

Intestinal Amino Acid Transport and Metabolic Health

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Keywords

digestion, protein restriction, membrane transport, amino acids, epithelial cells

Abstract

Amino acids derived from protein digestion are important nutrients for the growth and maintenance of organisms. Approximately half of the 20 proteinogenic amino acids can be synthesized by mammalian organisms, while the other half are essential and must be acquired from the nutrition. Absorption of amino acids is mediated by a set of amino acid transporters together with transport of di- and tripeptides. They provide amino acids for systemic needs and for enterocyte metabolism. Absorption is largely complete at the end of the small intestine. The large intestine mediates the uptake of amino acids derived from bacterial metabolism and endogenous sources. Lack of amino acid transporters and peptide transporter delays the absorption of amino acids and changes sensing and usage of amino acids by the intestine. This can affect metabolic health through amino acid restriction, sensing of amino acids, and production of antimicrobial peptides.

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1. PROTEIN DIGESTION

Protein is one of the three macronutrients. In contrast to carbohydrates and fat, which are mostly used as fuels, a significant part of protein nutrition is used for the maintenance of tissue protein, which is in constant turnover. Only excess protein is used to generate energy, requiring the urea cycle to dispose of nitrogen. Approximately 30 g of protein are required daily to replace unavoidable losses from metabolism and loss through feces. Nutritional intake typically exceeds this essential requirement by 40–70 g. Digestion of protein occurs in stages involving pepsin, pancreas-derived peptidases, and brush-border peptidases. In combination, these enzymes ensure largely complete digestion of protein (digestibility ranging from 70% to 97%) into individual amino acids, dipeptides, and tripeptides. Digestion includes the recycling of endogenous proteins and peptides, which amounts to 50–60 g per day. As a result, approximately 150 g of protein is digested and absorbed every day (90). Adibi & Mercer (1) analyzed jejunal contents 3 h after a 50-g load of albumin and found that approximately 10 times more amino acids were found as peptides as opposed to individual amino acids. At first glance, this would suggest that most protein amino acids are absorbed as peptides. However, for small peptides (2–4 residues) substantial peptidase activity is located in the brush border (147), which further degrades short peptides close to the cell surface, releasing amino acids. The close proximity between brush-border peptidases generates instantaneous amino acid transport and elevates local amino acid concentration. As a result, it is difficult to quantify the ratio between uptake of individual amino acids, dipeptides, and tripeptides. When sampled in the ileum, close to 90% of amino acids are absorbed (132), with tryptophan and glycine having the lowest digestibility. Between meals, free amino acid concentration in the lumen of the intestine was found to be 50–300 μ M, while after a protein-rich meal, the concentration increased to 0.6–6 mM (1). As mentioned, the local concentration of peptides and amino acids seen by transporters may be higher than that. These results suggest that amino acid transporters are exposed to millimolar concentrations of amino acids after a meal and micromolar concentrations of amino acids between meals. Digestion of a protein-rich meal may take hours but still remains complete, with only trace amounts remaining in feces.

2. AMINO ACID TRANSPORT IN THE SMALL INTESTINE

The absorption of amino acids along the small intestine occurs through transporters that cover groups of related amino acids. The groups were discovered either through functional studies or through rare aminoacidurias affecting both renal and intestinal amino acid transporters. Transport processes in intestinal epithelia are vectorial, that is, an electrochemical driving force is available for the translocation of amino acids across the apical membrane followed by a passive transport process across the basolateral membrane. This principle, first formulated by Robert K. Crane (41) for glucose transport, applies with some modifications also to the transport of amino acids. In the following paragraphs, the transporters of the small and large intestine are described, followed by the description of health aspects related to amino acid transporters. For reference, a list of all transporters covered in this review and their properties is presented in **Table 1**.

2.1. Neutral Amino Acids

2.1.1. Neutral amino acid transport across the apical membrane. A transporter in the apical membrane that accepts a broad variety of neutral amino acids was originally defined by functional studies in tissue (120), brush-border membrane vesicle studies (149), and the pattern of amino acids in the urine of individuals with Hartnup disorder (42), a rare aminoaciduria that also affects intestinal amino acid transport (139). The activity has been referred to as system B⁰ (broad neutral) or NBB (neutral brush border). This was subsequently confirmed through the molecular cloning of the transporter (27), followed by the identification of mutations associated with Hartnup disorder (80, 136). The transporter was named B⁰AT1 (broad neutral amino acid transporter 1, gene designation *SLC6A19*; see **Figure 1**). The transporter requires ancillary proteins for surface expression and catalytic activity (44, 83). This was discovered serendipitously in a collectrin-deficient mouse, which showed a urine amino acid profile similar to that observed in Hartnup disorder (44). In lieu of kidney collectrin, ACE2 (angiotensin converting enzyme II) was identified as the corresponding ancillary protein in the intestine (34, 83). In addition to its role in the inactivation of the hormone angiotensin II, ACE2 is a general carboxypeptidase in the brush border (163). Coexpression of ACE2 and B⁰AT1 generates a metabolon that allows the instantaneous transport of amino acids upon exposure to peptides (83). The transporter accepts all neutral amino acids but prefers branched-chain amino acids and methionine. K_M values for preferred substrates range between 0.5 and 1 mM (19, 33, 36). Consistent with its location in the apical membrane, neutral amino acids are cotransported with 1Na⁺, ensuring vectorial transport across the apical membrane driven by the Na⁺ electrochemical gradient. Discussion of the structure and pharmacology B⁰AT1 goes beyond the scope of this article, and the reader is referred to recent articles in this area (45, 49, 180, 182).

No other transporter for neutral amino acids has been identified in the apical membrane of the small intestine (26). ASCT2 (alanine-serine-cysteine transporter 2), which has been suggested as another neutral amino acid transporter in the small intestine (9), is rather expressed in the large intestine (172). B⁰AT1 expression is low in the duodenum, steeply increasing toward the ileum in the pig where expression in individual sections of the intestine has been investigated (62, 89). This gradient was also observed at the protein level in rats (72). ACE2 showed a more even distribution. In rats, no change of B⁰AT1 protein expression was observed in response to a high protein diet (45% casein versus 18% casein). However, protein expression increased significantly during the feeding period on a normal protein diet (72), accompanied by increased amino acid uptake activity in the proximal jejunum. The upregulation followed a diurnal rhythm. The tissue-isoleucine content was high in the proximal jejunum and low in the middle and distal jejunum, independent

