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Metabolic fuel selection by intestinal epithelium

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Sélection des substrats énergétique par l'épithélium intestinal

RÉSUMÉ

Les nutriments présents dans la lumière intestinale après un repas sont absorbés sélectivement par l'épithélium intestinal. L'absorption varie avec le mode d'alimentation et avec les conditions physiologiques (croissance, lactation, vieillissement) et avec les conditions pathologiques liées ou non à la nutrition. En principe, ces variations d'absorption pourraient s'expliquer, au moins en partie, par des variations du passage sélectif de substrats à travers l'épithélium intestinal. Cette sélectivité est due à la présence de transporteurs qui sont membranaires pour les substances hydrosolubles et intracellulaires pour les substances liposolubles. Les cellules épithéliales sont caractérisées par une asymétrie de composition membranaire que l'on retrouve au niveau des transporteurs. Cette asymétrie de composition permet d'expliquer le transport vectoriel de la lumière intestinale vers le sang. Comme ces transporteurs sélectifs sont des protéines, il n'est pas étonnant de constater que leur expression est sous contrôle génétique et métabolique. Bien que le rôle de ces transporteurs dans les variations de l'absorption soit encore peu étudié, certains exemples tirés de la physiologie (modification de la composition alimentaire) et de la pathologie (diabète) montrent clairement que la régulation de l'expression des transporteurs membranaires peut participer au contrôle de la sélection des substrats énergétiques au moment de l'absorption.

Nutrients present in the intestinal lumen are selectively absorbed by the intestinal epithelium. The concept of selectivity reflects the concept of a barrier and has been extensively studied by biophysicists, biochemists, and geneticists (Powell, 1994). Recently, the three strategies have been merged into one integrated concept that could be useful to nutritionists. This development can be traced back to 1986 when chloride selective conductance through the luminal membrane of epithelial cells was found to be supported by transmembrane protein having phosphorylation sites under the genetic control of a gene called CFTR (Frizzell *et al.* 1986; Collins *et al.* 1987; Drumm *et al.* 1991).

In bacteria, the cytoplasmic membrane is the structure that selects nutrients required for growth and reproduction. The genetic control of transmembrane transport proteins is essential for bacteria to grow in their environment. In mammals, the intestinal epithelium shows some resemblance to the bacterial membrane but has a higher degree of complexity: (1) each cell is surrounded by a luminal membrane and a basolateral

membrane; both membrane domains differing in structure and function. In addition, the intestinal epithelium is a monolayer of epithelial cells attached by a specific structure called *zonula occludens*. This epithelial asymmetric structure is the support for vectorial transport; (2) the intestinal epithelium not only covers the entire surface of the intestinal lumen but is continuously renewed; i.e. the epithelial function is surface-and-time-dependent; (3) the environment of the intestinal epithelium comprises the luminal content, the composition of which keeps changing with time, and somatic systems on the serosal side, including endocrine, nerve, immune and vascular systems in the sub-epithelial space.

Intestinal nutrient absorption is subject to variation (1) associated with feeding, including feeding pattern, diet composition, route of administration (enteral *v.* parenteral feeding), (2) with physiological conditions, including genetic constitution, ontogeny, growth, lactation, ageing and (3) with pathological conditions including malnutrition, diabetes mellitus, ileal resection, abdominal irradiation, sepsis, intestinal infection (Gardiner & Barbul, 1993; Thomson *et al.* 1994). The mechanisms involved in the alteration of intestinal absorption, which may be regarded as an adaptable response, include alterations in morphology, membrane and intracellular composition of enterocytes, cell kinetics, digestive enzyme activities and endocrine, nerve, immune and vascular system functions.

My aim is to present our current knowledge on the selection of metabolic substrates by the intestinal epithelium. Selectivity across the intestinal epithelium is achieved by specific transporters that are under genetic and metabolic control.

TRANSPORTERS AND SELECTIVE PERMEABILITY ACROSS THE INTESTINAL EPITHELIUM

At the luminal membrane of the enterocytes, the nutrients are in the form of monosaccharides, amino acids or small peptides, monoacylglycerols and free fatty acids. Water-soluble substrates are transported across lipidic membranes by transmembrane transporters, while lipid-soluble substrates require transporters to migrate within the cell.

The transport of water-soluble substrates

Monosaccharides and amino acids are absorbed into the body by the mature enterocytes lining the upper villi of the small intestine. Absorption occurs by a two-stage process: glucose and galactose are co-transported with Na^+ from the gut lumen into the enterocytes across the brush-border membrane by co-transporter SGLT1, followed by facilitated transport out of the cell by transporter GLUT2 (Wright *et al.* 1988).

