

TOPICAL REVIEW

Oxygen in the regulation of intestinal epithelial transport

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Abstract The transport of fluid, nutrients and electrolytes to and from the intestinal lumen is a primary function of epithelial cells. Normally, the intestine absorbs approximately 9 l of fluid and 1 kg of nutrients daily, driven by epithelial transport processes that consume large amounts of cellular energy and O₂. The epithelium exists at the interface of the richly vascularised mucosa, and the anoxic luminal environment and this steep O₂ gradient play a key role in determining the expression pattern of proteins involved in fluid, nutrient and electrolyte transport. However, the dynamic nature of the splanchnic circulation necessitates that the epithelium can evoke co-ordinated responses to fluctuations in O₂ availability, which occur either as a part of the normal digestive process or as a consequence of several pathophysiological conditions. While it is known that hypoxia-responsive signals, such as reactive oxygen species, AMP-activated kinase, hypoxia-inducible factors, and prolyl hydroxylases are all important in regulating epithelial responses to altered O₂ supply, our understanding of the molecular mechanisms involved is still limited. Here, we aim to review the current literature regarding the role that O₂ plays in regulating intestinal transport processes and to highlight areas of research that still need to be addressed.

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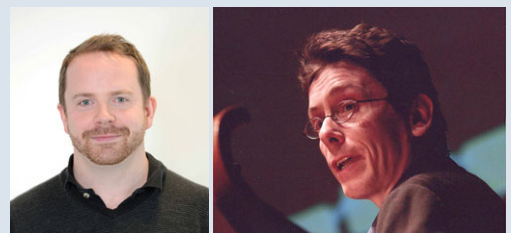
Abbreviations AMI, acute mesenteric ischaemia; AMPK, AMP kinase; ASCT2, sodium-dependent neutral amino acid transporter type 2; B⁰AT1, system B(0) neutral amino acid transporter 1; CaMKK, calcium-calmodulin-dependent kinase kinase; CFTR, cystic fibrosis transmembrane conductance regulator; DMT1, divalent metal transporter 1; DRA, down regulated in adenoma; ENaC, epithelial sodium channel; GI, gastrointestinal; GLUT, glucose transporter; HIF, hypoxia-inducible factor; IC, ischaemic colitis; I/R, ischaemia-reperfusion; KCNN4, potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4; KCNQ1, potassium voltage-gated channel, KQT-like subfamily, member 1; MCT, monocarboxylate transporter; NEC, necrotising enterocolitis; NFκB, nuclear factor κB; NHE, sodium hydrogen exchanger; NKCC1, sodium potassium chloride cotransporter 1; PAT-1, proton amino acid transporter 1; PepT1, peptide transporter 1; PHD, prolyl hydroxylase; ROS, reactive oxygen species; SGLT1, sodium glucose cotransporter 1.

Introduction

The entire surface of the gastrointestinal (GI) tract is lined by the epithelium, a single layer of cells that separates

us from the harsh and changeable environment of the intestinal lumen. The primary role of the epithelium is to recognise nutrients and other useful substances

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in the lumen and to transport them into the body, while at the same time excluding potentially harmful substances and acting as a barrier against the entry of pathogens. Under physiological conditions the intestinal epithelium is exposed to one of the highest oxygen gradients in the body. Although the serosa is supplied with amply oxygenated blood by the splanchnic circulation, the epithelium is invariably exposed to the anoxic environment of the lumen (Taylor & Colgan, 2007). Add to this the highly dynamic nature of the splanchnic circulation, and the result is a hugely fluctuating oxygen supply to the intestinal mucosa, which at times may not meet the demands of the tissue, leading to a state of hypoxia. While pathophysiological conditions, such as intestinal inflammation or splanchnic hypoperfusion, are known to be associated with mucosal hypoxia, recent studies highlight that even in normal conditions the intestinal epithelium exists in a low-level state of hypoxia, which has come to be referred to as 'physiological hypoxia' (Karhausen *et al.* 2004; Mastrogiannaki *et al.* 2009; Colgan & Taylor, 2010). We propose that this state of physiological hypoxia is responsible for homeostatic regulation of intestinal transport. This is achieved by maintaining a state of low energy consumption during fasting conditions, thereby reducing the activity of ATP-dependent transporters, such as Na^+/K^+ -ATPase pumps, which generate the electrochemical gradients that drive the secondary active transport of fluid, nutrients and electrolytes. Conversely, ATP-independent transport remains unaffected and crucial processes, such as iron transport, appear to be facilitated by hypoxia while others, such as glutamine absorption (Kles *et al.* 2001), appear to be independent of hypoxic regulation. Although O_2 supply to the intestine clearly has important implications in physiological regulation of epithelial transport function, there is still a poor understanding of the molecular mechanisms involved. Here, we aim to review the relevant literature regarding the role of O_2 in regulating intestinal epithelial transport, and how such processes can become dysregulated in conditions of hypoxic disease.

Intestinal epithelial transport

Two important characteristics of epithelial cells that enable them to transport substances to and from the gut lumen are their ability to form tight junctions with one another and their functional polarity (Keely *et al.* 2009). Tight junctions are complex structures located towards the apical pole of the cell through which adjacent cells make contact with one another (Shen *et al.* 2011). Tight junctions also constitute a physical barrier that separates epithelial cells into distinct apical and basolateral domains. It is this functional polarity that enables epithelial cells to differentially express channels, pumps, exchangers and cotransporters in the apical and basolateral domains,

thereby enabling the vectorial transport of nutrients, ions and fluid to occur (Barrett & Keely, 2000).

Nutrient transport. Through the actions of proteases and peptidases, proteins are degraded into amino acids and short peptides (2–6 amino acids), which are then absorbed by distinct amino acid and peptide transporters. Di- and tripeptides are absorbed by a single broad-specificity H^+ -coupled co-transporter, called peptide transporter 1 (PepT1) (Thwaites & Anderson, 2007), while a number of different transport proteins, such as system B(0) neutral amino acid transporter 1 ($\text{B}^0\text{AT1}$), sodium-dependent neutral amino acid transporter (ATB^0) and excitatory amino acid transporter 3, exist for the uptake of neutral, cationic and anionic amino acids (Broer, 2008). A separate set of transporters exists on the basolateral membrane that mediates the export of amino acids into the portal circulation.

Glucose and galactose are the main products of carbohydrate digestion by amylases and membrane-bound saccharidases and these are transported across the apical membrane via the sodium glucose co-transporter (SGLT) 1. The Na^+ electrochemical gradient generated by the basolateral Na^+/K^+ -ATPase pump is the key value that drives SGLT1 activity (Wright *et al.* 2011). Another product of carbohydrate digestion, fructose, is taken up by a Na^+ -independent mechanism through the glucose transporter (GLUT) 5 and the exit of these monosaccharides across the basolateral membrane is mediated by GLUT2.

Dietary lipids are digested in the stomach and small intestine by lipases to yield fatty acids and 2-monoacylglycerol. These molecules are incorporated into mixed micelles with bile acids, cholesterol, fat-soluble vitamins and phospholipids. Micelles can then diffuse to the epithelium where the fatty acids can enter epithelial cells by passive diffusion or through the activity of specific transporters in the small intestine and colon (Stahl *et al.* 1999; Sun *et al.* 2009).

