Chapter 6
Digestion and Absorption of Carbohydrates and Proteins

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1 Introduction

The pathways used for the digestion and absorption of carbohydrates and proteins share several important common features. Proteins and starch, one of the major dietary carbohydrates, are both polymers that are initially broken down into smaller compounds by enzymes secreted into the intestinal lumen, principally by the pancreas. Further digestion occurs by a variety of enzymes present in the small intestinal brush-border membrane, generating the small molecules (monosaccharides, amino acids, di- and tripeptides) that are capable of being absorbed by the enterocytes. Transport of these molecules through the intestine occurs mainly via various membrane carrier proteins. In some cases, there is an **active transport mechanism** by which the movement of the nutrient through the membrane is coupled directly or indirectly to a source of metabolic energy, permitting concentrative transport against an electrochemical gradient. Other carrier systems accelerate the rate of movement of a nutrient through the membrane, but they are not linked to an energy source and therefore do not result in concentration against an electrochemical gradient. This type of transport process is known as **facilitated diffusion**. **Simple passive diffusion** either through the plasma membrane or between the enterocytes (the *paracellular pathway*) is quantitatively important only when the luminal concentration of nutrients is very high.

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2 Carbohydrates

Dietary carbohydrates consist of complex carbohydrates, starch or dietary fiber, and various sugars, including ***monosaccharides*** (glucose, galactose, fructose) and ***disaccharides*** (sucrose, lactose, maltose). Adult males consume approximately 300 g/day of carbohydrates, and adult females, approximately 200 g/day, half as starch and half as various sugars. Carbohydrates provide about 45–50 % of the energy contained in the average American diet.

2.1 Structure of Dietary Starch

Two principal types of dietary starch are present in the human diet. **Amylose** is composed almost entirely of **α-1,4-linked linear chains** of glucose, with an average chain length of about 600 glucose residues and a molecular weight of about 100 kd. **Amylopectin** is a branched starch with a molecular weight of approximately 1,000 kd and contains about 6,000 glucose residues. Amylopectin has both **α-1,4-linked straight chains** and **α-1,6-linked branch points** occurring approximately every 20 glucose residues (Fig. 6.1). The ratio of amylose to amylopectin varies in different foods, but it is usually about 1:4.

   In food, starches are present in association with proteins, many of which are hydrophobic. The starch tends to be in the interior of the complex particle, limiting access to enzymes responsible for starch hydrolysis. Starch digestion is facilitated by physical processing, such as cracking grains and milling, and by cooking, which converts the starch from a crystalline to a gel structure. Certain foods contain protein inhibitors of starch digestion.
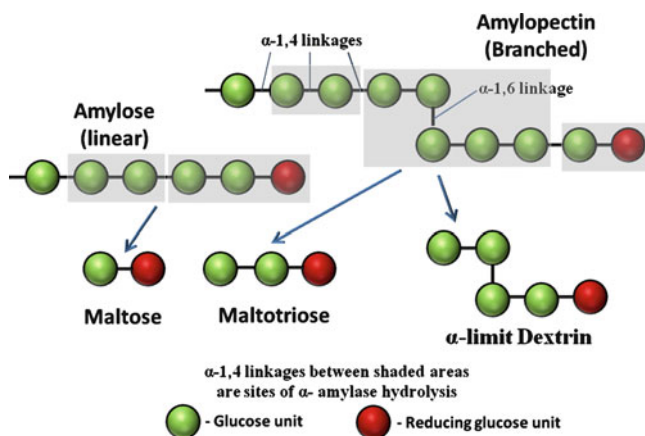
Fig. 6.1 Digestion of starch by pancreatic of alpha-amylase. The action of alpha-amylase on amylose and amylopectin, the two major forms of starch (Adapted from Alpers [1])

2.2   Starch Digestion by α-Amylases

The enzymes responsible for intraluminal starch digestion, **α-amylases**, are present in both *salivary* and *pancreatic* secretions. Human salivary amylase is 94 % identical with pancreatic amylase, although the two enzyme forms are the products of different genes. **Salivary amylase** tends to be degraded at acidic pH (less than pH 3-3.5), but the rate of degradation decreases when salivary amylase is accompanied by its starch substrate or by its oligosaccharide breakdown products. The substrate interacts with the active hydrolytic site and maintains the amylase in a more favorable conformation that allows some active enzyme to survive passage into the duodenum. Thus, while **pancreatic amylase** is responsible for most of the intraluminal starch hydrolysis in adults, some active salivary amylase is usually present in jejunal fluid. In neonates with immature pancreatic function and limited secretion of pancreatic amylase, salivary amylase supports a significant fraction of starch digestion.

The five glucose residues adjacent to the terminal-reducing glucose unit of amylose and amylopectin bind to specific catalytic subsites of α-amylase. There is then cleavage between the second and third α-1,4-linked glucose residues. The disaccharide **maltose** and the trisaccharide **maltotriose** are therefore the *main products of amylose digestion* (Fig. 6.1). **α-amylase** has no activity against the α-1,6-linkages in amylopectin and the capacity to break α-1,4 links adjacent to branch points is sterically hindered. In addition to maltose and maltotriose, therefore, about one third of the *degradation products of amylopectin* are **α-limit dextrins** and **branched oligosaccharides** with one or more α-1,6 bonds and an average molecular weight of 800–1,000 kd (5–10 glucose units) (Fig. 6.1).

2.3   Brush-Border Membrane Digestion of Oligosaccharides

The oligosaccharide products of α-amylase starch digestion are hydrolyzed by enzymes on the brush-border membrane surface, and only free glucose is transported into the enterocyte. These oligosaccharidases are large glycoproteins, with most of the protein, including the catalytic domain, residing at the lumen-cell interface. The enzymes are anchored into the membrane by a short hydrophobic segment. The activities of the oligosaccharidases are greatest in the proximal small intestine and decline distally; starch digestion is normally complete in the jejunum. Membrane hydrolysis of starch-derived oligosaccharides is normally very rapid and is not rate-limiting in the overall process of starch digestion and absorption.