Table 1 Intestinal amino acid transport systems and their mediators

Protein	Name	Solute carrier	Substrate(s) ^a	Affinity ^b	Mechanism	Ion(s)	Expression ^c
ASCT2	Ala/Ser/Cys Tp	<i>SLC1A5</i>	A, S, C, T, Q, N	High	A	Na ⁺	Ub
ATB ^{0,+}	AA Tp broad neutral (0) and cationic (+)	<i>SLC6A14</i>	AA ⁰ , AA ⁺ , β-Ala	High	S	Na ⁺ , Cl ⁻	LI, Lu
B ⁰ AT1	Broad neutral (0) AA Tp	<i>SLC6A19</i>	AA ⁰	Low	S	Na ⁺	K, SI
EAAT3	Excitatory AA Tp	<i>SLC1A1</i>	E, D, C	High	S	Na ⁺ , H ⁺ (S), K ⁺ (A)	K, SI, B, L, Lu
GlyT1	Glycine Tp	<i>SLC6A9</i>	G	High	S	Na ⁺ , Cl ⁻	Ub
LAT2/4F2hc	Large neutral AA Tp	<i>SLC3A2/SLC7A8</i>	AA ⁰ (except P)	Medium	A	NA	Ub (except L)
LAT4		<i>SLC43A2</i>	L, I, M, F	Low	U		K, I, M, Bl
PAT1	Proton AA Tp (imino acid carrier)	<i>SLC36A1</i>	P, G, A, GABA, β-Ala	Low	S	H ⁺	SI, LI, Bl, B
rBAT/b ^{0,+} AT	Broad neutral (0) and cationic AA (+) Tp	<i>SLC3A1/SLC7A9</i>	R, K, O, CysC	High	A	NA	K, SI
SIT1	System IMINO Tp	<i>SLC6A20</i>	P, HO-P	Medium	S	Na ⁺ , Cl ⁻	K, SI, B
SNAT2	Sodium neutral AA Tp	<i>SLC38A2</i>	G, P, A, S, C, L, Q, N, H, M	Medium	S	Na ⁺	Ub
SNAT3		<i>SLC38A3</i>	Q, N, H	Low		Na ⁺ (S), H ⁺ (A)	K, L
SNAT4		<i>SLC38A4</i>	G, A, S, C, Q, N, M	Medium		Na ⁺	L
SNAT5		<i>SLC38A5</i>	Q, N, H, S, G	Low		Na ⁺ (S), H ⁺ (A)	Ub
TAT1	T-type AA Tp	<i>SLC16A10</i>	F, Y, W	Low	U	NA	M, P, S, (SI), (K)
TauT	Taurine Tp	<i>SLC6A6</i>	Tau, β-Ala	High	S	Na ⁺ , Cl ⁻	Ub
y ⁺ LAT1/4F2hc	Cationic (y ⁺) and large neutral AA Tp	<i>SLC3A2/SLC7A7</i>	K, R, Q, H, M, L, I	High	A	Na ⁺ (S-AA ⁰)	K, SI, Bl, Lu
y ⁺ LAT2/4F2hc		<i>SLC3A2/SLC7A6</i>	K, R, Q, H, M, L, I, C			Na ⁺ (S-AA ⁰)	Ub

Abbreviations: A, antiport; AA, amino acid; AA⁰, neutral amino acid; AA⁺, cationic amino acid; CysC, cystine; HO-P, hydroxyproline; NA, not applicable; O, ornithine; S, symport; S-AA⁰, symport together with neutral amino acids; Tp, transporter; U, uniport.

^aAmino acids are indicated by one-letter codes.

^bHigh, <100 μM; medium, 100 μM to 1 mM; low, >1 mM.

^cLow expression shown in parentheses. Abbreviations: B, brain; Bl, blood; K, kidney; L, liver; LI, large intestine; Lu, lung; M, muscle; P, pancreas; SI, small intestine; Ub, ubiquitous.

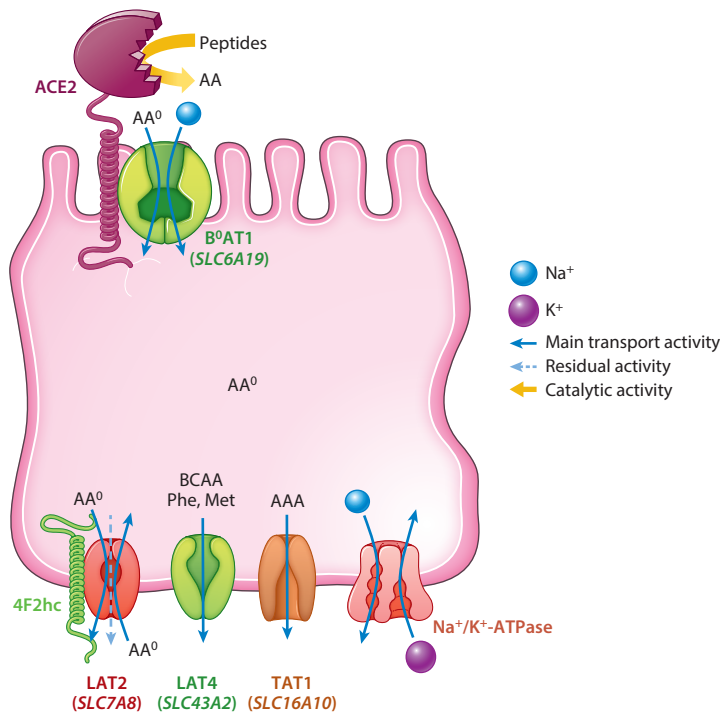


Figure 1

Vectorial transport of neutral amino acids in enterocytes. B⁰AT1 (broad neutral amino acid transporter 1, *SLC6A19*) forms a complex with angiotensin converting enzyme II (ACE2) in the apical membrane. It cotransports neutral amino acids (AA⁰) with Na⁺ ions. LAT2 (large neutral amino acid transporter 2, *SLC7A8*), LAT4 (*SLC43A2*), and TAT1 (system T amino acid transporter 1, *SLC16A10*) mediate the transport of neutral amino acids across the basolateral membrane. LAT2 forms a complex with 4F2hc and has been shown to exchange neutral amino acids. Indirect evidence suggests a limited capability to mediate uniport (*dashed arrow*). LAT4 and TAT1 facilitate diffusion of branched-chain amino acids (BCAA), phenylalanine, methionine, and aromatic amino acids (AAA). Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

of the protein content of the nutrition. This suggests that absorption is largely complete in the proximal jejunum.

The B⁰AT1 and ACE2 mRNA and protein expression increase along the crypt-to-villus axis (162). Thus, as enterocytes move from their point of differentiation in the crypts to the villus tip, genes involved in nutrient absorption are increasingly expressed. The main drivers of *SLC6A19* gene expression are the transcription factors *HNF1a* and *HNF4a*. In the crypts, *SLC6A19* expression is suppressed by several mechanisms. First, CpG dinucleotides in the proximal promoter are highly methylated in the crypts but fully demethylated in the villus. Histone modifications of active promoters such as H3K27Ac are absent in the crypt, becoming more prevalent during migration to the villus, and, lastly, transcription factor *SOX9* acts as a repressor in the crypt but is not found in mature enterocytes at the tip of the villus.

2.1.2. Mouse models related to apical neutral amino acid transport. The role of B⁰AT1 in the intestinal absorption of neutral amino acids has been studied in two different mouse models: first, a B⁰AT1 global knockout (26, 73–75) and, second, an ACE2 knockout that lacks B⁰AT1 in the intestine (66, 140). B⁰AT1^{-/-} mice show a mild malnutrition phenotype. The animals show

reduced body weight and growth after weaning. Histological staining did not reveal abnormalities. Reduced absorption was demonstrated in inverted sections of mouse intestine (26) and also through uptake of radiolabeled amino acids in living mice (74). The radiolabeled uptake experiments suggested a delayed absorption instead of a complete lack of absorption. When uptake of amino acids was analyzed in intestinal rings of $B^0AT1^{-/-}$ mice, Na^+ -dependent uptake of Gln, Leu, His, and Trp was completely abolished. However, Na^+ -independent uptake, most likely mediated by rBAT/b⁰⁺AT, remained the same. Interestingly, uptake of D-glucose was reduced by ~50% as well (26). This observation has been confirmed in kidney, where expression of SGLT2 was significantly reduced, resulting in low-level glucosuria (107). $B^0AT1^{-/-}$ mice show two main effects on amino acid homeostasis (75): first, elevated levels of amino acids in the lumen of the intestine and, second, a reduced amount of amino acids reaching the liver, particularly Thr and Trp (73, 184), which caused an amino acid stress response (75, 184). In the fasting state, no significant differences of plasma amino acid levels were observed between wild-type and $B^0AT1^{-/-}$ mice. However, use of amino acids as energy metabolites was reduced, as evidenced by lower urea production. The reduced absorption increased the luminal and fecal content, particularly of branched-chain and aromatic amino acids (10- to 20-fold change) (74). This resulted in the increased production of incretins, such as GLP-1 and GIP (75).

