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Sweet talk - insights into the nature & importance of glucose transport in lung epithelium

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Abstract

For over 50 years glucose has been recognised to cross the lung epithelial barrier and be transported by lung epithelial cells. However until recently, research into these processes was focused on their effects on lung liquid volume. Here we consider a newly-identified role for pulmonary glucose transport in maintaining low airway surface liquid (ASL) glucose concentrations and propose that this contributes to lung defence against infection.

Glucose diffuses into ASL via paracellular pathways at a rate determined by paracellular permeability and the transepithelial glucose gradient. Glucose is removed from ASL in proximal airways via facilitative glucose transporters (GLUTs), down a concentration gradient generated by intracellular glucose metabolism. In distal lung, glucose transport via sodium-coupled glucose transporters (SGLTs) predominates. These processes vary between species but universally maintain ASL glucose at 3 to 20-fold lower concentrations than plasma.

ASL glucose concentrations are increased in respiratory disease and by hyperglycaemia. Elevated ASL glucose in intensive care patients was associated with increased *Staphylococcus aureus* infection. Diabetic patients with and without chronic lung disease are at increased risk of respiratory infection. Understanding of mechanisms underlying lung glucose homeostasis could identify new therapeutic targets for control of ASL glucose and prevention and treatment of lung infection.

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Introduction

Our understanding of the role of glucose transport in the lung, and mechanisms that regulate glucose movement across the human lung epithelium, lags far behind that of the gut and kidney. Since the mid-1960s, it has been known that there are energy dependent, sodium-coupled glucose transporter (SGLT) and energy independent, facilitative glucose transporter (GLUT) pathways for glucose uptake in the lung [1], and that glucose can permeate the alveolar epithelial barrier [2]. However, much subsequent research in this area was focused on the role of pulmonary SGLT transport as a modifier of lung liquid volume [3, 4] and the effects of starvation and diabetes on glucose transport [1, 5]. It is only recently that we and others have begun to investigate glucose transport as an important mechanism for maintaining a nutrient-depleted environment in the lung lumen to limit the growth of pathogenic organisms.

Normal glucose concentrations in airway surface liquid

Glucose concentrations are 3-20 times lower in the fluid that lines the lung epithelium (airway surface liquid, ASL) than in plasma. This is in contrast to conditions in the gut and kidney, where luminal glucose concentrations regularly exceed plasma glucose concentrations [6, 7]. In humans, glucose concentrations were <1 mM in nasal ASL [8] and 0.4 ± 0.2(SD) mM in the lower respiratory tract, 12.5 times lower than plasma concentrations, which are normally maintained at ~ 5 mM [9]. In a perfused, fluid-filled, adult rat lung model, ASL glucose was estimated to be 0.5 mM when perfusate glucose was 10 mM [4]. In the chronically catheterized sheep fetus between 122 and 143 days gestation, lung liquid glucose was <0.01mM with plasma glucose 0.19 mM [3]. *In vitro* studies in resistive human lung epithelial cell monolayers grown at air-liquid-interface have supported these *in vivo* observations. In immortalised human airway cells (H441) with 10 mM glucose in the basolateral medium, apical ASL glucose was 0.24 ± 0.07 mM [10]. In primary cultures of human bronchial epithelial cells (HBECs) with 16.6 ± 0.4 mM glucose in the basolateral medium, ASL glucose was 2.2 ± 0.5 mM [11].

Processes that determine ASL glucose concentrations

ASL glucose concentrations are the net result of diffusion of glucose from blood/interstitial fluid across the respiratory epithelium into ASL and removal of glucose from ASL by epithelial glucose transport processes. Glucose diffusion is determined by epithelial permeability to glucose, transepithelial glucose gradient and surface area. Glucose removal is determined by cellular glucose uptake and metabolism (Figure1) [4, 12-14].

Glucose diffusion into ASL

Movement of glucose from blood/interstitial fluid into ASL occurs primarily by paracellular pathways. Early i*n vivo* studies found that rabbit respiratory epithelium and pig tracheal epithelium were permeable to L-glucose, an isoform of glucose which is neither transported nor metabolised. In rat lungs, increasing paracellular permeability with protamine increased luminal glucose concentrations [15, 16]. More recent work has shown that L-glucose, applied to the basolateral side of resistive human airway epithelial cell monolayers grown on permeable filters at air-liquid-interface, moved into the apical compartment in a time- and concentration-dependent manner, consistent with paracellular diffusion [10-12]. Moreover, apical appearance of L-glucose increased exponentially with a decrease in transepithelial electrical resistance (R_t) [10, 12]. These findings imply that glucose diffuses passively across the epithelium via paracellular pathways and that this process is affected by the permeability of epithelial tight junctions to glucose and by the glucose concentration gradient (Figure 1).

Paracellular pathways

Tight junctions between epithelial cells predominantly determine the permeability of the epithelium [17]. They comprise transmembrane proteins, including junctional adhesion molecules, claudins and occludins, which are linked to cytoskeletal proteins and each other by scaffolding proteins such as zonula occludens (ZO). The protein components and their localisation contribute to the formation of different populations of size- and charge-limiting transcellular diffusion pathways. These pathways have been broadly classified into pore and leak types [18-22]. The pathway(s) by which glucose crosses the epithelial tight junction barrier are currently unknown.

ZO-1, occludin and claudins are the main junction proteins determining paracellular permeability [21, 22]. Claudins are critical for tight junction formation affecting both pathways and charge selectivity of tight junction pores. In airway epithelial cells, differential expression of claudin-1, 3 and 5 altered epithelial permeability to large molecules. Claudin 2 is important for cation conductivity but had no effect on flux of mannitol (similar size to glucose) [23-25]. Overexpression of occludin increased epithelial permeability to small hydrophilic molecules whilst reducing ion movement [26]. The role of tight junctions in regulating glucose movement through epithelial tight junctions in the lung will undoubtedly be complex. However, identification of proteins and signaling pathways that restrict glucose movement could provide potential therapeutic targets to reduce glucose in ASL.