Three enzymes are responsible for the brush-border membrane hydrolysis of oligosaccharides to glucose. (**1**) **Glucoamylase** (**maltase**) removes single glucose residues sequentially from the nonreducing end of the α-1,4 chain, preferring substrates of 5–9 saccharide units; however, like α-amylase, it is blocked when an α-1,6-linked glucose is at the terminal end of the saccharide. *Sucrase-isomaltase* is initially synthesized in the enterocyte as a single glycoprotein chain and, after

Fig. 6.2 Digestion of α-limit dextrins by brush-border membrane oligosaccharidases (Adapted from Gray [8])
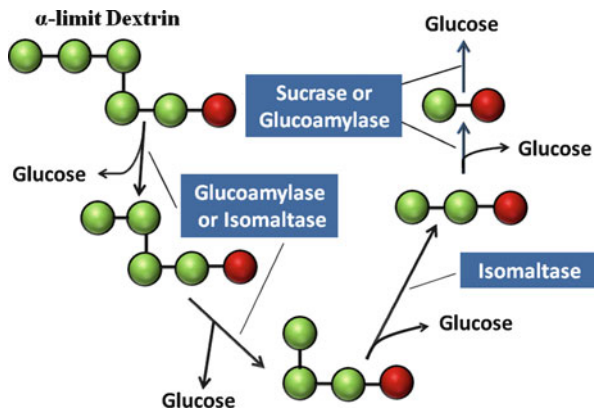
insertion in the brush-border membrane, is cleaved by pancreatic proteases into sucrase and isomaltase units, which then reassociate noncovalently at the intestinal surface (see below). (**2**) **Sucrase** is highly-efficient at hydrolyzing short α-1,4-linked oligosaccharides, such as *maltose* and *maltotriose*. (**3**) **Isomaltase** is the only enzyme that can cleave the nonreducing, terminal α-1,6 bond once it becomes uncovered. As shown in Fig. 6.2, the digestion of a typical α-limit dextrin involves the initial removal of glucose residues from the nonreducing end mainly by glucoamylase, the cleavage of the α-1,6 branching link by isomaltase, and finally the digestion of maltotriose and maltose to glucose principally by sucrase. In general, glucoamylase activity determines the rate of starch digestion.

The **oligosaccharidases** are synthesized as **large glycosylated precursors** that are then processed to the mature enzyme form. For example, sucrase-isomaltase is first synthesized as a single polypeptide chain with a molecular weight of 240–260 kd. O-linked and N-linked sugar chains are added and the precursor is transferred to the brush-border membrane by a vesicular pathway moving along microtubules and inserted into the membrane through its amino terminus. Pancreatic enzymes, mainly *trypsin*, cleave the protein into **two subunits**, the *isomaltase* subunit that is membrane-anchored near its amino-terminus and *sucrase* that is noncovalently bound to isomaltase. Various mutations in the sucrase-isomaltase gene affecting the processing of the precursor protein, the intracellular transport to the apical membrane, and the active catalytic site have been reported. In contrast to sucrase-isomaltase, human glucoamylase is synthesized as a single polypeptide chain that is N- and O-glycosylated and inserted into the brush-border membrane without intracellular or extracellular proteolytic processing.

Brush-border membrane oligosaccharidase activities are affected by many factors and their regulation is complex, involving alterations in transcriptional and post-transcriptional events. In humans, sucrase and glucoamylase mRNA and protein appear in the fetal intestine at 10–14 weeks' gestation and are fully developed at birth. The regulation of enzyme activities along the crypt-villus axis also appears to generally reflect the mRNA abundances. For example, sucrase-isomaltase mRNA is
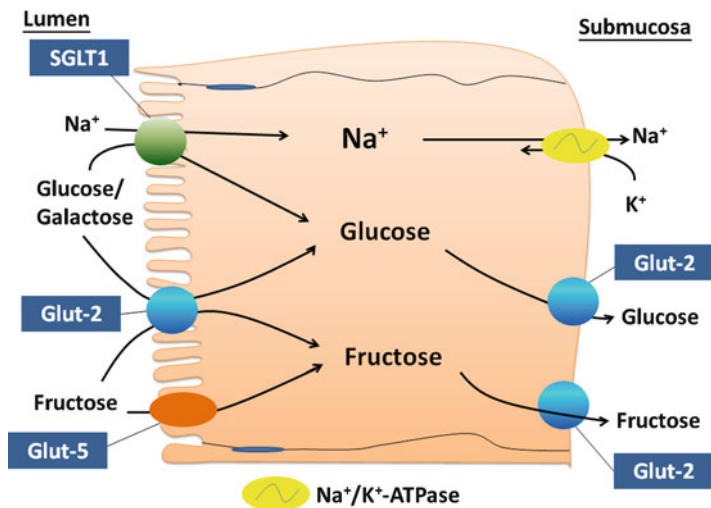
Fig. 6.3 Enterocyte transport of monosaccharides (Adapted from Wright et al. [19])

absent in **crypt cells**, but *detectable* at the **crypt-villus junction**, and enzyme activity is *greatest* in the **mid-upper villus**. Dietary modifications produce alterations in oligosaccharidase activities that may be caused by changes in enzyme synthesis, enzyme degradation, or the activity of the catalytic site. For example, *sucrose feeding* increases sucrase mRNA content, whereas feeding a *carbohydrate-free diet* has been shown to cause conversion of brush-border membrane sucrase to an inactive molecular species. Pancreatic enzymes participate in the degradation of oligosaccharidases and may account, at least in part, for the observed diurnal variation in enzyme activities. **Epidermal growth factor** (**EGF**), an important peptide regulator of intestinal function, affects the intracellular processing and degradation of sucrase.

2.4 Intestinal Glucose Transporters

The efficient intestinal absorption of glucose occurs through the coordinated action of transport proteins located in the **brush-border** and **basolateral membranes** (Fig. 6.3). One pathway of glucose uptake into the enterocyte occurs by a **Na^+-dependent active transport mechanism**. Two sodium ions enter the enterocyte for each molecule of glucose transported, and this absorptive process is therefore electrogenic (net charge transfer across the membrane). Na^+ increases the affinity of the transporter for glucose, and the energy for glucose accumulation is derived from the transmembrane electrochemical Na^+ gradient. The Na^+ gradient is maintained by the action of **Na^+,K^+-ATPase** (**sodium pump**) located on the basolateral
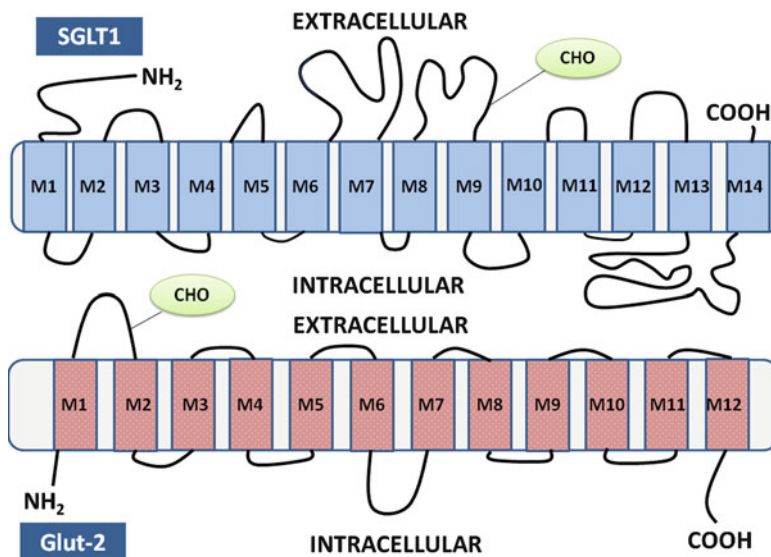
Fig. 6.4 Proposed membrane structure for SGLT1 and Glut2. CHO, glycosylation site; M1-14, membrane-spanning domains (Adapted from Wright et al. [19])

membrane surface, and glucose uptake is therefore a secondary active transport system in which the ATPase indirectly provides the energy for uptake.

