Original Article

MOLECULAR METABOLISM

Intestinal invalidation of the glucose transporter GLUT2 delays tissue distribution of glucose and reveals an unexpected role in gut homeostasis

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ABSTRACT

Objective: Intestinal glucose absorption is orchestrated by specialized glucose transporters such as SGLT1 and GLUT2. However, the role of GLUT2 in the regulation of glucose absorption remains to be fully elucidated.

Methods: We wanted to evaluate the role of GLUT2 on glucose absorption and glucose homeostasis after intestinal-specific deletion of GLUT2 in mice (GLUT2$^{\Delta IEC}$ mice).

Results: As anticipated, intestinal GLUT2 deletion provoked glucose malabsorption as visualized by the delay in the distribution of oral sugar in tissues. Consequences of intestinal GLUT2 deletion in GLUT2$^{\Delta IEC}$ mice were limiting body weight gain despite normal food intake, improving glucose tolerance, and increasing ketone body production. These features were reminiscent of calorie restriction. Other adaptations to intestinal GLUT2 deletion were reduced microvillus length and altered gut microbiota composition, which was associated with improved inflammatory status. Moreover, a reduced density of glucagon-like peptide-1 (GLP-1) positive cells was compensated by increased GLP-1 content per L-cell, suggesting a preserved enteroendocrine function in GLUT2$^{\Delta IEC}$ mice.

Conclusions: Intestinal GLUT2 modulates glucose absorption and constitutes a control step for the distribution of dietary sugar to tissues. Consequently, metabolic and gut homeostasis are improved in the absence of functional GLUT2 in the intestine, thus mimicking calorie restriction.

Keywords Malabsorption; Glucose homeostasis; Intestinal adaptation; Microbiota; GLP-1

1. INTRODUCTION

The intestine responds to the external environment by complex interplays between cells in the epithelium, the immune system, and microbes. Gut homeostasis allows a permanent adaptation to nutritional, metabolic, or bacterial signals through continuous renewal and differentiation of epithelial cells. After carbohydrate intake and digestion, sugars are absorbed in the proximal small intestine by several specialized transporters. The Na^+/glucose cotransporter SGLT1 triggers glucose absorption at the food-facing membrane. GLUT2 facilitates the passage of dietary sugars, glucose, fructose, and galactose towards the bloodstream [1]. In enterocytes, GLUT2 is consistently located in the basolateral membrane but, during a sugar rich meal, can be recruited transiently to the apical membrane, where it remains permanently in case of insulin resistance [2–4]. GLUT2 is expressed in enteroendocrine cells, especially in L-cells [5]. L-cells express nutrient transporters and receptors that can sense luminal nutrients at their apical membrane and thereby influence their own functions [6,7]. Enteroendocrine cells are scattered in the intestinal epithelium and constitute the largest population of hormone-producing

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Abbreviations: 2FDG, 2-deoxy-2-[^{18}F]fluoro-D-glucose; DPP-IV, dipeptidyl-peptidase IV; GLP-1, glucagon-like peptide-1; GLUT1-7, glucose transporter 1–7; IEC, intestinal epithelial cells; IL, interleukin; IPGTT, intraperitoneal glucose tolerance test; ITT, insulin tolerance test; OGTT, oral glucose tolerance test; PET-CT, Positron Emission Tomography-Computed Tomography; SGLT1, sodium-glucose transporter 1

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cells of the body. L-cells produce incretins such as glucagon-like peptide-1 (GLP-1), a potent activator of glucose-induced insulin secretion [8]. It is now thought that sufficient L-cells are present in the proximal intestine to account for early rises of GLP-1 after a meal. However, when absorption is reduced, the higher nutrient delivery to distal intestine may bring more L-cells in contact with nutrients and/or their microbial fermentation products, resulting in increased plasma GLP-1 levels [9].

Physiological studies of genetically modified mice have revealed a role for GLUT2 in several regulatory mechanisms. A total knockout of GLUT2 in mouse indicated that GLUT2 is dispensable for intestinal glucose absorption because of the existence of compensatory mechanisms [10]. However, Glut2$^{-/-}$;RIPGlut1 mice rescued by GLUT1 expression in pancreatic β cells displayed impaired plasma GLP-1 levels after glucose gavage, which was attributed to reduced intestinal GLP-1 content [11], suggesting that GLUT2 is of importance in L-cell function. In the present study, our hypothesis was that intestinal GLUT2 could contribute to both metabolic and gut homeostasis. Impaired glucose absorption in the small intestine could cause disturbed glucose delivery, modified gut microbiota composition, and altered L-cell function. To test this hypothesis, we generated an inducible model of GLUT2 invalidation in intestinal epithelial cells.

2. MATERIAL AND METHODS

2.1. Generation of transgenic mice with targeted deletion of *Slc2a2* in the intestine

Heterozygous *Slc2a2*$^{tm1a(KOMP)Wtsi}$ mice were generated by the KnockOut Mouse Project (KOMP) at the University of California, Davis (Project ID#CSD40514), using the targeting vector described in Figure 1A(a). This vector contains the lacZ expressing cassette with polyA tail inserted between Exon 3 and Exon 4 of *Slc2a2*, generating a null allele that produces β-galactosidase in cells with active *Slc2a2* gene transcription (Figure 1A(a)). We could observe that heterozygous mice survived and expressed β-galactosidase in GLUT2 expressing tissues including the intestine (data not shown). These founder mice were crossed with mice that express the Flippase recombinase to remove the lacZ and neomycin cassettes generating *Slc2a2*$^{FLOX/FLOX}$ mice (Figure 1A(b)). *Slc2a2*$^{FLOX/FLOX}$ mice were then crossed with a *Villin-CreERT2* mouse line [12] (gift from S. Robine) to generate *Slc2a2*$^{FLOX/FLOX}$ × *Villin-CreERT2*$^{+/-}$ and *Slc2a2*$^{FLOX/FLOX}$ × *Villin-CreERT2*$^{-/-}$ mice. In the *Slc2a2*$^{FLOX/FLOX}$ × *Villin-CreERT2*$^{+/-}$ mice, Cre recombinase was activated by tamoxifen gavage (1 mg/mouse; Sigma–Aldrich) for 3 consecutive days to induce a specific *Slc2a2* deletion in intestinal epithelial cells (GLUT2$^{\Delta IEC}$ mice, Figure 1A(c)).

