

## Is the Small Intestine a Gluconeogenic Organ?

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*Gluconeogenesis is responsible for the maintenance of blood glucose levels as hepatic glycogen stores become depleted. Traditionally, only liver and kidney have been believed to be capable of gluconeogenesis, but a gluconeogenic capacity for the small intestine has recently been proposed. This possibility is supported by the expression of key gluconeogenic enzymes and radiolabeled tracer experiments, but these data are not unequivocal and alternative roles can explain the presence of gluconeogenic enzymes in this organ.*

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doi: 10.1301/nr.2005.oct.356–360

Glucose is the universal fuel of the body, and key tissues, notably the brain, have an absolute requirement for glucose that can only be met by maintaining blood glucose levels above 3 mM. Since we eat discrete meals, this means that the maintenance of glucose homeostasis is carefully orchestrated to store glucose when it is abundant and to mobilize those stores as required. Hepatic glycogen storage during the absorptive period is followed by the controlled release of that glycogen in the postabsorptive period, with a gradually increasing contribution from gluconeogenesis; the synthesis of glucose from non-carbohydrate precursors.

Traditionally, the only two organs believed to be capable of gluconeogenesis have been the liver and the kidney, with the latter being of importance only at times of metabolic acidosis and during prolonged starvation (when hepatic gluconeogenesis has decreased considerably). Occasionally, the presence of gluconeogenesis in other tissues has been suggested, and recently extensive work has been published claiming such a role for the small intestine during starvation and insulin-dependent

diabetes.<sup>1–4</sup> The presence of intestinal gluconeogenesis would have profound consequences on how we view glucose homeostasis, but the evidence is far from unequivocal and there are alternative explanations for the presence of gluconeogenic enzymes in the small intestine.

The major substrates for gluconeogenesis are lactate and pyruvate, glycerol, and most of the common amino acids (lysine and leucine being exceptions). In addition, propionate can be converted to glucose, but since the only source is fermentation of dietary carbohydrate in the colon, which is not likely to be important in the starved state, it will not be considered further in this discussion. For a cell to carry out gluconeogenesis, it must express some or all of four key enzymes to bypass the irreversible steps of glycolysis (Figure 1):

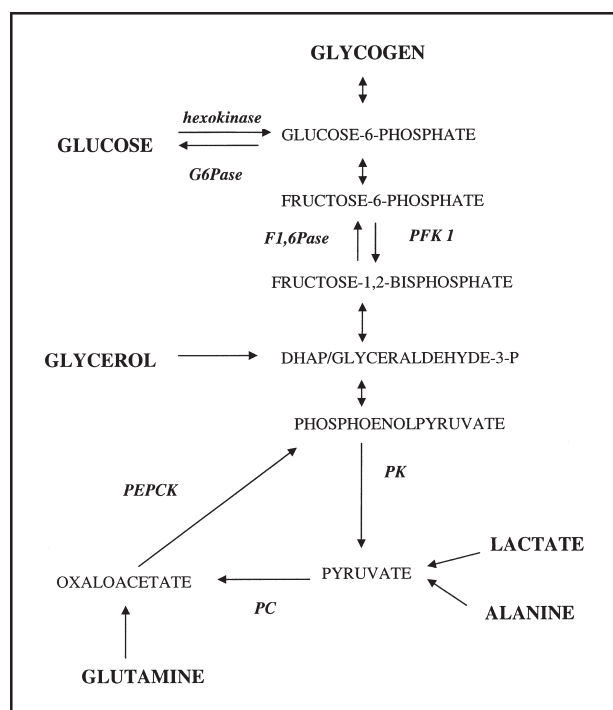
- Glucose 6-phosphatase (G6Pase) to bypass hexokinase (HK),
- Fructose 1,6-bisphosphatase (F1,6Pase) to bypass phosphofructokinase 1 (PFK 1), and
- Pyruvate carboxylase (PC) together with phosphoenolpyruvate carboxykinase (PEPCK) to bypass pyruvate kinase (PK).