The only mechanism for iron uptake is in the intestine and dysregulated iron homeostasis can lead to conditions of iron deficiency, such as anaemia, or iron overload, such as haemochromatosis, the most common genetic disorder in man. Ferrous iron (Fe^{2+}) is co-transported with H^+ across the intestinal epithelium by divalent metal transporter 1 (DMT1). The co-transport with H^+ down its electrochemical gradient ensures a concentrative uptake of divalent cations (Gunshin *et al.* 1997). There can be slippage of the ratio of Fe^{2+} and H^+ uptake by DMT1 at low luminal pH, such that Fe^{2+} uptake may also cause a mild acidification of the cells requiring efflux of H^+ (Mackenzie *et al.* 2006). Fe^{2+} is then either stored in the enterocyte bound to ferritin, or transported to the blood via the sole basolateral transporter, ferroportin. DMT1 is also responsible for the transport of an array of other

divalent cations such as Zn^{2+} , Co^{2+} and Cd^{2+} amongst others (Illing *et al.* 2012).

Fluid and electrolyte transport. Between digestive secretions and ingested water, approximately 9 l of fluid enters the proximal intestine on a daily basis, and of this only ~200 ml is lost in the faeces. All of this water movement occurs passively in response to ionic gradients established by active transport processes (Fig. 1).

Absorption normally predominates and is driven mainly by the absorption of cations, most notably Na^+ , with several mechanisms for Na^+ absorption being present along the intestine (Keely *et al.* 2009). In the small intestine, SGLT1 accounts for approximately 6 l of absorbed fluid each day (Wright *et al.* 2011), while another critical mechanism driving Na^+ and fluid absorption in the intestine is that of Na^+/H^+ exchange (Zachos *et al.* 2009; Kato & Romero, 2011). Sodium hydrogen exchanger (NHE) 3, the most important NHE for Na^+ absorption

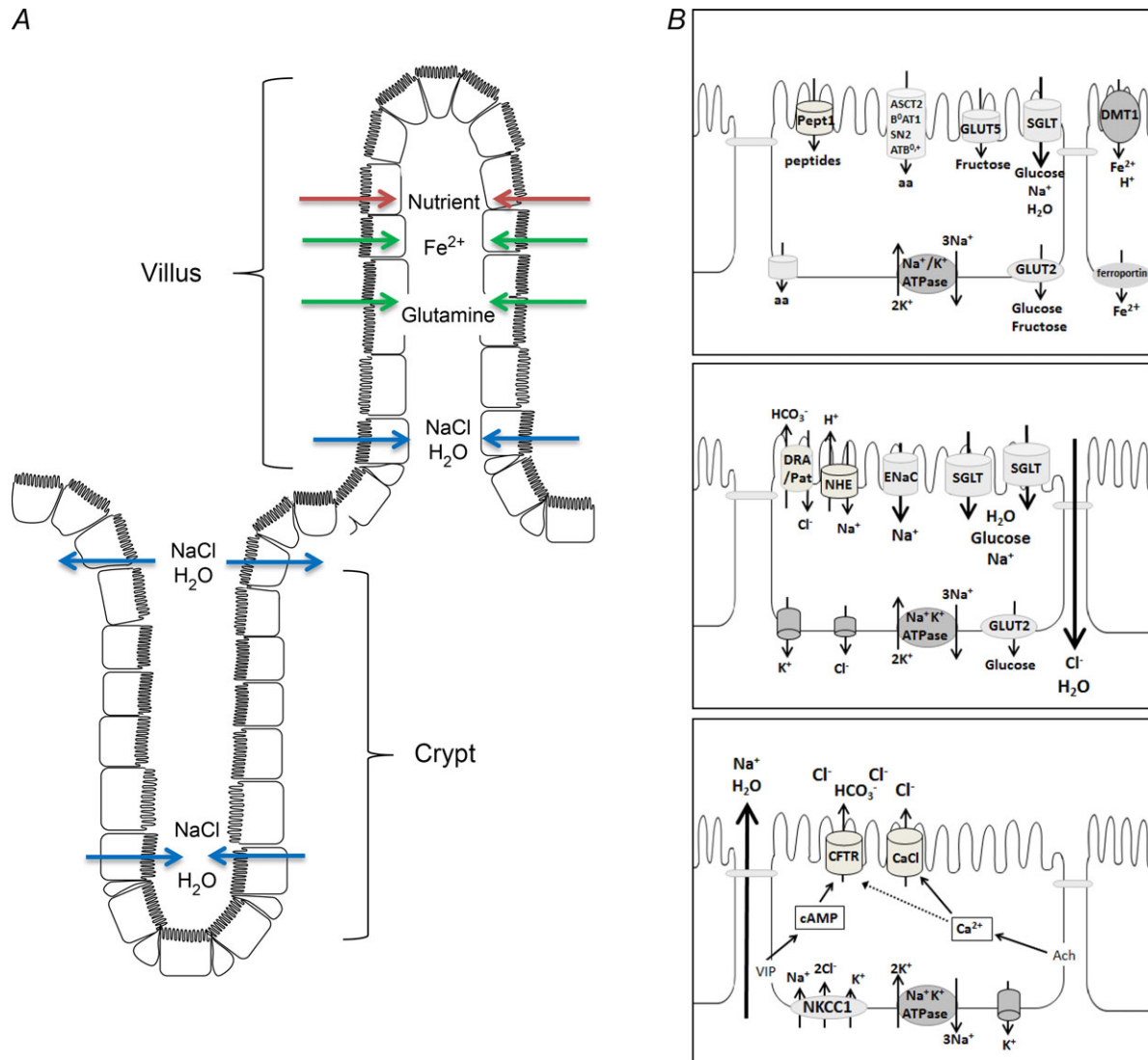


Figure 1. Schematic representation of intestinal absorptive and secretory mechanisms
 A, transport processes occur in a spatially distinct manner along the crypt–villus axis. While transporters responsible for nutrient and fluid absorption are primarily expressed in villus cells and upper crypt cells, Cl^- and fluid secretion occur primarily from the base of the crypt. B: upper panel, intestinal epithelial cell with a complement of transporters involved in nutrient absorption; middle panel, cell expressing transporters responsible for fluid absorption; lower panel, a crypt cell expressing the transport proteins involved in Cl^- and fluid secretion. aa, amino acid; VIP, vasoactive intestinal polypeptide. It should be noted that these figures are purely schematic representations, designed to summarise various transport mechanisms that can be expressed in intestinal epithelial cells. They do not take into account that transport proteins are differentially expressed along the crypt villus axis and in different intestinal sections.

across the apical membrane in the intestine, extrudes H^+ in exchange for Na^+ into the cell. The activity of NHE3 is coupled to that of Cl^-/HCO_3^- exchangers in the apical membrane, of which multiple isoforms exist, including down regulated in adenoma (DRA) and proton amino acid transporter 1 (PAT-1) (Soleimani, 2006). These proteins extrude HCO_3^- in exchange for Cl^- and so the net activity of the two transport mechanisms serves to absorb $NaCl$ from the lumen, with H_2O following by passive diffusion. A third mechanism for Na^+ absorption in the intestine is that of electrogenic Na^+ absorption and in humans this process occurs predominantly in the distal colon and rectum through channels in the apical membrane known as epithelial sodium channels (ENaCs) (Kashlan & Kleyman, 2012).

