity and the absorption of liver cobalamins. *Scand J Gastroenterol* 22: 156-160, 1987.
97. Kittang E, Schjønsby H. Effect of gastric anacidity on the release of cobalamins from food and their subsequent binding to R-protein. *Scand J Gastroenterol* 22: 1031-1037, 1987.
98. Kohrogi K, Imagawa E, Muto Y, Hirai K, Migita M, Mitsubuchi H, Miyake N, Matsumoto N, Nakamura K, Endo F. Biotin-responsive basal ganglia disease: A case diagnosed by whole exome sequencing. *J Hum Genet*. 60: 381-385, 2015.
99. Kornerup LS, Juul CB, Fedosov SN, Heegaard CW, Greibe E, Nexo E. Absorption and retention of free and milk protein-bound cyano- and hydroxocobalamins. An experimental study in rats. *Biochimie* 126: 57-62, 2016.
100. Krause KH, Bonjour J, Berlit P, Kochen W. Biotin status of epileptics. *Ann New York Acad Sci* 447: 297-313, 1985.
101. Kumar CK, Moyer MP, Dudeja PK, Said HM. A protein-tyrosine kinase regulated, pH-dependent carrier-mediated uptake system for folate by human normal colonic epithelial cell line NCM 460. *J Biol Chem* 272: 6226-6231, 1997.
102. Kumar JS, Subramanian VS, Kapadia R, Kashyap ML, Said HM. Mammalian colonocytes possess a carrier-mediated mechanism for uptake of vitamin B3 (niacin): Studies utilizing human and mouse colonic preparations. *Am J Physiol* 305: G207-G213, 2013.
103. Kuroishi T, Endo Y, Muramoto K, Sugawara S. Biotin deficiency up-regulates TNF-α production in murine macrophages. *J Leukoc Biol* 83: 912-920, 2008.
104. Kuroishi T, Kinbara M, Sato N, Tanaka Y, Nagai Y, Iwakura Y, Endo Y, Sugawara S. Biotin status affects nickel allergy via regulation of interleukin-1β production in mice. *J Nutr* 139: 1031-1036, 2009.
105. Kuroishi T. Regulation of immunological and inflammatory functions by biotin. *Can J Physiol Pharmacol* 93: 1091-1096, 2015.
106. Labay V, Raz T, Baron D, Mandel H, Williams H, Barrett T, Szargel R, McDonald L, Shalata A, Nosaka K, Gregory S, Cohen N. Mutations in *SLC19A2* cause thiamin-responsive megaloblastic anaemia associated with diabetes mellitus and deafness. *Nat Genet* 22: 300-304, 1999.
107. Lagarde WH, Underwood LE, Moats-Staats BM, Calikogl AS. Novel mutation in the SLC19A2 gene in an African-American female with thiamine-responsive megaloblastic anemia syndrome. *Am J Med Genet* 125A: 299-305, 2004.

108. Lahner E, Annibale B. Pernicious anemia: New insights from a gastroenterological point of view. *World J Gastroenterol* 15: 5121-128, 2009.
109. Lakhan R, Said HM. Lipopolysaccharide inhibits colonic biotin uptake via interference with membrane expression of its transporter: A role for a casein kinase 2-mediated pathway. *Am J Physiol Cell Physiol* 312: C376-C384, 2017.
110. Lakhan R, Subramanian VS, Said HM. Role of MicroRNA-423-5p in post-transcriptional regulation of the intestinal riboflavin transporter-3. *Am J Physiol Gastrointest Liver Physiol* 313(6): G589-G598, 2017.
111. Lee ES, Corfe BM, Powers HJ. Riboflavin depletion of intestinal cells in vitro leads to impaired energy generation and enhanced oxidative stress. *Eur J Nutr* 52: 1513-1521, 2013.
112. Levine AS, Doscherholmen A. Vitamin B12 bioavailability from egg yolk and egg white: Relationship to binding proteins. *Am J Clin Nutr* 38: 436-439, 1983.
113. Levine JS, Nakane PK, Allen RH. Immunocytochemical localization of human intrinsic factor: The nonstimulated stomach. *Gastroenterology* 79: 493-502, 1980.
114. Liang WJ, Johnson D, Jarvis SM. Vitamin C transport systems of mammalian cells. *Mol Membr Biol* 18: 87-95, 2001.
115. Liang WJ, Johnson D, Ma LS, Jarvis SM. Regulation of the human vitamin C transporters expressed in COS-1 cells by protein kinase C. *Am J Physiol* 283: C1696-C1704, 2002.
116. Linkswiler H, Baumann CA, Snell EE. Effect of aureomycin on the response of rats to various forms of vitamin B6. *J Nutr* 43: 565-573, 1951.