In ACE2^{-/-}-deficient mice, plasma amino acids were normal, with the exception of glycine and tryptophan (140). Na^+ -dependent transport of Pro and Trp was abolished, suggesting that B^0AT1 and SIT1 (System IMINO transporter 1, *SLC6A20*) surface expression was lacking. Glucose transport was unaffected. When a mixture of unlabeled and labeled amino acids was administered orally, the content of Gly, Ile, and Trp in the ileum was elevated after 1 h. Complete amino acid analysis showed increased amounts of all neutral amino acids except Pro. On a 7% low-protein diet without niacin supplementation, weight gain after initial weight loss was significantly slower than that of wild-type mice despite increased food consumption. Low-protein diet had a more significant influence on plasma amino acid levels than the genotype of the mice. Tissue Thr levels were elevated on a low-protein diet, suggesting a downregulation of Thr catabolism. The results suggest that low protein intake either through low-protein diet or lack of B^0AT1 is compensated by reduced growth and reduced use of amino acids for energy to maintain amino acid levels.

2.1.3. Neutral amino acid transport across the basolateral membrane. In contrast to the apical membrane, multiple neutral amino acid transporters have been identified in the basolateral membrane, namely LAT2 (large neutral amino acid transporter 2, *SLC7A8*), TAT1 (system T amino acid transporter 1, *SLC16A10*), and LAT4 (large neutral amino acid transporter 4, *SLC43A2*) (**Figure 1**). LAT2 forms a complex with the trafficking subunit 4F2hc (60), which directs it to the basolateral membrane. When expressed in *Xenopus laevis* oocytes, it has been characterized as an obligatory exchanger (97). However, modelling of amino acid transport across the microvillous plasma membrane of the placenta suggests that LAT2 may carry out facilitative diffusion to some extent (175). The low temperature of oocyte experiments could increase the ratio between antiport and uniport due to higher activation energy of the latter. LAT2 transports all neutral amino acids with the exception of proline. K_M values range from 50–200 μM for extracellular binding, but intracellular K_M values are approximately 200-fold higher (97). The substrate specificity and extracellular K_M values have been confirmed for the isolated protein (183). The structure of the 4F2hc/LAT2 complex has been determined (129, 183). LAT2 is expressed in the basolateral membrane throughout the small intestine, with the highest expression in the jejunum (46).

LAT4 has very low affinity for its substrates and is a uniporter. The K_M values are ≈ 4 mM for the substrates Leu, Ile, Val, Phe, and Met (10, 17). LAT4 is well suited to equilibrate cytosolic and

blood plasma amino acid concentrations. LAT4 expression in the intestine is critical for the net efflux of neutral amino acids absorbed from the intestine, at least in newborn animals (64).

TAT1 is functionally similar to LAT4. It is selective for aromatic amino acids (K_M range of 2.5–7 mM) (79). It is a uniporter allowing facilitated diffusion of its substrates. As a result, TAT1 and LAT4 allow a net efflux of neutral amino acids across the plasma membrane (126, 127). Computational modelling suggests that LAT4 and TAT1 mediate the efflux of their respective amino acid substrates. LAT2, in contrast, mediates the uptake of Gln, Ile, Leu, Met, Phe, Tyr, Trp, and Val to allow efflux of the smaller and polar amino acids Ala, Asn, Cys, Gly, His, Ser, and Thr (**Figure 1**). In humans, LAT4 expression is correlated with plasma citrulline levels, confirming a role of this transporter for the efflux of neutral amino acids (93).

In addition to transporters involved in the vectorial transport of amino acids, the basolateral membrane also contains neutral amino acid transporters that can provide nutrients from the blood circulation, particularly during fasting. In particular, glutamine is removed from splanchnic circulation to produce citrulline and to provide energy for the enterocytes (176). Several glutamine transporters, notably SNAT1, SNAT2, and SNAT4 (sodium neutral amino acid transporters 1/2/4; *SLC38A1*, *SLC38A2*, and *SLC38A4*), are expressed in the intestine (122). Expression levels are low, however, compared with transporters involved in vectorial transport. SNAT5 (sodium neutral amino acid transporter 5, *SLC38A5*) is also expressed in the intestine but appears to have a role in glutamine supply to stem cells in the crypt (141).

There is limited information about the regulation of epithelial transporters. LAT4 is regulated by phosphorylation/dephosphorylation at Ser274 and Ser297. Single dephosphorylation of Ser274 severely curtails activity of the transporter, while double dephosphorylation restores activity (111). Phosphorylation affects the relocalization from the membrane into intracellular stores and the stability of the protein. In mice at the start of the feeding period, phosphorylation of Ser274 decreased and that of Ser297 increased (112). This is consistent with an increased activity of LAT4 in anticipation of food intake. At the mRNA level, amino acid transporters show regulation in dependence of the protein content of the food and along the feeding–fasting cycle. B^0 AT1, LAT2, TAT2, and γ^+ LAT2 mRNA were higher during the restricted feeding time compared with resting time (111).

2.1.4. Mouse models related to basolateral neutral amino acid transport. LAT2^{-/-} mice show slightly elevated aminoaciduria for a number of neutral amino acids such as Gly, Ser, Thr, Gln, Leu, and Val (24). Surprisingly, serum levels of the same amino acids were elevated, suggesting reduced metabolism. Growth and weight gain of LAT2^{-/-} mice were normal, suggesting that intestinal absorption of amino acids remained normal.

LAT2^{-/-} mice, however, gained significantly less weight on a 14-week high-fat diet compared with wild-type mice (118). In particular, subcutaneous adipose tissue was refractory to weight increase. Adipocytes remained smaller in the knockout mice, and infiltration of white adipose tissue with macrophages was reduced. Liver weight also increased only slightly on a high-fat diet, and lipid accumulation in hepatocytes was reduced. No differences were observed on the control diet. Glucose tolerance was strongly reduced in the wild type after 14 weeks of a high-fat diet, while remaining stable in the LAT2^{-/-} mice. Muscle atrophy and perimuscular adipose tissue on the high-fat diet were reduced in LAT2^{-/-} mice. Reduced lipid deposits were also observed in other organs such as heart, kidney, and brain.

The metabolic phenotype of LAT2^{-/-} mice was most likely driven by the lack of increased food consumption on the high-fat diet, which led to the weight gain and increased adipose tissue in wild-type mice. A smaller weight gain was also observed in the LAT2^{-/-} mice, due to increased weight of perigonadal adipose tissue. LAT2 is widely expressed, and as a result it is difficult to

assess which organ plays the dominant role in this phenotype. However, LAT2 is not expressed in liver, suggesting that the reduced accumulation of fat in the liver is an indirect effect of reduced amino acid absorption from the intestine or reduced free fatty acid release from adipose tissue (118). LAT2 is expressed in the brain and could also modulate neuronal signaling and feeding behavior (29).

TAT1^{-/-} mice breed normally and have normal organ histology (94). Similar to other knockout mice, no upregulation of other transporters was observed as a compensation. Consistent with the substrates of the transporter, plasma levels of aromatic amino acids were elevated between twofold (Phe) and eightfold (Tyr). This suggests that lack of TAT1 in the liver reduced the metabolism of aromatic amino acids. Plasma levels of several other amino acids were slightly lower than in wild-type mice. No significant changes of this pattern were observed on a high-protein diet. Transepithelial transport of Phe was reduced (94).