There is no doubt that some of these enzymes are present in non-hepatic/non-renal tissues, but since one or more is usually lacking, such tissues have not been considered to be capable of gluconeogenesis. Examples include the high levels of F1,6Pase in some skeletal muscles, which are believed to play a role, through substrate cycling, in the regulation of glycolysis,<sup>5</sup> and the presence of PEPCK in adipose tissue, where it functions in glyceroneogenesis.<sup>6</sup> The mucosa of the small intestine appears to be an exception in that it clearly expresses all four activities at relatively high levels during the neonatal period in the rat, rabbit, and mouse.<sup>7–13</sup> This has led to proposals that this organ could be a site of gluconeogenesis in the newborn, but since expression of key gluconeogenic enzymes decreases to very low levels after weaning, intestinal gluconeogenesis has not been considered to be of physiological significance in the adult. Recently, however, Mithieux et al.<sup>1–4</sup> have argued that there is sufficient expression of all gluconeogenic enzymes in the rat during starvation or streptozotocin diabetes for this organ to be a significant site of glucone-

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**Figure 1.** Gluconeogenesis. For clarity, only key enzymes are shown, substrates (glutamine, lactate, alanine, and glycerol) are shown in bold and the relationship to glycogen is illustrated as a double-headed arrow. For detailed explanation, see text.

ogenesis, and using tracer methodology they claim that intestinal glucose release may represent between 19% and 35% of total body glucose production under such conditions.<sup>1,3</sup>

It has long been recognized that the four enzymes of gluconeogenesis are present in the small intestinal mucosa during the neonatal period, but most reports show a dramatic decline after weaning.<sup>1,3,4,7-15</sup> F1,6Pase has received little attention, but does appear to be expressed in the intestine of the adult rat, as does PC. Most attention has focused on the expression of G6Pase and PEPCK, where, again, the highest levels of expression have been reported for the neonatal period. In the rat, expression of G6Pase has been shown to be increased in adult intestine in response to both starvation and streptozotocin diabetes (a model of type 1 diabetes).<sup>3,4,14</sup> Similarly, both starvation and streptozotocin diabetes result in an increase in intestinal PEPCK mRNA abundance and activity,<sup>1,3,12,14,15</sup> although the magnitude of the changes may depend on how the results are expressed or even on which species is studied.<sup>7</sup> The mucosa of the small intestine is subject to rapid turnover, and the amount of mucosa, especially when expressed relative to submucosal tissue or body weight, can change relatively quickly with the physiological and pathological state.<sup>16,17</sup> For example, starvation results in a decrease in mucosal mass, while diabetes is accompanied by a massive increase in both the mass and cellularity of

mucosal layers and a decrease in the mass of submucosal layers. Therefore, it is difficult to know exactly what changes in intestinal enzyme activities mean in terms of the whole animal. The very low levels of expression in fed adult intestine, however, has usually meant that the idea of gluconeogenesis in the intestine has been dismissed, and any changes in activities in starvation or diabetes usually discounted as simply adaptive in nature (see comments on glutaminase below).

Mithieux<sup>1-4</sup> proposed that the changes in enzyme activity seen in the small intestine during starvation and diabetes could be sufficient to allow flux through gluconeogenesis, and proceeded to try to quantify intestinal glucose production by a combination of arteriovenous difference measurements and isotope dilution across the mesenteric drained viscera. Since the small intestine contains cells that utilize glucose, it is very difficult to detect glucose output or uptake as changes in glucose concentration across the organ, particularly when small arteriovenous differences are multiplied by a relatively large blood flow. To overcome such limitations, this group infused [ $3\text{-}^3\text{H}$ ] glucose to steady state and then determined glucose specific activity in the artery and mesenteric vein.<sup>1,3</sup> If there was no intestinal production of glucose, then the specific activity in the two vessels would be the same, although the total amount of radioactivity may be lower in the vein, reflecting glucose utilization by the tissue. If, however, the tissue was producing new glucose, then this would not be radiolabeled and thus the specific activity of the glucose in the vein would be lower than that in the artery. In theory, an increase in specific activity across the tissue is impossible, since this would mean either synthesis of radiolabeled glucose or isotopic selective utilization of non-radioactive glucose. In such experiments, some animals do yield increases in specific activity, and these investigators (to their credit) include such values in their analyses, recognizing that this is representative of experimental error (just as some of the lower values are due to experimental error).

