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**Title:** Putative tissue location and function of the SLC5 family member SGLT3

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**Running Title:** Developments in SGLT3 biology

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attention. The purpose of this overview and review of SGLT3 biology is to provide an update, highlight the gaps in our knowledge, and try to signpost potential ways forward to define its likely function in vivo.

**New Findings:** • What is the topic of this review? This review summarizes the evidence on sodium glucose transporter 3 (SGLT3) localization, electrophysiological properties, agonist specificity, and its putative physiological role. • What advances does it highlight? Published information is reviewed in some detail by comparing human and rodent isoforms, as well as advances in testing hypotheses for its physiological role as a glucose sensor or incretin release mediator. We provide a critical overview of available published data and discuss a putative functional role for SGLT3 in human and mouse physiology.

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## **Putative tissue location and function of the SLC5 family member SGLT3**

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Running title: Developments in SGLT3 biology

Key words: SGLT3, glucose, sodium, kidney, intestine

### New findings:

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## Abstract

Sodium glucose transporter 3 (SGLT3) has attracted interest because of its putative role as a glucose sensor, rather than a sugar transporter, in contrast to its SGLT1 and SGLT2 co-family members. Significant progress has been made in characterizing the electrophysiological properties *in vitro* of the single human SGLT3 isoform and the two mouse isoforms, SGLT3a and SGLT3b. Although early reports indicated SGLT3 expression in the small intestinal myenteric and submucosal neurones, hypothalamic neurones, portal vein, and kidney, a lack of reliable antibodies has left unanswered its exact tissue and cellular localization. Several hypotheses for a role of SGLT3 in glucose sensing, gastric emptying, GLP-1 release, and post-Roux en-Y gastric bypass remodelling have been explored, but so far there is only limited and indirect supportive evidence using non-specific agonists/antagonists, but with no firm conclusions. There are no published or available data in knockout animals and translation is difficult because of its different isoforms in human *versus* rodent, as well as a lack of selective agonists or antagonists, all making SGLT3 challenging to study. However, its unique electrophysiological properties, ubiquitous expression at the mRNA level, enrichment in the small intestine, and potential, but uncertain, physiological role demand more attention. The purpose of this overview and review of SGLT3 biology is to provide an update, highlight the gaps in our knowledge, and try to signpost potential ways forward to define its likely function *in vivo*.

## Introduction

The SLC5 family is exemplified by the SGLT1 isoform (*Slc5a1*) that is responsible for active glucose absorption in the intestine, but is also present in the kidney proximal tubule, and the SGLT2 isoform (*Slc5a2*) that is almost exclusive to the renal epithelium and is responsible for the bulk of glucose reabsorption by the kidney. SGLT2 has become topical recently, because it is an effective therapeutic target for lowering blood glucose levels in poorly controlled diabetes mellitus. Targeting this transport pathway in the kidney has been approved by some authorities as a second-line therapy in type 2 diabetes after metformin. However, the advances in SGLT1 and SGLT2 biology and function have been reviewed comprehensively elsewhere (Poulsen *et al.*, 2015; Song *et al.*, 2016; Lehmann & Hornby, 2016) and in the present review we focus on recent developments in our understanding of the distribution and function of another SLC5 family member and putative glucose sensor, SGLT3. Its function and topographical distribution have been less well studied and are still incompletely defined. We review its gene structure, electrophysiological and transport properties, differences between human and rodent isoforms, tissue and cellular distribution, and putative physiological role(s). Finally, we will emphasize the gaps in current knowledge and outline some future perspectives.

## SGLT3 (*Slc5a4*)

### Structure

Human *SLC5A4* gene is located on chromosome 22 at locus 22q12.3. The gene contains 17 exons; *SLC5A1* coding SGLT1 is close by on the same chromosome. In contrast, rat and mouse have 2 isoforms, *Slc5a4a* and *Slc5a4b*. In rodents both isoforms are in close proximity on the same chromosome, chromosome 10 and 20 in the mouse and rat, respectively. So far, mouse and rat are the only species known to have 2 isoforms of *Slc5a4*, making it more difficult to extrapolate findings from rodent models to human. Human SGLT3 cDNA was fully cloned by Diez-Sampedro *et al.* in 2003. In mouse the SGLT3b isoform was the first to be cloned (Tabatabai *et al.*, 2003), followed by SGLT3a, and then rat SGLT3a (Barcelona *et al.*, 2012). Rat SGLT3b has not been fully cloned and its sequence is predicted from the genomic sequence.