To study the role of LAT4 in intestinal amino acid absorption, a global knockout was generated. These mice died within 10 days after birth (64). LAT4^{-/-} mice have reduced plasma nonessential amino acids and low fasting plasma glucose, suggesting that they were starving due to a defect of intestinal absorption. To investigate the phenotype in mature mice, a tissue-specific and an inducible knockout strain were generated (125). In addition, a TAT1/LAT4 (inducible) double knockout was generated. In the inducible LAT4^{-/-} mice, the knockout in the intestine was 98% complete (125) but did not lead to any changes of body weight or fasting plasma amino acid levels. No compensatory changes of other transporters were observed at the mRNA level. Surprisingly, newborn intestine-specific LAT4^{-/-} mice gained weight normally and did not show a starvation phenotype. Of note, the knockout was 85–95% efficient, leaving some residual transport activity. Enterocyte accumulation of labeled leucine after oral gavage was elevated in the early sections of the small intestine, and the remaining luminal leucine was elevated in later sections of the intestine. This is consistent with intact apical transport and reduced transepithelial transport. Appearance of labeled leucine in blood plasma was delayed but not reduced overall. In summary, it appears that individual basolateral transporters are redundant for absorption of neutral amino acids.

Consequently, LAT4^{-/-intestine}/TAT1^{-/-} mice were used to test the role of the two basolateral uniporters in transepithelial movement of neutral amino acids (125). The LAT4 knockout was almost 100% complete in the intestine at mRNA and protein levels. Surprisingly, amino acid absorption was similar to wild-type animals, and body weight and lean mass were similar to the wild type. One reasonable explanation of this surprising result would be that LAT2 carries out exchange most of the time but can mediate some uniport, particularly when alternative routes are missing. This has also been proposed for the placenta (175). A triple knockout has not been reported.

2.2. Cationic Amino Acids

2.2.1. Cationic amino acid transport across the apical membrane. In contrast to the standard model of vectorial transport, where a Na⁺-dependent transporter is found in the apical membrane and facilitated diffusion occurs in the basolateral membrane, cationic amino acids use antiporters in both membranes (60). A transporter for cationic amino acids was identified by functional studies using intestinal tissue (101) and vesicles (148). The transporter was Na⁺ independent and interacted with neutral amino acids. A specific carrier for cationic amino acids and cystine was also suggested by the aminoaciduria observed in cystinuria (113), a rare disorder known to also affect intestinal transport (98). These unusual characteristics were confirmed by the cloning of, initially, the trafficking subunit rBAT (related to b^{0,+} amino acid transporter, *SLC3A1*) (114) and, later, the catalytic subunit b^{0,+}AT (sodium independent blastocyst neutral and cationic amino acid

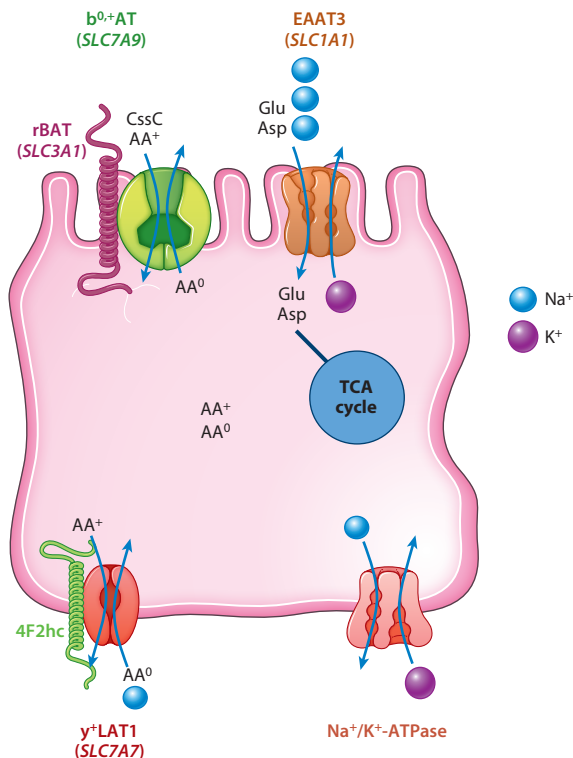


Figure 2

Vectorial transport of cationic and anionic amino acids in enterocytes. The transporter $b^{0,+}AT$ (sodium independent blastocyst neutral and cationic amino acid transporter, *SLC7A9*) forms a complex with rBAT (related to $b^{0,+}$ amino acid transporter, *SLC3A1*) in the apical membrane. It mediates the import of cationic amino acids (AA^+) and cystine (CysC) in exchange for neutral amino acids (AA^0). EAAT3 (excitatory amino acid transporter 3, *SLC1A1*) mediates the active uptake of anionic amino acids through a complex transport mechanism involving Na^+ and K^+ ions. In the basolateral membrane, y^+LAT1 (cationic and large neutral amino acid transporter 1, *SLC7A7*) forms a complex with 4F2hc, mediating the efflux of cationic amino acids in exchange with neutral amino acids. Anionic amino acids are largely metabolized in enterocytes, with limited vectorial transport occurring. Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

transporter, *SLC7A9*) (113) (**Figure 2**). The antiporter activity, involving an exchange of cationic with neutral amino acids, was first identified by electrophysiological experiments (31) and later by flux studies (38) and vesicle experiments (128). The substrate specificity was elucidated in more detail for the human isoform (100) and the purified transporter (181). The transporter accepts most neutral and all cationic amino acids including cystine, with K_M values ranging from 100–300 μM . Preferred substrates are Arg, Lys, Ala, Cys and cystine, Leu, Met, Phe, and Tyr. The affinity of the antiporter is asymmetric, with cytosolic K_M values being ~ 20 times higher than luminal K_M values (158). When investigated in vesicles containing the reconstituted transporter, the difference was even larger, with leucine having K_M values of 0.5 $\mu mol/L$ versus 2,500 $\mu mol/L$ (128). The structure of the rBAT and $b^{0,+}AT$ complex has been resolved by cryogenic electron microscopy (cryo-EM) (178, 181).

No other transporter has been identified in the small intestine for cationic amino acids or cystine. $ATB^{0,+}$ (sodium dependent blastocyst neutral and cationic amino acid transporter, *Slc6a14*),

which transports cationic and neutral amino acids, is expressed in the distal jejunum and colon (67). Physiologically, rBAT/b^{0,+}AT takes up cationic amino acids in exchange for neutral amino acids (117), which are recaptured by B⁰AT1 located in cells downstream. Consistently, cationic amino acids are slightly elevated in the urine and feces of B⁰AT1^{-/-} mice (74). Cystine is most likely transported by rBAT/b^{0,+}AT as a neutral amino acid. It is partially anionic at neutral pH but is largely neutral at the local acidic pH close to the apical membrane.

2.2.2. Mouse models related to apical cationic amino acid transport. Due to the focus on the renal phenotype of cystinuria, the intestinal phenotype of b^{0,+}AT^{-/-} mice has received limited attention. Hyperexcretion of cystine, lysine, arginine, and ornithine in the kidney suggests a similar deficiency of absorption in the intestine. Plasma amino acid levels were normal, with histidine, serine, and glutamate/glutamine levels slightly elevated. Ussing chamber experiments demonstrated a lack of lysine-induced inward currents in sections of the jejunum (53). Arginine- and ornithine-induced currents, by contrast, were only partially suppressed, suggesting some redundancy in mouse jejunum. In the absence of Na⁺ ions, lysine-induced currents were very small, and no differences between wild type and b^{0,+}AT^{-/-} intestines were observed. This may be caused by the coupling of apical and basolateral transport, in which the exit of lysine through the basolateral membrane requires Na⁺ for the exchange with neutral amino acids. Alternatively, it may indicate expression of ATB^{0,+} in this section of mouse intestine.