The transport protein responsible for **Na$^+$-dependent glucose transport** (**SGLT1**) has been cloned and characterized (Fig. 6.4). The human protein contains 664 amino acids and has a molecular weight of about 73 kd. It has **14 membrane spanning domains** with both extracellular amino- and carboxyl-termini. SGLT1 contains an **N-linked glycosylation site** in a hydrophilic, extracellular domain, but glycosylation does not appear to alter the properties of the carrier protein significantly. SGLT1 has several **phosphorylation sites** that appear to play regulatory roles. Activation of protein kinase A or protein kinase C alters the glucose transport rate. SGLT1 is a high affinity, but rather low capacity glucose transporter. In addition to glucose, galactose and other sugars, but not fructose, are transported by SGLT1. SGLT1 expression is high in neonatal intestine, and increases as enterocytes migrate from the crypt to the villus tip (Fig. 6.5). SGLT1 mRNA is increased by feeding a high sugar diet and in *diabetes mellitus*.

Glucose-galactose malabsorption is a rare genetic defect in monosaccharide absorption that is manifested as *severe watery diarrhea* in newborn children when milk or sugar water is ingested. A variety of mutations ranging from missense mutations to mutations that impair trafficking of SGLT1 to the brush-border membrane causes glucose-galactose malabsorption.

Oral rehydration therapy is used to treat the dehydration associated with cholera and other diarrheal diseases, and this simple approach has markedly reduced mortality due to childhood diarrhea. Patients are given solutions containing *glucose*,
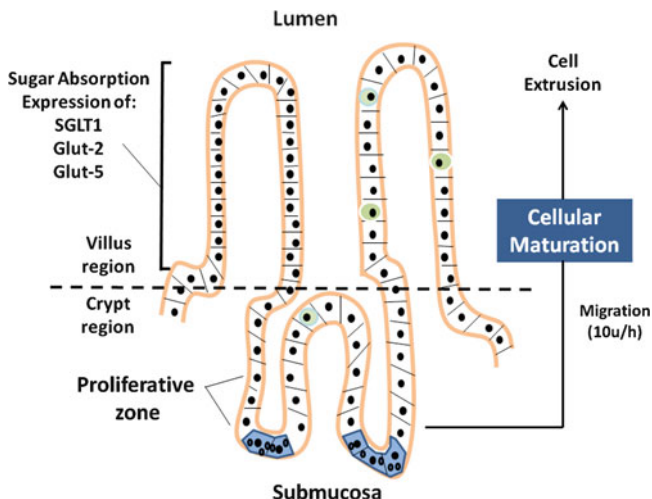
Fig. 6.5 Sites of sugar absorption along the intestinal crypt-villus axis (Adapted from Wright et al. [19])

sodium, *chloride*, *and potassium*. Oral administration of glucose upregulates SGLT1 and absorption of glucose and Na^+ results in increased water absorption by a mechanism that is still uncertain.

Glucose and other sugars are transported out of the enterocyte into the portal circulation by a **Na^+ independent facilitative diffusion mechanism**. Kinetic studies using basolateral membrane vesicles have shown that this process has a relatively low affinity and high capacity for glucose transport. This membrane transporter, **Glut-2**, is a 524-amino-acid protein that has no homology to SGLT1 (Fig. 6.4). Glut-2 has **12 membrane-spanning regions** with intracellular amino and carboxyl terminals and **sites for glycosylation and phosphorylation**. In addition to glucose, Glut-2 can also transport galactose, fructose, and mannose. Glut-2 expression increases as cells migrate from the intestinal crypt to the villous tip, and increases with sugar feeding (Fig. 6.5). Evidence exists for a second pathway for glucose exit from the enterocyte involving glucose phosphorylation, transport to the endoplasmic reticulum, dephosphorylation, and exit via microsomal transport and trafficking.

Glut-2 can also be detected in the enterocyte brush-border membrane. Current evidence suggests that transport of glucose into the enterocyte via SGLT1 promotes the rapid insertion of Glut-2 into the brush-border membrane via a signal transduction pathway involving **protein kinase C βII** (**PKCβII**). The hypothesis is that brush-border membrane Glut-2 is low when the luminal glucose level is low. When luminal glucose increases there is transport of glucose into the enterocyte by SGLT1 which induces recruitment of Glut-2 to the brush-border membrane, providing a high capacity system for glucose uptake. This model explains the **severe sugar malabsorption** observed with mutation of SGLT1, as recruitment of Glut-2 to the brush-border membrane would be impaired in

this condition. Apical Glut-2 insertion is regulated by many factors, including sugars, long-term dietary carbohydrate intake, glucocorticoids, the gastrointestinal hormone GLP-2, cellular metabolic needs, and certain medications used for treating diabetes.

2.5 Digestion and Absorption of Dietary Sugars

Dietary sugars represent approximately half of the digestible carbohydrate in the human diet. Important dietary sugars include the **monosaccharides** glucose and fructose and the **disaccharides** lactose, sucrose, and maltose. The disaccharides must be digested by brush-border membrane **disaccharidases** to their component monosaccharides that are then transported through the enterocytes.

The milk sugar **lactose** (glucose-β-1,4-galactose) is the major carbohydrate consumed in the neonatal period. Human milk contains 7 % lactose, more than most other mammalian species. The brush-border membrane disaccharidase **lactase** is responsible for the digestion of lactose to glucose and galactose, which is then absorbed using the brush-border and basolateral-membrane glucose transporters, SGLT1 and Glut-2. In contrast to other disaccharides, hydrolysis by lactase is the rate-limiting step in the overall process of intestinal lactose absorption. Lactase also is responsible for the hydrolysis of glycolipids present in the intestinal lumen, utilizing a different catalytic site on the protein than that responsible for lactose hydrolysis.

