Na(+)–D-glucose cotransporter SGLT1 is pivotal for intestinal glucose absorption and glucose-dependent incretin secretion.

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To clarify the physiological role of Na\(^{+}\)-D-glucose cotransporter SGLT1 in small intestine and kidney, Sglt1\(^{-/-}\) mice were generated and characterized phenotypically. After gavage of D-glucose, small intestinal glucose absorption across the brush-border membrane (BBM) via SGLT1 and GLUT2 were analyzed. Glucose-induced secretion of insulinotropic hormone (GIP) and glucagon-like peptide 1 (GLP-1) in wild-type and Sglt1\(^{-/-}\) mice were compared. The impact of SGLT1 on renal glucose handling was investigated by micropuncture studies. It was observed that Sglt1\(^{-/-}\) mice developed a glucose-galactose malabsorption syndrome but thrive normally when fed a glucose-galactose–free diet. In wild-type mice, passage of D-glucose across the intestinal BBM was predominantly mediated by SGLT1, independent the glucose load. High glucose concentrations increased the amounts of SGLT1 and GLUT2 in the BBM, and SGLT1 was required for upregulation of GLUT2. SGLT1 was located in luminal membranes of cells immunopositive for GIP and GLP-1, and Sglt1\(^{-/-}\) mice exhibited reduced glucose-triggered GIP and GLP-1 levels. In the kidney, SGLT1 reabsorbed \(~3\%\) of the filtered glucose under normoglycemic conditions. The data indicate that SGLT1 is 1) pivotal for intestinal mass absorption of D-glucose, 2) triggers the glucose-induced secretion of GIP and GLP-1, and 3) triggers the upregulation of GLUT2.

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Obesity, type 2 diabetes, and the metabolic syndrome are some of the major medical and economical challenges of modern societies. Dysregulation of carbohydrate management, increased consumption of carbohydrates and fat, and reduced insulin receptor sensitivity contribute to the metabolic derangements. Drugs that reduce small intestinal uptake of D-glucose and/or reabsorption of D-glucose from the renal filtrate or that modulate secretion of insulinotropic enterohormones can provide new therapeutic strategies (1,2). To further explore these options a better understanding of the underlying molecular mechanisms is required.

The primary transporters that mediate transcellular movements of D-glucose in small intestine have been identified and include the Na\(^{+}\)-D-glucose cotransporter SGLT1 and GLUT2 (3,4). It is generally accepted that SGLT1 mediates uptake of low concentrations of D-glucose across the brush-border membrane (BBM) of the small intestine and that D-glucose leaves enterocytes across the basolateral membrane (BLM) via GLUT2 (3). However, their relative contributions to D-glucose absorption after a carbohydrate-rich meal remain controversial (5–7). Kellet and coworkers suggested that under these conditions GLUT2 is incorporated into the BBM allowing mass absorption of D-glucose via GLUT2 (5,6,8). However, the observation that small intestinal mass absorption of D-glucose in mice was not significantly changed when GLUT2 was removed (9) contradicts this hypothesis.

Among other dietary stimulants, D-glucose can trigger the intestinal secretion of glucose-dependent insulinotropic peptide (GIP) by K-cells as well as the secretion of glucagon-like peptide 1 (GLP-1) by L-cells (10–12). Potential glucose sensing systems expressed in these enteroendocrine cells include sweet taste receptors and glucose transporters like SGLT1, but their roles in glucose-induced secretion of GLP-1 and GIP have not been fully established (7,13–16).

There is agreement that the bulk of D-glucose filtered in the glomeruli of the kidney is reabsorbed in the S1 and S2 segments of proximal tubules via SGLT2 in the BBM and GLUT2 in the BLM (17), and it is generally assumed that the remaining glucose is reabsorbed in the S2 and S3 segments via SGLT1 in BBM and GLUT1 in the BLM. However, the physiological significance and quantitative contribution of SGLT1 for renal reabsorption of D-glucose has not been directly determined (17).