The identity of the amino acid sequence of SGLT3 is about 70% compared with SGLT1, and 55-60% compared with SGLT2 in each species (see **Table 1**). Interestingly, mouse and rat SGLT3a isoforms are closer (79-80%) to human SGLT3 than SGLT3b isoforms (76%). Basic phylogram analysis confirmed a closer relationship of mouse and rat SGLT3a isoforms to human SGLT3. Consistent with this, amino acid residues in the conserved ligand binding sites of rodent SGLT3a isoforms are more similar to

human SGLT3 (only residue F101 in the outer gate is different - Table 2), suggesting a distinct functional role of the two rodent isoforms. Indeed, mSGLT3b is able to partially transport glucose in a similar way to SGLT1. In contrast, mSGLT3a does not transport glucose or generate currents at pH 7.4 (Barcelona *et al.*, 2012). That these rodent isoforms may have distinct roles is also consistent with the high level of identity between the corresponding mouse and rat SGLT3a (93%), and SGLT3b (90%) isoforms, respectively, while identity between the SGLT3a and SGLT3b isoforms for mouse and rat is only about 75%. Similarly, conservation in the ligand bindings sites is very high between rat and mouse isoforms (**Table 2**).

Based on *in silico* predictions and the solved crystal structure of the *Vibrio parahaemolyticus* sodium/galactose symporter (vSGLT), SGLT proteins are composed of 14 transmembrane helices (Faham *et al.*, 2008). Actual structural differences between SGLT3 and other isoforms are not known, but the key amino acid in the sugar-binding site has been identified. In isoforms able to transport glucose (SGLT1 and 2), the residue within TM11 in the position 457 is glutamine. In contrast, SGLT3 isoforms that do not transport glucose have glutamate at this position (**Table 2**), except for the SGLT3b isoform of mouse and rat, which can partially transport glucose and has glycine or serine, respectively. Indeed, it was shown experimentally that in both hSGLT3 and mSGLT3a a change of glutamate to glutamine restored the ability of the protein to transport the glucose analogue  $\alpha$ -MDG in a  $\text{Na}^+$ -dependent manner, and resulted in similar apparent affinities for glucose analogues to hSGLT1. Similarly, mutated hSGLT1 with glutamate at the position where glutamine is normally present loses the coupling to  $\text{Na}^+$  and has a lower  $K_{0.5}$  than wildtype hSGLT1 (Díez-Sampedro *et al.*, 2001; Bianchi & Díez-Sampedro, 2010; Barcelona *et al.*, 2012). The glutamine at position 457 in SGLT1 is also important for binding of the competitive SGLT inhibitor phlorizin, since mutated hSGLT3 with glutamine instead of glutamate (E457Q-hSGLT3) exhibits 100x higher affinity for phlorizin (Bianchi & Díez-Sampedro, 2010). Interestingly, there is full conservation at the  $\text{Na}^+$  binding residues between SGLT1 and SGLT3 (Table 2). The difference in F453 in SGLT3a isoform in the outer gate, which closes after sugar binding and makes the sugar binding site inaccessible (Sala-Rabanal *et al.*, 2012), may be responsible for the inability of SGLT3a to transport sugars.

#### Tissue distribution and localization

In humans, mRNA expression screening revealed highest SGLT3 expression in the small intestine, followed by significant expression in skeletal muscle and testis. However, mRNA has also been detected at lower levels in many other tissues, including adrenal gland, bone marrow, heart, kidney, lung, prostate, spinal cord, stomach, thyroid gland, trachea, uterus, brain, and blood vessels

(Nishimura & Naito, 2005; Chen *et al.*, 2010). Expression has also been detected in several human colonic and ovarian cancers, and in various cancer cell lines (Veyhl *et al.*, 1998).

Human SGLT3 protein has been detected by western blotting and immunohistochemistry in small intestine, skeletal muscle, and kidney (Diez-Sampedro *et al.*, 2003). Immunofluorescence staining localised the protein to discrete patches in the submucosa of the small intestine, which co-localized with the nicotinic acetylcholine receptor, suggesting hSGLT3 expression is in cholinergic neurones of the submucosal and myenteric plexus. Moreover, similar co-localization was found at the neuromuscular junction of skeletal muscle (Diez-Sampedro *et al.*, 2003). SGLT3 protein has also been reported in human kidney homogenates and the HK-2 proximal tubule cell line (Kothinti *et al.*, 2012). Of note, there is a discrepancy between the sizes of the bands detected by these groups. Diez-Sampedro *et al.* (2003) detected the band at ~60kDa, which is smaller than the predicted protein size (72 kDa) that was detected by the other group in kidney (Kothinti *et al.*, 2012). The antibodies of both groups were made to order and raised against very similar peptides with 95% shared sequence homology. Nevertheless, both groups did provide appropriate controls using over-expressing cell line/oocytes, empty vectors, blocking peptides, and negative cross-reactivity with SGLT1.