117. Liu D, Zempleni J. Low activity of LSD1 elicits a pro-inflammatory gene expression profile in riboflavin-deficient human T Lymphoma Jurkat cells. *Genes Nutr* 9: 422, 2014
118. Liu XY, Witt TL, Matherly LH. Restoration of high-level transport activity by human reduced folate carrier/ThTr1 thiamine transporter chimaeras: Role of the transmembrane domain 6/7 linker region in reduced folate carrier function. *Biochem J* 369: 31-37, 2003.
119. Lo PK, Chen JY, Tang PP, Lin J, Lin CH, Su LT, Wu CH, Chen TL, Yang Y, Wang FF. Identification of a mouse thiamin transporter gene as a direct transcriptional target for p53. *J Biol Chem* 276: 37186-37193, 2001.
120. MacDonald L, Thumser AE, Sharp P. Decreased expression of the vitamin C transporter SVCT1 by ascorbic acid in a human intestinal epithelial cell line. *Br J Nutr* 87: 97-100, 2002.
121. Madsen CT, Sylvestersen KB, Young C, Larsen SC, Poulsen JW, Andersen MA, Palmqvist EA, Hey-Mogensen M, Jensen PB, Treebak JT, Lisby M, Nielsen ML. Biotin starvation causes mitochondrial protein hyper-acetylation and partial rescue by the SIRT3-like deacetylase Hst4p. *Nat Commun* 6: 7726, 2015.
122. Marchant JS, Subramanian VS, Parker I, Said HM. Intracellular trafficking and membrane targeting mechanisms of the human reduced folate carrier in mammalian epithelial cells. *J Biol Chem* 277: 33325-33333, 2002.
123. Marcoullis G, Rothenberg SP, Labombardi VJ. Preparation and characterization of proteins in the alimentary tract of the dog which bind cobalamin and intrinsic factor. *J Biol Chem* 255: 1824-1829, 1980.
124. Mason JB, Choi SW. Effects of alcohol on folate metabolism: Implications for carcinogenesis. *Alcohol* 35: 235-241, 1971.
125. Mathews FS, Gordon MM, Chen Z, Rajashankar KR, Ealick SE, Alpers DH, Sukumar N. Crystal structure of human intrinsic factor: Cobalamin complex at 2.6-A resolution. *Proc Natl Acad Sci USA* 104: 1731-1736, 2007.
126. Maulén NP, Henriquez EA, Kempe S, Cárcamo JG, Schmid-Kotsas A, Bachem M, Grünert A, Bustamante ME, Nualart F, Vera JC. Up-regulation and polarized expression of the sodium-ascorbic acid transporter SVCT1 in post-confluent differentiated Caco-2 cells. *J Biol Chem* 278: 9035-9041, 2003.
127. Mazur-Bialy AI, Buchala B, Plytycz B. Riboflavin deprivation inhibits macrophage viability and activity: A study on the RAW 264.7 cell line. *Br J Nutr* 110: 509-514, 2013.
128. Michels AJ, Hagen TM. Hepatocyte nuclear factor 1 is essential for transcription of sodium-dependent vitamin C transporter protein 1. *Am J Physiol* 297: C1220-C1227, 2009.
129. Middleton HM. Uptake of riboflavin by rat intestinal mucosa *in vitro*. *J Nutr* 120: 588-593, 1985.
130. Mock DM, deLorimer AA, Liebma WM, Sweetman L, Baker H. Biotin deficiency: An unusual complication of parenteral alimentation. *N Engl J Med* 304: 820-823, 1981.
131. Monteverde DR, Gomez-Consarnau L, Suffridge C, Sanudo-Wilhelmy SA. Life's utilization of B vitamins on early earth. *Geobiology* 15: 3-18, 2017.
132. Mozzillo E, Melis D, Falco M, Fattorusso V, Taurisano R, Flanagan SE, Ellard S, Franzese A. Thiamine responsive megaloblastic anemia: A novel SLC19A2 compound heterozygous mutation in two siblings. *Pediatr Diabetes* 14: 384-387, 2013.
133. Mutti E, Ruetz M, Birn H, Kräutler B, Nexo E. 4-Ethylphenyl-cobalamin impairs tissue uptake of vitamin B12 and causes vitamin B12 deficiency in mice. *PLoS One* 8: e75312, 2013.
134. Nabokina SM, Inoue K, Subramanian VS, Valle JE, Yuasa H, Said HM. Molecular identification and functional characterization of the human colonic thiamine pyrophosphate transporter. *J Biol Chem* 289: 4405-4416, 2014.
135. Nabokina SM, Kashyap ML, Said HM. Mechanism and regulation of human intestinal niacin uptake. *Am J Physiol* 289: C97-C103, 2005.
136. Nabokina SM, Ramos MB, Said HM. Mechanism(S) involved in the colon-specific expression of the thiamine pyrophosphates (Tpp) transporter. *PLoS One* 11: 1-14, 2016.