Transcellular pathways

There is some evidence that glucose can move by transcellular pathways from blood/interstitial fluid into ASL [11]. Pezzulo and colleagues compared bidirectional flux of L-glucose, which diffuses by paracellular pathways as it is not transported, and 2-deoxyglucose (2-DOG), which can be transported and moved by paracellular and transcellular pathways. 2-DOG flux in both basolateral-apical and apical-basolateral directions was identified, indicating the presence of transcellular pathways for glucose movement across airway epithelium. (Figure 1).

Transepithelial glucose gradient

The airway epithelium maintains the glucose concentration gradient, with blood/interstitial glucose concentrations being higher than ASL glucose concentrations (see above, [3, 8, 9]. However, if this gradient is increased by elevation of blood/interstitial glucose concentrations, this increases movement of glucose into ASL. In healthy human volunteers, experimental elevation of blood glucose from ~5mM to 12-15mM increased glucose in nasal ASL from <1 mM to 4.8 ± 2.2 (SD) mM [27] and in lower respiratory tract ASL from 0.36 ± 0.27 (SD) mM to 0.75 ± 0.39 (SD) mM [9]. In support of these findings, people with diabetes mellitus had elevated nasal (4 (2-7) mM (median (interquartile range)) [8] and lower respiratory tract (1.2 ± 0.7 (SD) mM) ASL glucose concentrations [9].

The role of glucose transport in limiting ASL glucose

As glucose is able to diffuse across airway epithelium, with time ASL glucose might be expected to equilibrate with blood glucose concentrations. As this is not the case, additional mechanisms must exist to remove glucose from ASL and/or restrict diffusion and transcellular transport. In the next section we will expand on the nature and role of glucose transport in lung epithelium.

Glucose transporters

Glucose transport across mammalian cell membranes is mediated either by facilitative glucose transporters (GLUTs; SLC2 gene family) or by sodium-coupled glucose transporters (SGLTs; SLC5 gene family). Glucose moves through GLUTs by passive diffusion down a concentration gradient generated by hexokinases and glucokinases, which phosphorylate intracellular glucose. By contrast, SGLT-mediated transport is driven by Na^+ and glucose gradients and therefore can move glucose against its concentration gradient.

The human SLC2 family consists of 14 known isoforms (SLC2A1-14) encoding glucose transporter proteins, each with different tissue expression patterns, substrate (sugar) specificity and transport kinetics. According to their sequence homology, GLUTs can be divided into 3 distinct classes: Class I comprises the well-characterised transporters GLUT1-4 [28-31] and GLUT14 (a gene duplication of GLUT3)[32]; Class II includes the fructose transporter GLUT5 [33], and GLUT7 [34], GLUT9 [35] and GLUT11 [36]; Class III comprises the recently identified transporters GLUT6 [33], GLUT8 [37], GLUT10 [38], GLUT12 [39] and the proton-driven myoinositol transporter HMIT (or GLUT13) [40]. Their affinities for glucose vary greatly. GLUT 2 has a lower affinity for glucose than other members of the glucose transporter family (K_m of ~ 32 mM when expressed in *Xenopus* oocytes); [41], while GLUT 10 has the highest affinity (K_m of ~ 0.3 mM)[42]. The human SLC5 gene family consists of 11 isoforms (SLC5A1-11), of which SLC5A1 (SGLT1) [43] and SLC5A2 (SGLT2) [44] are known to encode sodium-coupled glucose transporter proteins.

GLUT- and SGLT-mediated glucose transport have been well characterised in many tissues and cell types, although relatively little is known about their roles in the lung. Understanding

of this is greatly complicated by regional, species and developmental differences in pulmonary glucose transport.

Glucose transport in airway epithelium

In human airway epithelium (trachea, bronchi and bronchioles), GLUTs are the predominant glucose transporter type expressed (tables 1 and 2). GLUT 10 is present in immortalised human airway epithelial cells (H441) [10]and GLUT2 protein was detected in both apical and basolateral membranes of H441 and in epithelial cells in human bronchial biopsies [13]. In primary cultured human bronchial epithelial cells (HBECs), GLUT10 was similarly detected in the apical membrane but GLUT1 protein was detected in basolateral membrane [11]. In H441 cell monolayers, phloretin (an inhibitor of GLUT mediated transport) inhibited apical glucose uptake and increased appearance of D-glucose in the apical compartment [12]. In HBEC monolayers, 2-deoxyglucose (a substrate for GLUT but not SGLT transport) was taken up across the apical membrane [11]. Taken together these findings indicate that apical glucose uptake through GLUTs plays an important role in maintaining low ASL glucose concentrations.

As GLUT transporters only transport glucose down its concentration gradient, this poses the question as to how GLUTs can maintain low ASL glucose concentrations. Two mechanisms have been proposed from recent research. First, glucose taken up into airway epithelial cells is metabolised rapidly [12]. This would maintain low intracellular glucose concentrations, providing a driving force for glucose uptake across basolateral and apical membranes. Rapid metabolism of glucose would also be predicted to limit transcellular movement of glucose that could occur given the basolateral to apical glucose concentration gradient. Second, glucose uptake from the basolateral membrane and intercellular clefts could modify glucose concentration in localised microdomains and decrease glucose concentration gradients close to routes of paracellular diffusion. In support of this, basolateral phloretin increased appearance of glucose in the apical compartment of polarised H441 cell monolayers [12]. In this scenario, combined paracellular and transcellular glucose movement would be less than cell glucose uptake, allowing generation of low ASL glucose concentrations [11, 12]. We also speculate that the different apical and basolateral localization of GLUT transporters may play an important role in regulating

glucose uptake from these apical and basolateral domains. For example, the low Km of GLUT 10 compared to the higher Km's of GLUT 1 and 2 would preferentially favour glucose uptake from the apical side at low glucose concentrations. These findings indicate that airway epithelial GLUTs could be targeted to enhance depletion of glucose in ASL in respiratory disease. Moreover, it could be envisaged that genetic mutations that decrease glucose transporter function, could lead to compromised airway glucose homeostasis and result in recurrent pulmonary or upper airway infections.