Expression of lactase begins in late gestation, and high levels of the enzyme are expressed in the neonatal period, particularly in the proximal and mid-jejunum. In most mammalian species, lactase activity markedly declines after weaning. In humans, however, two general patterns of lactase expression are observed post-weaning. In most humans, a pattern similar to other mammals is observed, with lactase activity declining after weaning, and by age 5-10 years enzyme specific activity drops to the adult level, approximately 10 % of the neonatal value. Individuals with nonpersistence of lactase (**adult-type hypolactasia**) frequently develop *abdominal pain*, *cramping*, *distention*, *flatulence*, *diarrhea*, *nausea*, and occasionally *vomiting* after consuming significant amounts of lactose-containing dairy products. These symptoms are caused by malabsorbed lactose drawing water into the intestinal lumen, producing an ***osmotic diarrhea***, and by the gut bacterial flora metabolizing the unabsorbed lactose, forming gases such as *hydrogen*, *methane*, and *carbon dioxide* (Fig. 6.6). A minority of humans, however, demonstrate persistence of a high level of lactase activity in the adult small intestine. This is a genetically determined trait with an **autosomal recessive mode** of inheritance of lactase persistence. It has been suggested that lactase persistence offers a ***selective advantage*** to individuals living in population groups that practice dairy farming. The prevalence of lactose malabsorption therefore varies widely in different ethnic or racial groups (Table 6.1).
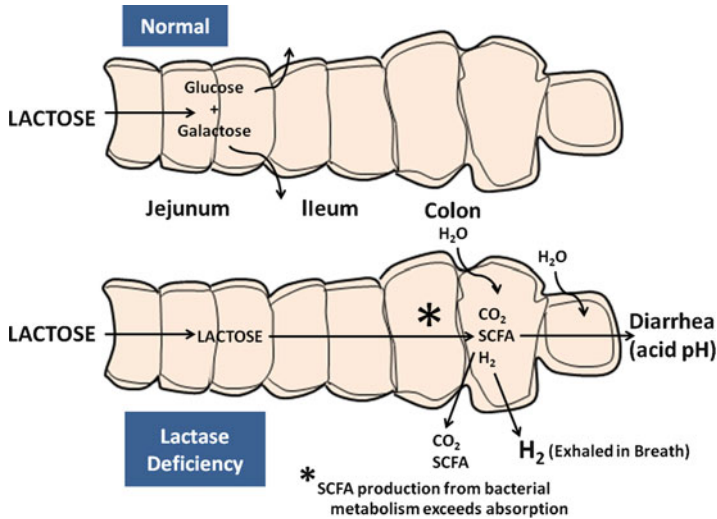
Fig. 6.6 Pathophysiology of lactose intolerance. SCFA, short chain fatty acids

Table 6.1 Prevalence of late-onset lactose malabsorption among various ethic and racial groups

Group	Prevalence of lactose malabsorption (%)
Orientals in the United States	100
American Indians (Oklahoma)	95
Ibo and Yoruba (Nigeria)	89
Black Americans	81
Italians	71
Aborigines (Australia)	67
Mexican Americans	56
Greeks	53
White Americans	24
Danes	3
Dutch	0

(From Buller and Grand [4])

The molecular regulation of lactase expression has been intensely investigated, particularly with respect to the factors that determine the postweaning decline in lactase levels or lactase persistence. The proenzyme is synthesized as a single polypeptide containing 1,927 amino acids, including a 19-amino-acid signal peptide. **Prolactase** undergoes a series of N- and O-glycosylations that affect intracellular transport and enzymatic activity. Prolactase is converted by intracellular proteolysis and extracellular cleavage by pancreatic proteases to the mature enzyme form (approximately 160 kd, 1,060 amino acids) present in the brush-border membrane. The C-terminus is intracellular and the N-terminus is found

on the luminal side of the brush-border membrane. Lactase is anchored into the brush-border membrane by a hydrophobic amino acid sequence located near the carboxyl-terminus.

In adults with persistence of lactase activity, lactase mRNA and protein are present in essentially all of the intestinal villus cells in the proximal jejunum. The **mRNA** is most abundant in the cells at the *crypt-villus junction*, whereas the **protein** is highest in the *midvillus region*. Lactase mRNA is highest in the distal jejunum and proximal ileum.

Gene polymorphisms have been described in the five-prime region flanking the lactase gene that are associated with adult-type hypolactasia or lactase persistence. In the Finnish population, individuals homozygous for a C at position -13910 upstream of the lactase gene have hypolactasia, whereas those homozygous for a T at that position have lactase persistence. Heterozygotes have an intermediate level of lactase that is sufficient for lactose digestion. A G at position -22018 is more than 95 % associated with hypolactasia, whereas an A at that position correlates with lactase persistence. Chromosomes with a T at position -13910 all have an A at -22018. The C -13910 allele has been reported to result in reduced transcription of the lactase gene, but this is controversial. The T-13910 and A-22018 alleles, however, are not found in some African and Bedouin populations with lactase persistence; other mutations in the five prime region have been identified. A small number of individuals with non-persistence of lactose have been showed to have an abnormality in the intracellular processing of newly synthesized lactase.

Lactase is expressed at high levels in the human neonatal intestine. **Congenital lactase deficiency** is a rare **autosomal recessive** disorder characterized by a marked reduction in intestinal lactase activity resulting in *watery diarrhea*, *dehydration*, *acidosis*, and *weight loss* when the neonate is fed breast milk or lactose-containing formula. Mutations in the lactase gene causing premature truncation of lactase and missense mutations have been described in children with congenital lactase deficiency.

In humans, lactose ingestion has little effect on intestinal lactase activity. Hormones, particularly **thyroid hormone** and **glucocorticoids**, appear to play modulatory roles in the developmental regulation of lactase expression.

Fructose is present in the diet in several forms, as part of the disaccharide sucrose (see below), as a monosaccharide in foods such as honey and certain fruits, and as fructose sweeteners formed by the enzymatic conversion of glucose in cornstarch to fructose. Fructose is taken up into the enterocyte by a Na^+-**independent**, **facilitated diffusion mechanism. Glut-5** has been identified as the intestinal brush-border membrane fructose transporter (Fig. 6.3). This transporter is a 501-amino-acid protein that has 41 % amino acid identity and a similar structure to Glut-2. Glut-5 knockout mice have severely impaired fructose absorption. Glut-2 in the brush-border membrane may also be responsible for some fructose uptake, particular at high fructose loads. Glut-5 is expressed at low levels at birth and increases post-weaning, perhaps modulated in part by glucocorticoids. Glut-5 mRNA expression is increased by fructose feeding. Glut-5 expression increases as enterocytes migrate from the crypt to the villus (Fig. 6.5). Fructose is transported from the enterocyte into the portal circulation via the basolateral-membrane Glut-2 transporter.