137. Nabokina SM, Ramos MB, Valle JE, Said HM. Regulation of basal promoter activity of the human thiamine pyrophosphate transporter SLC44A4 in human epithelial cells. *Am J Physiol* 308: C750-C757, 2015.
138. Nabokina SM, Reidling JC, Said HM. Differentiation-dependent up-regulation of intestinal thiamin uptake: Cellular and molecular mechanisms. *J Biol Chem* 280: 32676-32682, 2005.
139. Nabokina SM, Said HM. Characterization of the 5′-regulatory of the human thiamin transporter SLC19A3: *In vitro* and *in vivo* studies. *Am J Physiol Gastrointest Liver Physiol.* 287: G822-G829, 2004.
140. Nabokina SM, Said HM. A high-affinity and specific carrier-mediated mechanism for uptake of thiamine pyrophosphate by human colonic epithelial cells. *Am J Physiol* 303: G389-G395, 2012.
141. Nabokina SM, Senthilkumar SR, Said HM. Tspan-1 interacts with thiamine transporter-1 in human intestinal epithelial cells and modulates its stability. *Am J Physiol* 301: G808-G813, 2011.
142. Nabokina SM, Subramanian VS, Said HM. Association of PDZ-containing protein PDZD11 with the human sodium dependent multivitamin transporter gene. *Am J Physiol* 280: C548-C555, 2001.
143. Nabokina SM, Subramanian VS, Said HM. Comparative analysis of ontogenic changes in renal and intestinal biotin transport in the rat. *Am J Physiol* 284: F737-F742, 2003.
144. Nabokina SM, Subramanian VS, Said HM. Effect of clinical mutations on functionality of the human riboflavin transporter-2 (hRFT-2). *Mol Genet Metab* 105: 652-657, 2012.
145. Nabokina SM, Subramanian VS, Said HM. The human colonic thiamine pyrophosphate transporter (hTPPT) is a glycoprotein and N-linked glycosylation is important for its function. *Biochim Biophys Acta* 1858: 866-871, 2016.
146. Nabokina SM, Subramanian VS, Valle JE, Said HM. Adaptive regulation of human intestinal thiamine uptake by extracellular substrate level: A role for THTR-2 transcriptional regulation. *Am J Physiol* 305: G593-G599, 2013.
147. Najjar VA, Holt LE. The biosynthesis of thiamin in man and its implication in human nutrition. *JAMA* 123: 683-684, 1943.
148. Najmi M, Zhoa R, Riser A, Goldman ID. Role of the tryptophan residues in proton-coupled folate transporter (PCFT-SLC46A1) function. *Am J Physiol* 311: C150-C157, 2016.
149. Namour F, Dobrovoljski G, Chery C, Audonnet S, Feillet F, Sperl W, Gueant JL. Luminal expression of cubilin is impaired in Imerslund-Grasbeck syndrome with compound AMN mutations in intron 3 and exon 7. *Haematologica* 96: 1715-1719, 2011.
150. Naughton CA, Chandler CJ, Duplantier RB, Halsted CH. Folate absorption in alcoholic pigs: *In vitro* hydrolysis and transport at the brush border membrane. *Am J Clin Nutr* 50: 1436-1441, 1989.
151. Neufeld EJ, Fleming JC, Tyartaglini E, Steinkamp MP. Thiamin-responsive megaloblastic anemia syndrome: A disorder of high-affinity thiamin transport. *Blood Cells Mol Dis* 27: 135-138, 2001.
152. Nexo E. Cobalamin binding proteins. In: Krautler B, Arigoni D, Golding BT, editors. *Vitamin B12 and B12-Proteins*. Germany: Wiley-VCH, 1997, p. 459-475.
153. Nexo E, Hansen MR, Konradsen L. Human salivary epidermal growth factor, haptocorrin and amylase before and after prolonged exercise. *Scand J Clin Lab Invest* 48: 269-273, 1988.
154. Nielsen MJ, Rasmussen MR, Andersen CB, Nexo E, Moestrup SK. Vitamin B12 transport from food to the body's cells—A sophisticated, multistep pathway. *Nat Rev Gastroenterol Hepatol* 9: 345-354, 2012.
155. Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: From experimental models to human disease. *Kidney Int* 89: 58-67, 2016.
156. Nyberg W. Diphyllobothrium latum and human nutrition, with particular reference to vitamin B12 deficiency. *Proc Nutr Soc* 22: 8-14, 1963.
157. Obeid R, Fedosoy SN, Nexo E. Cobalamin coenzyme forms are not likely to be superior to cyano- and hydroxyl-cobalamin in prevention or treatment of cobalamin deficiency. *Mol Nutr Food Res* 59: 1364-1372, 2015.
158. Pawlak R, Lester SE, Babtunde T. The prevalence of cobalamin deficiency among vegetarians assessed by serum vitamin B12: A review of literature. *Eur J Clin Nutr* 68: 541-548, 2014.