Both of these hypotheses predict that glucose metabolism in airway epithelial cells is a key driver of GLUT transport and determinant of ASL glucose concentrations. Once taken up into the cell, conversion of glucose to glucose-6-phosphate is catalysed by hexokinases. As glucose phosphorylation is considered to be the rate-limiting step in glucose metabolism [45, 46] this is likely to be an important process in lung glucose homeostasis. Hexokinases (HK) I, II and III have been identified in rat lung [47, 48] and HKII was present in human lung tumours and A549 alveolar epithelial cells [49, 50]. How hexokinase activity might regulate glucose uptake, transepithelial glucose transport and glucose concentrations in ASL has yet to be explored. However, it could be speculated that upregulation of hexokinase activity could increase glucose uptake and reduce ASL glucose. There is already interest in activators and inhibitors of hexokinase activity for the treatment of diabetes. These enzymes may also provide potential therapeutic targets for the treatment and prevention of lung disease associated with elevated ASL glucose.

In human airway epithelium, sodium-coupled glucose transport does not appear to contribute to maintenance of low ASL glucose concentrations. SGLT protein was not detected in human airway epithelium (table 1). In human tracheal epithelium mounted in Ussing chambers, I_{sc} was not increased by luminal glucose or reduced by phlorizin (SGLT inhibitor) [11]. There was no consistent effect of apically applied phlorizin on glucose uptake or I_{sc} in human cell monolayers. Ussing chamber studies comparing sheep and human tracheal epithelium (from diseased lung) showed phlorizin produced a small reduction in I_{sc} in ovine airways (~5%), but had no significant effect in human tissue [51].

While GLUTs dominate apical glucose transport in the surface epithelium of the airways, there is some enticing evidence that SGLTs could be present in the submucosal glands

(SMG). In polarised cultures of serous SMG cells (Calu-3 cells), the addition of phlorizin (200μM) inhibited ~20% of basal I_{sc}, which could be mimicked by removal of glucose from the apical bath solution [52], consistent with the presence of apical sodium-coupled glucose transport in these cells [53, 54]. Whether SGLTs are present in submucosal glands *in vivo* remains to be tested but it seems unlikely so far that they have a significant contribution to airway glucose transport given the lack of functional evidence (see above).

Glucose transport in distal lung epithelium

In distal lung (alveolar) epithelium, sodium-coupled glucose transport appears to be the major mechanism responsible for apical glucose uptake from the lumen. As studies have not been performed in human distal lung epithelium, understanding of this area comes from animal studies.

SGLT 1, but not SGLT 2, mRNA has been identified in distal lung epithelium from adult rats [55, 56] and from mouse and human whole lung tissue [57, 58]. Bodega and colleagues have recently identified SGLT protein in type I and type II alveolar cells of rat and sheep lung [59]. However, SGLT1 protein could not be detected in adult rabbit lung tissue. In rat, rabbit and sheep distal lung, glucose removal from the lumen is prevented by phlorizin, which inhibits sodium-coupled glucose transporters [3, 4, 60]. SGLT-mediated transport was demonstrated in freshly isolated alveolar type II (ATII) cells from guinea pig [60]. In airway epithelial cells the intracellular Na^+ concentration is low (~23mM) compared to Na^+ concentration in ASL (~120mM) or blood plasma (~140mM) [61, 62]. SGLT utilises this Na^+ gradient to drive glucose uptake from ASL (low glucose) to interstitium/blood (high glucose) against its concentration gradient.

By contrast, there is little evidence of luminal GLUT-mediated transport by the distal lung epithelium. Phloretin in the lung instillate did not inhibit glucose or fluid absorption from rabbit [15, 63] or rat whole lung [4], and there was little luminal 2-deoxy-D-glucose (GLUT-specific) uptake in fetal sheep lung [3]. However, both phlorizin and phloretin inhibited glucose uptake in isolated type II alveolar cells from guinea pig lung [60], indicating that GLUT transporters could be present on the basolateral membrane.

The observation that airway and alveolar epithelium utilise diverse glucose transport mechanisms has led to speculation as to the role of apical glucose transport in different lung regions. In the airway, which is the first line of defence against infection, generation of low ASL glucose concentrations may be crucial for maintenance of airway sterility. In distal lung, where gas exchange occurs, sodium-coupled glucose transport may be an important driver of fluid reabsorption and regulation of ASL volume, with generation of low ASL glucose concentrations as a secondary beneficial effect. Developmental changes in lung epithelial glucose transport also indicate differences in the role of these transport process in fetal and adult lung.

Developmental differences in lung glucose transport

Developmental changes in lung glucose transporter expression have best been characterised in rat lung (table 2). In fetal rats, GLUT1 mRNA expression increases to maximal levels at gestational day 20 and falls to very low levels by postnatal day 8 [64]. Immunohistochemical staining of the rat fetus from gestational day 13 to 21 showed weak GLUT12 expression in the epithelial cell membrane of bronchioles at gestational day 19, which increased in intensity by day 21. Consistent with mRNA expression studies, GLUT1 protein was also observed in the airways at gestational day 19, decreasing in intensity at day 21 [65]. GLUT1 protein has also been shown in alveolar epithelium of the fetal rat, although to date GLUT1 protein has not been identified in either the airways or distal lung of the adult rat [66]. SGLT1 mRNA expression has been seen in the distal lung of both fetal and adult rats [55, 56].

In fetal sheep, the maximum rate of phlorizin-sensitive glucose uptake from the lung lumen increased with gestational age [3], indicating increased pulmonary expression of sodium-coupled glucose transporters towards birth. In humans, amniotic fluid glucose concentrations fall towards the end of pregnancy [67], consistent with increased pulmonary glucose reabsorption.

It is interesting to speculate on the role of glucose transport in fetal lungs. In fetal sheep, both addition of glucose to lung liquid [3] and maternal hyperglycaemia decreased net lung liquid secretion, probably by driving glucose-coupled sodium absorption. However as

glucose concentrations are very low in normal fetal lung liquid, this process is unlikely to be a major determinant of lung sodium transport unless there is gestational diabetes. An alternative role could be to scavenge glucose from amniotic fluid which could have the dual functions of conserving nutrients and reducing the risk of infection.

Species and tissue differences in lung glucose transport

The investigation of lung glucose transport in humans *in vivo* is challenging and so animal and cell culture models have been used to explore this field. There are important differences in expression and function of transporters between species, cell types and experimental conditions that should be appreciated when choosing a model.

