Original article

MOLECULAR METABOLISM

GLP-2 receptor signaling controls circulating bile acid levels but not glucose homeostasis in *Gcgr*$^{-/-}$ mice and is dispensable for the metabolic benefits ensuing after vertical sleeve gastrectomy

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ABSTRACT

Objective: Therapeutic interventions that improve glucose homeostasis such as attenuation of glucagon receptor (Gcgr) signaling and bariatric surgery share common metabolic features conserved in mice and humans. These include increased circulating levels of bile acids (BA) and the proglucagon-derived peptides (PGDPs), GLP-1 and GLP-2. Whether BA acting through TGR5 (Gpbar1) increases PGDP levels in these scenarios has not been examined. Furthermore, although the importance of GLP-1 action has been interrogated in *Gcgr*$^{-/-}$ mice and after bariatric surgery, whether GLP-2 contributes to the metabolic benefits of these interventions is not known.

Methods: To assess whether BA acting through Gpbar1 mediates improved glucose homeostasis in *Gcgr*$^{-/-}$ mice we generated and characterized *Gcgr*$^{-/-}$:*Gpbar1*$^{-/-}$ mice. The contribution of GLP-2 receptor (GLP-2R) signaling to intestinal and metabolic adaptation arising following loss of the Gcgr was studied in *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ mice. The role of the GLP-2R in the metabolic improvements evident after bariatric surgery was studied in high fat-fed *Glp2r*$^{-/-}$ mice subjected to vertical sleeve gastrectomy (VSG).

Results: Circulating levels of BA were markedly elevated yet similar in *Gcgr*$^{-/-}$:*Gpbar1*$^{+/+}$ vs. *Gcgr*$^{-/-}$:*Gpbar1*$^{-/-}$ mice. Loss of GLP-2R lowered levels of BA in *Gcgr*$^{-/-}$ mice. *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ mice also exhibited shifts in the proportion of circulating BA species. Loss of *Gpbar1* did not impact body weight, intestinal mass, or glucose homeostasis in *Gcgr*$^{-/-}$ mice. In contrast, small bowel growth was attenuated in *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ mice. The improvement in glucose tolerance, elevated circulating levels of GLP-1, and glucose-stimulated insulin levels were not different in *Gcgr*$^{-/-}$:*Glp2r*$^{+/+}$ vs. *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ mice. Similarly, loss of the GLP-2R did not attenuate the extent of weight loss and improvement in glucose control after VSG.

Conclusions: These findings reveal that GLP-2R controls BA levels and relative proportions of BA species in *Gcgr*$^{-/-}$ mice. Nevertheless, the GLP-2R is not essential for i) control of body weight or glucose homeostasis in *Gcgr*$^{-/-}$ mice or ii) metabolic improvements arising after VSG in high fat-fed mice. Furthermore, despite elevations of circulating levels of BA, Gpbar1 does not mediate elevated levels of PGDPs or major metabolic phenotypes in *Gcgr*$^{-/-}$ mice. Collectively these findings refine our understanding of the relationship between Gpbar1, elevated levels of BA, PGDPs, and the GLP-2R in amelioration of metabolic derangements arising following loss of Gcgr signaling or after vertical sleeve gastrectomy.

Keywords Glucagon; GLP-1; GLP-2; Bile acids; Bariatric surgery; Glucose; Diabetes; Obesity

1. INTRODUCTION

Although multiple organ systems contribute to control of energy balance, the complex network of enteroendocrine cells has received increasing attention as physiological regulators of metabolic homeostasis [1,2]. Notably, L cells that produce the proglucagon-derived peptides (PGDPs) have been extensively studied, as PGDPs exert pleiotropic actions regulating appetite, gastrointestinal motility,

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nutrient absorption, gut epithelial integrity, gallbladder emptying, and the uptake and assimilation of nutrients in peripheral tissues [3,4]. Indeed, PGDP secretion from enteroendocrine L cells is stimulated by a range of nutrients, microbial metabolites, and bile acids (BA), through direct and indirect mechanisms [2].