Fructose is not as well absorbed as is glucose, and there is considerable interindividual variation in the efficiency of fructose absorption. Patients with *chronic gastrointestinal symptoms*, such as *diarrhea*, *gas*, and *bloating*, due to a very limited capacity for fructose absorption have been identified.

Sucrose (**table sugar**) is the disaccharide glucose-α-1,2-fructose. The brush-border membrane **disaccharidase sucrase** hydrolyzes sucrose into the monosaccharides glucose and fructose, which are then absorbed as previously described. Intestinal fructose absorption is considerably more efficient when the fructose is administered as sucrose rather than as an equivalent amount of the monosaccharide. Furthermore, co-administration of glucose with fructose enhances fructose absorption. These findings may be explained by recruitment of Glut-2 to the apical membrane during glucose absorption.

2.6   Dietary Fiber

The term *dietary fiber* operationally refers to plant material that resists digestion by the enzymes of the human GI tract. With the *exception* of *lignin*, the components of dietary fiber are carbohydrates that are mainly constituents of **plant cell walls**, such as cellulose, hemicellulose, and pectins, among others. These polysaccharides, with the exception of wood cellulose, are partially metabolized by enzymes present in gut bacteria, producing short-chain fatty acids (acetate, propionate, and butyrate), hydrogen, carbon dioxide, and methane. The short-chain fatty acids are absorbed to some extent by the distal small intestine and colon and can be utilized as a metabolic fuel. **Butyrate**, in particular, appears to be the principal energy source for the large intestine. Starches that resist efficient hydrolysis by α-amylases and some poorly absorbed dietary sugars and sugar alcohols will also be converted to short-chain fatty acids by gut bacteria, permitting salvage of some of the energy contained in those compounds.

The components of dietary fiber have many diverse effects on gastrointestinal function. For example, fiber can alter intestinal absorption of various nutrients by several mechanisms, including changing the physical characteristics of the luminal contents via the hydratability of fiber and by binding cations and organic compounds. Certain types of fiber, such as wheat bran, decrease gastrointestinal transit time, whereas other fiber components, such as pectin and guar gum, increase gastrointestinal transit time. Short-chain fatty acids produced from dietary fiber stimulate colonic salt and water absorption and regulate colonocyte proliferation and differentiation. Dietary fiber alters the gastrointestinal flora, influencing the diversity and numbers of microorganisms.

Epidemiologic studies have repeatedly demonstrated an association between low fiber diets and common diseases such as *cardiovascular disease*, *type 2 diabetes*, *colorectal* and other *cancers*, and *obesity*. The specific components of dietary fiber and the mechanisms of action resulting in these putative health effects remain controversial.
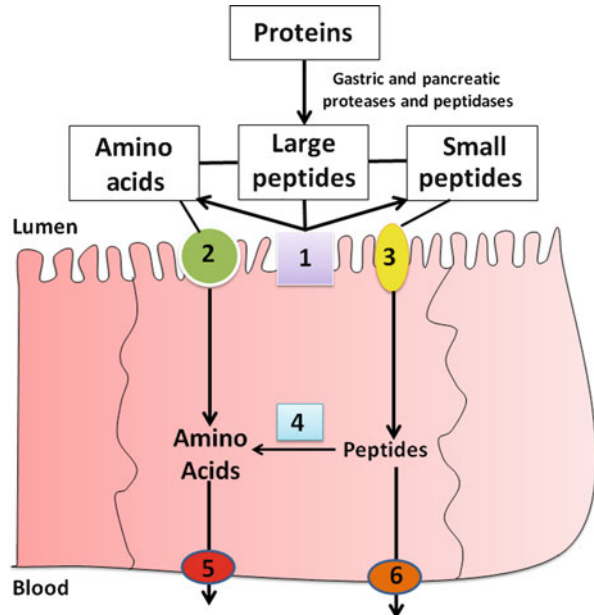
3   Protein

The normal adult human requires approximately 0.75 g/kg body weight per day of highly digestible, high-quality protein to ensure maintenance of nitrogen balance and an adequate supply of essential amino acids. Higher protein intakes are needed to support normal **growth**, **pregnancy**, and **lactation**. Nine of the 20 amino acids commonly found in food, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine are not synthesized by mammals. They are therefore termed **essential amino acids**, because they must be obtained from dietary sources. Other amino acids may become conditionally essential in disease because of impaired conversion from precursors, and protein requirements are generally increased with illnesses such as *sepsis* and *trauma*.

Individual dietary proteins differ in their amino acid compositions, but all food proteins, *except gelatin*, contain some of each amino acid. The digestibility of different proteins varies considerably, with plant proteins generally digested more poorly than animal proteins. Protein digestibility is influenced by methods of food processing. In addition to proteins contained in the diet, approximately 35 g/day of protein is secreted into the intestine in various digestive juices, and 30 g/day enter the lumen as part of desquamated intestinal cells. These endogenous proteins are digested and absorbed along with the dietary proteins. Overall, protein absorption is quite efficient, with the normal adult excreting fecal nitrogen equivalent to only 6–12 g/day of protein. The digestion of protein begins with intraluminal hydrolysis by proteases secreted by the stomach and pancreas and continues as peptidases in the brush-border membrane of the small intestine digest the protein to amino acids and di- and tripeptides that are taken up by the enterocytes (Fig. 6.7).

3.1   Acid-Peptic Digestion

Gastric juice contains a family of proteolytic enzymes known as **pepsins**. In the acid environment of the stomach, these enzymes are capable of hydrolyzing a broad range of peptide bonds. Proteolysis within the stomach not only contributes to overall protein digestion, but the peptides and amino acids that are generated are important stimulators of gastrin and cholecystokinin release and are regulators of gastric acid secretion and gastric emptying. In the neonatal stomach, **chymosin**, which very effectively digests milk protein, is the predominant enzyme. In adults, two families of enzymes, **pepsin A** and **pepsin C**, predominate. Pepsin A activity comprises five closely related isoenzymes coded for by genes on chromosome 11, whereas pepsin C represents two isoenzymes coded for on chromosome 6. These enzymes are irreversibly denatured in a mildly alkaline fluid, and therefore little peptic digestion occurs past the duodenum. Acid-peptic hydrolysis is not essential for efficient protein digestion, as patients who have undergone total gastrectomy have little impairment of protein utilization. In patients with pancreatic disease and limited secretion of pancreatic proteases, however, acid-peptic proteolysis is an important facilitator of protein digestion.