The two main anions driving intestinal fluid secretion in the intestine are HCO_3^- and Cl^- and there is an aboral gradient in their relative contributions. In the duodenum, HCO_3^- secretion predominates in order to afford protection against acid-induced mucosal ulceration (Akiba & Kaunitz, 2011). HCO_3^- enters duodenocytes through a $Na^+-HCO_3^-$ cotransporter on the basolateral side of the cell, or is generated intracellularly by the activity of carbonic anhydrase, and is secreted apically by predominantly two mechanisms: an electrogenic pathway mediated by cystic fibrosis transmembrane conductance regulator (CFTR) channels, and an electroneutral pathway involving DRA and PAT-1 (Seidler *et al.* 2011). Cl^- enters the cell through the $Na^+-K^+-2Cl^-$ cotransporter (NKCC1) on the basolateral side and exits through channels in the apical membrane. CFTR is the predominant pathway for Cl^- exit across the apical membrane but other channels, such as the Ca^{2+} -dependent Cl^- channel transmembrane protein 16A (TMEM16A), are also expressed (Barrett & Keely, 2000; Flores *et al.* 2009).

The balance between absorptive and secretory processes in the intestine is highly dynamic, with absorption normally predominating in order to conserve the large volumes of fluid passing through the intestine each day. However, many pathological conditions exist that can disrupt this finely tuned balance so that there is an over-expression of secretion, an underexpression of absorption, or vice versa, leading to the clinical manifestation of diarrhoea or constipation.

O_2 and the Na^+/K^+ -ATPase. One protein that is common to all of the transport processes described above is the Na^+/K^+ -ATPase pump. This protein can be considered as the 'engine' that drives all epithelial transport mechanisms. When one considers that the intestinal epithelium covers an area equivalent to a tennis court and that it must actively pull 9 l of fluid and approximately 1 kg of nutrients from the lumen each day, one can get an appreciation of the energy requirement for the Na^+/K^+ -ATPase to perform

its functions. Thus, transepithelial transport processes are critically dependent on the availability of O_2 to generate ATP by oxidative phosphorylation. Hence, there is an increase in blood flow through the splanchnic circulation after ingestion of a meal, when energy requirements to drive epithelial transport processes are at their highest (Nowicki & Granger, 2009). Conversely, in the fasting state, energy requirements of the epithelium are at their lowest and mucosal blood flow is diminished. With this in mind, it is not hard to envision how disruptions in mucosal blood flow, and O_2 supply, can have drastic consequences for the ability of the intestine to appropriately handle fluid, electrolytes and nutrients.

While aerobic conditions facilitate ATP generation by oxidative phosphorylation, hypoxic conditions result in considerably less ATP generation and this is exacerbated by the reversal of ATP synthase activity in order to maintain mitochondrial membrane potential (Jennings *et al.* 1991; Fig. 2). Thus, as a consequence of the diminished supply of molecular energy in hypoxia, cells must regulate their metabolism accordingly. Consequently, Na^+/K^+ -ATPase activity is reduced and non-essential transport processes are shut down.

Physiology and pathophysiology of splanchnic circulation

The splanchnic circulation perfuses the organs of the GI tract and stems from the anterior aspect of the abdominal aorta where it diverges into three main arteries, the coeliac, superior mesenteric and inferior mesenteric arteries, that together perfuse the liver, stomach, pancreas and

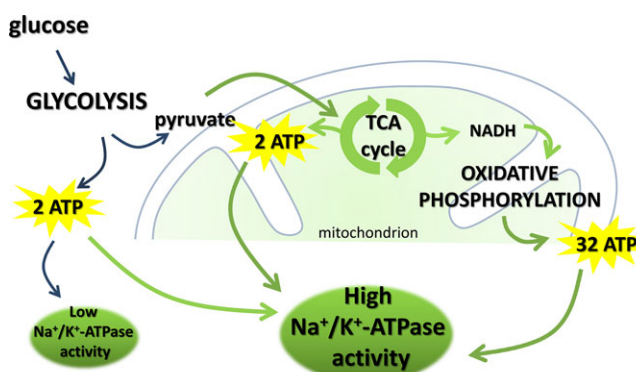


Figure 2. ATP generation in normoxic and hypoxic conditions

When oxygen supply is sufficient to meet cellular demands, pyruvate formed by glycolysis enters the tricarboxylic acid (TCA) cycle which yields 2 molecules of ATP and NADH. NADH is then used in the process of oxidative phosphorylation, yielding 32 molecules of ATP. In normoxia there is sufficient ATP available to drive Na^+/K^+ -ATPase activity. However, in hypoxic conditions lower levels of ATP production result in reduced Na^+/K^+ -ATPase activity. Arrows in green only occur in normoxic conditions.

intestines (Takala, 1996; Green & Tendler, 2005; Mensink *et al.* 2011; van Wijck *et al.* 2012). The intestines are supported by a vast network of interconnected collateral blood vessels within the splanchnic circulation, which imparts redundancy and thus protection from vascular occlusion and ischaemic insults (Mensink *et al.* 2011). However, a more global reduction in splanchnic flow can be caused by events ranging from strenuous exercise to superior mesenteric artery embolism (Alhan *et al.* 2012). Disruptions in flow can result in intestinal ischaemia and hypoxia, with sometimes fatal outcomes resulting from intestinal gangrene, sepsis and multi-organ failure (Wyers, 2010). Such is the case in acute mesenteric ischaemia (AMI) where mortality rates are as high as 71% (McKinsey & Gewertz, 1997; Brandt & Boley, 2000). Since, under normal circumstances the colonic epithelium already exists in a state of physiological hypoxia, such global reductions in splanchnic perfusion pose a significant threat to intestinal epithelial barrier integrity and transport function (Karhausen *et al.* 2004; Mastrogiannaki *et al.* 2009; Colgan & Taylor, 2010).

Physiological regulation. At rest, the intestines are supplied by approximately 20% of the total cardiac output, from which they consume 10–20% of the available oxygen (Rowell *et al.* 1964; ter Steege & Kolkman, 2012). In healthy individuals the volume of the splanchnic circulation changes throughout the day depending on various factors, most notably, feeding. It is well established that GI blood flow increases after meals, a phenomenon known as postprandial hyperaemia which has two phases. Firstly, the anticipatory phase occurs prior to ingestion and is regulated by the sympathetic nervous system (Vatner *et al.* 1970). The anticipatory phase is transient, lasting up to 30 min and causes only minor changes in GI vascular resistance (Vatner *et al.* 1970; Nowicki & Granger, 2009). On the other hand, the digestive/absorptive phase is characterised by GI hyperaemia that progresses along the GI tract in association with the aboral movement of food (Nowicki & Granger, 2009). Mesenteric blood flow increases rapidly within 5–15 min of ingestion, reaching a maximum level between 30 and 90 min, and returning to pre-fed states within several hours. The duration of postprandial hyperaemia depends on the food type ingested (Vatner *et al.* 1970; Gallavan *et al.* 1980; Qamar & Read, 1988; Sieber *et al.* 1991; Sidery *et al.* 1994; Kozar *et al.* 2002a; Mensink *et al.* 2011). Increased blood flow to the proximal intestine precedes that of the ileum, whereas colonic postprandial hyperaemia does not appear to occur (Bond *et al.* 1979; Gallavan *et al.* 1980). The biochemical stimuli that induce postprandial hyperaemia are not well understood, but are known to involve several factors that act in an orchestrated, region-specific, and synergistic fashion. Constituents of chyme, such as luminal bile, are