Species differences. Where it has been investigated, adults of most species have been found predominantly to express GLUT transporters in airway and SGLT in distal lung epithelium. Glucose uptake by isolated primary guinea pig alveolar type II epithelial cells could be reduced both by phlorizin and by phloretin, indicating that not only SGLT, but also GLUT transporters are present in guinea pig distal lung cells [60]. By contrast in adult sheep, glucose uptake across the tracheal epithelium could be inhibited by phlorizin and required external sodium, consistent with sodium-coupled glucose transport [68]. Similarly in isolated equine trachea, phlorizin addition to the apical (but not the basolateral) membrane produced a significant decrease in I_{sc} [69]. However, these differences between guinea-pig and sheep/horse more likely reflect the experimental preparation. In intact epithelium SGLT is predominantly present on the luminal membrane, but in isolated cells there is a contribution from basolateral localized GLUT transporters.

Cell types. Most *in vitro* studies of human airway epithelial glucose transport have used immortalised H441 cells or primary cultured human bronchial airway epithelial cells. H441 cells derive from a papillary adenocarcinoma of the bronchiolar epithelium. When cultured at air interface, these cells form an absorptive epithelial monolayer, exhibit vectorial ion transport processes and have similar morphological and phenotypic characteristics to human bronchiolar epithelium [70]. Both H441 and HBEC cells express GLUTs and do not exhibit sodium-coupled glucose transporter expression. However there may be differences

in the GLUT isoform expressed, with H441 cells predominantly expressing GLUT2 and GLUT10 [10, 13] and HBECs expressing GLUT1 and GLUT10 [11].

Experimental conditions. Experimental conditions in both animal and *in vitro* studies may influence channel expression and function. In animals, investigation of distal lung glucose transport requires use of a fluid filled lung model which may alter transport processes. In cell culture, non polarised H441 cells expressed GLUT4, which could not be identified in H441 cells polarised at air-liquid-interface [13].

Effects of elevated ASL glucose concentrations

So what are the implications of lung glucose homeostasis for human health and disease? Clinical observation has shown that ASL glucose concentrations are normally ~12.5 times lower in human ASL than in plasma. However ASL glucose concentrations are elevated in respiratory disease and when blood glucose is elevated. Nasal glucose concentrations were undetectable (<1 mM) in healthy volunteers, but detectable in 90% of people with diabetes mellitus (4 (2-7) mM) [8]. Ventilated patients on intensive care were more likely to have glucose detectable in bronchial aspirates when they had elevated blood glucose [7]. ASL glucose concentration, estimated using an exhaled breath condensate technique (breath glucose), was 0.4 ± 0.2 mM in healthy volunteers but was elevated to 2.0 ± 1.1 mM in patients with cystic fibrosis, to 1.2 ± 0.7 mM in patients with diabetes mellitus and by the greatest amount (to 4.0 ± 2.0 mM) in people with both cystic fibrosis and diabetes mellitus [9]. In patients undergoing bronchoscopy, glucose was elevated in bronchoalveolar lavage fluid (BAL) from patients with chronic obstructive pulmonary disease and chronic severe asthma compared to levels in BAL fluid from healthy controls [71, 72]. These scenarios involve hyperglycaemia as a result of stress, Type I and Type II diabetes mellitus. Any specific effect(s) of these individual disorders on glucose transport and homeostasis is, as yet, unclear. However, this may be important to understand, particularly with Type II diabetes becoming more prevalent in the western population.

There is both direct and indirect evidence that elevated ASL glucose concentrations promote lung infection. The respiratory pathogens *Staphylococcus aureus* and *Pseudomonas aeruginosa* both utilise glucose as a growth substrate [73]. Patients intubated and ventilated on an intensive care unit who had detectable glucose in bronchial aspirates were more likely to have methicillin-resistant *S. aureus*, isolated from aspirates than those without detectable glucose [74]. Diabetes mellitus is a predisposing factor for nasal colonisation with *S. aureus* [75]. Patients with chronic obstructive pulmonary disease (COPD) who also had diabetes mellitus were more likely to have gram negative organisms cultured from sputum than those without diabetes [76]. During acute exacerbations of COPD, increasing admission blood glucose concentrations were associated with increasing likelihood of isolating *S. aureus* and multiple pathogens from sputum [72]. The growth of *Ps. aeruginosa* was increased in the lungs of hyperglycaemic mice *in vivo* [11]. In humans with cystic fibrosis, coexisting diabetes mellitus was associated with an increased risk of infection with multiple antibiotic-resistant *Ps. aeruginosa* [77]. Cystic fibrosis patients with diabetes mellitus had more pulmonary exacerbations [78], which were less likely to respond to intravenous antibiotics [79], than those without diabetes

Questions for the future

Whilst there are several mechanisms that regulate glucose homeostasis across the pulmonary epithelium, glucose transport undoubtedly plays a key role in maintaining a low glucose concentration in the ASL and thus restricting the growth of respiratory pathogens. This newly-identified role for glucose transport raises some important questions, particularly regarding our classic understanding of GLUT-mediated transport. For example, is GLUT abundance regulated in response to changes in ASL glucose concentration? Why is SGLT only detectable in the distal lung? (SGLT can transport glucose against its concentration gradient which would more efficiently reduce glucose in ASL). In addition, in the gut, SGLT was proposed to have an important role as a glucose-sensor with consequent up-regulation of GLUT mediated transport [80]. What are the roles of the different transporters especially GLUT 1, 2 and 10 in pulmonary glucose homeostasis? Is pulmonary glucose transport compromised in respiratory disease and diabetes (particularly in Type II

diabetes) and does this contribute to increased ASL glucose and infection? Finally, could we target glucose transport in the lung to ameliorate some of the consequences of respiratory disease?

Abbreviations

ASL, airway surface liquid; DOG, deoxyglucose; GLUT, facilitative glucose transporter; HBEC, primary cultured human bronchial epithelial cells; HK, hexokinase; H441, immortalized human airway epithelial cells; K_m, the Michaelis constant; SD, standard deviation; SGLT, sodium-coupled glucose transporter; SMG, submucosal gland; ZO, zona occludens.