In mouse and rat, mRNA expression of SGLT3a and SGLT3b seems to be broadly similar. Using reverse transcriptase PCR both isoforms have been found in mouse small intestine and kidney (Gribble *et al.*, 2003; Tabatabai *et al.*, 2003). Quantitative PCR revealed much higher mRNA expression of SGLT3a and SGLT3b in small intestine compared with kidney (~150,000- and 700-fold, respectively) (Barcelona *et al.*, 2012). In the hepatic portal area, which itself is not well defined and includes the portal vein, hepatic artery and common bile duct, the expression levels for both isoforms were similar to levels found in the kidney, with SGLT3a ~4 times higher than SGLT3b (Delaere *et al.*, 2013). The first attempt to profile SGLT3 mRNA expression in rat intestine was reported by Freeman *et al.* (2006). The authors detected transcripts in duodenum, jejunum, and colon, with relatively similar levels detected in each segment. However, the primers and probe sequence for SGLT3 were based on the human sequence for *SLC5A4*, and although it shared substantial similarities with rat, it cannot distinguish between the rodent SGLT3a and SGLT3b isoforms. Studies designed to differentiate between the two isoforms have detected expression in hypothalamus, kidney and duodenum in Sprague-Dawley rats (O'Malley *et al.*, 2006), while SGLT3b was also detected in jejunum and proximal ileum (Pal *et al.*, 2015). In addition, SGLT3b was detected in different parts of the jejunum after Roux-en-Y gastric bypass (RYGB) surgery in Sprague-Dawley and Zucker diabetic fatty (ZDF) rats (Bhutta *et al.*, 2014). In cell lines, expression was found in the intestinal endocrine GLUTag cell line (Gribble *et al.*, 2003) and in primary cultures of mouse cortical kidney cells (Tabatabai *et al.*, 2001). Overall, from published microarray datasets reporting expression across most tissues, it is clear that the highest

expression occurs in the small intestine for both isoforms. Low-level expression is detected in most other tissues with moderate expression of SGLT3b occurring in testes and ovary (GEO datasets: GDS3142, GDS3357, GDS4319).

In rodents there are limited data on SGLT3 at the protein level. It has been localized by western blot to kidney, liver, ileum, and portal vein area. However, the specificity of the antibody is uncertain, because the authors interpreted two bands of 71 and 72kDa as staining for both SGLT3a and SGLT3b isoforms, but did not describe how the antibody was generated (Delaere *et al.*, 2013). SGLT3 has also been detected by western blot in various brain structures, including cortex, hypothalamus, hippocampus, midbrain, striatum, medulla, olfactory bulb, and cerebellum (Yamazaki *et al.*, 2014). The antibody used was raised against the peptide for mouse SGLT3b and again the authors could not distinguish between the SGLT3a and SGLT3b isoforms. Using immunofluorescence, the authors detected SGLT3 in neurones, but not astrocytes of the striatum and cortex; detection was performed using another SGLT3 antibody, although details of its specificity were not provided. However, the staining strongly co-localized with choline acetyltransferase, which is consistent with the co-localization of hSGLT3 and the  $\beta$ -subunit of acetylcholine receptor reported in human skeletal muscle and intestine (Diez-Sampedro *et al.*, 2003), suggesting that cholinergic neurones are an important site of mSGLT3 protein localization. While, neuronal localization is likely in these tissues, wider neuronal localization has not been explored.

Taken together, the highest expression among tissues is convincingly reported at the mRNA level in different regions of the small intestine. Ubiquitous expression at low levels seems to be present in most tissues, with possibly more significant expression in ovaries, testis, and skeletal muscle. Though expression has been detected in kidney, its level seems to be very low and the localization of mRNA expression to specific cell types is lacking. Western blotting has revealed SGLT3 protein in small intestine, kidney, brain structures, liver, and portal vein area. Human SGLT3 protein has been localized to cholinergic neurones of the small intestine submucosal and myenteric nerve plexuses, and neuromuscular junction of skeletal muscle. In mouse, cholinergic neurones of brain cortex and striatum also show positive immunofluorescence staining. However, localization data at the protein level needs to be considered with some caution, since the antibodies used do not always show convincing specificity or distinguish between isoforms, and additional studies on cellular localization are required. The expression of all isoforms is summarized in **Table 3**.