2.2.3. Cationic amino acid transport across the basolateral membrane. Cationic amino acids are transported across the basolateral membrane by the heteromeric transporter 4F2hc-y⁺LAT1 (cationic and large neutral amino acid transporter 1, *Slc3a2/Slc7a7*) (**Figure 2**). The closely related transporter y⁺LAT2 (cationic and large neutral amino acid transporter 2, *Slc3a2/Slc7a6*) is more widely expressed, and its transport activity was first described in placenta and erythrocytes (52). However, there appears to be no redundancy in the intestine (131). The transport activity of y⁺LAT1 was defined by the ability of leucine to stimulate the absorption of cationic amino acids by epithelial cells and by the aminoaciduria observed in the rare disorder lysinuric protein intolerance (LPI) (51). An exchange process in the basolateral membrane involving cationic and neutral amino acids was first proposed for frog intestine (35). Thus, efflux of cationic amino acids is mediated by an exchange against neutral amino acids plus a Na⁺ ion. This mechanism was confirmed after the molecular cloning of the y⁺LAT1 light chain (116, 159). The presence of Na⁺ increases the affinity for neutral amino acids by two orders of magnitude. When Na⁺ is absent, protons can sustain transport activity (76). The affinity of the transporter ranges from 44 μM for leucine to 340 μM for arginine. Vectorial transport of cationic amino acids was demonstrated by coexpression of rBAT/b^{0,+}AT and 4F2hc/y⁺LAT1 in polarized MDCK (Madin–Darby canine kidney) cells (15). However, in the absence of B⁰AT1, it is accompanied by a vectorial transport of leucine in the opposite direction.

2.2.4. Mouse models related to basolateral cationic amino acid transport. Initial attempts to create y⁺LAT1^{-/-} mice were hampered by intrauterine growth restriction, resulting in cannibalism of the newborns (146). An inducible knockout mouse model was subsequently generated to study the physiological role of y⁺LAT1 in more detail (18). LPI is characterized by low serum levels of lysine, arginine, and ornithine, resulting in urea cycle deficiency. This can be ameliorated by citrulline supplementation, an amino acid absorbed by neutral amino acid transporters. Because of the catalytical nature of the urea cycle, citrulline can restore its activity. In the absence of citrulline, 50% of the induced y⁺LAT1^{-/-} mice died within the first month. Body weight and adipose tissue weight were dramatically reduced after 7–10 days of knockout induction. Citrulline supplementation improved both parameters. Intestinal absorption of lysine was absent, while

absorption of glucose was normal. γ^+ LAT1^{-/-} mice showed reduced plasma levels of arginine and lysine but not of ornithine. Citrulline supplementation normalized arginine levels and increased ornithine levels, due to metabolic conversion. γ^+ LAT1^{-/-} mice have a complex pathology involving lungs, liver, spleen, bones, and brain, which is not considered here in the context of intestinal absorption. An alternative mouse model was generated by Stroup et al. (151), who noted improved perinatal survival on a mixed 129/SvEv × C57BL/6 background. Plasma levels of cationic amino acids were significantly reduced, particularly that of arginine. The model replicated several of the complex phenotypes of LPI, but the intestinal phenotype was not investigated.

2.3. Anionic Amino Acids

EAAT3 (excitatory amino acid transporter 3, *SLC1A1*) is the dominant transporter for the absorption of anionic amino acids in the intestine (58) (**Figure 2**). In the rabbit ileum, ASCT2 (*SLC1A5*) contributes to the absorption of anionic amino acids (91, 104), which are transported by ASCT2 at low pH (164). EAAT3 has a complex mechanism involving the cotransport of aspartate or glutamate with 3Na⁺ and 1H⁺ followed by return of the carrier with K⁺ bound to it (166). Glutamate and aspartate are transported with K_M values of <100 μM. In contrast to other glutamate transporters of the EAAT family, EAAT3 can also transport cysteine (188). The structure of the human EAAT3 has been determined by cryo-EM (123).

Similar to neutral and cationic amino acids, a rare aminoaciduria (dicarboxylic aminoaciduria) exists, which is caused by inactivating mutations of EAAT3 affecting renal and intestinal anionic amino acid transport (12). While the renal phenotype is readily detected as an aminoaciduria, the intestinal phenotype is difficult to assess because glutamate and aspartate are largely metabolized in enterocytes (179). As a result, very little vectorial transport is observed (121). A mouse model lacking EAAT3 has been reported, but the intestinal phenotype was not investigated (115). Basolateral transporters that release glutamate have not been identified; in fact, an anionic amino acid transporter similar to the apical transporter has been described (22).

2.4. Transport of Glycine, Proline, and β-Amino Acids

Due to their short side chains, special conformation, and distance between the α-amino and α-carboxyl group, glycine, proline, and β-amino acids are poorly transported by other neutral amino acid transporters. Three separate transport mechanisms were identified for glycine and proline (177). These correspond to the neutral amino acid transporter B⁰AT1, the proline and glycine transporter PAT1 (proton amino acid transporter 1, *Slc36a1*) (4), and the proline transporter SIT1 (*Slc6a20*) (28) (**Figure 3**). PAT1 is a low-affinity, high-capacity transporter for glycine, betaine, proline, alanine, the β-amino acid taurine, and the γ-amino acid GABA. K_M values range from 3.1–7.5 mM for these substrates. In contrast to other amino acid transporters, it is not stereo specific, accepting D-amino acids with similar affinity (20). The transporter is proton dependent and has the highest transport activity at the acidic pH of the intestinal brush border (88). PAT1 serves as a lysosomal transporter in other tissues.

SIT1 is an Na⁺- and Cl⁻-dependent transporter for proline, betaine, and hydroxyproline. The transporter has been intensively characterized in brush-border membrane vesicles (150), and its properties match that of the cloned transporter (82, 152). Proline transport is electrogenic due to the cotransport of 2Na⁺ and 1Cl⁻ with each substrate. Proline, hydroxyproline, and betaine are transported with K_M values of 0.1–0.2 mM. SIT1 is expressed in all sections of the small intestine (130, 154). Like B⁰AT1, it requires ACE2 to be expressed at the apical membrane of the intestine. The structure of the SIT1/ACE2 complex has been resolved by cryo-EM (138). The expression of both proteins in the intestine is overlapping without showing a strong gradient (169).

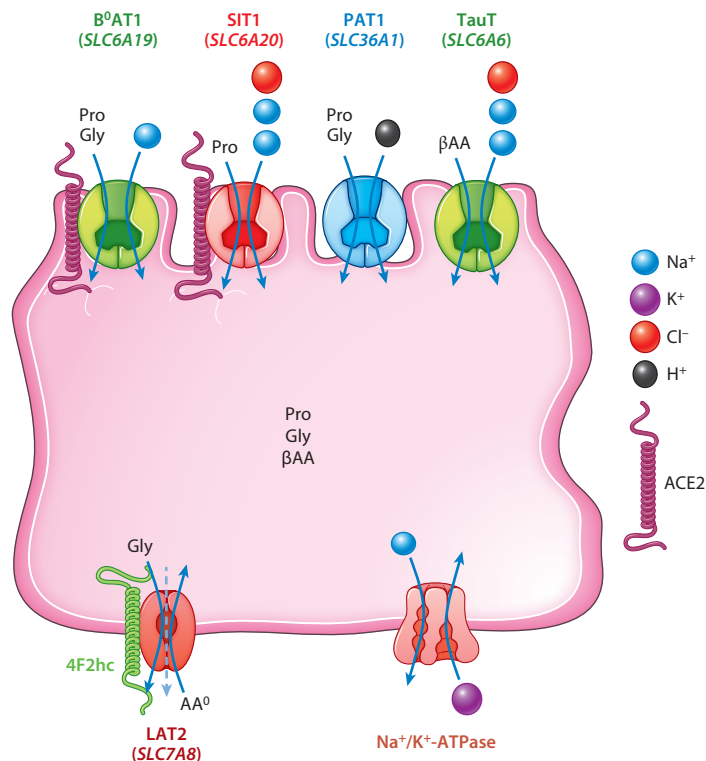


Figure 3

Vectorial transport of proline, glycine, and β -amino acids (β AA) in enterocytes. B⁰AT1 (broad neutral amino acid transporter 1, *SLC6A19*) and SIT1 (System IMINO transporter 1, *SLC6A20*) form a complex with angiotensin converting enzyme II (ACE2) in the apical membrane. B⁰AT1 cotransports neutral amino acids (AA⁰) with Na⁺ ions. SIT1 mediates the cotransport of proline with 2Na⁺ and Cl⁻. PAT1 (proton amino acid transporter 1, *SLC36A1*) facilitates the cotransport of H⁺ with glycine and proline. TauT (taurine transporter, *SLC6A6*) mediates the cotransport of β AA with 2Na⁺ and Cl⁻. LAT2 (large neutral amino acid transporter 2, *SLC7A8*) mediates the efflux of glycine in exchange with other neutral amino acids across the basolateral membrane. No efflux pathway for proline has been identified. Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