In the present work we generated and characterized the phenotype of an Sglt1\(^{-/-}\) mouse model to gain new insights. We report that these mice show symptoms of the glucose-galactose malabsorption (GGM) syndrome (OMIM 182380) that appears to be cured by a diet low in glucose and galactose (18,19). The experiments identify SGLT1 as the primary pathway for the transport of D-glucose across the BBM during D-glucose mass absorption and show that...
SGLT1 is essential for the glucose-induced release of GIP and GLP-1 into the peripheral circulation. Finally, we establish a small but significant contribution of SGLT1 to renal D-glucose reabsorption under normoglycemic conditions.

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RESEARCH DESIGN AND METHODS

Animal handling. Mice were handled in compliance with Institutional guidelines and German, U.K., and U.S. laws. Comparison between Sglt1−/− and wild-type mice was performed between the 8th and 12th generation of backcrossing of Sglt1−/− (129/OlaC57Bl/6 background) with wild-type (C57Bl/6 background). Animals were kept in a temperature-controlled environment with a 12-h-light/12-h-dark cycle.

Diets. Standard maintenance Chow (Sniff V1534–000 R/M-H, 10 mm) was obtained from Spezialdiäten GmbH, Soest, Germany. It contained 12.8 MJ kg−1 metabolizable energy and was composed of 36.4% starch, 19% protein, 4.9% fiber, 4.7% mono- and disaccharides, and 3.8% fat, minerals, and vitamins. The glucose-galactose fiber-free diet was prepared by Altromin Spezialfutter GmbH, Lage, Germany and contained 13.2 MJ kg−1 metabolizable energy. It was composed of 33.8% protein, 30.7% fiber, and 20.5% fat, minerals, and vitamins.

Antibodies. The antibodies used are described in Table 1.

Immunohistochemistry. Immunofluorescence histochemistry was performed with cryo-sections of p-formaldehyde-fixed tissue as described (22). For double staining sections were first incubated with GIP-Ab or GLP-1 Ab and respective secondary antibody and washed. Thereafter sections were incubated overnight at 4°C with SGLT1-Ab, rinsed with PBS, incubated with GAR-AF555 for 60 min at room temperature, and washed. Laser scanning fluorescence microscopy was performed with a confocal laser-scanning imaging system, LSM-510 using the argon laser for green fluorochromes (λex = 488 nm, LP505 nm), and the helium-neon laser for red fluorochromes (λex = 543 nm, BP560–615 nm) (Zeiss, Jena, Germany). The Zeiss LSM-510 software 2.5 SP2 stack, multi track, 8 bit scan mode was used.

Gavage with glucose and oil. After a 16- to 18-h fasting period mice were gavaged with a 10% d-glucose solution (2 mg/g body wt) in PBS, a 40% d-glucose solution (6 mg/g body wt) in PBS, or with olive oil (10 mL/g body wt). Control animals were gavaged with PBS.

Measurements of glucose, insulin, and enterohormones in the plasma. For determination of d-glucose and insulin blood was immediately mixed with a dipeptidyl peptidase (DPP)-IV inhibitor yielding a glucose determination blood was immediately mixed with a dipeptidyl peptidase (DPP)-IV inhibitor yielding a glucose determination. Concentrations of D-glucose were determined with an Ascensia CONTOUR Meter using Ascensia MICROFIL Test Strips (Bayer Vial GmbH, Leverkusen).

Measurements of glucose, insulin, and enterohormones in the intestine. Glucose uptake into everted rings. Glucose uptake into intestinal BBMVs. Glomerular filtration rate (GFR) and renal glucose reabsorption. Oocytes. GIP characterization. Mice were killed 30 min after gavage. BBM vesicles (BBMVs) were isolated by magnesium precipitation and the resulting pellet was suspended in 35 mL vesicle buffer (100 mM/l Mannitol, 200 mM/l HEPES, Tris [pH 7.4]). After 30 min centrifugation at 27,000 g, the pellet was suspended in 300 µL vesicle buffer. The BBMVs were snap-frozen in liquid nitrogen and stored at −70°C until use.