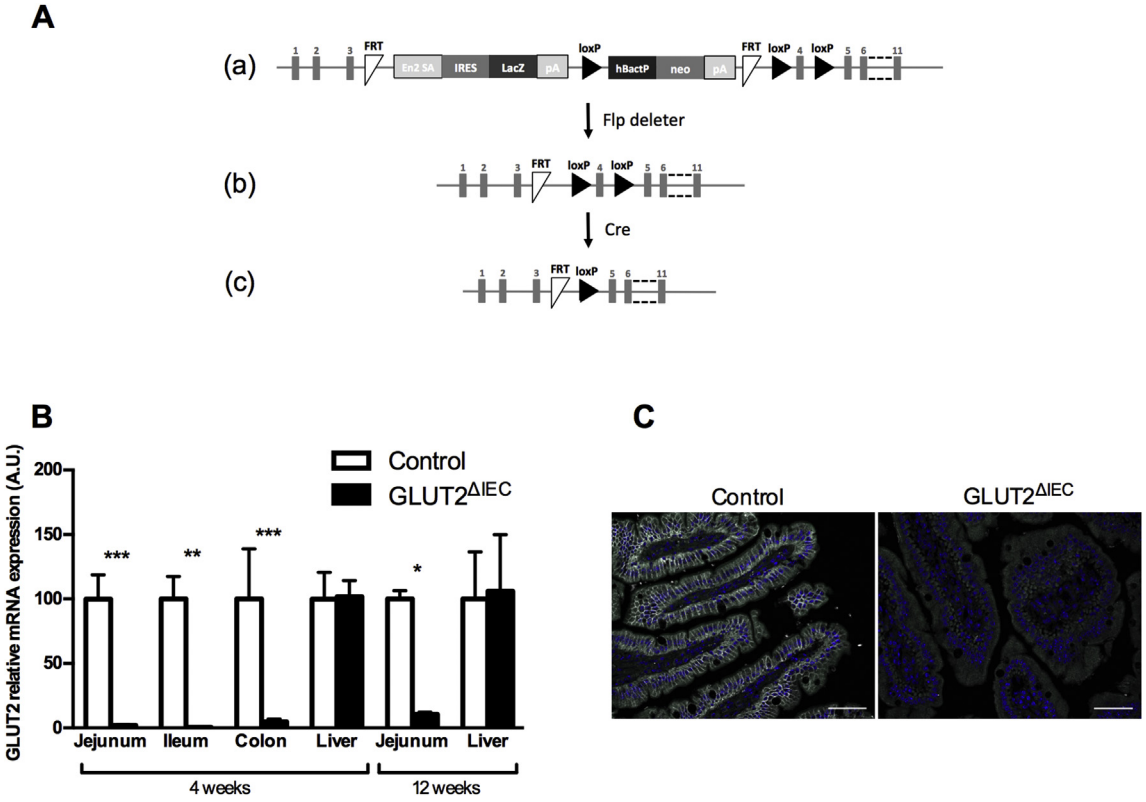

Figure 1: Generation of GLUT2$^{\Delta IEC}$ mice. (**A**) (a) The *Slc2a2*/lacZ knock-in allele (*Slc2a2*(KOMP)Wtsi) is shown to illustrate the mutated *Slc2a2* gene, in which the lacZ and neomycin expression cassettes were inserted between Exon 3 and Exon 4 and which is flanked by FRT (Flippase Recognition Target) sites. This cassette contains the splice acceptor of mouse engrailed 2 exon 2 (En2 SA), an internal ribosome entry sequence (IRES) to initiate lacZ translation, and polyadenylation (pA) to terminate transcription after the lacZ gene. The neo gene is driven by human beta actin promoter (hBactP) and contains its own pA. Additionally, Exon 4 is flanked by loxP sites. (b) The resulting *Slc2a2* conditional knockout gene structure is shown to illustrate removal of the lacZ and neomycin cassettes; it is generated by crossing Slc2a2$^{tm1a(KOMP)Wtsi}$ mice with mice that express the Flp recombinase. (c) The resulting *Slc2a2* knockout allele generated after crossing with Cre expressing mice and activation by tamoxifen gavage is shown. (**B**) Relative mRNA levels of sugar transporter GLUT2 in the jejunum, ileum, colon, and liver 4 weeks after tamoxifen administration and in the jejunum and liver 12 weeks after tamoxifen administration in control (white bars, n = 4–8) and GLUT2$^{\Delta IEC}$ (black bars, n = 3–9) mice. Values are means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001; control *vs* GLUT2$^{\Delta IEC}$ mice (Mann–Whitney *U* test). (**C**) Jejunal sections from control (left panel) and GLUT2$^{\Delta IEC}$ (right panel) mice 4 weeks after tamoxifen administration were immunostained for GLUT2 (white) and DAPI (blue). Scale bars = 50 μm. Note the absence of GLUT2 labeling in jejunum of GLUT2$^{\Delta IEC}$ mice.