#### Functional properties

Functional studies in *Xenopus laevis* oocytes expressing human SGLT3 showed that hSGLT3 does not transport glucose in the absence or presence of  $\text{Na}^+$  at pH 7.5 or 5 (Diez-Sampedro *et al.*, 2003);

however, D-glucose and  $\alpha$ -MDG were able to generate a phlorizin-sensitive,  $\text{Na}^+$ -dependent depolarization of oocyte cell membrane potential, while D-galactose, D-fructose, and mannitol had no effect. The glucose-induced depolarization shows saturation with a  $K_{0.5}$  of 20 mM and a maximum depolarization of 23 mV, increasing as resting membrane potential is lowered (Diez-Sampedro *et al.*, 2003). No glucose/ $\alpha$ -MDG-induced currents were observed in the absence of  $\text{Na}^+$  at pH 7.4, although glucose/ $\alpha$ -MDG-induced currents are substantially greater at lower pH (pH 5) when the charge carrier is likely to be  $\text{H}^+$ . In addition, at low pH, glucose/ $\alpha$ -MDG is able to induce currents even in  $\text{Na}^+$ -free media. This is supported by the observation that there is no increase in  $\text{Na}^+$  uptake at pH 5, but there is significant intracellular acidification, consistent with  $\text{H}^+$  as the charge carrier at low pH. Interestingly, the imino sugars, 1-deoxynojirimycin (DNJ), N-hydroxyethyl-1-deoxynojirimycin (miglitol), and N-butyl-1-deoxynojirimycin (miglustat), which are all potent inhibitors of  $\alpha$ -glucosidase enzymes of the intestinal brush border membrane, are potent agonists at hSGLT3 with a  $K_{0.5}$  of 0.5 - 5  $\mu\text{M}$ , which is up to a 40,000 times greater affinity than for glucose (Voss *et al.*, 2007). However, imino sugars are not of mammalian origin and are not expected to be the natural agonists. Nonetheless, it provides a pharmacological tool to study a functional role of SGLT3, although their higher affinities for  $\alpha$ -glucosidases prevent their use as specific agonists, at least in the small intestine.

In rodents, the two isoforms of SGLT3 exhibit different electrophysiological properties, as well as different responses to glucose and glucose analogue-induced stimulation. At pH 7.4 in the presence of  $\text{Na}^+$ , mSGLT3a expressed in oocytes does not depolarize the cell membrane when exposed to D-glucose,  $\alpha$ -MDG, or DNJ, unlike with hSGLT3. In contrast, at pH 7.4 glucose,  $\alpha$ -MDG, 1-deoxyglucose, and 6-deoxyglucose induce a strong depolarization in mSGLT3b-expressing oocytes (Aljure & Díez-Sampedro, 2010; Barcelona *et al.*, 2012). Like mSGLT3a, mSGLT3b is also not activated by DNJ, but unlike hSGLT3 and mSGLT3a, mSGLT3b is able to transport sugar to a small extent, although the uptake of the glucose analogue  $\alpha$ -MDG is about 60 times less than uptake by mSGLT1 (Aljure & Díez-Sampedro, 2010). In addition, mSGLT3b shows only a small pH-dependency; glucose-induced currents are present at neutral and acidic pH (Barcelona *et al.*, 2012). Rat SGLT3a exhibits similar functional properties to mSGLT3a. Oocytes expressing rSGLT3a exposed to glucose showed minimal currents at pH 7.4, but inward currents are much larger at lower pH, and in the presence of glucose (Barcelona *et al.*, 2012). These characteristics of mSGLT3a, and mSGLT3b in oocytes have been confirmed in mammalian CHO (Chinese Hamster Ovary) cells expressing these isoforms (Barcelona *et al.*, 2012). It seems that in rodents the properties of the SGLT3 isoforms can be separated according to external pH; however, the significance of this *in vivo* is unknown.

Overall there are substantial differences between human and rodent SGLT3 electrophysiological characteristics that are summarized in **Table 4**. While hSGLT3 does not transport glucose, it can

induce a small depolarization at pH 7 and it is pH-sensitive. In contrast, mouse and rat SGLT3a do not respond to glucose at pH 7, but at low pH can generate currents carried most probably by H<sup>+</sup>, and these glucose-induced currents are larger than those for H<sup>+</sup> alone. In contrast, mSGLT3b can transport glucose and generates currents in response to glucose, rather than H<sup>+</sup>.