known to be potent vasodilators and play a key role in intestinal hyperaemia. While bile alone can stimulate ileal hyperaemia without having direct vasoactive effects in the jejunum, at concentrations of up to 33% of gallbladder bile, it also has the ability to render food molecules vasoactive. For example, at normal postprandial luminal concentrations in the proximal bowel, bile can render long chain fatty acids and amino acids vasoactive and it can potentiate the vasoactive effects of luminal glucose (Chou *et al.* 1978; Kvietyts *et al.* 1980, 1981). The mechanisms of tissue hyperaemia induced by bile are poorly understood but with respect to fatty acids they are thought to be due to increased bioavailability as a consequence of their solubilisation in the aqueous phase (Gallavan *et al.* 1985). Other studies have shown that intra-ileal bile administration causes an increase in plasma vasoactive intestinal polypeptide (VIP), an effect that can be blocked by atropine and is thus suggestive of either an indirect neuronally mediated effect or a direct effect of bile on muscarinic receptors (Chijiwa *et al.* 1986). Tissue metabolic activity also plays an important role in regulating postprandial vasoactivity. Postprandial hyperaemia is accompanied by enhanced intestinal O₂ uptake (Madsen *et al.* 2006), presumably to fuel the increased activity of processes involved in digestion and absorption. In fact, nutrient absorption from the colon has been shown to increase O₂ uptake in a manner that is mediated entirely by enhanced O₂ extraction from the blood, as opposed to increased O₂ supply (Kvietyts & Granger, 1981). Such increases in O₂ extraction from the available blood supply can be achieved by increasing capillary density and modulation of precapillary sphincters, events that have been postulated to occur postprandially (Shepherd, 1982a, b).

Other factors can also affect splanchnic perfusion, such as exercise (Qamar & Read, 1987; Perko *et al.* 1998), stress (Veenstra *et al.* 2007), medication (Stadeager *et al.* 1989) and recreational drug abuse (Elramah *et al.* 2012). For example during exercise, splanchnic vasoconstriction rapidly redistributes blood to where it is required in the lungs, muscle and skin (Perko *et al.* 1998; van Wijck *et al.* 2012), with splanchnic blood flow dropping to as low as 4% of cardiac output (Rowell *et al.* 1964). Although the intestines are able to utilise oxygen more efficiently during hypoperfusion, strenuous exercise has been associated with gut dysfunction in healthy individuals (van Wijck *et al.* 2011, 2012; ter Steege & Kolkman, 2012). It should also be noted that the intestinal villi are more susceptible than crypts to alterations in splanchnic perfusion. Villus atrophy can ensue with a concomitant reduction in the absorptive function of the gut (Niinikoski *et al.* 2004).

Pathophysiological regulation. Regulation of the splanchnic circulation is highly dynamic and it can

become disrupted in many pathophysiological conditions, such as AMI, chronic mesenteric ischaemia, colonic ischaemia, necrotising enterocolitis (NEC), hypertension (King *et al.* 2007; Osborn *et al.* 2011), vasculitis (Geboes & Dalle, 2002), thrombosis (De Stefano & Martinelli, 2010), and surgical procedures, such as cardiopulmonary bypass surgery (Wilmore *et al.* 1988). Pathophysiological reductions in intestinal perfusion lead to ischaemia, the most common form of which is ischaemic colitis (IC), which is reported to be responsible for 50–60% of all GI ischaemic episodes (Brandt & Boley, 2000; Green & Tendler, 2005; Zou *et al.* 2009). IC is most frequent in the elderly due to low flow states associated with cardiac dysfunction or hypovolaemia, but is also evident when other risk factors are present, such as the use of medications (Seon *et al.* 2011; Ajani *et al.* 2012) and recreational drugs, such as cocaine (Elramah *et al.* 2012). A deficit in O₂ availability is thought to be a major risk factor in the development of NEC, which is the most frequent life-threatening GI emergency of neonatal intensive care units (Kafetzis *et al.* 2003; Hunter *et al.* 2008). The diminished O₂ availability associated with NEC is thought to arise from both splanchnic hypoperfusion and increased intestinal epithelial metabolic demand.

While it is clear that dramatic alterations in the flow of the splanchnic circulation can occur under both physiological and pathophysiological circumstances, there are also mechanisms in place to prevent the occurrence of mucosal hypoxia. For example, there is considerable redundancy in the vascular network, which helps to prevent the loss of mucosal perfusion when blood vessels become occluded. Furthermore, in order to cope with the dynamic nature of the splanchnic circulation, cells and tissues in the intestine have evolved mechanisms that

enable them to survive under hypoxic conditions. This ability for adaptation to fluctuating levels of available O₂ is facilitated by a complex interplay of intracellular signalling pathways and intricate molecular interactions.

Epithelial responses to hypoxia

Hypoxia is defined as a state of cellular oxygen deprivation whereby the O₂ requirements of a cell exceed that of available O₂. Cellular energy status is a key factor in cell survival and metabolic stresses, such as hypoxia, which deplete intracellular ATP trigger adaptive responses (Laderoute *et al.* 2006). Epithelial responses to oxidative stress elicited by hypoxia can have biochemical consequences, including generation of reactive oxygen species (ROS), or can induce compensatory protective mechanisms, such as activation of AMP-activated protein kinase (AMPK) 1, inhibition of hypoxia-inducible factor (HIF) prolyl hydroxylases (PHDs) and activation of the master regulators of transcription, HIF and nuclear factor κ B (NF κ B) (Fig. 3).

Biochemical responses to hypoxia. One of the most rapid cellular responses to hypoxia is the generation of ROS due to the incomplete reduction of O₂ at the Q₀ site of complex III within mitochondria (Bell *et al.* 2007). ROS induces elevations in intracellular Ca²⁺, which in turn lead to the activation of AMPK (Mungai *et al.* 2011). ROS also activates other pathways associated with hypoxia, such as those involved in promoting energy and redox homeostasis and enhancing cellular survival (Liu *et al.* 2008).

Compensatory responses to hypoxia. The key role of AMPK is to act as a cellular energy sensor and in conditions

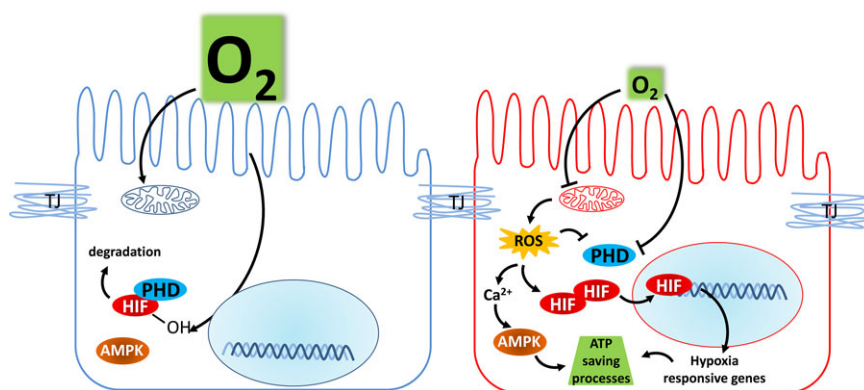


Figure 3. Effects of hypoxia on epithelial signalling pathways

In conditions where oxygen supply meets demand, PHDs can hydroxylate target proteins, such as HIF- α , which is subsequently targeted for proteasomal degradation. However, in hypoxic conditions, insufficient oxygen availability prevents PHDs from hydroxylating HIF α , leading to its stabilisation, nuclear translocation, dimerisation with HIF β and ultimately, the transcription of HIF-responsive genes. Hypoxia also induces mitochondrial ROS production which can regulate transport protein expression and activity in several ways, including elevation of intracellular Ca²⁺ and AMPK activation. TJ, tight junction.

of hypoxia it becomes rapidly activated due to increases in cellular levels of AMP, oxidants and ROS. ROS exerts its actions through increases in intracellular Ca^{2+} levels, and activation of Ca^{2+} release-activated Ca^{2+} channels, with subsequent activation of the upstream Ca^{2+} -dependent regulator of AMPK, Ca^{2+} -calmodulin-dependent kinase kinase (CaMKK) β (Dyck *et al.* 1996; Mungai *et al.* 2011). Upon its activation, AMPK induces a cellular switch from ATP-consuming anabolic pathways to ATP-generating catabolic pathways as an efficient means to conserve cellular ATP at times of cellular stress (Mungai *et al.* 2011; S. Wang *et al.* 2012).