159. Pedersen GA, Chakraborty S, Steinhauser AL, Traub LM, Madsen M. AMN directs endocytosis of the intrinsic factor-vitamin B(12) receptor cubam by engaging ARH or Dab2. *Traffic* 11: 706-720, 2010.
160. Pratt JM. *Inorganic Chemistry of Vitamin B12*. New York: Academic Press, 1972, p. 250-251.
161. Pupavac M, Watkins D, Petrella F, Fahiminiya S, Janer A, Cheung W, Gingras AC, Pastinen T, Muenzer J, Majewski J, Shoubridge EA, Rosenblatt DS. Inborn error of cobalamin metabolism associated with the intracellular accumulation of transcobalamin-bound cobalamin and mutations in ZNF143, which codes for a transcriptional activator. *Hum Mutat* 37: 976-982, 2016.
162. Qiu A, Jansen M, Sakaris A, Min SH, Chattopahyay S, Tsai E, Sandoval C, Zhao R, Akabas MH. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. *Cell* 127: 917-928, 2006.
163. Qiu A, Min SH, Jansen M, Usha M, Tsai E, Cabelof DC, Matherly LH, Zhao R, Akabas MH, Goldman ID. Rodent intestinal folate transporters (SLC46A1): Secondary structure functional properties and response to dietary folate restriction. *Am J Physiol* 293: C1669-C1678, 2007.
164. Quadros EV, Regec AL, Khan KM, Quadros E, Rothenberg SP. Transcobalamin II synthesized in the intestinal villi facilitates transfer of cobalamin to the portal blood. *Am J Physiol* 277: G161-G166, 1999.
165. Quadros EV, Sequeira JM. Cellular uptake of cobalamin: Transcobalamin and the TCblR/CD320 receptor. *Biochinie* 95: 1008-1018, 2004.
166. Rajgopal A, Edmondson A, Goldman D, Zhao R. SLC19A3 encodes a second thiamin transporter ThTr2. *Biochim Biophys Acta* 1537: 175-178, 2001.
167. Rappazzo ME, Salmi HA, Hall CA. The content of vitamin B12 in adult and foetal tissue: A comparative study. *Br J Haematol* 18: 425-433, 1970.
168. Reidling JC, Balamurugan K, Nabokina S, Said HM. Adaptive regulation of intestinal thiamin uptake: Molecular mechanisms in wild-type and transgenic mice. *Gastroenterol* 126: 488, 2004.
169. Reidling JC, Lambrecht N, Kassir M, Said HM. Impaired intestinal vitamin B1 (thiamin) uptake in thiamin transporter-2-deficient mice. *Gastroenterol* 138: 1802-1809, 2010.
170. Reidling JC, Nabokina SM, Balamurugan K, Said HM. Developmental maturation of intestinal and renal thiamin uptake: Studies in wild-type and transgenic mice carrying human THTR-1 and 2 promoters. *J Cell Physiol* 206: 371-377, 2006.
171. Reidling JC, Nabokina SM, Said HM. Molecular mechanisms involved in the adaptive regulation of human intestinal biotin uptake: A study of the hSMVT system. *Am J Physiol* 292: G275-G281, 2007.
172. Reidling JC, Rubin SA. Promoter analysis of the human ascorbic acid transporters SVCT1 and SVCT2: Mechanism of adaptive regulation in liver epithelial cells. *J Nutr Biochem* 22: 344-350, 2010.
173. Reidling JC, Said HM. *In vitro and in vivo* characterization of the minimal promoter region of the human thiamin transporter *SLC19A2*. *Am J Physiol* 285: C633-C641, 2003.
174. Reidling JC, Said HM. Regulation of the human biotin transporter hSMVT promoter by KLF-4 and AP-2: Confirmation of promoter activity in vivo. *Am J Physiol* 292: C1305-C1312, 2007.
175. Reidling JC, Subramanian VS, Dudeja PK, Said HM. Expression and promoter analysis of SLC19A2 in the human intestine. *Biochim Biophys Acta* 1561: 180-187, 2002.
176. Reizenstein P, Ek G, Matthews CM. Vitamin B12 kinetics in man. Implications on total-body-B-12-determinations, human requirements, and normal and pathological cellular B12 uptake. *Phys Med Biol* 1: 295-306, 1966.
177. Rindi G, Laforenza U. Thiamin intestinal transport and related issues: Recent aspects. *Proc Soc Exp Biol Med* 224: 246-255, 2000.
178. Robertson JA, Gallagher ND. Increased intestinal uptake of cobalamin in pregnancy does not require synthesis of new receptors. *Biochim Biophys Acta* 757: 145-150, 1983.
179. Rodriguez-Melendez R, Zempleni J. Regulation of gene expression by biotin. *J Nutr Biochem* 14: 680-690, 2003.
180. Rong N, Selhub J, Goldin BR, Rosenberg I. Bacterially synthesized folate in rat large intestine is incorporated into host tissue folylpolyglutamates. *J Nutr* 121: 1955-1959, 1991.