### Physiological role

Although the electrophysiological properties of human and mouse SGLT3 have been well described, their physiological function is unknown. The inability of SGLT3 protein to transport glucose (or its analogues) and its ability to generate membrane currents in the presence of glucose and Na<sup>+</sup> or H<sup>+</sup> have led to the hypothesis that SGLT3 might act as a glucose sensor. Another hypothesis is that SGLT3 controls gastric emptying, which may be related to the pH-dependency of SGLT3's membrane current generation (see earlier): chyme released from the stomach will progressively lower duodenal luminal pH (Miller *et al.*, 1978) causing SGLT3 to generate larger currents at this lower pH. Intraduodenal perfusion of glucose and its analogues 3-O-methylglucose (3-OMG), and  $\alpha$ -methyl-D-glucose ( $\alpha$ -MDG) can inhibit gastric emptying in conscious rats, while 2-deoxyglucose (2-DG, a substrate for facilitative glucose transporters) cannot (Freeman *et al.*, 2006). In addition, intraduodenal perfusion with glucose inhibits gastric motility in anesthetised rats, while galactose has the opposite effect. The authors (Freeman *et al.*, 2006) used the difference in glucose and galactose affinity for SGLT1 and SGLT3 - both glucose and galactose are substrates of SGLT1, whereas galactose has negligible affinity for SGLT3 (Díez-Sampedro *et al.*, 2000; Voss *et al.*, 2007) - to conclude that SGLT3 delays gastric emptying. However, the low affinity of galactose for hSGLT3 was seen at pH 7.4, whereas galactose's ability to induce currents via mSGLT3a at pH 5 is similar to glucose and other glucose analogues (Barcelona *et al.*, 2012). While the same authors consider 3-OMG and  $\alpha$ -MDG as substrates for both SGLT1 and SGLT3, others have reported data showing 3-OMG is not a substrate for pig SGLT3 (Díez-Sampedro *et al.*, 2000) and interpret the difference between 3-OMG and  $\alpha$ -MDG to distinguish the effects between SGLT1 and SGLT3 in rat (Delaere *et al.*, 2013; Pal *et al.*, 2015). Thus, the interpretation of SGLT3 as a mediator of gastric emptying by Freeman *et al.* (2006) seems less certain.

The role of SGLT3 as a glucose sensor has also been proposed and tested in the hepatic portal vein of the rat (Delaere *et al.*, 2013). Detection of glucose in hepatic portal vein after protein-enriched, diet-induced intestinal gluconeogenesis has been shown to decrease food intake (Mithieux *et al.*, 2005). Delaere and colleagues (2013) excluded GLUT2 and taste receptors as mediators of the sensing mechanism and proposed SGLT3 as the sensing mediator. They showed that glucose or  $\alpha$ MDG infusion reduced food intake, while 3-OMG did not. The effect of glucose on food intake was

abolished by phlorizin and following exposure of the portal vein to capsaicin to denervate sensory nerves. The effect of glucose was not changed after ventral vagotomy. SGLT3 mRNA and protein were detectable in the portal vein area; however, the reported mRNA levels were similar to those found in kidney, which are much lower than in intestine (unpublished observations). Since the presence of SGLT3 can be detected, and sensory afferents are involved, and because 3-OMG is not a substrate for SGLT3, at least in pig, these authors concluded that SGLT3 is the portal glucose sensor.

The main glucose sensing mechanism in the body that leads to insulin secretion depends on closure of  $K_{ATP}$  channels in pancreatic beta cells. A similar mechanism has been proposed for glucose-stimulation of neurones. However, only a small proportion (9%) of cultured hypothalamic glucose-stimulated neurones show any response to the  $K^+$  channel inhibitor tolbutamide (O'Malley *et al.*, 2006), suggesting that other mechanisms exist. The majority of glucose-excitabile cultured hypothalamic neurones show a rise in cytosolic  $Ca^{2+}$  in response to the  $\alpha$ MDG, a substrate of the SGLTs, but not the GLUTs; an effect blocked by phlorizin (O'Malley *et al.*, 2006). A higher proportion of neurones are activated by  $\alpha$ MDG (67%) than by 3-OMG (45%). This difference may be accounted for by SGLT3 and is supported by the finding of SGLT3a and SGLT3b expression in rat hypothalamus and cultured hypothalamic neurones (O'Malley *et al.*, 2006). Moreover, hSGLT3 expressed in sensory neurons of *C. elegans* is able to mediate preferential glucose-induced chemotaxis in low pH and this effect is blocked by phlorizin (Bianchi & Díez-Sampedro, 2010). In addition, glucose and DNJ, an hSGLT3 agonist, activate rat intestinal enterochromaffin cells and myenteric neurons (Vincent *et al.*, 2011).