Another important consequence of cellular hypoxia is that it leads to the induction of an array of genes that are pivotal to the ability of cells to survive and function in low O_2 environments (Ratcliffe *et al.* 1998; Semenza, 1998). Many of these genes are regulated by the HIF proteins (G. L. Wang *et al.* 1995; G. L. Wang & Semenza, 1995). Known for their importance in carcinogenesis (Semenza, 2000), HIFs also have protective roles to play against inflammatory diseases (Semenza *et al.* 2000). Under conditions of normal cellular O_2 supply, HIF levels are constitutively suppressed by proteasomal degradation. The initiating step in this process is the iron-dependent proline hydroxylation of HIF-1 α (Huang *et al.* 1998) by the HIF PHDs (Epstein *et al.* 2001). This then mediates the association of the von Hippel–Lindau E3 ligase complex, culminating in the proteasomal degradation of HIF- α (Epstein *et al.* 2001; Jaakkola *et al.* 2001). However, when cellular O_2 is depleted, PHDs become inactive with a consequent rise in cytosolic HIF- α levels. Hypoxic stabilisation of HIF- α in this way initiates a multi-step pathway of activation that includes nuclear translocation and dimerisation with HIF- β subunits. The HIF complex then interacts with cognate hypoxia-response elements on the promoters of target genes, to induce transcriptional responses (Semenza, 2001).

Interestingly, there is also a complex interplay between HIF and other mediators of the hypoxic responses that allow for fine-tuning of cellular responses to reduced O_2 availability. Thus, while PHDs are known to be responsible for stabilisation of cytosolic HIF levels, they have also been implicated in regulation of AMPK activity (Yan *et al.* 2012). Furthermore, AMPK is essential for the transcriptional activity of HIF and for HIF-induced responses to occur (Lee *et al.* 2003). PHDs have also been shown to be important in regulating cellular expression of NF κ B. During hypoxia, NF κ B becomes activated and this is due to an inability of PHDs to hydroxylate and inhibit the activity of I κ B kinase, an activating kinase of NF κ B. Given that NF κ B is a master regulator of epithelial responses to inflammation, it is not surprising that its activation in conditions of hypoxia has important consequences for intestinal barrier and transport function.

Regulation of epithelial transport processes in hypoxia

Given the adaptable nature of intestinal epithelial cells and their inherent ability to evoke homeostatic responses to changes in their environment, it is not surprising that alterations in O_2 supply induce dramatic changes in transport function. Indeed, it is well documented that intestinal disorders that cause hypoxia are often associated with the onset of diarrhoea. At their most basic level, changes in intestinal fluid movement occur either as a consequence of a loss of epithelial barrier function or changes in transepithelial osmotic gradients. Reduced absorption (Kles & Tappenden, 2002) and/or increased Cl^- secretion (Matthews *et al.* 1995) is disturbed and as a consequence can result in a net efflux of fluid as seen in diarrhoea. Likewise, any change in motility of the GI tract causing a reduction in transit time and subsequent reduction in the intestinal epithelial capacity for absorption could also result in diarrhoea (Spiller, 2006). The effects of hypoxia on intestinal epithelial barrier function are complex, depend on the duration of exposure to hypoxia, and are mediated by numerous factors. Loss of epithelial barrier function during hypoxia, such as that of tight junction dysregulation (Matthews *et al.* 1994), is also an important cause of diarrhoea and can be exacerbated by subsequent inflammatory responses (Binder, 2009). While the actions of hypoxia on epithelial barrier function have been reviewed elsewhere (Karhausen *et al.* 2003; Glover & Colgan, 2011), here we focus on its consequences for epithelial transport function.

Impact of hypoxia on Na^+/K^+ -ATPase function. Many of the transporting functions of the intestinal epithelium are highly energy-demanding, none more so than that of the Na^+/K^+ -ATPase pump. The oxygen demand of the Na^+/K^+ -ATPase is such that in the sigmoid colon, it is reported to consume 26% of available oxygen under basal conditions (Carra *et al.* 2011), whereas it can demand 20% of available ATP in resting duodenum (Milligan & McBride, 1985). Despite the fact that the pump is the primary driving force for intestinal epithelial transport processes, there is still little known of its regulation in conditions associated with intestinal hypoxia. However, studies in a variety of models have shown that Na^+/K^+ -ATPase activity becomes downregulated when O_2 supply is reduced. For example, studies in rats have shown that fasting for 48 h is associated with a 33% reduction in jejunal Na^+/K^+ -ATPase activity, with activity of the pump increasing when feeding is restored (Murray & Wild, 1980). This is likely to be an important regulatory mechanism under physiological circumstances that prevents excessive consumption of cellular energy by the pump in the interdigestive period when intestinal blood

supply is at its lowest (Fronek & Stahlgren, 1968). Similarly, pathological conditions associated with mucosal hypoxia are also associated with decreased Na^+/K^+ -ATPase activity (Berant *et al.* 1986; DuVall *et al.* 1998; Barrett & Keely, 2000). In this context, inhibition of pump activity might represent a homeostatic response whereby cellular energy is directed away from transport processes to those that are more important for cell survival.

The molecular mechanisms by which cellular hypoxia regulates intestinal epithelial Na^+/K^+ -ATPase activity are poorly defined. Studies in animal models and cultured colonic epithelia suggest that the generation of ROS with subsequent decreases in cellular levels of ATP or downregulation in expression of the catalytic α subunit of the pump may be involved (DuVall *et al.* 1998; Orsenigo *et al.* 2007). Mechanisms by which ROS inhibit Na^+/K^+ -ATPase activity in the intestine have not been defined but studies from airway epithelial cells suggest that AMPK may have a role to play (Gusarova *et al.* 2009). In these cells, ROS produced during hypoxia activate a signalling cascade involving CaMKK β , AMPK1 and protein kinase C ζ (Gusarova *et al.* 2009), which then phosphorylates Na^+/K^+ -ATPase α_1 causing its clathrin-dependent endocytosis (Dada *et al.* 2003; Chen *et al.* 2006). However, it remains to be determined if intestinal epithelial Na^+/K^+ -ATPase pumps are also regulated in such a fashion.