Glucose uptake into BBMVs. BBMVs were thawed in a water bath, and uptake of radiolabeled substrates was determined at 22°C using the rapid filtration technique (23). To measure sodium-dependent and phlorizin-inhibitable uptake of 1-β-thiogluco-side (AMG), vesicles were incubated with vesicle buffer containing 0.1 mM [14C]AMG plus either 100 mM/l NaSCN or 100 mM/l NaSCN and 0.2 mM/l L-phlorizin or 100 mM/l KScn. Uptake was stopped with ice-cold vesicle buffer containing 100 mM/l NaSCN plus 0.2 mM/l L-phlorizin (stop solution), and vesicles were washed on nitrocellulose filters with stop solution. The radioactivity on the filters was measured and phlorizin-inhibitable or sodium-dependent AMG uptake was calculated.

Glucose uptake into everted rings. Glucose uptake into everted rings of duodenum and jejunum were incubated for 2 min in Krebs-Ringer buffer containing 7 µmol/L [14C] AMG and with or without 0.2 mM/l L-phlorizin. Uptake was stopped with ice-cold Ringer buffer containing 0.2 mM/l L-phlorizin. The length of individual segments was measured under a microscope, the segments were solubilized, and radioactivity was analyzed.

Glucose uptake by SGLT1 expressed in oocytes. SGLT1 was expressed in oocytes of Xenopus laevis, and measurements of AMG uptake were performed as described (25). The cDNA of mouse Sglt1 (accession number BC003845) was cloned into the vector pFSpSS, and mgI(5′)G-capped cRNA was prepared. Oocytes were injected with 5 ng of mouse Sglt1 cRNA and incubated 2 days for expression. For transport measurements the oocytes were incubated for 20 min at room temperature with [14C]AMG or [3H]glucose in the absence and presence of 100 µmol/L L-phlorizin, and phlorizin-inhibited uptake was analyzed.

Renal glucose reabsorption. Glomerular filtration rate (GFR) and renal glucose reabsorption were determined in awake and anesthetized mice as described previously (20,26,27).

TABLE 1 Description of antibodies

| Name   | Antigen                  | Host     | Source (Cat. No.) | Properties (use)          |
|--------|--------------------------|----------|-------------------|---------------------------|
| SGLT1-Ab1 | mouse SGLT1, aa 586 to 601 (KDYTIDTEAPQKKKK) | Rabbit   | raised for this study | see article (pA, WB, IH) |
| SGLT2-Ab1 | rat SGLT1, aa 592–609 (AMGIEEVQPSPAGDRRC) | Rabbit   | raised previously (20) | cross-reacts with mouse SGLT2 (pA, WB, IH) |
| GLUT2-Ab | peptide of human GLUT2 (pep C-19, from COOH terminus) | Goat     | cross-reacts with mouse GLUT2 (sc-7580) | (pA, WB, IH) |
| GIP-Ab  | human GIP (pep T-20, from internal region) | Goat     | cross-reacts with mouse GIP GLUT2 (sc-23554) | (pA for IH) |
| GLP-1-Ab | human GLP-1 (pep C-17, from COOH terminus) | Goat     | cross-reacts with mouse GLP-1 (sc-7728) | (pA, IH) |
| GAR-AF555 | rabbit IgG | Goat     | Invitrogen GmbH (A21428) | Alexa Fluor 555-labeled (SA, IH) |
| CAG-488F | goat IgG | Chicken  | Invitrogen GmbH (A21467) | Alexa Fluor 488-labeled (SA, IH) |
| DAR-HRP | rabbit IgG | Donkey   | Dianova (711–035–152) | Horseradish peroxidase-labeled (SA, WB) |
| DAG-HRP | goat IgG | Donkey   | Dianova (705–035–147) | Horseradish peroxidase-labeled (SA for WB) |

aa, Amino acids; WB, Western blot; IH, immunohistochemistry; pA, primary antibody; pep, peptide; sA, secondary antibody. Affinity-purified via the respective antigenic peptide as described (20).
RESULTS