181. Rose RC. Intestinal absorption of water-soluble vitamins. In: Johnson LR, editor. *Physiology of the Gastrointestinal Tract*. New York: Raven Press, 1987, p. 1581-1596.
182. Rosenfeld L. Vitamine—vitamin. The early years of discovery. *Clin Chem* 43: 680-685, 1997.
183. Sabui S, Bohl J, Kapadia R, Cogburn K, Ghosal A, Lambrecht N, Said HM. Role of sodium-dependent multivitamin transporter (SMVT) in the maintenance of intestinal mucosal integrity. *Am J Physiol* 311: G561-G570, 2016.
184. Sabui S, Ghosal A, Said HM. Identification and characterization of 5′-flanking region of the human riboflavin transporter 1 gene (*SLC52A1*). *Gene* 553: 49-65, 2014.
185. Said HM, Balamurugan K, Subramanian VS, Marchant JS. Expression and functional contribution of hTHTR-2 in thiamin absorption in human intestine. *Am J Physiol* 286: G491-G498, 2003.
186. Said HM. Cellular uptake of biotin: Mechanisms and regulation. *J Nutr* 129: 490S-493S, 1999.
187. Said HM. Recent advances in carrier-mediated absorption of water-soluble vitamins. *Ann Review Physiol* 66: 419-446, 2004.
188. Said HM. Thiamin. In: Coats PM, Betz JM, Blackman MR, editors. *Encyclopedia of Dietary Supplements*. New York: Informa Healthcare, 2010, p. 752-757.
189. Said HM. Intestinal absorption of water-soluble vitamins in health and disease. *Biochem J* 437: 357-372, 2011.
190. Said HM, Blair JA, Lucas ML, Hilburn ME. Intestinal surface acid microclimate in vitro and in vivo in the rat. *J Lab Clin Med* 107: 420-424, 1986.
191. Said HM, Chatterjee H, Haq RU, Subramanian VS, Ortiz A, Matherly LH, Sirotnak FM, Halsted C, Rubin SA. Adaptive regulation of intestinal folate uptake: Effect of dietary folate deficiency. *Am J Physiol* 279: C1889-C1895, 2000.
192. Said HM, Hollander D, Khani R. Uptake of riboflavin by intestinal basolateral membrane vesicles: A specialized carrier-mediated process. *Biochim Biophys Acta* 114: 263-268, 1993.
193. Said HM, Khani R. Uptake of riboflavin across the brush border membrane of rat intestine: Regulation by dietary vitamin levels. *Gastroenterol* 105: 1294-1298, 1993.
194. Said HM, Ma TY. Mechanism of riboflavin uptake by Caco-2 human intestinal epithelial cells. *Am J Physiol* 266: G15-G21, 1994.
195. Said HM, Ma TY, Grant K. Regulation of riboflavin intestinal uptake by protein kinase A: Studies with Caco-2 cells. *Am J Physiol* 267: G955-G959, 1994.
196. Said HM, Ma TY, Ortiz A, Tapia A, Valerio CK. Intracellular regulation of intestinal folate uptake: Studies with cultured IEC-6 epithelial cells. *Am J Physiol* 272: C729-C736, 1997.
197. Said HM, Mock DM, Collins J. Regulation of intestinal biotin transport in the rat: Effect of biotin deficiency and supplementation. *Am J Physiol* 256: G306-G311, 1989.
198. Said HM, Nylander W, Redha R. Biotin transport in human intestine: Site of maximum transport and effect of pH. *Gastroenterol* 95: 1312-1317, 1988.
199. Said HM, Ortiz A, Kumar CK, Chatterjee N, Dudeja PK, Rubin SA. Transport of thiamin in the human intestine: Mechanism and regulation in intestinal epithelial cell model Caco-2. *Am J Physiol* 277: C645-C651, 1999.
200. Said HM, Ortiz A, Ma TY. A carrier-mediated mechanism for pyridoxine uptake by human intestinal epithelial Caco-2 cells: Regulation by a PKA-mediated pathway. *Am J Physiol* 285: C1219-C1225, 2003.
201. Said HM, Ortiz A, McCloud E, Dyer D, Moyer MP, Rubin S. Biotin uptake by the human colonic epithelial cells NCM460: A carrier-mediated process shared with pantothenic acid. *Am J Physiol* 44: C1365-C1371, 1998.
202. Said HM, Ortiz A, Moyer MP, Yanagawa N. Riboflavin uptake by human-derived colonic epithelial NCM460 cells. *Am J Physiol* 278: C270-C276, 2000.