Other transporters with substrate specificity have recently been identified: SAAT1 is a mammalian Na^+-dependent neutral amino acid transporter identified as a protein of 90 KDa with properties characteristic of system A, i.e. transporting alanine (Kong *et al.* 1993). A Na^+-dependent neutral L-α-amino-acid transporter characteristic of system B has been described (Nakanishi *et al.* 1994). Pep T1 may be a major mechanism for absorption of the products of protein digestion; it is a proton-coupled organic solute transporter (Fei *et al.* 1994). GLUT5 is a Na^+-independent transporter for fructose and, to a lesser degree, glucose at the luminal membrane (Burant *et al.* 1994).

At the basolateral membrane, GLUT2 is a Na^+-independent transporter for glucose, galactose and fructose (Burant *et al.* 1994), but no comparable transporter for amino acids has been identified. MCT1 is a monocarboxylate transporter at the basolateral membrane. Its main function may be to export lactate from epithelial cells (Garcia *et al.* 1994).

Other water-soluble transporters have been identified with undefined nutritional function. NBAT is a Na-independent neutral and basic amino acid transporter. It is located on the luminal membrane of the enterocyte and, probably in greater quantity, within enteroendocrine cells and submucosal neurons (Pickel *et al.* 1993). Another transporter (NAA-Tr) with Na-independent amino transport properties has been identified (Yan *et al.* 1992).

In addition, the presence of a common carrier or multichannel transporter for glucose and amino acids suggested by Alvardo (1966) has not been established (Metel'skiol, 1992). To be functional, transporters may be in the form of hetero-oligomers. For example, mammalian Na^+–D-glucose co-transport systems are hetero-oligomers containing two SGLT1-type and one or two RS1-type subunits (Koepsell & Spangenberg, 1994). Different combinations of SGLT1-type subunits and RS1-type subunits may form the heterogeneous Na^+–D-glucose co-transport systems in kidney and intestine. In the same line, it is possible that the D_2 molecule is part of a larger system involved in the transport of cystine and other amino acids (Kanai & Hediger, 1992).

The transport of lipid-soluble substrates

Dietary triacylglycerols are hydrolysed in the intestine by lipases to fatty acids and monoacylglycerols which are re-esterified to form triacylglycerols in enterocytes. Triacylglycerols are assembled in the endoplasmic reticulum with other lipids and proteins to form the core of nascent chylomicrons. Dietary cholesterol also is largely esterified in these cells and incorporated into the core of the particles. After assembly in the Golgi apparatus is completed, nascent chylomicrons are secreted into the interstitium of intestinal villi and enter the lacteals. The protein components of the nascent chylomicrons are synthesized in the enterocytes and include Apo B-48 and the A apoproteins (A-I, A-II, A-IV; Havel & Kane, 1989).

The B-apoproteins are the products of a single gene. The larger form produced by the liver, designated apo B-100 ('100' represents 100% of the gene product), is a major component of VLDL and LDL and contains a binding domain for the LDL receptor. The smaller form, apo B-48, is found in chylomicrons of intestinal origin. Both apo B-48 and apo B-100 are translated from the same messenger RNA sequence, with the tissue-specific post-transcriptional or co-transcriptional insertion of a stop-codon limiting translation to apo B-48 synthesis in the intestine.

Fatty acid-binding proteins (FABP) are abundant cytosolic proteins whose level is responsive to nutritional and endocrine conditions and to a variety of pathological states. They may have a role in fatty acid and cholesterol absorption (Schroeder *et al.* 1993).

METABOLIC FUEL SELECTION IS UNDER GENETIC CONTROL

Most of our information on this subject comes from human congenital and selective transport defects (for recent review, see Desjeux, 1993). These rare diseases involve

most nutrients including energy substrates and the vitamins and minerals required for their metabolism. Clinical features vary according to the nature of the substrate whose selective absorption by the intestine is impaired. The severity of the disease is an indication that the substrate is essential. The genetic defect for water-soluble substrate absorption may be at the luminal or basolateral membrane of the enterocyte. The most studied genetic transport defect of the luminal membrane is glucose–galactose malabsorption (GGM). The main symptom is neonatal diarrhoea which is relieved by a glucose- and galactose-free diet. No significant glucose metabolism dysfunction is observed. It is characterized by functional deficiency of glucose–Na^+ co-transporter, even though the protein is present in the membrane. A wide variety of mutations have been identified including point mutations which alter residues conserved among other Na^+ co-transporters (Martin *et al.* 1994; Turk *et al.* 1994). Another example of a genetic defect of transport is lysinuric protein intolerance (LPI). In this defect in selective transport of lysine and other dibasic amino acids there are severe metabolic consequences including postprandial hyperammonaemia and growth retardation. *In vivo*, malabsorption of the three amino acids, lysine, arginine and ornithine, given as free amino acids or dipetides, by the small intestine and the kidney (Simell, 1989) and plasma concentrations of dibasic amino acids are low. *In vitro*, entry of the amino acids across the luminal membrane of the epithelial cells is not impaired but the exit permeability across the basolateral membrane to the blood is grossly impaired (Desjeux *et al.* 1980). In addition, the transport defect is also found in other plasma membranes (fibroblast, hepatocyte). Gene coding for lysine transport across the plasma membrane has not been identified.