Table 1. **GLUT and SGLT expression (mRNA and protein), in human cultured epithelial cells (H441 and primary human bronchial epithelial cells), human bronchial tissue and whole lung.** HBEC = human primary cultured bronchial epithelial cells; RNA = mRNA expression; protein = protein expression; √ = present; x = absent; blank = no evidence; numbers are references.

	H441 Non-polarised		H441 Polarised		HBEC		Bronchial tissue		Whole lung	
Transporter	mRNA	protein	mRNA	protein	mRNA	protein	mRNA	protein	mRNA	protein
GLUTs										
GLUT1	√[13]	X[13]		X[13]	√[11]	√[11]	√[11]	X[81]	√[82]	X[81, 83]
GLUT2	√[13]	√[13]		√[10, 13]	√[11]	X[11]	√[11]	√[13, 83]		
GLUT3	X[13]	X[13]		X[13]	√[11]	X[11]	√[11]	X[81]	√[82]	X[81, 83]
GLUT4	√[13]	√[13]		X[13]	√[11]	X[11]	√[11]	X[81]		X[81, 83]
GLUT5					√[11]		√[11]	X[81]	√[82]	X[81, 83]
GLUT6					√[11]		√[11]			
GLUT7					√[11]		√[11]			
GLUT8					√[11]	X[11]	√[11]			
GLUT9					√[11]		√[11]			
GLUT10				√[10]	√[11]	√[11]	√[11]		√[84]	
GLUT11					√[11]		√[11]			

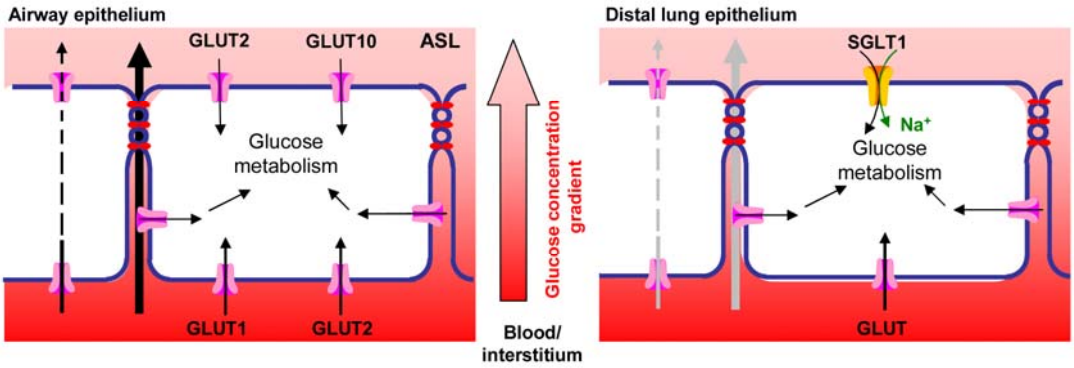
GLUT12					√[11]		√[11]			
SGLTs										
SGLT1					√[11]	X[11]	√[11]		√[58]	
SGLT2					√[11]	X[11]	√[11]		√[58]	

Table 2. GLUT and SGLT expression (mRNA and protein), in whole lung, bronchial and alveolar (type II cells) epithelium in fetal and adult stages. Expression in rats unless otherwise stated. M = mouse; P = pig; R = rabbit; S = sheep. RNA = mRNA expression; Prot = protein expression; √ = present; x = absent; blank = no evidence; numbers are references.

	Whole lung				Bronchial epithelium				Alveolar epithelium			
	Fetal		Adult		Fetal		Adult		Fetal		Adult	
Transporter	RNA	Prot	RNA	Prot	RNA	Prot	RNA	Prot	RNA	Prot	RNA	Prot
GLUTs												
GLUT1	√[85] [64]	√[85]	√[47, 64]			√[65] x[86]M		x[86]M		√[66] x[86]M	√[56]	x[86]M
GLUT2			√[87] P					√[81]			x[56]	
GLUT3												
GLUT4			√[47]								√[56]	
GLUT5											√[56]	
GLUT6												
GLUT7												
GLUT8												
GLUT9												
GLUT10												
GLUT12						√[65]						

SGLTs												
SGLT1			√[47, 55] √[57] **M**	x[81] **R**							√[56]	√[59] √[59] **S**
SGLT2											X[56]	

Figure 1. Current model of the mechanisms controlling glucose concentrations in the surface liquid lining the airway and distal lung epithelium. *Airway epithelium*: The glucose concentration of the airway surface liquid (ASL) is the net effect of paracellular diffusion (and to a lesser extent, the transcellular flux of glucose) from blood/interstitial fluid across respiratory epithelium into ASL and removal of glucose from ASL by uptake into epithelial cells**.** Glucose uptake across the apical membrane is mediated by GLUT2 and GLUT10, and across the basolateral membrane by GLUT2 and GLUT1. *Distal lung (alveolar) epithelium*: Glucose uptake across the apical membrane is via SGLT1 and the basolateral membrane via unidentified GLUTs.



References

1. Chaisson CF, Massaro D. 2-Deoxy-D-glucose uptake by lung slices from fed and fasted rats. *J Appl Physiol* 1978; 44:380-3.

2. Taylor AE, Guyton AC, Bishop VS. Permeability of the Alveolar Membrane to Solutes. *Circ Res* 1965; 16:353-62.

3. Barker PM, Boyd CA, Ramsden CA, Strang LB, Walters DV. Pulmonary glucose transport in the fetal sheep. *J Physiol* 1989; 409:15-27.

4. Saumon G, Martet G, Loiseau P. Glucose transport and equilibrium across alveolar-airway barrier of rat. *Am J Physiol* 1996; 270:L183-L90.

5. Fricke RF, Longmore WJ. Effects of insulin and diabetes on 2-deoxy-D-glucose uptake by the isolated perfused rat lung. *J Biol Chem* 1979; 254:5092-8.

6. Ferraris RP, Yasharpour S, Lloyd KC, Mirzayan R, Diamond JM. Luminal glucose concentrations in the gut under normal conditions. *Am J Physiol* 1990; 259:G822-37.

7. DeFronzo RA, Davidson JA, Del Prato S. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. *Diabetes Obes Metab* 2012; 14:5-14.