Studies of a potential role for HIF in regulating intestinal epithelial Na^+/K^+ -ATPase activity are also lacking, although studies from other systems suggest it may have a role to play in endocytosis and degradation of the pump under hypoxic conditions (Comellas *et al.* 2006; Zhou *et al.* 2008). Interestingly, studies from our own laboratory have shown that pharmacological inhibition of HIF PHDs reduces Na^+/K^+ -ATPase activity in colonic epithelial cells (Ward *et al.* 2011). However, in this case the response did not involve alterations in the expression or cellular location of the pump. These experiments suggest that while PHD-dependent activation of HIF may be sufficient to inhibit Na^+/K^+ -ATPase activity, additional hypoxia-induced signals are necessary for internalisation and degradation of the pump.

Overall, while it seems clear that hypoxic signals downregulate epithelial Na^+/K^+ -ATPase activity in the intestine, there is still much work to be done to elucidate the molecular mechanisms involved. Nevertheless, given its central role as the driving force for intestinal transport, such negative effects on pump activity would be expected to have significant consequences for fluid and nutrient movement in conditions of both health and disease.

Impact of hypoxia on fluid and electrolyte transport.

As discussed above, fluid movement in the intestine is driven by osmotic gradients, which are in turn established by active transepithelial ion transport. Fluid secretion

into the lumen is primarily driven by the secretion of Cl^- ions and studies from several models have shown that this process is attenuated in conditions of hypoxia (Taylor *et al.* 1998; Ibla *et al.* 2006; Thwaites & Anderson, 2007; Keely *et al.* 2009; Sun *et al.* 2009; Zheng *et al.* 2009). While inhibition of Na^+/K^+ -ATPase pump activity is likely to play an important role in this effect, several studies indicate that other transport proteins that comprise the secretory pathway are also affected. For example, hypoxia has been shown to inhibit colonic epithelial CFTR (Zheng *et al.* 2009) and NKCC1 function (Ibla *et al.* 2006), thereby preventing both the entry and exit of Cl^- ions from epithelial cells. Both of these effects appear to be mediated by HIF-dependent transcriptional repression of the transport proteins. However, it should be noted that our studies using the HIF PHD inhibitor dimethyloxalylglycine revealed no effects on the activity of CFTR in colonic epithelial cells (Ward *et al.* 2011), suggesting that while HIF-1 is required for hypoxia-induced downregulation of the channel, other hydroxylase-independent signals are also likely to be involved. Interestingly, studies from rat small intestine and cultured colonic epithelia suggest that cAMP-dependent secretory responses are more sensitive to downregulation by hypoxia than those stimulated by Ca^{2+} -dependent agonists, although the physiological/pathophysiological significance of this remains to be defined (Taylor *et al.* 1998; Thwaites & Anderson, 2007).

In addition to transcriptional regulation by HIF-1, it is likely that ROS and AMPK also have important roles to play in mediating the effects of hypoxia on intestinal secretion. In support of this idea, many previous studies have established ROS as important regulators of epithelial Cl^- secretion. While exposure of voltage-clamped colonic epithelial cells to ROS can rapidly and transiently induce Cl^- secretion, their more long-term (>30 min) actions appear to be to render the epithelium refractory to subsequent stimulation with secretagogues (DuVall *et al.* 1998). The antisecretory effects of ROS appear to be mediated by inhibition of the activity of multiple transport proteins (DuVall *et al.* 1998; Chappell *et al.* 2008). It is interesting to note that, similar to hypoxia, responses to cAMP-dependent secretagogues appear to be more sensitive to ROS than those stimulated by Ca^{2+} -dependent agonists. Several studies suggest that such selectivity may be conferred through activation of AMPK, which has been shown to function downstream of ROS generation. Activation of AMPK exerts antisecretory effects on intestinal epithelial cells, at least in part, by directly phosphorylating and inhibiting CFTR channels (Hallows *et al.* 2000; Kongsuphol *et al.* 2009a; King *et al.* 2012), thereby attenuating cAMP-dependent Cl^- secretory responses (Hallows *et al.* 2003; Walker *et al.* 2003; Kongsuphol *et al.* 2009b). Furthermore, hypoxia-induced inhibition of Cl^- secretion in human colon can be reversed

by AMPK inhibitors (Collins *et al.* 2011). Whether AMPK regulates other components of the secretory pathway in intestinal epithelia is not known, although studies from airway and renal epithelia suggest it can regulate the activity of the calcium-activated K^+ channel KCNN4, and the voltage-gated K^+ channel KCNQ1 (Alzamora *et al.* 2010).

In addition to the direct mechanisms described above, intestinal epithelial secretory function could also be regulated by hypoxia in a more indirect fashion. For example, a recent study has shown that hypoxia and ischaemia–reperfusion (I/R) induce apoptosis of intestinal Paneth cells, important producers of anti-microbial defensins (Grootjans *et al.* 2011; van Wijck *et al.* 2012). Defensins have the ability to form apical Cl^- conductive channels in colonic epithelial cells (Lencer *et al.* 1997; Kunzelmann & Mall, 2002), while also being capable of regulating cholinergic responses in the colon (Himmerkus *et al.* 2010). Thus, the possibility that hypoxia-induced reductions in defensin production may have an indirect role in regulating intestinal secretory function warrants further investigation.

While Cl^- secretion is the primary driving force for fluid secretion in the distal intestine, in the proximal small intestine, HCO_3^- is the main driving force for secretion. This process is necessary for alkalinisation of the duodenal mucosa and protection against acid from the stomach. Similar to Cl^- secretion, this process also appears to be dependent on cellular O_2 availability, since it has been shown that decreased oxygenation of human duodenal biopsies *in vitro* significantly inhibits HCO_3^- secretion (Pratha *et al.* 1998). Such an effect probably explains the propensity of patients with mesenteric ischaemia to develop ulceration of the duodenal mucosa (Gomez-Rubio *et al.* 1995). However, there is a disconnect between the production and the secretion of HCO_3^- , since carbonic anhydrase IX is expressed in the GI tract and is highly inducible by hypoxia (Wykoff *et al.* 2000). Molecular mechanisms underlying attenuated duodenal HCO_3^- secretion under conditions of low O_2 availability remain to be determined, but could conceivably involve similar pathways to those described above for hypoxic regulation of CFTR activity, since this channel is a primary exit pathway for HCO_3^- in epithelial cells (Clarke & Harline, 1998).

As described earlier, fluid absorption in the intestine is driven by electrogenic and electroneutral Na^+ absorption, through SGLT1, ENaCs and NHEs. Although early studies in rat ileum *in vitro* showed that reduced O_2 supply inhibits intestinal salt and water absorption (Curran, 1960), how absorptive processes are regulated under conditions of physiological or pathophysiological hypoxia have since received scant research attention. One study shows that Na^+ absorption is decreased after 2 h of hypoxia in rat jejunum (Berant *et al.* 1986), while

another shows that jejunal SGLT1 activity is attenuated by a mechanism involving altered membrane trafficking of the protein (Kles & Tappenden, 2002). Other studies show that hypoxia-induced signalling intermediates can also attenuate intestinal absorptive function. For example, ROS generated by H_2O_2 treatment of Caco-2 colonic epithelial cells, inhibits Cl^-/OH^- exchange, a component of the electroneutral absorptive pathway (Saksena *et al.* 2008). Some additional clues may come from studies of hypoxia, and hypoxia-induced signalling mechanisms, in other tissues. For example, hypoxia and AMPK activation have been shown to downregulate ENaC activity in airway epithelia by mechanisms that are independent of changes in gene expression (Carattino *et al.* 2005; Bhalla *et al.* 2006), while generation of ROS has been reported to upregulate ENaC activity in airway and renal epithelia (Ma, 2011; Downs *et al.* 2013). Whether such effects occur in the hypoxic intestine is not yet known and is an area requiring a great deal of study in the future.