203. Said HM, Ortiz A, Subramanian VS, Neufeld EJ, Moyer MP, Dudeja PK. Mechanism of thiamin uptake by human colonocytes: Studies with cultured colonic epithelial cell line NCM460. *Am J Physiol* 281: G144-G150, 2001.
204. Said HM, Redha R. Biotin transport in basolateral membrane vesicles of human intestine. *Gastroenterol* 94: 1157-1163, 1988.
205. Said HM, Redha R. Ontogenesis of the intestinal transport of biotin in the rat. *Gastroenterol* 94: 68-72, 1988.
206. Said HM, Redha R, Nylander W. A carrier-mediated, Na^+ gradient-dependent transport system for biotin in human intestinal brush border membrane vesicles. *Am J Physiol* 253: G631-G636, 1987.
207. Said HM, Redha R, Nylander W. Biotin transport in the human intestine: Inhibition by anticonvulsant drugs. *Am J Clin Nutr* 49: 127-131, 1987.
208. Said ZM, Subramanian VS, Vaziri ND, Said HM. Pyridoxine uptake by colonocytes: A specific and regulated carrier-mediate process. *Am J Physiol* 294: C1192-C1197, 2008.
209. Said HM, Thuy LP, Sweetman L, Schatzman B. Transport of the biotin dietary derivative biocytin (N-biotinyl-L-lysine) in rat small intestine. *Gastroenterol* 104: 75-80, 1993.
210. Sassoon CS, Zhu E, Fang L, Subramanian VS, Said HM. Inhibition of intestinal thiamin transport in rat model of sepsis. *Crit Care Med* 44: e875-e881, 2016.
211. Schell DA, Bode AM. Measurement of ascorbic acid and dehydroascorbic acid in mammalian tissue utilizing HPLC and electrochemical detection. *Biomed. Chromatography* 7: 267-272, 1993.
212. Schilling RF. Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity after the oral administration of radioactive vitamin B12, 1953. *J Lab Clin Med* 15: 761-767, 1990.
213. Schramm M, Wiegmann K, Schramm S, Gluschko A, Herb M, Utermohlen O, Kronke M. Riboflavin (vitamin B2) deficiency impairs

NADPH oxidase 2 (Nox2) priming and defense against Listeria monocytogenes. *Eur J Immunol* 44: 728-741, 2014.
214. Seekamp A, Hultquist DE, Till GO. Protection by vitamin B2 against oxidant-mediated acute lung injury. *Inflamm* 23: 449-460, 1999.
215. Shao J, Sartor RB, Dial E, Lichtenberger LM, Schepp W, Alpers DH. Expression of intrinsic factor in rat and murine gastric mucosal cell lineages is modified by inflammation. *Am J Pathol* 157: 1197-1205, 2000.
216. Shibata K, Gross CJ, Henderson LM. Hydrolysis and absorption of pantothenate and its coenzymes in the rat small intestine. *J Nutr* 113: 2107-2115, 1983.
217. Siliprandi L, Vanni P, Kessler M, Semenza G. Na^+-dependent electroneutral L-ascorbate transport across brush-border membrane vesicles from guinea pig small intestine. *Biochim Biophys Acta* 552, 129-142, 1979.
218. Simpson KW, Alpers DH, De Wille J, Swanson P, Farmer S, Sherding RG. Cellular localization and hormonal regulation of pancreatic intrinsic factor secretion in dogs. *Am J Physiol* 265: G178-G188, 1993.
219. Sklan D, Trostler N. Site and extend of thiamin absorption in the rat. *J Nutr* 107: 353-356, 1977.
220. Sorrell MF, Frank O, Thomson AD, Aquino A, Baker H. Absorption of vitamins from the large intestine. *Nutr Res Int* 3: 143-148, 1971.
221. Subramanian VS, Chatterjee N, Said HM. Folate uptake in the human intestine: Promoter activity and effect of folate deficiency. *J Cell Physiol* 196: 403-408, 2003.
222. Subramanian VS, Ghosal A, Kapadia R, Nabokina SM, Said HM. Molecular mechanisms mediating the adaptive regulation of intestinal riboflavin uptake process. *PLoS One* 10: 1-13, 2015.
223. Subramanian VS, Ghosal A, Subramanya SB, Lytle C, Said HM. Differentiation-dependent regulation of intestinal vitamin B2 uptake: Studies utilizing human-derived intestinal epithelial Caco-2 cells and native rat. *Am J Physiol* 304: G741-G748, 2013.
224. Subramanian VS, Lambrecht N, Lytle C, Said HM. Conditional (intestinal-specific) knockout of the riboflavin transporter-3 (RFVT-3) impairs riboflavin absorption. *Am J Physiol* 310: G285-G292, 2016.
225. Subramanian VS, Marchant JS, Boulware MJ, Ma TY, Said HM. Membrane targeting and intracellular trafficking of the human sodium-dependent multivitamin transporter in polarized epithelial cells. *Am J Physiol* 296: C663-C671, 2009.