#### Roux-en-Y gastric bypass surgery (RYGB)

Roux-en-Y gastric bypass surgery is a highly effective treatment for severe obesity, especially in diabetic patients. Isolation of the proximal small intestine and its luminal glucose sensing capacity from nutrients, and the presence of non-digested luminal nutrients in more distal parts of the small intestine, is thought to be crucial in the weight-reducing and anti-diabetic effects of surgery; although the mechanisms have not been defined. SGLT3b mRNA is expressed differentially along the small intestine after RYGB surgery in a rat model (Bhutta *et al.*, 2014). Expression was significantly higher in the *common limb* (distal to the Y-intersection) of both Sprague-Dawley and diabetic ZDF rats compared with the *biliopancreatic* (bypassed portion of stomach and duodenum) and *Roux* (anastomosis of fundal gastric remnant with jejunum) limbs. In contrast, no differential mRNA expression was detected for SGLT1, GLUT2, and taste receptor T1R2. Furthermore, saccharin, which stimulates taste receptors T1R2/3, infused into the biliopancreatic limb down-regulated expression of SGLT3b in the common limb of ZDF rats, though not significantly in Sprague-Dawley rats (Bhutta *et*

*et al.*, 2014). The data suggest a possible role for SGLT3 in the post-RYGB intestinal changes. The involvement of SGLT3-mediated intraluminal glucose sensing in proximal small intestine has also been proposed in a foregut exclusion model resembling RYGB (Pal *et al.*, 2015). Here the proximal duodeno-jejunal part of the intestine was isolated so that different glucose analogues could be administered to the excluded segment. At the same time, a glucose bolus was administered to the jejunum so that the effect on absorption could be measured.  $\alpha$ MDG administration caused significantly higher glucose absorption than 3-OMG or saccharin, and this effect was blocked by the SGLT1/SGLT3 inhibitor phlorizin. The authors conclude that SGLT3, and not SGLT1 or taste receptors, mediates the higher glucose absorption in the RYGB model. Taken together it seems that SGLT3 may have a role in the beneficial outcomes of RYGB, although more direct evidence is required.

#### GLP-1 secretion

SGLT3 has been thought to play a role in the secretion of the incretin hormone glucagon-like peptide-1 (GLP-1). In the foregut exclusion model, GLP-1 secretion (measured as the difference between portal and systemic concentration) was significantly higher after treatment with the SGLT1/3 agonist  $\alpha$ MDG, while no difference was detected after treatment with the SGLT1-only agonist 3-OMG (Pal *et al.*, 2015), suggesting SGLT3 might mediate GLP-1 secretion. The effect of  $\alpha$ MDG was blocked by phlorizin and prior vagotomy, supporting SGLT participation and indicating involvement of vagal innervation.

An indirect role for SGLT3 in mediating GLP-1 secretion was proposed recently by Lee *et al.* (2015). To stimulate SGLT3, the authors used miglitol, an iminosugar that is used as an  $\alpha$ -glucosidase inhibitor and it is a potent agonist of human SGLT3 (Voss *et al.*, 2007; Lee *et al.*, 2015). Miglitol was able to activate duodenal enteroendocrine cells more effectively than acarbose, another  $\alpha$ -glucosidase inhibitor. However, miglitol administration alone failed to increase GLP-1 secretion enough to alter plasma concentrations and was only effective when co-administered with maltose. Since no GLP-1 stimulation was seen after giving acarbose with maltose, it was concluded that miglitol may have a direct effect on SGLT3 to stimulate GLP-1 secretion (Lee *et al.*, 2015).

#### Some controversies and limitations

Since there is still a lack of suitable tool compounds for studying SGLT3 function directly, so far all reports on its likely function *in vivo* are based on indirect evidence. Several authors (O'Malley *et al.*, 2006; Delaere *et al.*, 2013; Pal *et al.*, 2015) argue for discrimination between SGLT1 and SGLT3 on the basis of differing affinities for  $\alpha$ MDG and 3-OMG:  $\alpha$ MDG is a substrate for both SGLT1 and SGLT3,

whereas 3-OMG is a substrate for SGLT1 only. However, this selectivity is based only on data from pig SGLT3 (Diez-Sampedro *et al.*, 2003) and we do not know if it extends to other species. Similarly, the putative SGLT3 agonist miglitol was shown to activate human SGLT3 (Voss *et al.*, 2007; Lee *et al.*, 2015), although evidence for this in rodents is lacking, despite its use in rats (Lee *et al.*, 2015). Moreover, some authors have not tried or been able to distinguish between the SGLT3a and SGLT3b rodent isoforms in their studies, particularly when using antibodies where its isoform specificity is unclear, or they have focused on only one isoform (SGLT3b).