Taurine transport is mediated by two transporters, namely PAT1 and the Na⁺- and Cl⁻-dependent taurine transporter TauT (*SLC6A6*) (5). The properties of the taurine transporter TauT match those of taurine and the β -alanine transport in brush-border membrane vesicles of rat (13) and rabbit intestine (99). It is a high-affinity, low-capacity transporter with a K_M for taurine of 7 μ M (5). In rabbit and guinea-pig intestine, this transporter appears to be nonredundant, while in the rat intestine a low-affinity, high-capacity carrier is observed (105), namely PAT1 (156). In the distal jejunum and colon, β -alanine but not taurine is transported by the general amino acid transporter ATB⁰⁺ (5). Transcripts of PAT1 and TauT were found in all sections of the human intestine, with highest expression in stomach, duodenum, and jejunum. ATB⁰⁺, by contrast, was found in stomach, duodenum, and colon.

It has been proposed that the nutritional dependence on taurine is related to the type of diet. Carnivores such as cats have little endogenous taurine biosynthesis and require absorption in the

intestine, which is mediated by TauT and PAT1. Most vertebrates conjugate bile acids exclusively to taurine, a major metabolic sink for this amino acid (5, 70).

Transport of glycine across the apical membrane would be mainly mediated by the general neutral amino acid transporter B⁰AT1 and in addition by PAT1. Both transporters have been described in previous sections.

Transport of glycine across the basolateral membrane is mediated by GlyT1A (39, 69). This transporter would accumulate glycine inside enterocytes, and it is assumed that the efflux via LAT2 (135) is of higher capacity, therefore dominating during nutrient absorption. In times of nutrient limitation, GlyT1A can be upregulated via the GCN2/ATF4 (general control nonderepressible 2/activating transcription factor 4) pathway to support enterocyte function.

2.5. Peptide Transport

Peptide transport across the apical membrane has received significant attention and has been covered by several comprehensive reviews (43, 63, 145). It is covered in this review only briefly as an alternative important route of amino acid absorption because the peptides, with few exceptions, are fully hydrolyzed in the cytoplasm of enterocytes and are released as individual amino acids. The transporter accepts di- and tripeptides and uses the proton gradient to drive transport across the apical membrane. Transport is electrogenic and includes peptides with charged side chains. Peptides containing proline in the N-terminal position have a low affinity for the transporter. Peptides of D-amino acids are not transported. The minimal essential features of a substrate are two oppositely charged head groups with a distance of 5.5–6.3 Å. These are clamped by electrostatic interactions with lysine and arginine residues in the C-terminal pocket and glutamate and asparagine residues in the N-terminal pocket (78). As a result, PepT1 transports a variety of non-peptide and pseudopeptide molecules, including β-lactam antibiotics, ACE inhibitors, antiviral peptides, prodrugs, and δ-aminolevulinic acid (23, 25).

3. AMINO ACID TRANSPORT IN THE LARGE INTESTINE

While the absorption of nutritional protein is completed in the small intestine, the large intestine is an important site for the absorption of amino acids derived from endogenous secretions such as mucus, shed epithelial cells, and microbial amino acids (165). It has been estimated that 2–7% of the daily protein uptake enters the large intestine. The mRNA expression of the major transporters involved in vectorial transport, such as B⁰AT1, b^{0,+}AT, and LAT2, drops several orders of magnitude from the jejunum to the colon. PAT1, by contrast, shows an even expression along the intestine (5). Dominant amino acid transporters in the large intestine are ATB^{0,+} (*Slc6a14*) and ASCT2 (*Slc1a5*), which are both located in the apical membrane (3, 8) (**Figure 4**). The activities of both transporters were initially detected in the distal ileum of rabbits (102, 103). Subsequently, ATB^{0,+} was identified as the molecular correlate of the intestinal β-alanine carrier (3). It is mainly expressed in the distal ileum and colon (67). The transporter virtually accepts all neutral and cationic amino acids but accumulates mainly glycine and branched-chain amino acids (57). The cotransport stoichiometry is 2Na⁺/1Cl⁻ for most neutral amino acids except glycine, where it is 3Na⁺/1Cl⁻ (86), which explains the predominant accumulation of glycine when expressed heterologously (57). K_M values range from 6–600 μM (142). ASCT2 is an obligatory antiporter exchanging small neutral amino acids including glutamine and asparagine with high affinity (164). As such, it would have limited use in vectorial transport; however, the transporter does accept glutamate and aspartate at low pH. Thus, it could mediate removal of glutamate and aspartate in the acidic microclimate of the brush border in exchange for nonessential neutral amino acids (**Figure 4**).

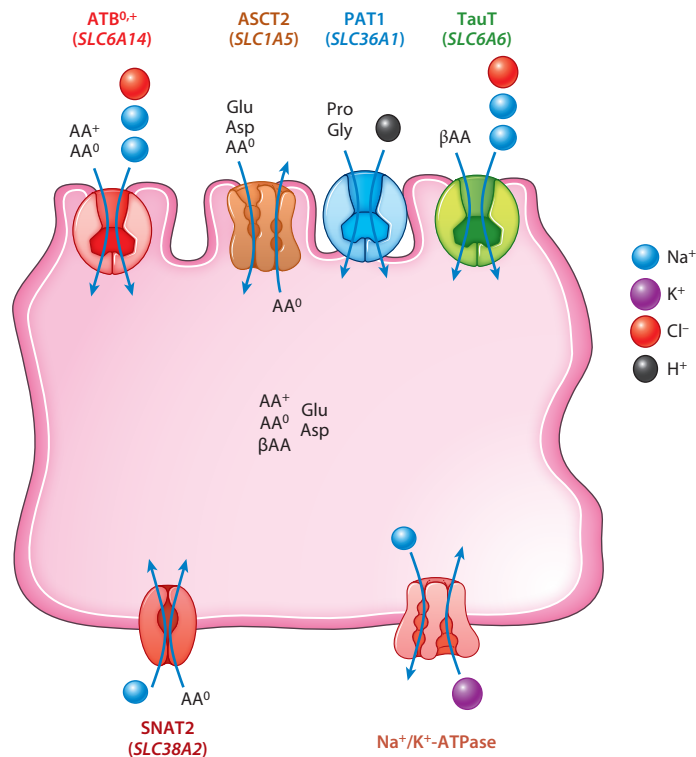


Figure 4

Amino acid transport in the large intestine. In the large intestine, the apical membrane contains mainly four amino acid transporters, namely $ATB^{0,+}$ (sodium dependent blastocyst neutral and cationic amino acid transporter, *SLC6A14*), ASCT2 (alanine-serine-cysteine transporter 2, *SLC1A5*), PAT1 (proton amino acid transporter 1, *SLC36A1*), and TauT (taurine transporter, *SLC6A6*). $ATB^{0,+}$ and TauT cotransport their substrates with $2Na^+$ and Cl^- . ASCT2 is an exchanger that normally mediates antiport of neutral amino acids but in the acidic climate of the intestine can mediate the uptake of glutamate and aspartate in exchange with neutral amino acids. PAT1 (*SLC36A1*) facilitates the cotransport of H^+ with glycine and proline. SNAT2 (sodium neutral amino acid transporter 2, *SLC38A2*) is found in the basolateral membrane. Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

4. METABOLIC EFFECTS OF PROTEIN RESTRICTION AND OF SPECIFIC AMINO ACIDS

Low-protein diets have been shown to promote leanness, glycemic control, and overall health, whereas high-protein diets do the opposite (59, 85, 87, 92, 143, 144, 185). These effects appear to be mainly mediated by essential amino acids that are limiting. In particular, isoleucine (185), tryptophan, and threonine (30, 184) have been reported as drivers in low-protein diets, and their respective roles may depend on the precise amino acid composition. Consistently, elevated isoleucine has been identified as part of a metabolomic profile that is associated with increased mortality (47). Restriction of methionine has also been associated with improved health (30).