Removal of SGLT1 leads to GGM syndrome. The strategy to generate mice lacking part of the Sglt1 promoter and the first exon of Sglt1 is outlined in Supplementary Fig. 1A. Sglt1+/− mice and Sglt1−/− mice were identified by Southern blots and PCR (Supplementary Fig. 1B and C). To verify effects on glucose transport, we expressed murine Sglt1 or murine Sglt1 mutant without exon 1 in Xenopus laevis oocytes and measured phlorizin (100 μmol/L)-inhibitable uptake of 50 μmol/L AMG, a SGLT-specific substrate that is not transported by GLUT transporters. Whereas a high transport activity was observed after expression of SGLT1 wild type, no significant AMG uptake was observed with the SGLT1 mutant (Supplementary Fig. 2). Breeding of Sglt1 heterozygote mice demonstrated Mendelian type inheritance (from 531 outcomes with values obtained from Western blots were increased 2.4-fold (Fig. 2C). The corresponding amount of SGLT1 protein in BBMVs estimated from Western blots was increased 2.4-fold (Fig. 2D). In BBMVs of Sglt1−/− mice no uptake of the SGLT-specific substrate AMG was detectable without and with d-glucose gavage (Fig. 2C). The data indicate upregulation of SGLT1 expression in the BBM after gavage with d-glucose in mice.

Maximal transport activity (V_max) of GLUT2 across the BBM was determined by measuring the uptake of 100 nmol/L d-glucose (partially [3H]-labeled) that could be blocked by the GLUT2 specific inhibitor glucosamine (100 mmol/L) (24). In oocytes expressing mouse SGLT1 we verified that glucosamine does not inhibit SGLT1 (Supplementary Fig. 4). V_max of GLUT2-mediated d-glucose uptake into BBMVs in wild-type mice without glucose gavage was similar to Sglt1+/− mice without or with glucose gavage (43–70 pmol × mg−1 × s−1; Fig. 2E). Gavage of d-glucose increased V_max of GLUT2-mediated d-glucose uptake into BBMVs in wild-type mice without glucose gavage was 2.7-fold (168 ± 31 pmol × mg−1 × s−1). Western blots using antibodies against GLUT2 from human (GLUT-Ab) that cross-react with mouse GLUT2 (21) showed a similar magnitude in the increase of GLUT2 protein expression in BBMVs of wild-type mice after the d-glucose bolus (Fig. 2F). The increase in both the GLUT2 protein expression and the GLUT2-mediated d-glucose uptake in response to the glucose bolus were blunted in Sglt1−/− mice. These findings are consistent with a role of SGLT1 in the upregulation of GLUT2 activity in the BBM by high glucose concentration.

Without d-glucose bolus the V_max of AMG uptake via SGLT1 was 5.5 times higher than maximal d-glucose uptake via GLUT2, and after glucose gavage the V_max of AMG
uptake via SGLT1 was 8.7 times higher compared with D-glucose uptake via GLUT2 (compare Fig. 2C and E). This indicates that D-glucose uptake via the luminal membrane of small intestinal enterocytes is predominantly (>80%) mediated via SGLT1.

SGLT1 is expressed in L-cells and K-cells. We investigated the expression of SGLT1 in enteroendocrine L-cells and K-cells, which are known to secrete GLP-1 and GIP, respectively (Fig. 4). Immunohistochemistry was performed using double staining with specific primary antibodies raised against SGLT1 in enteroendocrine L-cells and K-cells. Immunohistochemistry was performed using double staining with specific primary antibodies raised against SGLT1 in enteroendocrine L-cells and K-cells.
AMG was measured after incubation for 2 s in the absence and presence of 100 mM/L phlorizin, and the differences were calculated. Uptake of 0.1 mmol/L NaSCN or 100 mmol/L KSCN, and the differences were calculated.

For 5 s in the presence of an inwardly directed gradient of 100 mmol/L D-glucosamine, and the differences were calculated.