226. Subramanian VS, Marchant JS, Boulware MJ, Said HM. A C-terminal region dictates the apical plasma membrane targeting of the human sodium-dependent vitamin C transporter-1 in polarized epithelia. *J Biol Chem* 279: 27719-27728, 2004.
227. Subramanian VS, Marchant JS, Parker I, Said HM. Intracellular trafficking/membrane targeting of human reduced folate carrier expressed in Xenopus oocytes. *Am J Physiol* 281: G1477-G1486, 2001.
228. Subramanian VS, Marchant JS, Parker I, Said HM. Cell biology of the human thiamin transporter-1 (hTHTR1): intracellular trafficking and membrane targeting mechanisms. *J Biol Chem* 278: 3976-3984, 2003.
229. Subramanian VS, Marchant JS, Reidling JC, Said HM. N-Glycosylation is required for Na+-dependent vitamin C transporter functionality. *Biochem Biophys Res Commun* 374: 123-127, 2008.
230. Subramanian VS, Marchant JS, Said HM. Biotin-responsive basal ganglia disease-linked mutations inhibit thiamine transport via hTHTR2: Biotin is not a substrate for hTHTR2. *Am J Physiol* 291: C851-C859, 2006.
231. Subramanian VS, Marchant JS, Said HM. Targeting and trafficking of the human thiamine transporter-2 in epithelial cells. *J Biol Chem* 281: 5233-5245, 2006.
232. Subramanian VS, Marchant JS, Said HM. Apical membrane targeting and trafficking of the human proton-coupled folate transporter in polarized epithelia. *Am J Physiol* 294: C233-C240, 2008.
233. Subramanian VS, Nabokina SM, Patton JR, Marchant JS, Moradi H, Said HM. Glyoxalate reductase/hydroxypyruvate reductase interacts with the sodium dependent vitamin C tranporter-1 to regulate cellular vitamin C homeostasis. *Am J Physiol* 304: G1079-G1086, 2013.
234. Subramanian VS, Nabokina SM, Said HM. Association of TM4SF4 with the human thiamine transporter-2 in intestinal epithelial cells. *Dig Dis Sci* 59: 583-590, 2014.
235. Subramanian VS, Rapp L, Marchant JS, Said HM. Role of cysteine residues in cell surface expression of the human riboflavin transporter-2 (hRFT2) in intestinal epithelial cells. *Am J Physiol* 301: G100-G109, 2011.
236. Subramanian VS, Reidling JC, Said HM. Differentiation-dependent regulation of the intestinal folate uptake process: Studies with Caco-2 cells and native mouse intestine. *Am J Physiol* 295: C828-C835, 2008.
237. Subramanian VS, Subramanya SB, Ghosal A, Said HM. Chronic alcohol feeding inhibits physiological and molecular parameters of intestinal and renal riboflavin transport. *Am J Physiol Cell Physiol* 305: C539-C546, 2013.
238. Subramanian VS, Subramanya SB, Rapp L, Marchant JS, Ma TY, Said HM. Differential expression of human riboflavin transporters -1, -2, and -3 in polarized epithelia: A key role for hRFT-2 in intestinal riboflavin uptake. *Biochim Biophys Acta* 1808: 3016-3021, 2011.
239. Subramanya SB, Subramanian VS, Kumar JS, Hoiness R, Said HM. Inhibition of intestinal biotin absorption by chronic alcohol feeding: Cellular and molecular mechanisms. *Am J Physiol* 300: G494-G501, 2011.
240. Subramanya SB, Subramanian VS, Said HM. Chronic alcohol consumption and intestinal thiamin absorption: Effects on physiological and molecular parameters of the uptake process. *Am J Physiol* 299: G23-G31, 2010.
241. Stark M, Gonen N, Assaraf YG. Functional elements in the minimal promoter of the human proton-coupled folate transporter. *Biochem Biophys Res Commun* 388: 79-85, 2009.
242. Storm T, Zeitz C, Cases O, Amsellem S, Verroust PJ, Madsen M, Benoist JF, Passemard S, Lebon S, Jønsson IM, Emma F, Koldsø H, Hertz JM, Nielsen R, Christensen EI, Kozyraki R. Detailed investigations of proximal tubular function in Imerslund-Gräsbeck syndrome. *BMC Med Genet* 14: 111, 2013.
243. Stupperich E, Nexo E. Effect of the cobalt-N coordination on the cobamide recognition by the human vitamin B12 binding proteins intrinsic factor, transcobalamin and haptocorrin. *Eur J Biochem* 199: 299-303, 1991.
244. Sweetman L, Nyhan WL. Inheritable biotin-treatable disorders and associated phenomena. *Ann Rev Nutr* 6: 314-343, 1986.