#### Future perspectives

A glucose sensing mechanism for SGLT3 has been proposed from *in vitro* models and based on SGLT3's ability to generate membrane currents in the presence of glucose, Na<sup>+</sup> and/or H<sup>+</sup>. However, direct evidence for glucose sensing *in vivo* is still lacking. Neuronal protein expression has been shown in human submucosal/myenteric tissue, and mRNA expression has been detected in scrapped mucosa, and by microarray gene expression analysis in enterocytes and enteroendocrine cells. Tissue localization of rodent isoforms remains controversial at the protein level, because of antibody specificity, and poorly defined mRNA expression. A more definitive examination of SGLT3's organ and tissue distribution is still needed, as well as better selective agonists and antagonists, or its function will remain intriguing, but increasingly speculative. Knockout mice for both rodent isoforms have been reported from one source to have no obvious phenotype, but they have not been investigated in any detail and are not widely available for study. Emerging technologies such as CRISPR make it possible to produce isoform-specific knockout mice or even rats, which in combination with imino sugars and specific SGLT inhibitors, should enable us to explore and define better the physiological and pathophysiological function of SGLT3, at least in rodents.

**Table 1.** Protein sequence identity matrix of human and rodent sodium glucose transporter (SGLT) isoforms 1, 2, and 3.

	hSGLT3	mSGLT3a	rSGLT3a	mSGLT3b	rSGLT3b	hSGLT1	mSGLT1	rSGLT1	hSGLT2	mSGLT2	rSGLT2
hSGLT3	100.0	79.2	80.3	76.5	76.8	69.6	71.6	71.9	56.5	56.4	55.3
mSGLT3a	79.2	100.0	92.8	75.3	75.0	69.0	70.1	69.7	56.4	56.2	56.3
rSGLT3a	80.3	92.8	100.0	76.4	76.7	69.8	71.5	71.5	56.4	56.1	56.4
mSGLT3b	76.5	75.3	76.4	100.0	90.2	70.4	71.8	71.5	58.4	59.5	57.9
rSGLT3b	76.8	75.0	76.7	90.2	100.0	71.3	72.4	72.3	58.4	58.6	57.3
hSGLT1	69.6	69.0	69.8	70.4	71.3	100.0	88.0	87.8	58.9	60.9	58.9
mSGLT1	71.6	70.1	71.5	71.8	72.4	88.0	100.0	95.8	59.0	60.2	59.5
rSGLT1	71.9	69.7	71.5	71.5	72.3	87.8	95.8	100.0	59.3	60.6	59.8
hSGLT2	56.5	56.4	56.4	58.4	58.4	58.9	59.0	59.3	100.0	91.0	91.2
mSGLT2	56.4	56.2	56.1	59.5	58.6	60.9	60.2	60.6	91.0	100.0	90.1
rSGLT2	55.3	56.3	56.4	57.9	57.3	58.9	59.5	59.8	91.2	90.1	100.0

**Table 2.** A comparison of corresponding amino acid residues in putative ligand binding sites and gates.

	Sugar binding								Na <sup>+</sup> binding					Outer gates			Inner gates
	H83	E102	A105	K321	T287	W291	Q457	N78	A76	I79	S389	S392	S393	L87	F101	F453	Y290
hSGLT3	H	E	<b>S</b>	K	A	W	<b>E</b>	N	A	I	S	S	S	L	F	<b>I</b>	Y
mSGLT3a	H	E	<b>S</b>	K	A	W	<b>E</b>	N	A	I	S	S	S	L	F	<b>V</b>	Y
rSGLT3a	H	E	<b>S</b>	K	A	W	<b>E</b>	N	A	I	S	S	S	L	F	<b>I</b>	Y
mSGLT3b	H	E	A	K	A	W	<b>G</b>	N	A	I	S	S	S	L	<b>V</b>	F	Y
rSGLT3b	H	E	A	K	A	W	<b>S</b>	N	A	I	S	S	S	L	<b>V</b>	F	Y
hSGLT1	H	E	A	K	T	W	Q	N	A	I	S	S	S	L	F	F	Y
mSGLT1	H	E	A	K	A	W	Q	N	A	I	S	S	S	L	F	F	Y
rSGLT1	H	E	A	K	A	W	Q	N	A	I	S	S	S	L	F	F	Y
hSGLT2	H	E	A	K	S	W	Q	N	A	I	A	S	S	L	F	F	Y
mSGLT2	H	E	A	K	S	W	Q	N	A	I	A	S	S	L	F	F	Y
rSGLT2	H	E	A	K	S	W	Q	N	A	I	A	S	S	L	F	F	Y
conservation	*	*	:	*	:	*		*	*	*	:	*	*	*	.	.	*

The conserved ligand binding sites and gates were adopted from alignment of hSGLT1 to vSGLT (Sala-Rabanal *et al.*, 2012). The residue numbering is based on hSGLT1 and corresponding residues were obtained by multiple sequence alignment CLUSTAL O (1.2.3). \*, fully conserved residue; :, conservation strongly similar - scoring > 0.5 in the Gonnet PAM 250 matrix. . (period), conservation weakly similar - scoring =< 0.5 in the Gonnet PAM 250 matrix. Residues in SGLT3 different from SGLT1 are in bold.