These examples show the importance of the genetic control of substrate selectivity by the enterocyte. In addition, they indicate that genetic control at the basolateral membrane is a limiting step in amino acid absorption.

Similarly, the study of fat malabsorption related to altered secretion of B-apoprotein-containing lipoprotein may provide information on the genetic control of fatty acid absorption. However, the findings have not been very informative. The molecular basis of abetalipoproteinaemia is unknown. The initial belief, that impaired apo-B synthesis was responsible for this disorder, may not account for all cases and it has been challenged in the light of recent results obtained with specific and sensitive methodology (Dullaart *et al.* 1986; Glickman *et al.* 1991). These findings indicate immunologically recognizable, normal size and normally glycosylated apo B-48 and B-100 are synthesized even in abetalipoproteinaemia.

METABOLIC CONTROL OF TRANSPORTERS

Factors controlling the expression of transporters also control metabolic fuel selection by the intestinal epithelium. This is illustrated by typical examples: amongst intracellular messengers, cAMP plays an interesting role in nutrition. In caco-2 intestinal cells, stimulation of cAMP production by forskolin increases fructose uptake 2-fold, and raises GLUT5 protein and mRNA levels 5- and 7-fold respectively (Mahraoui *et al.* 1992). As in response to hypoglycaemia, adrenaline and glucagon stimulate cAMP production, the increased fructose absorption may be part of the hypoglycaemic response (Cryer, 1993).

Extracellular molecules also participate in the control of nutrient absorption. Epidermal growth factor (EGF) which stimulates cell replication and increases the DNA

content of the small intestine, also stimulates Na^+-dependent glutamine and alanine uptake by the brush-border membrane vesicles of enterocytes. This effect is not observed for glucose (Salloum *et al.* 1993).

In sepsis intestinal absorption may be reduced. Recently, it has been suggested that the release of cytokines induced by systemic bacteria or endotoxin may lead to reduction in the synthesis of transporter proteins (Salloum *et al.* 1991; Gardiner & Barbul, 1993).

Nutrients may also be involved in the control of transporters. Fructose in the intestinal lumen may upregulate GLUT2 expression (Cheeseman, 1992). Regulation of cholesterol uptake by enterocytes has recently been studied by Safonova *et al.* (1994). They found that loading the cells with non-lipoprotein-cholesterol reduced cholesterol uptake, while treatment of cells with an inhibitor of cholesterol synthesis had the opposite effect (Schroeder *et al.* 1993). Carbohydrate-rich diets stimulate Na^+–D-glucose co-transporter activity, phloridzin binding and the amount of SGLT1-homologous mRNA. This upregulation is post-transcriptional since it is correlated with the concentration of SGLT1-homologous mRNA. Post-transcriptional regulation may be due to chemical modification of SGLT1 which may alter membrane insertion or protein turnover, or may be mediated by the RS1 component (Shirazi-Beechey *et al.* 1991; Ferraris & Diamond, 1992; Koepsell & Spangenberg, 1994).

WHAT IS THE ROLE OF GENETIC CONTROL OF METABOLIC FUEL SELECTION BY INTESTINAL EPITHELIUM?

As mentioned in the introduction, nutrient absorption is altered in many physiological and pathological conditions. Glucose absorption is increased in experimentally streptozotocin-induced diabetes in rats. In the meantime, SGLT1, GLUT5 and GLUT2 are increased (Burant *et al.* 1994). Insulin reverses the increase in transporter-protein expression seen after induction of diabetes. *In situ* hybridization shows that after the induction of diabetes there is new hybridization in lower villus and crypt enterocytes. Thus, the increase in total hexose transport caused by diabetes is due to premature expression of hexose transporters by enterocytes along the crypt–villus axis, causing a cumulative increase in enterocyte transporter protein during maturation. These changes are likely to represent an adaptable response by the organism to increase nutrient absorption in a perceived state of tissue starvation, including starvation, lactation, hypophysectomy and unbalanced insulin-dependent diabetes.

In conclusion, results obtained in physiological (change in diet composition) and pathological conditions (diabetes) clearly indicate that the level of expression of specific transporters may participate in the control of metabolic fuel selection at the time of absorption.

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