Finally, when considering the impact of compromised O_2 availability on epithelial fluid and electrolyte transport, one must also consider the potential contribution of altered transport protein activity to the propagation of cellular responses to hypoxia. For example, it has been recently shown in renal epithelial cells that CFTR regulates HIF expression. Under hypoxic conditions, CFTR facilitates the exit of glutathione, a ROS scavenger, from the cell, thus leading to an accumulation of ROS within the cell (Duranton *et al.* 2012). ROS has been shown to cause stabilisation of HIF-1 α expression through transcriptional and translational mechanisms that are activated via phosphorylation of p70S6K, 4E-BP1 and eIF-4E, in addition to reducing its degradation by preventing its association with pVHL (Sasabe *et al.* 2010). Since HIF can, in turn, regulate the expression of CFTR, this mechanism may constitute a feedback loop, enabling fine-tuning of hypoxic and transport responses within epithelial cells. Again, whether such mechanisms are present in the intestine remains to be determined.

In summary, it is apparent that a primary response of intestinal epithelial cells to hypoxic stress is to downregulate the energy-consuming activities of transport proteins that control fluid and electrolyte movement to and from the gut. While such a response may allow the cells to redirect available cellular energy to survival processes, inhibition of absorptive function is likely to be a major contributing factor to the diarrhoea that is associated with conditions of mucosal hypoxia. On the other hand, it has been proposed that inhibition of Cl^- secretion under such conditions may be a beneficial protective mechanism that prevents excessive fluid loss from the intestine.

Impact of hypoxia on nutrient absorption. Decreased O_2 supply to the intestinal epithelium also impacts its capacity

to absorb nutrients. However, in contrast to most other transport processes which are suppressed by hypoxia, nutrient absorption persists in order to maintain barrier integrity. The physiology of nutrient absorption under hypoxic conditions has not been comprehensively studied, but there does appear to be cross talk between regulatory processes during normoxic and hypoxic states.

During hypoxic episodes, generation of ATP switches from the highly efficient process of oxidative phosphorylation, yielding 32 ATP per glucose molecule, to that of the poorly efficient process of glycolysis which yields just 2 ATP per glucose molecule. To facilitate further glucose uptake to meet cellular energy demand during hypoxia, HIF-dependent generation of transporters involved in glucose uptake and glycolysis occurs. Regulation of intestinal SGLT1 in response to hypoxia is likely to be complex, with post-translational regulation of SGLT1 in jejunal epithelial cells causing its rapid endocytosis within 1 h from the plasma membrane, coinciding with a reduction in glucose uptake (Kles & Tappenden, 2002).

Investigations of the effects of AMPK activation on sugar transporters in rat small intestine show that both GLUT2 and GLUT5 are inserted into the intestinal epithelial brush border membrane leading to enhanced galactose and fructose transport, respectively (Sakar *et al.* 2009). Studies of AMPK in mice have shown that, within 20 min of its activation, AMPK increases jejunal GLUT2-mediated glucose absorption, a facilitative transport process that does not require ATP (Walker *et al.* 2005), while on the other hand reducing the activity and expression of the ATP-dependent transporter SGLT1. Again, these data suggest that in conditions where O₂ and ATP availability are limited, the intestinal epithelium undergoes adaptation so that it favours energy-independent mechanisms of sugar transport. Studies from normoxic gut show that GLUT2 can partially compensate for a lack of SGLT1-mediated glucose absorption (Chaudhry *et al.* 2012). However, despite this compensatory mechanism, higher luminal glucose concentrations would be likely to enter the colon and alter microbial metabolism during hypoxia. Studies in rats and humans have found that in the presence of increased colonic glucose, resident bacteria anaerobically metabolise the glucose to short-chain fatty acids, which are then absorbed and oxidised by the host (Bond & Levitt, 1976). Such an effect would be expected to protect the host from the onset of diarrhoea by preventing free luminal glucose from creating a massive osmotic gradient.

While maintenance of glucose absorption is an important facet of hypoxic responses, so too is that of amino acid absorption. The activity and expression of amino acid transporters has been shown to be regulated under conditions of hypoxia and oxidative stress in several systems, including intestinal epithelial cells (Wasa *et al.*

2004; Huang *et al.* 2008). Inhibition of glutamine transport in intestinal epithelial cells under ischaemic conditions appears to involve downregulation of sodium-dependent neutral amino acid transporter type 2 (ASCT2) expression at the level of gene transcription (Huang *et al.* 2008). Reports have also shown that the apical abundance and activity of the intestinal epithelial peptide transporter PepT1 is attenuated by AMPK activation. Interestingly, although PepT1 activity is usually coupled to that of NHE3 in the intestine, AMPK was not found to alter NHE3 activity in these studies (Pieri *et al.* 2010). This suggests that an uncoupling of ion absorption from nutrient absorption may occur under conditions of hypoxia.

Glutamine, a primary metabolic fuel of enterocytes, has long been known to exert protective effects on the intestinal mucosa and its levels become depleted during periods of prolonged stress and intestinal hypoperfusion (Plauth *et al.* 1999; Ban & Kozar, 2010). The amount of energy glutamine provides to epithelial cells is similar to that provided by glucose (Mastrogiannaki *et al.* 2012) and is readily metabolised, whereas other amino acids, such as the non-metabolisable arginine can exacerbate intestinal hypoxic injury (Kozar *et al.* 2004b). Cell culture models have shown the glutamine transporters ASCT2 and B (ATB) to be negatively regulated by hypoxia and ischaemia (Wasa *et al.* 2004; Huang *et al.* 2008). However, this is in contrast to *in vivo* models showing intestinal absorption of glutamine to be unaffected by hypoxia (Kles & Tappenden, 2002; Li *et al.* 2008; Saha *et al.* 2012). Protective effects of luminal glutamine have also been demonstrated in intestinal I/R injury models (Kozar *et al.* 2002b, 2004a) through induction of anti-inflammatory molecules, such as peroxisome proliferator-activated receptor γ (PPAR γ) (Ban & Kozar, 2008), and by inhibiting apoptosis (Ban & Kozar, 2010), effects that are likely to be mediated by HIF-2. HIF-2 α has been shown to upregulate the metabolism of glutamine in a c-Myc-dependent manner (Gordan *et al.* 2007; Gao *et al.* 2009). Despite many other ATP-dependent transport processes being suppressed during hypoxic episodes, the intestinal absorption of glutamine, for the most part, appears to be maintained and is facilitated by several transporters. Together, these studies indicate that the hypoxic intestine preferentially utilises readily metabolisable amino acids, such as glutamine, to provide energy and to protect barrier function. The apparent discrepancies that have been observed between *in vitro* and *in vivo* models may be due to the simplicity of *in vitro* models compared to the more adaptive nature of whole intestinal tissues (Saha *et al.* 2012).