Glucagon-like peptide-1 (GLP-1), the best characterized PGDP, is a 30 amino acid peptide that exerts its actions through a single well-defined G protein-coupled receptor [4]. GLP-1 is physiologically essential for glucose control and energy homeostasis, as revealed in preclinical studies using GLP-1 receptor (GLP-1R) antagonists or *Glp1r*$^{-/-}$ mice [4]. GLP-1 also attenuates the rate of gastric emptying and small bowel motility, and promotes expansion of the intestinal mucosal epithelium, actions serving to optimize the efficiency of nutrient absorption [5]. Although less well studied, the related PGDP GLP-2 also controls the absorption of nutrients through reduction of gut motility, upregulation of nutrient transport and via optimization of mucosal surface area and gut integrity [6].

A number of experimental therapeutic manipulations resulting in improvement of glucose metabolism and either resistance to weight gain or development of weight loss are characterized by simultaneous elevation of circulating BA and PGDPs. Thus, partial or complete blockade of glucagon action, achieved through genetic loss of hepatic glucagon receptor (*Gcgr*) expression or pharmacological antagonism of GCGR signaling, is associated with a rapid rise in circulating levels of GLP-1 and GLP-2 [7,8]. Unexpectedly, reduction of Gcgr signaling is also associated with markedly increased circulating levels of BA [9,10]. Indeed plasma levels of PGDPs and BA are increased in humans with T2D following daily or chronic administration of GCGR antagonists [8,11].

Bariatric surgery represents a second therapeutic paradigm characterized by increased levels of circulating BA, GLP-1 and GLP-2. Both vertical sleeve gastrectomy (VSG) and Roux-en-Y gastric bypass (RYGB), when performed experimentally in animals, or therapeutically in humans, lead to elevated levels of BA and PGDPs [12–16]. Although BA are known stimulators of L cell PGDP secretion via signaling through TGR5 (Gpbar1) [17–19], the extent if any, to which elevated levels and action of BA contribute to increased levels of circulating PGDPs i) in animals or humans with loss of GCGR action or ii) following bariatric surgery, has not been yet determined.

The finding that enhanced circulating levels of BA and PGDPs are simultaneously associated with improved glucose control in the setting of loss of GCGR signaling or metabolic surgery raises important mechanistic questions [20–22]. Notably, preclinical studies implicate a role for BA, acting through changes in the gut microbiota and via the nuclear Farnesoid X Receptor (FXR), in the improvements in glucose control and weight loss ensuing following VSG [23]. Consistent with the importance of BA in this setting, the metabolic benefits ensuing from bariatric surgery are also attenuated in *Gpbar1*$^{-/-}$ mice [24,25]. Although somewhat controversial, GLP-1 contributes to improvements in β-cell function in some [26], but not all murine studies of metabolic surgery [20,27]. It seems likely that GLP-1 improves β-cell function in humans after bariatric surgery, and in rare instances, promotes development of hyperinsulinemic hypoglycemia [21].

In contrast to the extensive literature describing the metabolic roles of GLP-1, much less is known about the effects of GLP-2 on glucose control and body weight. Notably, GLP-2 inhibits ghrelin secretion [28], enhances hepatic insulin sensitivity [29], and suppresses food intake [30], actions mirroring some of the metabolic benefits ensuing after bariatric surgery or GCGR antagonism. Furthermore, GLP-2 enhances gut adaptation and barrier function while reducing metabolic endotoxemia [6,31], consistent with intestinal adaptation evident after VSG or RYGB [32,33]. Collectively, these observations raise the possibility that elevated levels of GLP-2, arising secondarily to or independent from increased levels of BA, may contribute to the metabolic benefits arising following i) reduction of GCGR signaling or ii) metabolic surgery. To interrogate the potential role of BA signaling through Gpbar1 and the importance of GLP-2R for the metabolic improvements in *Gcgr*$^{-/-}$ mice, we generated *Gcgr*$^{-/-}$:*Gpbar1*$^{-/-}$ and *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ mice. Simultaneously, we examined the importance of GLP-2R signaling in *Glp2r*$^{-/-}$ mice following experimental VSG.