8. Philips BJ, Meguer JX, Redman J, Baker EH. Factors determining the appearance of glucose in upper and lower respiratory tract secretions. *Intensive Care Med* 2003; 29:2204-10.

9. Baker EH, Clark N, Brennan AL, Fisher DA, Gyi KM, Hodson ME, Philips BJ, Baines DL, Wood DM. Hyperglycemia and cystic fibrosis alter respiratory fluid glucose concentrations estimated by breath condensate analysis. *J Appl Physiol* 2007; 102:1969-75.

10. Garnett JP, Nguyen TT, Moffatt JD, Pelham ER, Kalsi KK, Baker EH, Baines DL. Pro-inflammatory mediators disrupt glucose homeostasis in airway surface liquid. *J Immunol* 2012; May 23 published ahead of print.

11. Pezzulo AA, Gutierrez J, Duschner KS, McConnell KS, Taft PJ, Ernst SE, Yahr TL, Rahmouni K, Klesney-Tait J, Stoltz DA, Zabner J. Glucose depletion in the airway surface liquid is essential for sterility of the airways. *PLoS One* 2011; 6:e16166.

12. Kalsi KK, Baker EH, Fraser O, Chung YL, Mace OJ, Tarelli E, Philips BJ, Baines DL. Glucose homeostasis across human airway epithelial cell monolayers: role of diffusion, transport and metabolism. *Pflugers Arch* 2009; 457:1061-70.

13. Kalsi KK, Baker EH, Medina RA, Rice S, Wood DM, Ratoff JC, Philips BJ, Baines DL. Apical and basolateral localisation of GLUT2 transporters in human lung epithelial cells. *Pflugers Arch* 2008; 456:991-1003.

14. Pezzulo AA, Starner TD, Scheetz TE, Traver GL, Tilley AE, Harvey BG, Crystal RG, McCray PB, Jr., Zabner J. The air-liquid interface and use of primary cell cultures are important to recapitulate the transcriptional profile of in vivo airway epithelia. *Am J Physiol Lung Cell Mol Physiol* 2011; 300:L25-31.

15. Wangensteen D, Bartlett M. D- and L-glucose transport across the pulmonary epithelium. *J Appl Physiol* 1984; 57:1722-30.

16. Saumon G, Martet G. Effect of changes in paracellular permeability on airspace liquid clearance: role of glucose transport. *Am J Physiol* 1996; 270:L191-L8.

17. Farquhar MG, Palade GE. Junctional complexes in various epithelia. *J Cell Biol* 1963; 17:375-412.

18. Balda MS, Matter K. Tight junctions at a glance. *J Cell Sci* 2008; 121:3677-82.

19. Shimizu M, Fukunaga Y, Ikenouchi J, Nagafuchi A. Defining the roles of beta-catenin and plakoglobin in LEF/T-cell factor-dependent transcription using beta-catenin/plakoglobin-null F9 cells. *Mol Cell Biol* 2008; 28:825-35.

20. Ikenouchi J, Sasaki H, Tsukita S, Furuse M. Loss of occludin affects tricellular localization of tricellulin. *Mol Biol Cell* 2008; 19:4687-93.

21. Shen L, Weber CR, Raleigh DR, Yu D, Turner JR. Tight junction pore and leak pathways: a dynamic duo. *Annu Rev Physiol* 2011; 73:283-309.

22. Van Itallie CM, Holmes J, Bridges A, Gookin JL, Coccaro MR, Proctor W, Colegio OR, Anderson JM. The density of small tight junction pores varies among cell types and is increased by expression of claudin-2. *J Cell Sci* 2008; 121:298-305.

23. Coyne CB, Gambling TM, Boucher RC, Carson JL, Johnson LG. Role of claudin interactions in airway tight junctional permeability. *Am J Physiol Lung Cell Mol Physiol* 2003; 285:L1166-78.

24. Coyne CB, Vanhook MK, Gambling TM, Carson JL, Boucher RC, Johnson LG. Regulation of airway tight junctions by proinflammatory cytokines. *Mol Biol Cell* 2002; 13:3218-34.

25. Amasheh S, Meiri N, Gitter AH, Schoneberg T, Mankertz J, Schulzke JD, Fromm M. Claudin-2 expression induces cation-selective channels in tight junctions of epithelial cells. *J Cell Sci* 2002; 115:4969-76.

26. Aijaz S, Balda M, Matter K, . Tight junctions: molecular architecture and function. *Int Rev Cytol* 2006; 248:261-98.

27. Wood DM, Brennan AL, Philips BJ, Baker EH. Effect of hyperglycaemia on glucose concentration of human nasal secretions. *Clin Sci (Lond)* 2004; 106:527-33.

28. Mueckler M, Caruso C, Baldwin S, Panico M, Blench I, Morris H, Allard W, Lienhard G, Lodish H. Sequence and structure of a human glucose transporter. *Science* 1985; 229:941-5.

29. Fukumoto H, Seino S, Imura H, Seino Y, Eddy RL, Fukushima Y, Byers MG, Shows TB, Bell GI. Sequence, tissue distribution, and chromosomal localization of mRNA encoding a human glucose transporter-like protein. *Proc Natl Acad Sci U S A* 1988; 85:5434-8.

30. Kayano T, Fukumoto H, Eddy RL, Fan YS, Byers MG, Shows TB, Bell GI. Evidence for a family of human glucose transporter-like proteins. Sequence and gene localization of a protein expressed in fetal skeletal muscle and other tissues. *J Biol Chem* 1988; 263:15245-8.

31. James DE, Strube M, Muecdler M. Molecular cloning and characterization of an insulin-regulatable glucose transporter. *Nature* 1989; 338:83-7.

32. Wu X, Freeze HH. GLUT14, a Duplicon of GLUT3, Is Specifically Expressed in Testis as Alternative Splice Forms. *Genomics* 2002; 80:553-7.

33. Kayano T, Burant CF, Fukumoto H, Gould GW, Fan YS, Eddy RL, Byers MG, Shows TB, Seino S, Bell GI. Human facilitative glucose transporters. Isolation, functional characterization, and gene localization of cDNAs encoding an isoform (GLUT5) expressed in

small intestine, kidney, muscle, and adipose tissue and an unusual glucose transporter pseudogene-like sequence (GLUT6). *J Biol Chem* 1990; 265:13276-82.