Fig. 6.7 Protein digestion and absorption in the small intestine. (*1*) Brush-border membrane peptidases. (*2*) Brush-border membrane amino acid transporters. (*3*) Brush-border membrane di- and tripeptide transporters. (*4*) Intracellular peptidases. (*5*) Basolateral-membrane amino acid carriers. (*6*) Basolateral membrane di- and tripeptide carriers (Adapted from Ganapathy et al. [7])

Pepsins are synthesized in the **gastric chief cells** as inactive **pre-proenzymes**. A signal sequence of about 15 amino acids directs the protein to the interior of the endoplasmic reticulum, where the signal sequence is cleaved. The resulting **proenzyme** (**pepsinogen**) is transferred to the Golgi apparatus and condensed into secretory granules; in some cases it is also glycosylated. Secretion of the granules occurs by **exocytosis**, stimulated by agents that *increase cyclic adenosine monophosphate* (*cAMP*) (secretin, vasoactive intestinal polypeptide, epinephrine, prostaglandins) or that *increase intracellular calcium concentration* (acetylcholine, cholecystokinin, gastrin). Secretion of pepsinogen is initially very rapid, but then declines to a slower rate even before the chief cells are depleted of granules, suggesting feedback regulation or receptor desensitization. Synthesis of new preproenzyme occurs following stimulation of secretion. In the acid environment of the stomach, there is cleavage of an N-terminal peptide of 44 amino acids from pepsinogen, forming the active pepsin with a molecular weight of approximately 35 kd. The acid milieu appears to cause a conformational change in pepsinogen that permits intramolecular cleavage of the N-terminal activation peptide.

3.2   Pancreatic Proteases

Proteases are secreted by the **pancreas** as proenzymes (Chap. 4 for a detailed discussion of the regulation of pancreatic secretion). Activation of pancreatic proteases is initiated by the intestinal brush-border enzyme enteropeptidase (**enterokinase**),

which is stimulated by **trypsinogen** contained in pancreatic juice and released from the brush-border membrane by bile salts. Enteropeptidase has a single substrate, trypsinogen, and cleaves an N-terminal hexapeptide forming trypsin, which can then activate more trypsinogen and other pancreatic proenzymes.

Pancreatic secretions contain a variety of **endopeptidases** (trypsin, chymotrypsin, elastase) and **exopeptidases** (carboxypeptidase A and B). **Trypsin** cleaves peptide bonds on the carboxyl side of basic amino acids (lysine, arginine), **chymotrypsin** on the carboxyl side of aromatic amino acids (tyrosine, phenylalanine, tryptophan), and **elastase** on the carboxyl side of aliphatic amino acids (alanine, leucine, glycine, valine, isoleucine). The **carboxypeptidases** are zinc-containing metalloenzymes that remove single amino acids from the carboxyl-terminal ends of proteins and peptides. This array of enzymes with different substrate specificities results in the efficient hydrolysis of the different peptide linkages present in food and in endogenous proteins. The result of intraluminal hydrolysis is the production of 40 % free amino acids and 60 % oligopeptides in the small intestinal juice. Proline-containing peptides are resistant to hydrolysis by pancreatic enzymes.

3.3   Brush-Border Membrane Peptidases

Peptidases within the enterocyte brush-border membrane hydrolyze the oligopeptides generated by intraluminal digestion to free amino acids and the di- and tripeptides transported into the enterocyte (Fig. 6.7). About 20 different peptidases have been identified in the brush-border membrane, in contrast to the relatively few enzymes involved in brush-border carbohydrate digestion. These peptidases recognize specific amino acids at the amino- or carboxyl-terminus or internally within the oligopeptide and are necessary for the hydrolysis of the wide variety of peptide linkages found in food proteins. Many of these peptidases are **dimeric metalloenzymes** that are anchored into the brush-border membrane by either a hydrophobic membrane-spanning domain of the protein or through covalent attachment to membrane glycosyl-phosphatidylinositol. The active site of the enzyme is generally contained within a large hydrophilic portion of the protein that projects into the intestinal lumen. The brush-border membrane peptidases are generally classified into **four groups**: *endopeptidases*, *aminopeptidases*, *carboxypeptidases*, and *dipeptidases*. The most abundant peptidase is probably aminopeptidase N, which sequentially removes N-terminal amino acids from oligopeptides. The brush-border membrane contains at least four peptidases that are active against peptides with proline at the cleavage site. These enzymes are therefore complementary to the pancreatic proteases that have little ability to hydrolyze peptide bonds containing proline.

The enterocyte also contains at least **four intracellular peptidases** that are involved in the digestion of absorbed di- and tripeptides (Fig. 6.7). Ninety percent or more of the products of protein digestion appear in the portal blood as free amino acids.

In addition to participating in overall dietary protein digestion, several of the intestinal peptidases have other specific functions. **Folate conjugase** is a brush-border membrane carboxypeptidase that specifically degrades the polyglutamate side chain of dietary folates, producing the monoglutamate form that is transported into the enterocytes (Chap. 9). Other intestinal peptidases have been shown to be involved in the activation or catabolism of gastrointestinal peptide hormones or in the turnover of endogenous intestinal proteins. **Dipeptidyl aminopeptidase IV** (**DPP IV**) plays an important role in the metabolism of the **incretin GLP1** that enhances insulin secretion, and **DPP IV inhibitors** are used in the treatment of *type 2 diabetes*.

The mechanisms for biosynthesis and intracellular sorting of brush-border membrane peptidases are complex and differ significantly for the various enzymes. Complex glycosylation events, changes in protein folding, metabolism of proenzyme forms, and dimerization are important steps in the synthesis and incorporation into the brush-border membrane of peptidases. Most peptidases apparently have direct transport routes to the brush-border membrane, but aminopeptidase N has been shown to first appear in the basolateral membrane, followed by transit through the Golgi apparatus before insertion in the brushborder membrane.

The activities of brush-border membrane peptidases are highly regulated during development and cellular differentiation and by starvation or feeding, with specific patterns of enzyme expression observed for each peptidase. Aminopeptidase N is abundant in the *proximal jejunum*, whereas dipeptidyl aminopeptidase IV is higher in the *ileum*.

3.4 Brush-Border Membrane Amino Acid Transport

The intestinal brush-border membrane contains multiple transport systems for the uptake of amino acids. Systems capable of transporting classes of amino acids were defined based upon the physical-chemical properties, stereospecificity, size, and charge of the amino acids transported, as well as the ion and pH dependencies. **Transport systems** that have been identified include:

1. "**Neutral system**" or "**methionine preferring system**" that transports *all neutral amino acids*;
2. "**Basic system**" that transports *cationic amino acids* and *cysteine*;
3. "**Acidic system**" that transports *glutamate* and *aspartate*;
4. "**Iminoglycine system**" that transports *proline*, *hyroxyproline*, and *glycine*; and
5. "**β-amino acid system**" that transports *taurine*.