2. MATERIALS AND METHODS

2.1. Animals and vertical sleeve gastrectomy surgical procedure

Gcgr$^{-/-}$ mice provided by Maureen Charron [34], *Tgr5*$^{-/-}$ (*Gpbar1*$^{-/-}$) mice [35], obtained from Schering-Plough/Merck, and *Glp2r*$^{-/-}$ mice [36], all on a C57Bl/6 background were bred at the Toronto Centre for Phenogenomics animal facility. *Gcgr*$^{-/-}$:*Gpbar1*$^{-/-}$ and *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ double knockout mice were generated by crossing double heterozygous *Gcgr*$^{+/-}$:*Gpbar1*$^{+/-}$ *or* *Gcgr*$^{+/-}$:*Glp2r*$^{+/-}$ to obtain wild-type, single knockout and double knockout littermates. All experiments involving *Gcgr*$^{-/-}$:*Gpbar1*$^{-/-}$ and *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ double knockout mice and their single knockout and wild-type littermates were performed in male aged 12–26 weeks, housed up to 5 per cage, with free access to food (2018 Teklad global, Envigo Corp, Mississauga, ON, Canada) and water. VSG or sham surgeries were performed on male *Glp2r*$^{-/-}$ and wild-type littermate mice bred in-house at the University of Michigan. Four to 11 week old mice were placed on a 60% high fat diet (HFD) (D12492, Research Diets, New Brunswick, NJ, USA) were single housed and given ad libitum access to food. After 8 weeks, surgeries were performed as previously described [37]. Briefly, while mice were anesthetized under isoflurane inhalation, an abdominal midline laparotomy was made followed by incision of the underlying abdominal muscle and exteriorization of the stomach. For VSG the lateral 80% of the stomach was excised using an ETS 35-mm staple gun (Ethicon Endo-Surgery, Cincinnati, OH, USA) leaving a tubular gastric sleeve in continuity with the esophagus proximally and the pylorus distally. The sham procedure involved the application of light pressure on the stomach with blunt forceps. Mice were fed Osmolite 1.0 Cal liquid diet (Abbott Nutrition, Lake Forest, IL, USA) from 1 day prior to surgery to 3 days following surgery before returning to 60% HFD. *Glp2r*$^{-/-}$ pair-fed animals underwent sham surgery and were restricted to eating the daily average amount of food eaten by the *Glp2r*$^{-/-}$ mice subjected to VSG. Pair-feeding continued until sacrifice. Body composition was measured using an EchoMRI (Echo Medical Systems, Houston, TX, USA) 1 week prior to surgery and 6 and 8 weeks after surgery. Body weights were measured daily for 1 week after surgery and weekly for 10 weeks thereafter. All animal experiments performed in Toronto were conducted according to protocols approved by the Animal Care and Use Subcommittee at the Toronto Centre for Phenogenomics, Mt. Sinai Hospital, and were consistent with the ARRIVE guidelines. Studies on *Glp2r*$^{-/-}$ mice done in Ann Arbor were approved by the Institutional Animal Care & Use Committee at the University of Michigan (Animal Use Protocol #PRO00005678).

2.2. Glucose tolerance tests and measurement of plasma insulin, GLP-1 and bile acids

Glucose tolerance tests in *Gcgr*$^{-/-}$:*Gpbar1*$^{-/-}$ and *Gcgr*$^{-/-}$:*Glp2r*$^{-/-}$ double knockout mice and their single knockout and wild-type littermates were carried out in 12- to 15-week-old mice. Fed or overnight fasted (16–18 h in cages with wire grid flooring) mice were administered glucose (2 mg/g body weight) via either an oral gavage or i.p.