245. Tanner SM, Li Z, Bisson R, Acar C, Oner C, Oner R, Cetin M, Abdelaal MA, Ismail EA, Lissens W, Krahe R, Broch H, Gräsbeck R, de la Chapelle A. Genetically heterogeneous selective intestinal malabsorption of vitamin B12: Founder effects, consanguinity, and high clinical awareness explain aggregations in Scandinavia and the Middle East. *Hum Mutat* 23: 327-3233, 2004.
246. Tomei S, Yuasa H, Inoue K. Transport functions of riboflavin carrier in the rat small intestine and colon: Site difference and effects of tricyclic-type drugs. *Drug Deliv* 8: 119-124, 2001.
247. Tsukaguchi H, Tokui T, Mackenzie B, Berger UV, Chen X, Wang Y, Brubaker RF, Hediger MA. A family of mammalian Na^+-dependent L-ascorbic acid transporters. *Nature* 399: 70-75, 1999.
248. Tu BP, Ho-Schleyer SC, Travers KJ, Weissman JS. Biochemical basis of oxidative protein folding in the endoplasmic reticulum. *Science* 290: 1571–1574, 2000.
249. Unal ES, Zhao R, Chang MH, Fiser A, Romero MF, Goldman ID. The functional roles of the His247 and His281 residues in folate and proton translocation mediated by the human proton-coupled folate transporter SLC46A1. *J Biol Chem* 284: 17846-17857, 2009.
250. Varma S, Campbell CE, Kuo S. Functional role of conserved transmembrane segment 1 residues in human sodium-dependent vitamin C transporters. *Biochemistry* 47: 2952-2960, 2008.
251. Vera JC, Rivas CI, Fishbarg J, Golde DW. Mammalian facilitative hexose transporters mediates the transport of dehydroascorbic acid. *Nature* 364: 79-82, 1993.
252. Villanueva JA, Devlin AM, Halsted CH. Reduced folate carrier: Tissue distribution and effects of chronic ethanol intake in the micropig. *Alcohol Clin Exp Res* 25: 415-420, 2001.
253. Wang H, Mackenzie B, Tsukaguchi H, Weremowicz S, Morton CC, Hediger MA. Human vitamin C (L-ascorbic acid) transporter SVCT1. *Biochim Biophys Res Commun* 267: 488-494, 2000.
254. Wang Y, Zhao R, Russel RG, Goldman ID. Localization of the murine reduced folate carrier as assessed by immunohistochemical analysis. *Biochim Biophys Acta* 1513: 49-54, 2001.
255. Whetstine JR, Flatley RM, Matherly LH. The human reduced folate carrier gene is ubiquitously and differentially expressed in normal human tissues: Identification of seven non-coding exons and characterization of a novel promoter. *Biochem J* 367: 629-640, 2002.
256. Wilson MR, Kugel S, Huang J, Wilson LJ, Wloszczynski PA, Ye J, Matherly LH, Hou Z. Structural determinants of human proton-coupled folate transporter oligomerization: role of GXXXG motifs and identification of oligomeric interfaces at transmembrane domains 3 and 6. *Biochem J* 469: 33-44, 2015.
257. Wolf B, Heard GS, Secor-McVoy JR, Raetz H. M. Biotinidase deficiency: The possible role of biotinidase in the processing of dietary protein-bound biotin. *J Inherited Metab Dis* 7: 121-122, 1984.
258. Wrong OM, Edmonds CJ, Chadwich VS. Vitamins. In: *The Large Intestine: Its Role in Mammalian Nutrition and Homeostasis*. New York: Wiley, 1981, p. 157-166.
259. Wuerges J, Garau G, Geremia S, Fedosov SN, Petersen TE, Randaccio L. Structural basis for mammalian vitamin B12 transport by transcobalamin. *Proc Natl Acad Sci USA* 103: 4386-4391, 2006.
260. Yammani RR, Seetharam S, Seetharam B. Cubilin and megalin expression and their interaction in the rat intestine: Effect of thyroidectomy. *Am J Physiol Endocrinol Metab* 281: E900-E907, 2001.

261. Yang B, Feng L, Wang F, Wang L. Enterohemorrhagic Escherichia coli senses low biotin status in the large intestine for colonization and infection. *Nat Commun* 6: 6592, 2015.
262. Yonezawa A, Inui K. Novel riboflavin transporter family RFVT/SLC52, identification, nomenclature, functional characterization and genetic diseases of RFVT/SLC52. *Mol Aspects Med* 34: 693-701, 2013.
263. Zhao R, Matherly LH, Goldman ID. Membrane transporters and folate homeostasis: Intestinal absorption and transport into systemic compartments and tissues. *Expert Rev Mol Med* 11: 1-27, 2009.
264. Zhao R, Najmi M, Fiser A, Goldman DI. Identification of an extracellular gate for the proton-coupled folate transporter (PCFT-SLC46A1) by cysteine cross-linking. *J Bio Chem* 291: 8162-8172, 2016.