34. Li Q, Manolescu A, Ritzel M, Yao S, Slugoski M, Young JD, Chen X-Z, Cheeseman CI. Cloning and functional characterization of the human GLUT7 isoform SLC2A7 from the small intestine. *Am J Physiol Gastrointest Liver Physiol* 2004; 287:G236-G42.

35. Phay JE, Hussain HB, Moley JF. Cloning and Expression Analysis of a Novel Member of the Facilitative Glucose Transporter Family, SLC2A9 (GLUT9). *Genomics* 2000; 66:217-20.

36. Doege H, Bocianski A, Scheepers A, Axer H, Eckel J, G Joost HG, Schürmann A. Characterization of human glucose transporter (GLUT) 11 (encoded by SLC2A11), a novel sugar-transport facilitator specifically expressed in heart and skeletal muscle. *Biochem J* 2001; 359:443-9.

37. Doege H, Schürmann A, Bahrenberg G, Brauers A, Joost H-G. GLUT8, a Novel Member of the Sugar Transport Facilitator Family with Glucose Transport Activity. *J Biol Chem* 2000; 275:16275-80.

38. McVie-Wylie AJ, Lamson DR, Chen YT. Molecular Cloning of a Novel Member of the GLUT Family of Transporters, SLC2A10 (GLUT10), Localized on Chromosome 20q13.1: A Candidate Gene for NIDDM Susceptibility. *Genomics* 2001; 72:113-7.

39. Rogers S, Macheda ML, Docherty SE, Carty MD, Henderson MA, Soeller WC, Gibbs EM, James DE, Best JD. Identification of a novel glucose transporter-like protein-GLUT-12. *Am J Physiol Endocrinol Metab* 2002; 282:E733-8.

40. Augustin R. The protein family of glucose transport facilitators: It's not only about glucose after all. *IUBMB Life* 2010; 62:315-33.

41. Buchs A, Wu L, Morita H, Whitesell RR, Powers AC. Two regions of GLUT 2 glucose transporter protein are responsible for its distinctive affinity for glucose. *Endocrinology* 1995; 136:4224-30.

42. Zhao FQ, Keating AF. Functional properties and genomics of glucose transporters. *Curr Genomics* 2007; 8:113-28.

43. Hediger MA, Coady MJ, Ikeda TS, Wright EM. Expression cloning and cDNA sequencing of the Na+/glucose co-transporter. *Nature* 1987; 330:379-81.

44. Wells RG, Mohandas TK, Hediger MA. Localization of the Na+/Glucose Cotransporter Gene SGLT2 to Human Chromosome 16 Close to the Centromere. *Genomics* 1993; 17:787-9.

45. Salotra PT, Singh VN. Regulation of glucose metabolism in the lung: hexokinase-catalyzed phosphorylation, a rate-limiting step. *Life Sci* 1982; 31:791-4.

46. Salotra PT, Singh VN. Regulation of glucose metabolism in rat lung: subcellular distribution, isozyme pattern, and kinetic properties of hexokinase. *Arch Biochem Biophys* 1982; 216:758-64.

47. Allen CB, Guo XL, White CW. Changes in pulmonary expression of hexokinase and glucose transporter mRNAs in rats adapted to hyperoxia. *Am J Physiol* 1998; 274:L320-9.

48. Katzen HM, Soderman DD, Cirillo VJ. Tissue distribution and physiological significance of multiple forms of hexokinase. *Ann N Y Acad Sci* 1968; 151:351-8.

49. Riddle SR, Ahmad A, Ahmad S, Deeb SS, Malkki M, Schneider BK, Allen CB, White CW. Hypoxia induces hexokinase II gene expression in human lung cell line A549. *Am J Physiol Lung Cell Mol Physiol* 2000; 278:L407-16.

50. Mamede M, Higashi T, Kitaichi M, Ishizu K, Ishimori T, Nakamoto Y, Yanagihara K, Li M, Tanaka F, Wada H, Manabe T, Saga T. [18F]FDG uptake and PCNA, Glut-1, and Hexokinase-II expressions in cancers and inflammatory lesions of the lung. *Neoplasia* 2005; 7:369-79.

51. Steel DM, Graham A, Geddes DM, Alton EW. Characterization and comparison of ion transport across sheep and human airway epithelium. *Epithelial Cell Biol* 1994; 3:24-31.

52. Singh M, Krouse M, Moon S, Wine JJ. Most basal I(SC) in Calu-3 human airway cells is bicarbonate-dependent Cl- secretion. *Am J Physiol Lung Cell Mol Physiol* 1997; 272:L690-L8.

53. Lee MC, Penland CM, Widdicombe JH, Wine JJ. Evidence that Calu-3 human airway cells secrete bicarbonate. *Am J Physiol Lung Cell Mol Physiol* 1998; 274:L450-L3.

54. Krouse ME, Talbott JF, Lee MM, Joo NS, Wine JJ. Acid and base secretion in the Calu-3 model of human serous cells. *Am J Physiol Lung Cell Mol Physiol* 2004; 287:L1274-L83.

55. Lee WS, Kanai Y, Wells RG, Hediger MA. The high affinity Na+/glucose cotransporter. Re-evaluation of function and distribution of expression. *J Biol Chem* 1994; 269:12032-9.

56. Mamchaoui K, Makhloufi Y, Saumon G. Glucose transporter gene expression in freshly isolated and cultured rat pneumocytes. *Acta Physiol Scand* 2002; 175:19-24.

57. Icard P, Saumon G. Alveolar sodium and liquid transport in mice. *Am J Physiol* 1999; 277:L1232-8.

58. Ishikawa N, Oguri T, Isobe T, Fujitaka K, Kohno N. SGLT gene expression in primary lung cancers and their metastatic lesions. *Jpn J Cancer Res* 2001; 92:874-9.

59. Bodega F, Sironi C, Armilli M, Porta C, Agostoni E. Evidence for Na+-glucose cotransporter in type I alveolar epithelium. *Histochem Cell Biol* 2010; 134:129-36.

60. Kemp PJ, Boyd CA. Pathways for glucose transport in type II pneumocytes freshly isolated from adult guinea pig lung. *Am J Physiol* 1992; 263:L612-6.

