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Intestinal gluconeogenesis is crucial to maintain a physiological fasting glycemia in the absence of hepatic glucose production in mice

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ABSTRACT

Objective. Similar to the liver and kidneys, the intestine has been strongly suggested to be a gluconeogenic organ. However, the precise contribution of the intestine to endogenous glucose production (EGP) remains to be determined. To define the quantitative role of intestinal gluconeogenesis during long-term fasting, we compared changes in blood glucose during prolonged fasting in mice with a liver-deletion of the glucose-6 phosphatase catalytic (G6PC) subunit (LKO) and in mice with a combined deletion of G6PC in both the liver and the intestine (ILKO).

Materials/Methods. The LKO and ILKO mice were studied after 6 h and 40 h of fasting by measuring metabolic and hormonal plasmatic parameters, as well as the expression of gluconeogenic enzymes in the liver, kidneys and intestine.

Results. After a transient hypoglycemic episode (approximately 60 mg/dL) because of their incapacity to mobilize liver glycogen, the LKO mice progressively re-increased their plasma glucose to reach a glycemia comparable to that of wild-type mice (90 mg/dL) from 30 h of fasting. This increase was associated with a rapid induction of renal and intestinal gluconeogenic gene expression, driven by glucagon, glucocorticoids and acidosis. The ILKO mice exhibited a similar induction of renal gluconeogenesis. However, these mice failed to re-increase their glycemia and maintained a plasma glucose level of only 60 mg/dL throughout the 48 h-fasting period.

Conclusions. These data indicate that intestinal glucose production is essential to maintain glucose homeostasis in the absence of hepatic glucose production during fasting. These data provide a definitive quantitative estimate of the capacity of intestinal gluconeogenesis to sustain EGP during long-term fasting.

Abbreviations: EGP, endogenous glucose production; G6Pase, glucose-6 phosphatase; G6PC, glucose-6 phosphatase catalytic subunit; LKO, liver knockout mice; ILKO, intestine and liver knockout mice; PEPCK-c, phosphoenolpyruvate carboxykinase cytosolic form; WT, wild-type.

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1. Introduction

Endogenous glucose production (EGP) is a crucial physiological function that is essential for the maintenance of a plasma glucose concentration approximately 90–100 mg/dL in the absence of glucose supplied by food, i.e., between the periods of meal assimilation and during fasting [1–3]. The liver, kidneys and intestine are the three organs contributing to EGP, as they are the only organs known to express the catalytic subunit (G6PC) of the glucose-6 phosphatase (G6Pase) enzyme, which catalyzes the last biochemical step common to glycogenolysis and gluconeogenesis [4,5].

Although the liver has long been considered the major contributor to EGP in the post-absorptive state, which includes fasting, it is well-established that the kidney rapidly increases its contribution to EGP upon fasting [see [2,6] for review]. We demonstrated that the kidneys represent nearly 50% of the EGP after 24 h of fasting in rats instead of approximately 15%–20% in the post-absorptive state (6 h of fasting) [7]. This finding is consistent with the contribution of the kidneys to EGP in humans, which represents approximately 5%–20% of the EGP in the post-absorptive state [8,9] and roughly 25%–50% during long-term fasting [8,10]. The intestinal gluconeogenesis is enhanced more progressively during fasting, at least at the level of gluconeogenic gene expression [11]. However, we have suggested that the gut would increase its contribution to EGP from 5%–10% in post-absorptive rats to approximately 20%–25% after 48 h of fasting [12,13]. This suggests a model where both intestinal and renal gluconeogenesis could replace liver gluconeogenesis in the fasting rat [14,15]. In humans, extrahepatic gluconeogenesis could compensate for the absence of the liver during the anhepatic phase of liver transplantation [16,17]. In this case, the kidneys were suggested to contribute to approximately 70% of the EGP with the remaining 30% being attributed to the intestine [16].