Table 3. Tissue distribution of different isoforms expression.

	mRNA		protein	
	RT-PCR	qPCR	WB	IHC
human SGLT3	small intestine <sup>1</sup> , colon <sup>2</sup> , skeletal muscle <sup>1</sup> , kidney cell line <sup>2,3</sup>	small intestine <sup>4,5</sup> , kidney <sup>4,5</sup> , adipose <sup>5</sup> , adrenal <sup>4</sup> , blood vessel <sup>5</sup> , bone <sup>4</sup> , brain <sup>5</sup> , heart <sup>4,5</sup> , lung <sup>4</sup> , ovary <sup>5</sup> , prostate <sup>4</sup> , skeletal muscle <sup>5</sup> , spleen <sup>5</sup> , stomach <sup>4,5</sup> , testis <sup>4,5</sup> , thyroid <sup>4</sup> , trachea <sup>4</sup>	small intestine <sup>1</sup> , kidney <sup>3</sup> , kidney cell line <sup>3</sup> , skeletal muscle <sup>1</sup>	small intestine <sup>1</sup> , skeletal muscle <sup>1</sup>
mouse SGLT3a	small intestine <sup>6,7</sup> , intestinal cell line <sup>6,7</sup> , kidney <sup>8</sup>	small intestine <sup>9</sup> , kidney <sup>9,10</sup> , portal area <sup>10</sup>	small intestine <sup>10</sup> , kidney <sup>10</sup> , liver <sup>10</sup> , portal area <sup>10</sup>	
mouse SGLT3b	small intestine <sup>7</sup> , kidney <sup>8</sup> , kidney cell line <sup>11</sup>	small intestine <sup>9</sup> , kidney <sup>9,10</sup> , portal area <sup>10</sup>	small intestine <sup>10</sup> , kidney <sup>10</sup> , brain <sup>12</sup> , liver <sup>10</sup> , portal area <sup>10</sup>	brain <sup>12</sup>
rat SGLT3a	small intestine <sup>13</sup> , kidney <sup>9</sup> , brain <sup>13</sup>	small intestine <sup>14</sup> , colon <sup>14</sup>		
rat SGLT3b	small intestine <sup>13</sup> , brain <sup>13</sup>	small intestine <sup>15,16</sup>		

<sup>1</sup>Diez-Sampedro et al., 2003; <sup>2</sup>Veyhl et al., 1998; <sup>3</sup>Kothinti et al., 2012; <sup>4</sup>Nishimura et al., 2005; <sup>5</sup>Chen et al., 2010; <sup>6</sup>Gribble et al., 2003; <sup>7</sup>Lee et al., 2015; <sup>8</sup>Tabatabai et al., 2003; <sup>9</sup>Barcelona et al., 2012; <sup>10</sup>Delaere et al., 2012; <sup>11</sup>Tabatabai et al., 2001; <sup>12</sup>Yamazaki et al., 2014; <sup>13</sup>O'Malley et al., 2006; <sup>14</sup>Freeman et al., 2006; <sup>15</sup>Bhutta et al., 2014; <sup>16</sup>Pal et al., 2015.

**Table 4.** Table of sodium glucose transporter (SGLT) isoform 3 characteristics and differences

	hSGLT3	r/mSGLT3a	mSGLT3b
Glucose uptake	No	No	Small
pH sensitivity	Yes	Yes	No
Phlorizin sensitivity	Yes	No	Yes
Glucose/ $\alpha$ -MDG-induced currents at pH 7.4	Yes, small	No	Yes, large
Glucose/ $\alpha$ -MDG-induced currents at pH 5	Yes, large	Yes, large	Yes, small
H <sup>+</sup> -induced currents	Yes, medium	Yes, large	No
Na <sup>+</sup> requirement	Yes, partial	No	Yes, partial
DNJ-induced currents at pH 7.4	Yes	No	No
DNJ-induced currents at pH 5	Yes, large	Yes	n.d.

$\alpha$ -MDG,  $\alpha$ -methyl-D-glucose; DNJ, 1-deoxynojirimycin; n.d., not determined, data are not available. Sources: Diez-Sampedro *et al.*, 2003; Voss *et al.*, 2007; Aljure & Díez-Sampedro, 2010; Barcelona *et al.*, 2012.

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No conflict of interest.

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### Author contributions

MS and RJU designed, conceived and drafted manuscript. MS performed sequence alignment analyses. MS, JM, and RJU revised manuscript critically for important intellectual content, approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.