**FIG. 2.** Upregulation of small intestinal D-glucose absorption after application of a D-glucose bolus to the stomach. A: D-glucose concentrations in the plasma after gavage with D-glucose (2 mg/g body wt; Sglt1+/+ ○, Sglt1−/− •) or buffer (Sglt1+/+, ○). B: Properties of small intestine BBMVs of control Sglt1+/+ and Sglt1−/− mice (−bolus) and of Sglt1+/+ and Sglt1−/− mice that had received a D-glucose bolus (6 mg/g body wt) 30 min earlier (+bolus). B: K<sub>m</sub> values for Na<sup>+</sup>-dependent uptake of [14C]AMG into BBMVs. Uptake rates were measured after incubation for 5 s in the presence of an inwardly directed gradient of 100 mM/L NaSCN or 100 mM/L KSCN, and the differences were calculated.

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**V.**

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**SGLT1 is required for elevated systemic blood concentrations of GIP and GLP-1 after gavage with D-glucose.** Oral application of D-glucose stimulates the pancreatic secretion of insulin more than intravenous application. In humans, this surplus of insulin secretion is a result of the combined effects of GIP and GLP-1 (12,31). After exposure of the small intestine to high glucose the concentrations of these enterohormones increase in systemic blood.

Thirteen minutes after glucose gavage (2 mg/g body wt), plasma insulin was approximately two times higher in wild-type mice compared with Sglt1−/− mice (Fig. 5A). At the same time, GIP was increased ~10-fold in the systemic blood of wild-type mice but was not changed in Sglt1−/− mice (Fig. 5B). By comparison 13 min after gavage with olive oil (10 μL/g body wt), GIP increased four- to fivefold in the systemic blood of both wild-type and Sglt1−/− mice. Thirteen minutes after glucose gavage the active GLP-1 concentration in systemic blood was not increased in wild-type mice but in Sglt1−/− mice, whereas application of oil induced a SGLT1-independent twofold increase in active GLP-1 (Fig. 5C). Because the concentration of active GLP-1 in systemic blood may be reduced by degradation we also measured total GLP-1 in the systemic blood 5 min after gavage of a greater D-glucose load (6 mg/g body wt; Fig. 5D). Under these conditions a significant glucose-dependent increase of GLP-1 concentration was observed in wild-type mice but not in Sglt1−/− mice. Measuring glucose-dependent stimulation of GIP and GLP-1 secretion from primary intestinal cultures prepared from Sglt1−/− and Sglt1+/+ mice, we confirmed that SGLT1 is required for glucose-dependent stimulation of these enterohormones (Supplementary Fig. 5).

**SGLT1 in renal proximal tubules is required for complete reabsorption of filtered D-glucose.** In kidney the low affinity Na<sup>+</sup>-D-glucose cotransporter SGLT2 is located in BBMs of S1 segments of proximal tubules, whereas the high affinity Na<sup>+</sup>-D-glucose cotransporter SGLT1 is located in BBMs of the S2 and S3 segments (20,22). We confirmed that no expression of SGLT1 was observed in kidneys of Sglt1−/− mice (Supplementary Figs. 20, 22).
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6A and 7A) and determined at mRNA and protein levels that the expression of SGLT2 was not changed after removal of SGLT1 (Supplementary Figs. 6B and 7B and C). In wild-type and Sglt1−/− mice similar mRNA and protein concentrations of the passive glucose transporters GLUT1, GLUT2, and GLUT5 were determined (Supplementary Fig. 7B and C).

Spontaneous urine collections in awake male mice revealed that urinary glucose concentrations were significantly increased in Sglt1−/− compared with wild-type mice, whereas blood glucose levels were not different (Fig. 6A). Fractional glucose reabsorption was 100% in male wild-type and 97% in male Sglt1−/− mice (Fig. 6B). In male mice, GFR measured in awake and anesthetized animals, blood pressure, heart rate, plasma urea concentrations, food intake and fluid intake, and renal excretion of fluid, Na+, and Cl− were not changed after removal of SGLT1. Details and differences in food intake and fluid intake between males and females are described in the Supplementary Data.