High-protein diets are effective to improve satiety and weight loss (187), but long-term overactivation of mTORC1 appears to be associated with adverse health outcomes. Consistently, treatment of mice with the mTOR inhibitor rapamycin extended their life span (65, 108). Another important factor is the upregulation of autophagy when mTOR activity is reduced due to protein restriction (68).

In addition to mTOR, the second major readout of protein restriction is the GCN2/ATF4 pathway. This pathway detects uncharged transfer RNA molecules and stalled ribosomes (174). Activation of GCN2 and ATF4 causes increased expression of fibroblast growth factor 21 (FGF21), a metabolic hormone regulating lipid metabolism and energy balance (77, 109). FGF21 has a number of systemic effects, such as browning of adipose tissue, increased thermogenesis, and increased glucose uptake into muscle. In liver, it initiates ketogenesis (11, 119). Deletion of GCN2 abrogates some of the effects of amino acid restriction (84, 161).

Overall, protein restriction has direct cellular effects on mTOR, GCN2, and autophagy, and it has systemic effects through FGF21 (170). The effects of protein restriction are remarkably well recapitulated in mice lacking the intestinal neutral amino acid transporter B⁰AT1 (75). Depletion of the essential amino acid Thr has been suggested as a trigger for the metabolic effects observed in these mice (184). The mTOR activity is reduced in many tissues including liver, muscle, and intestine. Moreover, FGF21 is highly elevated, resulting in improved metabolic health (75). The mice are protected against diet-induced obesity and show improved glucose tolerance due to increased uptake of glucose into heart muscle and browning of subcutaneous adipose tissue. Liver triglycerides were reduced and animals had reduced hepatosteatosis when fed with a high-fat fructose/glucose diet for 16 weeks (180). As a result, B⁰AT1 has been recognized as a target to treat metabolic diseases (16, 49).

Surprisingly, the metabolic phenotype for PepT1^{-/-} mice is quite different (81). The mice also stay leaner on a high-fat diet, but this was caused by loss of energy in fecal matter, which did not occur in B⁰AT1^{-/-} mice. Plasma glucose levels were significantly lower in PepT1^{-/-} mice. The general malabsorption was possibly caused by a lack of adaptation of the upper small intestinal mucosa to the trophic effects of the diet, for instance by increasing the length of the villi. Mechanistically, this may be caused by the coupling of the epithelial proton fluxes of PepT1 and NHE3 (157) as indicated by a similar phenotype in NHE3-knockout mice (134). PepT1 was also involved in appetite control; food intake on a high-protein diet was reduced compared with wild-type mice. On a high-protein diet, the increase of branched-chain amino acids in blood plasma was reduced (106).

5. INTERACTION BETWEEN MICROBIOME AND AMINO ACID TRANSPORT

Several studies show that epithelial amino acid transporters are important for the maintenance of a balanced intestinal microbiome, which in turn affects many human diseases.

Chromodomain-helicase-DNA-binding protein 8 (CHD8) has been identified as one of the genes with the strongest association with autism. Mice haploinsufficient for CHD8 replicate many of the features of human autism spectrum disorder (ASD) and are used as a model to study neurodevelopmental changes associated with ASD (173). Gut microbiota have been shown to modulate neural function and behavior including ASD (137). Chd8^{+/-} mice have increased brain weight and a shortened small intestine similar to a certain subtype of autism (186). The mice had increased levels of Glu and Gln and decreased levels of Trp and His in brain. In the serum, Gln levels were increased, while Trp was decreased. In a search for possible causes, elevated expression of epithelial neutral amino acid transporters B⁰AT1 and LAT2 was detected. Fecal amino acid levels were reduced, including Trp and Gln. Reverting the microbiome to wild-type composition improved anxiety and learning and memory defects, and normalized brain glutamine and glutamate levels. Serum Gln and Trp levels also normalized together with expression levels of B⁰AT1 and LAT2. Administration of B⁰AT1 inhibitor S-benzyl-cysteine reduced brain glutamine but not glutamate levels. The treatment improved impaired social interaction and anxiety (186).

ACE2^{-/-} mice, which lack B⁰AT1 expression in the intestine, were shown to have an increased inflammatory reaction after administration of dextran sodium sulphate (DSS). DSS-induced colitis is a standard model to investigate intestinal inflammation. In ACE2^{-/-} mice it resulted in diarrhea, intestinal bleeding, and crypt damage (66). Administration of the soluble catalytic domain of ACE2 did not rescue the symptoms, suggesting that they were not associated with the renin–angiotensin system. Consistent with the lack of B⁰AT1, reduced tryptophan levels were observed in ACE2^{-/-} mice. Pellagra is often associated with colitis, which is treated with nicotinamide. In vivo, Trp and the vitamin niacin contribute to the synthesis of NAD(P). Administration of a Gly-Trp dipeptide rescued the enhanced DSS susceptibility and also reduced colitis in the wild type. Expression of multiple antimicrobial peptides was reduced in ACE2^{-/-} mice, including defensin α 1, potentially as a result of reduced mTORC1 activation. This, in turn, changed the ileocecal microbiome (Figure 5). Transplantation of the microbiota from ACE2^{-/-} mice into germ-free hosts transferred the susceptibility to DSS.

Together the studies show that tryptophan levels in the lumen and in enterocytes play an important role in regulating the intestinal microbiome. The delayed absorption of amino acids in