However, the individual contribution of the intestine and the kidneys in the EGP has yet to be clarified. While the role of the kidneys seems undeniable, the participation of the intestine in the EGP during fasting is uncertain, particularly from a quantitative viewpoint. Therefore, to investigate this point, we used a novel genetic approach in mice. We previously showed that liver-specific G6PC knockout (KO) mice are normoglycemic in the fed state and they resist fasting. These mice first undergo a transient hypoglycemic episode due to the absence of liver glycogenolysis [18] but eventually reach normal fasting glycemia of wild-type mice, i.e., 90–100 mg/dL. This steady state is due to a rapid induction of renal and intestinal gluconeogenesis [18]. To assess the respective contribution of the kidneys and the intestine in the maintenance of fasting plasma glucose during short- and long-term fasting periods, we generated a mouse model with a double KO of G6PC in both the liver and the intestine. If intestinal glucose production is essential to maintain glucose production during fasting, we hypothesized that the intestine/liver-KO mice would not maintain their blood glucose at 90–100 mg/dL during long-term fasting. Our data unequivocally demonstrate the key contribution of the gut in sustaining plasma glucose during fasting by comparing how liver-KO and intestine/liver-KO mice undergo fasting.

2. Methods

2.1. *Generation of liver and intestine G6pc knockout mice*

Transgenic B6.G6pc$^{lox/lox}$.SA$^{CreERT2/w}$ (liver-specific deletion) and B6.G6pc$^{lox/lox}$.Vill$^{CreERT2/w}$ mice (intestine-specific deletion) were crossed to generate a new mouse model (B6.G6pc$^{lox/lox}$.SA$^{CreERT2/w}$.Vill$^{CreERT2/w}$ mice) [19,20]. These new mice expressed the inducible Cre-ER^{T2} recombinase under the control of the serum albumin (SA) and villin (Vill) promoters. Additionally, their *G6pc* exon 3 was flanked by two *loxP* sites. The mouse genotypes were determined from tail genomic DNA by PCR with specific primers [19,20]. The genomic DNA was extracted from the tissues with a "Direct PCR" kit (Viagen, Los Angeles, CA, USA). Male adult (7–8 week-old) B6.G6pc$^{lox/lox}$.SA$^{CreERT2/w}$.Vill$^{CreERT2/w}$ and B6.G6pc$^{lox/lox}$.SA$^{CreERT2/w}$ mice were injected intraperitoneally with 100 μL tamoxifen (10 mg/mL, Sigma-Aldrich) once daily on five consecutive days to obtain the intestine and liver *G6pc* KO (ILKO) and liver *G6pc* KO (LKO) mice, respectively. The control C57BL/6 J (Charles River Laboratories, L'Arbresle, France) wild-type (WT) mice were treated with the same tamoxifen injections. The number of animals studied is indicated in the figure legends. All mice were housed in the animal facility of Lyon 1 University (Animaleries Lyon Est Conventionnelle et SPF) under controlled temperature (22 °C) conditions with a 12-h light/12-h dark cycle. The mice had free access to water and to a standard rodent starch-enriched diet (Safe, Augy, France). All procedures were performed in accordance with the principles and guidelines established by the European Convention for the Protection of Laboratory Animals. The animal care committee of the Lyon 1 University approved all experiments. The mice were killed by cervical dislocation in the post-absorptive state (6 h after food removal) or after 40 h of fasting, at the age of 12–13 weeks. The liver and kidneys were rapidly removed and flash-frozen in liquid nitrogen. The intestine (proximal jejunum) was rinsed and immediately frozen in liquid nitrogen. The frozen tissues were kept at −80 °C until use.

2.2. *Metabolic studies*

Blood was drawn under isoflurane anesthesia from the retro-orbital vein for the plasma metabolite and hormone determinations. The insulin, glucagon, corticosterone, epinephrine and norepinephrine concentrations were determined with mouse ELISA kits from Crystal Chem (Downers Grove, IL, USA), Yanaihara Institute (Shizuoka, Japan), Arbor Assays (Ann Arbor, MI, USA) and Cusabio (Wuhan, China), respectively. The β-hydroxybutyrate content was measured using Optium β-ketone test strips with Optium Xceed sensors (Abbott Diabetes Care, Alameda, CA, USA). The lactate concentrations were determined with bioMerieux (Marcy-l'Etoile, France) colorimetric kits. The blood glucose was determined with an Accu-Chek Go glucometer (Roche Diagnostics, Meylan, France) during the fasting experiments. The hepatic glycogen and glucose-6 phosphate determinations were carried out as described by Keppler and Decker [21]. The hepatic triglycerides content was measured using a