Quantitative free-flow collections of tubular fluid were performed from the last surface loop of proximal convoluted tubules. Mean values for single nephron GFR and the amount of glucose filtered per nephron were not different between male Sglt1−/− and wild-type mice (Fig. 7A and B). However, the concentration and delivery of glucose were increased in Sglt1−/− versus wild-type mice, whereas fractional glucose reabsorption was reduced (Fig. 7C and D). In contrast with fractional reabsorption of glucose, fractional reabsorption of fluid and chloride was not significantly different between male Sglt1−/− and wild-type mice (fluid: 0.48 ± 0.02 vs. 0.45 ± 0.03; chloride: 0.47 ± 0.03 vs. 0.45 ± 0.03; n = 17–25 nephrons in each group, not significant).

DISCUSSION

In the current study the role of SGLT1 for small intestinal glucose absorption and incretin secretion was investigated comparing nondiabetic mice without and with expression of SGLT1. Evidence is presented that SGLT1 mediates the majority of glucose transport across the BBM of enterocytes independent of the glucose load. SGLT3b, a second functional Na+-glucose cotransporter in the BBM of

![FIG. 3. The substrate dependence of SGLT1-mediated glucose uptake expressed in oocytes is different from the substrate dependence of SGLT1-mediated glucose uptake across small intestinal BBM. A and B: Transport measurements in oocytes. Oocytes were injected with 5 ng of mouse Sglt1 cRNA and incubated for 2 days for expression. Uptake of different concentrations of [14C]AMG (A) or [3H]D-glucose (B) was measured in the presence of 100 μM phlorizin. Phlorizin-inhibitable uptake rates of individual experiments were normalized to the uptake rates measured at 2 mmol/L AMG or D-glucose. Mean values ± SE of 25–30 oocytes from 3 independent experiments are shown. Similar apparent Km values of 0.17 ± 0.05 mmol/L and 0.13 ± 0.01 mmol/L were obtained for AMG and D-glucose, respectively. The Vmax values of SGLT1-mediated uptake of AMG and D-glucose were similar. In oocytes of the same batch, which were injected with 5 ng of Sglt1 cRNA and incubated for 2 days, Vmax values (measured at monosaccharide concentrations of 2 mmol/L without and with 100 μmol/L phlorizin in 5.0 ± 0.6 (AMG) and 4.8 ± 0.6 (D-glucose) pmol × oocyte−1 × min−1 were obtained (n = 25 each, not significant). C: Transport measurements in BBMVs. Wild-type mice fed with standard diet were starved for 18 h and killed at 3–4 P.M. BBMVs were prepared. BBMVs were incubated for 2 s at 37°C with different concentrations of [14C]AMG in the presence of an inwardly directed gradient of 100 mmol/L NaSCN or 100 mmol/L KSCN. The sodium-dependent uptake rates of individual experiments were normalized to the values obtained at 10 mmol/L AMG. Mean values ± SE of 12 measurements from three independent experiments are shown. A Km value of 1.20 ± 0.05 mmol/L (n = 5) was determined. D: Glucose-induced short circuit currents across small intestinal mucosa. Wild-type mice fed with standard diet were starved for 18 h and killed at 4 P.M. The jejunal small intestinal wall was then mounted to an Ussing chamber. The mucosal side was superfused with different concentrations of D-glucose, and D-glucose-induced short circuit currents (Isc) mediated by Na+-glucose cotransport was measured. Mean values ± SE of 7 experiments are shown. The Michaelis-Menten equation was fitted to the data. A Km value of 1.9 ± 0.6 mmol/L was determined.]

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mice does not contribute significantly. After high glucose load some GLUT2 is incorporated into the BBM, but this has minor impact on small intestinal glucose absorption. It is noteworthy that this translocation of GLUT2 was not observed in Sglt1−/− mice, indicating that SGLT1 rather than sweet taste receptors is pivotal in this upregulation (5,6,8). Without and with D-glucose load the capacity of SGLT1 versus GLUT2 for D-glucose transport across the BBMs was 5.5 and 8.7 times higher, respectively. In response to continuous feeding of sugar-rich diets and during diabetes, however, the contribution of GLUT2 to small intestinal glucose absorption may become more relevant. Under these conditions the expression of SGLT1 and GLUT2 is increased, and an increase of GLUT2 in the BBM was observed after gavage with glucose or fructose (32–35).