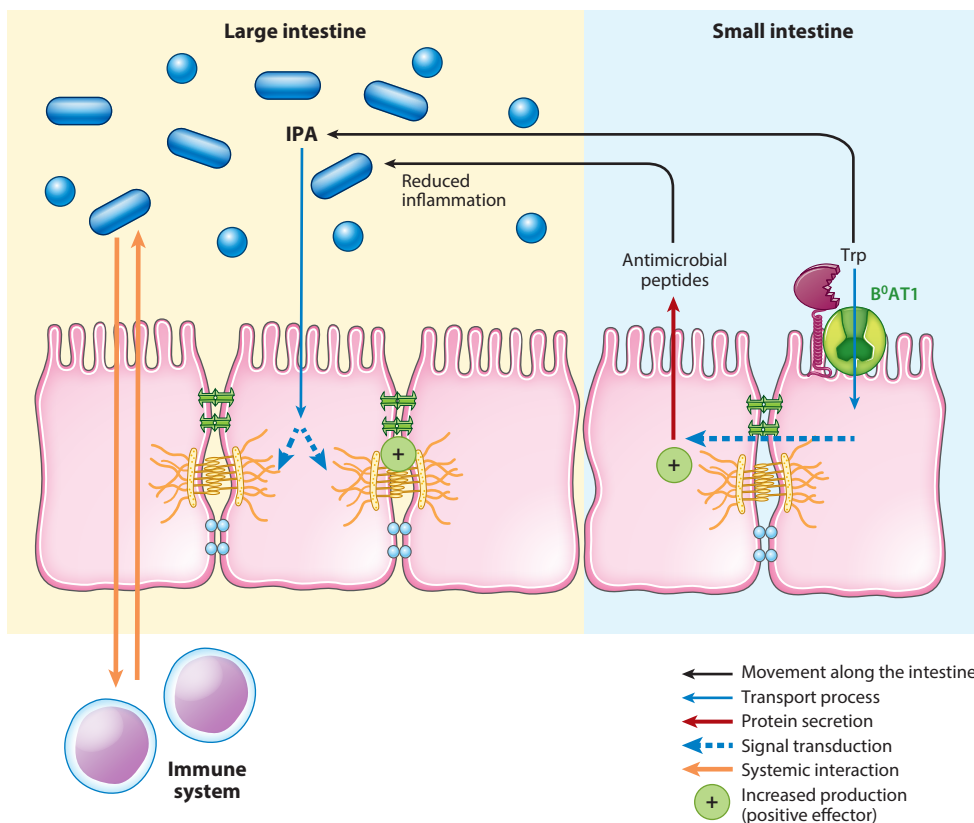


Figure 5

Interactions between amino acid transport and the microbiome. B⁰AT1 (broad neutral amino acid transporter 1) mediates the absorption of tryptophan in the intestine. Tryptophan (Trp) is crucial for the production of antimicrobial peptides in the small intestine, which regulate bacterial growth in the large intestine. The microbiome is under surveillance by the immune system. Unabsorbed Trp is converted by the microbiome into indole-3-propionic acid (IPA) among other metabolites, which have been shown to increase the expression of adherence junction proteins, thereby improving intestinal barrier function. Figure adapted from an image created using Servier Medical Art (CC BY 3.0 Unported).

B⁰AT1^{-/-} mice results in the fermentation of amino acids by the microflora into metabolites, such as isovaleric acid, valeric acid, and indole compounds (74). Bacterial Trp metabolism generates indole-3-propionic acid (IPA), which can be detected in the plasma of humans and mice (54, 74). IPA has been shown to improve intestinal barrier function through binding to the pregnane X receptor (167), which downregulates TNF- α and increases expression of junctional proteins (Figure 5). These results are at variance with the ACE2^{-/-} reported above.

Glycine is thought to protect intestinal cells from inflammation, possibly due to compensating against oxidative stress and through other mechanisms (189). Basolateral transport of glycine via GlyT1 provides enterocytes with elevated concentrations of glycine and may contribute to the protection against inflammation (96). In agreement with this notion, ATB^{0,+}, which highly accumulates glycine (57), is upregulated in inflammatory states, such as Crohn's disease (56) and ulcerative colitis (55). ATB^{0,+} has been identified as a modifier of cystic fibrosis, in particular the occurrence of bowel obstruction in infants (133). Multiple mechanisms have been proposed including the accumulation of arginine for the production of nitric oxide, which in turn regulates CFTR chloride channels (2), and the removal of amino acids to reduce bacterial growth.

Pathologic expression of PepT1 in the colon leads to the uptake of bacterial di- and tripeptides, causing chronic inflammation, and is associated with disorders such as inflammatory bowel disease and colon cancer (71, 168).

6. INHERITED DISORDERS OF AMINO ACID TRANSPORT

Inherited disorders of amino acid transport have been instrumental in the definition of epithelial transport activities in kidney and intestine. All disorders are rare, and treatment typically involves an adjustment of diets and supplements to stabilize amino acid homeostasis.

Hartnup disorder is a rare inherited renal aminoaciduria restricted to neutral amino acids (14) and is caused by mutations of B⁰AT1 (*SLC6A19*) (80, 136). It also affects intestinal amino acid uptake, as inferred from the presence of bacterial amino acid degradation products that are elevated in blood plasma, such as indole and tryptamine (7, 74). Despite malabsorption and loss of amino acids through the urine, clinical symptoms are typically minor when individuals are provided with a protein-rich diet. A shorter than normal stature has been reported (40). When clinical symptoms occur, they are similar to those observed in pellagra, namely a photosensitive skin rash and attacks of cerebellar ataxia. Very few severe cases have been reported in the recent literature (37, 171, 190) and may have been caused by unrelated comorbidities. The mild symptoms can be explained by compensation of the lack of neutral amino acid absorption through peptide transport (6) and some residual amino acid transport. The clinical symptoms of Hartnup disorder respond to treatment with NAD(P) precursor niacin. As a result, it is thought that the clinical symptoms are caused by the lack of tryptophan and of its metabolite nicotinamide.

Cystinuria is defined by the presence of excessive amounts of cystine, lysine, arginine, and ornithine in urine. The disease is caused by mutations in either of the two subunits of the epithelial cationic amino acid transporter rBAT/b^{0,+}AT (*SLC3A1/SLC7A9*) (32, 48). The clinical symptoms of the disease are caused by the formation of kidney stones due to the low solubility of cystine. Transport of the affected amino acids is also impaired in the intestine (95, 98, 155). This can be demonstrated by increased levels of cationic amino acids occurring in feces after oral loading and the generation of bacterial metabolites of cationic amino acids such as cadaverine, putrescine, and citrulline. The loss of cystine, arginine, and lysine transport in the intestine is complete, suggesting a lack of redundant transport capacity in the intestine (61). Expression of ATB^{0,+} (*SLC6A14*) in the large intestine cannot compensate for the lack of rBAT/b^{0,+}AT. This confirms the limited capacity of the large intestine in nutrient absorption (165) even when nutrient absorption in the small intestine is incomplete.

Lysinuric protein intolerance is characterized by hyperdibasic aminoaciduria and an associated protein intolerance. It is caused by mutations in the light chain of the heteromeric transporter 4F2hc/y⁺LAT1 (21, 160). The disease has a complex pathology affecting muscle, bones, liver, spleen, and lung. Plasma levels of cationic amino acids are severely reduced, affecting urea cycle activity, which explains the intolerance to protein ingestion (110). Although most readily detected by the defect in renal reabsorption, intestinal absorption of cationic amino acids is also lacking, resulting in malnutrition (124). In contrast to defects of apical amino acid absorption, peptide transport cannot compensate for the lack of basolateral transport. Consistently, transepithelial transport of lysine is reduced, while apical transport of lysine in enterocytes is unaffected (50). The defect can be partially compensated by provision of citrulline, which is transported by neutral amino acid transporters and can restore urea cycle activity but does not relieve lysine malabsorption.

Dicarboxylic aminoaciduria is a rare and benign autosomal recessive disorder of anionic amino acid reabsorption. Cystine is not affected, although a fraction of the amino acid is anionic at neutral pH. It is caused by mutations in the epithelial glutamate transporter EAAT3 (12). Intestinal transport is affected, as ascertained by glutamate loading followed by lack of an increase of plasma glutamate concentration (153). Because glutamate is rapidly metabolized by enterocytes, increases of plasma alanine, glutamine, and proline were also blunted.

7. CONCLUSION

The small intestine is equipped with a set of apical and basolateral transporters that ensure the vectorial transport of all 20 amino acids. Redundancy of brush-border transport is provided by peptide and amino acid transport occurring in parallel, while redundancy at the basolateral membrane is provided by a multitude of transporters with overlapping substrate specificities. An exception is the efflux of cationic amino acids across the basolateral membrane, for which no compensation occurs, resulting in a serious disturbance of amino acid homeostasis.

Reduced absorption of essential neutral amino acids is a novel principle that may be used to improve human health through a variety of mechanisms associated with amino acid signaling in the intestine, liver, and other organs.

The intestine maintains a balance between the needs of the organism and the microbiome. Higher-than-normal and lower-than-normal expression levels of intestinal amino acid transporters can facilitate disease states such as intestinal inflammation and also systemic disease.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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