It should be recognized that there is significant overlap of substrate specifies among the different transport systems. The various carrier systems may require Na^+, H^+, Cl^-, or K^+ as co-factors for amino acid transport, and may be electrogenic or neutral. Some of the transport proteins corresponding to these systems have been identified, and inherited defects in these transporters correspond to genetic abnormalities in amino acid metabolism. The association of some of the amino acid transporters to the apical membrane requires partner proteins, including members of the renin-angiotensin system, **collectrin** (Tmem27) and **angiotensin-converting enzyme 2**. Amino acid transport proteins also can transport orally administered amino-acid based drugs and derivatives.

3.5 *Brush-Border Membrane Peptide Transport*

Extensive evidence now exists that small peptides are transported across the brush-border membrane by a mechanism that is independent of the systems used for amino acid uptake (Fig. 6.7). A patient with a genetic defect in amino acid transport, such as *Hartnup disease* or *cystinuria*, may absorb certain free amino acids poorly, but absorption of those amino acids as part of small peptides is normal. There is a lack of competition between amino acid and peptide transport, often a faster absorption rate for amino acids presented in peptides rather than in the free forms, and differences in the developmental and dietary regulation of amino acid versus peptide uptake. The absorptive capacity for small peptides is greater in the jejunum than the ileum, whereas for amino acids it is greater in the ileum than the jejunum. Mainly di- and tripeptides are absorbed by the enterocyte, with very limited uptake of tetrapeptides and essentially no absorption of larger peptides.

PEPT1 is the major, if not only, di-tripeptide carrier expressed in the small intestinal brush-border membrane. The gene for PEPT1 is on chromosome 13, and the open reading frame encodes 708 amino acids. There are 12 transmembrane spanning domains with both the amino- and carboxy termini facing the cytoplasmic side. PEPT1 is an electrogenic symport system in which the inwardly directed proton gradient allows peptides to enter the cell against a concentration gradient. In some experimental situations, a **partial Na^+ dependency of peptide transport** was identified. This is apparently an indirect effect, as brush-border Na^+/H^+ exchange via the Na^+-proton antiporter NHE-3 is needed to maintain the inwardly directed proton gradient. A remarkable feature of PEPT1 is the ability of this one molecule to transport a myriad of possible di- and tripeptides generated from protein digestion. Evidence suggests that water in the vicinity of the carrier's binding domain plays a central role in shielding the electric charges of amino acid side chains, allowing charged, polar, and large apolar molecules to bind. In addition to peptide digestion products, PEPT1 can transport certain drugs, such as some *β-lactam antibiotics* and *ACE inhibitors*.

PEPT1 is acutely regulated by intracellular signaling pathways involving protein kinase A, protein kinase C, and intracellular calcium concentration. PEPT1 abundance and activity is also regulated by hormones such as insulin, growth hormone, thyroid hormone, and leptin, and is altered in diabetes. Dietary protein, certain amino acids, and peptides also alter PEPT1 expression. Normally PEPT1 is expressed throughout the **small intestine**, but is present at very low levels in the **colon**. In patients who have undergone major resections of the small intestine and have the short bowel syndrome and malabsorption, PEPT1 mRNA and protein is expressed in the **colonic mucosa**, and may permit colonic peptide absorption. Multiple mutations affecting various aspects of PEPT1 carrier function have been described.

3.6   Basolateral-Membrane Transporters

Amino acids transported across the brush-border membrane and generated intracellularly from proteolysis of absorbed peptides are transferred into the portal blood via multiple carrier systems with different electrolyte and pH requirements. As much as 10 % of absorbed protein may be delivered into the portal blood as small peptides (Fig. 6.7). A transport system similar, but not identical to PEPT1 has been found in the basolateral membrane. There is not likely to be a substantial H^+ gradient from the cytoplasm to the portal blood to drive transport, and it is possible that the high postprandial concentration of di- and tripeptides within the enterocyte cytoplasm compared with the minimal concentration of these peptides in blood is sufficient to cause efficient transfer into the circulation.

Transporters in the basolateral membrane can also mediate uptake of amino acids from the circulation into enterocytes during fasting that supports enterocyte protein synthesis.

3.7   Regulation of Amino Acid and Peptide Absorption

The absorption of amino acids and peptides is developmentally regulated and influenced by diet, hormones, and growth factors. The transport systems are generally present at birth, and transport rates for peptides and most amino acids decline with age, although the magnitude of change differs among individual carriers. **High dietary levels of protein or amino acids** generally result in upregulation of peptide and most amino acid transport. **Short**-**term fasting** also tends to increase the absorption rates, but **long**-**term starvation** causes decreased amino acid transport with little change in peptide absorption.

3.8 Role of Absorbed Amino Acids in Enterocyte Energy and Protein Metabolism

Absorbed amino acids, particularly *glutamine*, *glutamate*, and *aspartate*, are the major fuels for the small intestine. About 10 % of the absorbed amino acids is used for protein synthesis within the enterocyte. In fact, there is preferential utilization of absorbed amino acids versus those entering the enterocyte from the blood for energy metabolism and protein synthesis.

Clinical Correlations

Case Study 1

A 1-month-old infant is admitted with dehydration and a history of severe diarrhea since birth. Her pediatrician places her on lactose-free and sucrose-free infant formulas with no significant improvement in her condition. Stool examination demonstrates the presence of a large quantity of reducing substances, which are confirmed to be mainly glucose; the stool pH is acidic. There is no family history of a similar illness, but her parents are first cousins.

Questions

1. **What is the likely diagnosis?**
   Answer: This child most likely has **genetic glucose-galactose malabsorption**, an ***autosomal recessive*** disorder. Malabsorption of these monosaccharides causes a severe *osmotic diarrhea*, resulting in dehydration. Bacterial metabolism of the unabsorbed sugar produces organic acids and results in an *acidic stool pH*. **Congenital deficiencies of the disaccharidases sucrase-isomaltase** or **lactase** have similar clinical presentations, but this child failed to improve when sucrose or lactose were eliminated from her diet. **Monosaccharide malabsorption** can also occur following certain intestinal infections, but the onset of this child's symptoms at birth makes a genetic defect more likely.

2. **What is the molecular basis of this disorder?**
   Answer: Children with glucose-galactose malabsorption have ***impaired Na^+-dependent glucose and galactose uptake*** into the small intestine. **Mutations in the glucose transporter SGLT1** have been identified. The lack of glucose and Na^+ uptake via SGLT1 impairs recruitment of GLUT2 to the brush-border membrane, further impeding glucose and galactose absorption.