Removal of SGLT1 in mice resulted in GGM syndrome (OMIM 182380) that could be cured by a glucose-galactose-reduced diet as has been observed in patients.
with loss-of-function mutations in SGLT1 (SLC5A1) (18,19). GGM is characterized by severe diarrhea and dehydration as a result of osmotic loss by retention of nonabsorbed D-glucose, D-galactose, and sodium in the small intestinal lumen. In contrast with humans, where severe diarrhea and dehydration are observed in suckling neonates, suckling Sglt1<sup>−/−</sup> mice showed no obvious symptoms. This species difference may be explained by the three to four times higher concentrations of lactose in human milk compared with murine milk (36,37). In the small intestine, lactose is degraded by lactase-isomaltase and provides the only relevant source of D-glucose and D-galactose in suckling neonates.

Confirming previous results (15,16) we showed that SGLT1 is expressed in the luminal membrane of enteroendocrine cells staining positive for the incretin hormones, GLP-1 and GIP. Active forms of GLP-1 and GIP are detectable in the systemic blood of human subjects after glucose ingestion (38,39), but are rapidly inactivated in the circulation by DPP-IV (10). Both GIP and GLP-1 trigger glucose-dependent insulin secretion, and although the effectiveness of GIP is impaired in people with type 2 diabetes (40), the preserved insulinogetic effect of GLP-1 (40) has led to the successful development of GLP-1 mimetics and DPP-IV inhibitors as new classes of antidiabetic agent (41). Earlier in vitro studies have demonstrated that SGLT1-mediated monosaccharide transport triggers incretin secretion via the electrogenic coupled influx of Na<sup>+</sup>, which results in membrane depolarization, electrical activity, voltage-gated Ca<sup>2+</sup> entry, and exocytosis (16,42), although the contribution of this pathway to glucose-mediated secretion in vivo has remained controversial. Using the SGLT1<sup>−/−</sup> mice we now demonstrate the pivotal role of SGLT1 for both GLP-1 and GIP secretory responses triggered by oral glucose in vivo. This is consistent with the reported phlorizin sensitivity of glucose-induced incretin increases in the portal vein (13) showing the involvement of a phlorizin-sensitive SGLT-type transporter or channel (3,30,43), as well as with the finding that incretin secretion is triggered in vivo by substrates of SGLT1 (44), but not by other sweet tasting compounds (14). The results suggest that secretion of these hormones would be initiated after food ingestion by the early arrival of even small glucose loads in the small intestine, consistent with previous reports that GLP-1 release is initiated in humans when glucose infusion rates exceed the absorption capacity of the duodenum (45).

Whereas glucose is nearly completely reabsorbed by the kidneys in wild-type mice, Sglt1<sup>−/−</sup> mice lose ~3% of the filtered glucose in the urine, leading to a urinary D-glucose concentration of ~20 mmol/L. Notably Sglt2 null mice lose only ~60% of the filtered glucose in the urine although the expression of SGLT1 was downregulated by 50% (20). This suggests that wild-type mice do not use the maximal transport capacity of SGLT1 at normoglycemic conditions. This is different when the glucose load to the SGLT1-expressing
S2 and S3 segments is increased. In diabetic patients in which early proximal tubule glucose transport capacity is overwhelmed by high n-glucose concentrations, and even more in diabetic patients treated with an SGLT2 specific inhibitor, SGLT1 may operate at full transport capacity (17). The Sglt1 null mice provide a valuable tool for future studies of SGLT1 functions under pathological conditions as well as the therapeutic potential of SGLT1 inhibition.

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No potential conflicts of interest relevant to this article were reported.

V.G., A.S., V.V., F.M.G., and I.S. researched data, contributed to the discussion, and reviewed the manuscript. H.Ki., A.J., F.R., P.L., and M.S. researched data and contributed to the discussion. D.K., A.F., S.S., T.R., R.C., M.V.-W., A.S., D.Ba., D.Br., R.R., H.E.P., S.W. researched data. H.Ko. designed the study, researched data, wrote the manuscript, and takes full responsibility for the article and its originality.

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