Another source of energy particularly important to the colonic epithelium is fatty acids. Following a hypoxic switch to glycolytic metabolism, the energy required to convert fatty acids to triglycerides and glycerol would increase thus imparting a higher energy burden on the enterocyte. The monocarboxylate or short-chain fatty

Table 1. Effects of hypoxia on epithelial transport protein activity

Substrate	Transporter	Response to hypoxia	Mechanisms involved	Effect on protein	Source
Cl ⁻	CFTR	↓	HIF-1/AMPK	Transcription/phosphorylation	(Zheng <i>et al.</i> 2009)/ (Kongsuphol <i>et al.</i> 2009a)
	NKCC1	↓	HIF-1	Transcription	(Ibla <i>et al.</i> 2006)
Na ⁺	ENaC	↓↑	AMPK	Endocytosis	(Carattino <i>et al.</i> 2005; Bhalla <i>et al.</i> 2006)
	SGLT1	↓	Hypoxia	Endocytosis	(Kles & Tappenden, 2002)
	Na ⁺ /K ⁺ -ATPase	↓	ROS/AMPK /PHDs	Endocytosis/endocytosis /activity	(DuVall <i>et al.</i> 1998; Dada <i>et al.</i> 2003; Orsenigo <i>et al.</i> 2007)
	NKCC1	↓	HIF-1	Transcription	(Ibla <i>et al.</i> 2006)
K ⁺	KCNQ1	↓	AMPK	Ubiquitination	(Alzamora <i>et al.</i> 2010)
	Na ⁺ /K ⁺ -ATPase	↓	ROS/AMPK /HIF	Endocytosis/endocytosis /activity	(DuVall <i>et al.</i> 1998; Dada <i>et al.</i> 2003; Orsenigo <i>et al.</i> 2007)
	NKCC1	↓	HIF-1	Transcription	(Ibla <i>et al.</i> 2006)
Fe ²⁺	DMT1	↑	HIF/HIF-2	Transcription/transcription	(Romney <i>et al.</i> 2011)/ (Mastrogiannaki <i>et al.</i> 2009)
	Ferroportin	↑	HIF-2	Transcription	(Taylor <i>et al.</i> 2011)
Glucose	SGLT1	↓	Hypoxia	Endocytosis	(Kles & Tappenden, 2002)
	GLUT2	↑	Hypoxia	Localisation	(Sakar <i>et al.</i> 2009)
	GLUT5	↑	Hypoxia	Localisation	(Sakar <i>et al.</i> 2009)
Glutamine	B ⁰ AT1	↓	Inflammation	Abundance	(Saha <i>et al.</i> 2012)
	SN2	↑	Inflammation	Affinity for substrate	(Saha <i>et al.</i> 2012)
	ASCT2	↓	Ischaemia	mRNA expression	(Huang <i>et al.</i> 2008)
	ATB ⁰	↓	Ischaemia	mRNA expression	(Wasa <i>et al.</i> 2004)
Peptides	PepT1	↓	AMPK	Localisation	(Pieri <i>et al.</i> 2010)
Water	SGLT1	↓	Hypoxia	Endocytosis	(Kles & Tappenden, 2002)
	Aquaporin 4	↑	Traumatic brain injury	mRNA expression	(Duan <i>et al.</i> 2013)

Hypoxia differentially affects the activity of proteins involved in the transport of electrolytes, fluid and nutrients. While transport protein activity can be either increased or decreased during periods of hypoxia, in many cases the molecular mechanisms involved are not understood.

acid, butyrate, is produced by bacteria in the colonic lumen and is transported into the epithelium via the monocarboxylate transporters MCT1 and MCT4 (Goncalves *et al.* 2011; Kekuda *et al.* 2013). MCT4 is perhaps better known for mediating efflux of lactate from glycolytic cells, but it has also been shown to be transcriptionally promoted by HIF-1 under hypoxic conditions (Ullah *et al.* 2006). Conversely, other reports have shown that under such conditions, butyrate suppresses HIF-1 transcriptional activity in what may constitute a negative feedback loop (Miki *et al.* 2004). Furthermore, AMPK may also have the capacity to alter the abundance of MCT1 and 4 mRNA (Takimoto *et al.* 2013); however, to date no studies have been published of such effects in intestinal epithelia.

The intestinal epithelium is the only site of iron uptake into the body and transporters involved in iron homeostasis are known to be regulated by hypo-

xia. In fact, the activity of iron transport proteins has been shown to be dependent on the activity of HIFs (Shah *et al.* 2009; Romney *et al.* 2011). In an elegant study employing a conditional knockdown of HIF-2 α in murine intestinal epithelium it was demonstrated that lack of HIF activity resulted in impaired iron uptake and subsequent iron deficiency (Mastrogiannaki *et al.* 2009). Conversely, a model of haemochromatosis employing hepcidin knockout mice has shown that HIF-2 ablation in the enterocytes decreases the severity of tissue iron loading (Mastrogiannaki *et al.* 2012). Using the *Caenorhabditis elegans* model, under conditions of either iron depletion or hypoxia, it has been shown that the intracellular iron-sequestering protein ferritin is transcriptionally repressed, thus releasing intracellular Fe²⁺. Furthermore, the expression of both the apical iron transporter DMT1 (Romney *et al.* 2011) and the basolateral transporter, ferroportin, are rapidly increased

through HIF-dependent transcriptional pathways (Taylor *et al.* 2011). Thus, physiological hypoxia appears to be an important factor in the regulation of iron homeostasis.

In summary, exposure of epithelial cells to hypoxia triggers rapid adaptive responses that diminish the activity of many ATP-consuming transporters, while maintaining the activity of ATP-independent transporters, such as the GLUTs. Many of these rapid responses persist until O₂ levels normalise. However, additional signalling pathways, many of which involve HIF-mediated suppression of transporter expression and activity, can also be recruited to mediate additional adaptive responses (Table 1).

Summary and conclusion

There is now considerable evidence in the literature to suggest that fluctuating levels of O₂ in the splanchnic circulation play a critical role in regulating intestinal epithelial transport function. In response to altered O₂ availability, the epithelium is capable of evoking orchestrated responses which enable it to appropriately direct cellular energy consumption to, or from, costly transport processes. Here, we have highlighted how such dynamic changes in epithelial function are likely to be necessary in order to maintain intestinal homeostasis and to facilitate digestive function under normal conditions. On the other hand, pathophysiological states, such as AMI, IC or NEC, illustrate the detrimental effects that hypoxia can have on epithelial function, leading to dysregulated fluid, nutrient and electrolyte transport, and ultimately to the onset of diarrhoea. However, while it is clear that dramatic alterations in epithelial transport protein activity and expression occur in response to changes in O₂ availability, the molecular pathways involved still remain poorly understood. An important focus for future research in intestinal physiology should be on elucidating molecular mechanisms underlying hypoxia-induced regulation of transport protein expression and activity. In particular, given its central role as the driving force for trans-epithelial transport in the intestine, a greater understanding of how fluctuations in mucosal O₂ supply regulate the Na⁺/K⁺-ATPase pump is required. This is an area that is ripe for research and future studies investigating transcriptional, post-transcriptional, and post-translational mechanisms by which altered O₂ supply regulates epithelial transport function will be of great benefit in developing our understanding of the role of hypoxia in intestinal disease. Another aspect of intestinal biology that is currently receiving a great deal of research interest is that of epithelial/bacterial interactions in regulating intestinal function in health and disease. As a part of this growing field of research, it is important to also develop our understanding of how mucosal hypoxia

impacts on the intestinal microbiome and its production of metabolites, such as short-chain fatty acids, that regulate epithelial function. Overall, a more complete understanding of how mucosal O₂ supply regulates epithelial transport function will lead to a greater understanding of the pathogenesis of intestinal diseases and to the identification of new targets for their treatment.

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Additional information

Competing interests

The authors have no conflicts of interest to declare.

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