3. **What would you recommend as dietary treatment for this condition?**
   Answer: These children need to be placed on **infant formulas** that have *markedly reduced contents of glucose*, *galactose*, *disaccharides* that are ***hydrolyzed*** to glucose and galactose (lactose, sucrose), and starches or glucose polymers. Since fructose is absorbed by a different glucose transporter (Glut5 rather than SGLT1), **fructose-containing formulas** are effectively absorbed and are life-saving.

Case Study 2

A 30-year-old Chinese-American man complains of excessive gas and mild chronic diarrhea for many years. He has noted that eating ice cream and drinking milk tend to provoke these symptoms. He is otherwise healthy, has no other GI complaints, and has not lost weight.

Questions

1. **What is the most likely cause of this patient's symptoms?**
   Answer: The patient most likely has **adult-type hypolactasia**, resulting in *malabsorption of lactose* and *intolerance of dairy products*. The malabsorbed lactose causes an *osmotic diarrhea* and is metabolized by gut bacteria to *gases* such as carbon dioxide, hydrogen, and methane. ***Hypolactasia*** is extremely common in Chinese adults. Various small-intestinal diseases, for example, *celiac sprue*, can result in a reduction in brush-border lactase (***secondary hypolactasia***), but this patient's lack of other signs or symptoms of generalized malabsorption makes this diagnosis less likely.

2. **What tests could you use to make a definitive diagnosis?**
   Answer: Several tests have been developed to diagnose lactose malabsorption. A standard **lactose tolerance test** measures the rise in blood glucose after oral administration of lactose; the increase in blood glucose is diminished in patients with hypolactasia. **Breath-hydrogen testing** is currently the most convenient and accurate method for evaluating lactose absorption. A dose of lactose is given orally, and breath samples are serially collected for measurement of breath hydrogen. Individuals with lactase-persistence will digest the lactose to the component monosaccharides, which are efficiently absorbed, and there will be little change in breath hydrogen. Patients with hypolactasia will malabsorb lactose, which is then metabolized by gut bacteria to hydrogen gas that is absorbed and appears in the breath. Other tests that are used less commonly to diagnose lactose malabsorption include **intestinal biopsy** with measurement of lactase activity and **assessment of the appearance of galactose in blood or urine** after oral lactose administration.

3. **What treatment would you prescribe?**
   Answer: Avoidance of lactose-containing dairy foods will relieve the patient's symptoms. Dairy foods, however, are important sources of calcium, vitamin D, protein, and other nutrients. Some dairy foods, such as aged cheeses, have relatively little lactose. **Microbial lactases** are used to prepare **low-lactose milk**, and they can also be taken by mouth prior to eating dairy foods to enhance lactose digestion and diminish GI symptoms.

Case Study 3

A 7-year-old boy presents with recurrent scaly rashes on his face and arms, mild diarrhea, and ataxia. Laboratory testing demonstrates elevated urinary and fecal excretion of neutral amino acids, including tryptophan, and indoles. His parents are first cousins, and testing of his family demonstrates that his brother also has increased urinary and fecal amino acids and indoles, but is asymptomatic.

Questions

1. **What are the diagnosis and the pathogenetic basis of this syndrome?**
   Answer: The child has **Hartnup disease**, an *autosomal recessive* disorder in which there is a reduction of small intestinal and renal transport of certain neutral amino acids, including *tryptophan,* causing elevated amino acid excretion in urine and feces. The disease is caused by a **mutation** in the **neutral amino acid transporter SLC6A19**. **Malabsorbed tryptophan** is metabolized by gut bacteria to various indoles that are excreted in the feces and urine. The patient's symptoms are believed to be due, at least in part, to niacin deficiency that produces a pellagra-like syndrome, since tryptophan is a niacin precursor. **Abnormal production of serotonin** in the nervous system because of reduced tryptophan and altered protein synthesis because of amino acid deficiencies may also contribute to the patient's symptoms.

2. **How would you explain the observation that his brother has a similar metabolic abnormality but is asymptomatic?**
   Answer: There are multiple amino acid transport systems in the intestinal brush-border membrane with overlapping specificities. In addition, there is a separate carrier for di- and tripeptides PEPT1. A defect in a single transporter therefore is unlikely to result in a gross deficiency of essential amino acids, and most individuals with the Hartnup abnormality are asymptomatic. It is believed that other genetic, nutritional, or environmental factors are required in addition to the mutation in the amino acid transporter to cause clinically apparent metabolic abnormalities.

3. **What treatment would you recommend?**
   Answer: The clinical features of Hartnup disease, particularly the skin rash, will sometimes respond to **niacin supplementation**. These children will also often improve on a **high-protein diet**, which may result in the production of a large quantity of di- and tripeptides that are taken up via PEPT1, providing a sufficient supply of essential amino acids. **Special peptide-containing formula diets** that would be efficiently absorbed in patients with Hartnup disease may also be useful supplements for affected children.

Case Study 4

A 45-year-old woman complains of many years of excessive gas, bloating, flatulence, and watery diarrhea. Extensive evaluation has not revealed any gastrointestinal disease and a lactose hydrogen breath test was normal. A review of dietary records shows that her diarrhea and other symptoms occur when she eats fruits and other high fructose foods.

Questions

1. **What is the basis for her symptoms?**
   Answer: The patient likely has poor intestinal absorption of fructose. Studies have shown wide variation in the efficiency of absorption of fructose in healthy

individuals, and fructose malabsorption can produce symptoms of the irritable bowel syndrome. The malabsorbed fructose results in an osmotic diarrhea and is metabolized by intestinal bacteria forming hydrogen and other gases causing the bloating and flatulence.

2. **How could you make the diagnosis of fructose malabsorption?**
   Answer: A hydrogen breath test can be performed after fructose ingestion similar to the breath test that is done to detect lactose malabsorption. Excessive breath hydrogen after fructose ingestion is indicative of fructose malabsorption.

3. **Why is this patient able to tolerate eating large amounts of sucrose (a disaccharide containing glucose and fructose) without developing gastrointestinal symptoms?**
   Answer 3: Sucrose is hydrolyzed in the intestine to the monosaccharides glucose and fructose. Some fructose is absorbed across the brush-border membrane by the transporter GLUT-5. Glucose will be absorbed by the transporter SGLT-1. Glucose transport by SGLT-1 initiates a signal transduction cascade that recruits another transporter, GLUT-2, to the brush-border membrane that is a high capacity transport system for glucose and fructose. Fructose absorption as sucrose or when co-administered with glucose is, therefore, greater than when fructose alone is ingested.

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