61. Willumsen NJ, Boucher RC. Sodium transport and intracellular sodium activity in cultured human nasal epithelium. *Am J Physiol* 1991; 261:C319-31.

62. Knowles MR, Robinson JM, Wood RE, Pue CA, Mentz WM, Wager GC, Gatzy JT, Boucher RC. Ion composition of airway surface liquid of patients with cystic fibrosis as compared with normal and disease-control subjects. *J Clin Invest* 1997; 100:2588-95.

63. Basset G, Crone C, Saumon G. Significance of active ion transport in transalveolar water absorption: a study in isolated rat lung. *J Physiol* 1987; 384:311-24.

64. Werner H, Adamo M, Lowe WL, Jr., Roberts CT, Jr., LeRoith D. Developmental regulation of rat brain/Hep G2 glucose transporter gene expression. *Mol Endocrinol* 1989; 3:273-9.

65. Macheda ML, Kelly DJ, Best JD, Rogers S. Expression during rat fetal development of GLUT12--a member of the class III hexose transporter family. *Anat Embryol (Berl)* 2002; 205:441-52.

66. Hart CD, Flozak AS, Simmons RA. Modulation of glucose transport in fetal rat lung: a sexual dimorphism. *Am J Respir Cell Mol Biol* 1998; 19:63-70.

67. Weiss PA, Hofmann H, Winter R, Purstner P, Lichtenegger W. Amniotic fluid glucose values in normal and abnormal pregnancies. *Obstet Gynecol* 1985; 65:333-9.

68. Charon JP, McCormick J, Mehta A, Kemp PJ. Characterization of sodium-dependent glucose transport in sheep tracheal epithelium. *Am J Physiol* 1994; 267:L390-7.

69. Joris L, Quinton P. Evidence for electrogenic Na-glucose cotransport in tracheal epithelium. *Pflügers Arch* 1989; 415:118-20.

70. Hermanns MI, Unger RE, Kehe K, Peters K, Kirkpatrick CJ. Lung epithelial cell lines in coculture with human pulmonary microvascular endothelial cells: development of an alveolo-capillary barrier in vitro. *Lab Invest* 2004; 84:736-52.

71. Baker E, Akunuri S, Morgan M, Greenaway S, O'Connor B, Ratoff J. Effect of airways inflammation and prednisolone treatment on BAL glucose concentrations in asthma and COPD patients. *Thorax* 2008; (Suppl VII):A40.

72. Baker EH, Janaway CH, Philips BJ, Brennan AL, Baines DL, Wood DM, Jones PW. Hyperglycaemia is associated with poor outcomes in patients admitted to hospital with acute exacerbations of chronic obstructive pulmonary disease. *Thorax* 2006; 61:284-9.

73. Brennan A, Baines D, Woollhead A, Lindsay J, Hayes M, Philips B, Baker E. Development of an in vitro model to investigate the effect of glucose on the interaction between respiratory epithelia and bacterial pathogens. *Thorax* 2006; 61 :.101.

74. Philips BJ, Redman J, Brennan A, Wood D, Holliman R, Baines D, Baker EH. Glucose in bronchial aspirates increases the risk of respiratory MRSA in intubated patients. *Thorax* 2005; 60:761-4.

75. Tamer A, Karabay O, Ekerbicer H. Staphylococcus aureus nasal carriage and associated factors in type 2 diabetic patients. *Jpn J Infect Dis* 2006; 59:10-4.

76. Loukides S, Polyzogopoulos D. The effect of diabetes mellitus on the outcome of patients with chronic obstructive pulmonary disease exacerbated due to respiratory infections. *Respiration* 1996; 63:170-3.

77. Merlo CA, Boyle MP, Diener-West M, Marshall BC, Goss CH, Lechtzin N. Incidence and risk factors for multiple antibiotic-resistant Pseudomonas aeruginosa in cystic fibrosis. *Chest* 2007; 132:562-8.

78. Jarad NA, Giles K. Risk factors for increased need for intravenous antibiotics for pulmonary exacerbations in adult patients with cystic fibrosis. *Chron Respir Dis* 2008; 5:29-33.

79. Parkins MD, Rendall JC, Elborn JS. Incidence and risk factors for pulmonary exacerbation treatment failures in cystic fibrosis patients chronically infected with Pseudomonas aeruginosa. *Chest* 2011.

80. Kellett GL, Brot-Laroche E. Apical GLUT2: a major pathway of intestinal sugar absorption. *Diabetes* 2005; 54:3056-62.

81. Devaskar SU, deMello DE. Cell-specific localization of glucose transporter proteins in mammalian lung. *J Clin Endocrinol Metab* 1996; 81:4373-8.

82. Kurata T, Oguri T, Isobe T, Ishioka S, Yamakido M. Differential expression of facilitative glucose transporter (GLUT) genes in primary lung cancers and their liver metastases. *Jpn J Cancer Res* 1999; 90:1238-43.

83. Ito T, Noguchi Y, Satoh S, Hayashi H, Inayama Y, Kitamura H. Expression of facilitative glucose transporter isoforms in lung carcinomas: its relation to histologic type, differentiation grade, and tumor stage. *Mod Pathol* 1998; 11:437-43.

84. Dawson PA, Mychaleckyj JC, Fossey SC, Mihic SJ, Craddock AL, Bowden DW. Sequence and functional analysis of GLUT10: a glucose transporter in the Type 2 diabetes-linked region of chromosome 20q12-13.1. *Mol Genet Metab* 2001; 74:186-99.

85. Simmons RA, Flozak AS, Ogata ES. Glucose regulates glut 1 function and expression in fetal rat lung and muscle in vitro. *Endocrinology* 1993; 132:2312-8.

86. Mantych G, Devaskar U, deMello D, Devaskar S. GLUT 1-glucose transporter protein in adult and fetal mouse lung. *Biochem Biophys Res Commun* 1991; 180:367-73.

87. Zuo J, Huang Z, Zhi A, Zou S, Zhou X, Dai F, Ye H, Feng D. Cloning and Distribution of Facilitative Glucose Transporter 2 (SLC2A2) in Pigs: Asian-Autralasian Association of Animal Production Societies; 2010.