The results of such experiments are summarized in Table 1. In the post-absorptive (6 h after last food) state, the glucose concentration in the vein is lower than in the artery, indicating a net glucose utilization, but there is no difference in the specific activity of the glucose in the two vessels, and the net contribution of the small intestine to endogenous glucose synthesis is clearly not different from zero.<sup>1</sup> Similar results were found for 24-hour-starved animals.<sup>3</sup> In 48-hour<sup>1</sup> and 72-hour-starved<sup>3</sup> animals and in those rendered insulin-dependent diabetic (and sampled 6 hour after the last food),<sup>1</sup> the specific activities were lower in the vein than in the artery (although there were no differences in glucose concentration across the small intestine).

**Table 1.** Gluconeogenesis in Rat Small Intestine in Vivo<sup>1,3</sup>

Study	Glucose Concentration mM		Specific Activity dpm/μmol		IGR μmol/kg/min	EGP
	Artery	Vein	Artery	Vein		
Croset et al., 2001 <sup>1</sup>						
Control ( <i>n</i> = 6)	10.29 ± 0.58	9.49 ± 0.38*	20,715 ± 1357	20,366 ± 1045	−2.9 ± 2.4	75.3 ± 6.0
48-h ( <i>n</i> = 8)	7.79 ± 0.33	7.43 ± 0.28	20,903 ± 938	19,942 ± 780 <sup>†</sup>	8.8 ± 1.5	41.3 ± 1.0
Diabetes ( <i>n</i> = 10)	30.76 ± 1.42	31.03 ± 1.39	13,646 ± 946	13,464 ± 470 <sup>‡</sup>	22.4 ± 9.7	119 ± 5
Mithieux et al., 2004 <sup>3</sup>						
24-h ( <i>n</i> = 5)	8.2 ± 2.2	7.6 ± 0.5	17,225 ± 1096	17,691 ± 1115	−4.4 ± 3.0	39.3 ± 2.6
72-h ( <i>n</i> = 5)	9.1 ± 0.8	9.1 ± 0.8	20,227 ± 2568	18,630 ± 2264 <sup>†</sup>	16.9 ± 4.0	48.0 ± 3.6

IGR = intestinal glucose release; EGP = endogenous glucose production.

Results are means  $\pm$  SE. Control and diabetic animals were sampled 6 hours after the last meal. All other animals were starved for the times indicated. Venous values with a superscript were reported to show a statistically significant difference from the arterial values: \**p* < 0.02; <sup>†</sup>*p* < 0.01; <sup>‡</sup>*p* < 0.001.

Unfortunately, is difficult to evaluate these data because, although the authors claim statistical significance as low as *p* < 0.001 (Table 1), the very large variation and small number of animals raises doubts about exactly what data were analyzed or how. The important parameters are the individual differences for each animal, but such values are not shown and neither is it reported if such values are significantly different from zero, the usual test in arteriovenous difference experiments. However, taking the differences as shown in Table 1, the authors conclude that the small intestine is a site of glucose production in starvation and diabetes and calculate that it contributes 21%, 35%, and 19% of endogenous glucose production in 48-hour and 72-hour starved rats and diabetic rats, respectively.

These authors also tried to identify the substrates for intestinal gluconeogenesis. In 48-hour-starved and diabetic animals, there was evidence of glutamine and glycerol carbon incorporation into glucose across the small intestine, but no evidence of such for lactate or alanine.<sup>1</sup> By infusing U-<sup>14</sup>C glutamine to steady state, they reported that the arterial and venous glucose specific activities in 48-hour-starved animals were 669  $\pm$  36 and 692  $\pm$  35 dpm/ $\mu$ mol glucose, respectively. From this they calculated a precursor to glucose rate of 4.8  $\pm$  5.0  $\mu$ mol/min/kg body weight. Although this value does not appear to be significantly different from zero, the mean represents 57% of total intestinal glucose release.

Windmueller and Spaeth<sup>18,19</sup> also identified glucose as a product of intestinal glutamine and aspartate metabolism in vivo, but it represented a very small proportion (<5%) of the glutamine carbon. Experiments with labeled glutamine in diabetic animals yielded values of 45% of intestinal glucose output arising from glutamine, but the results also suggest that intestinal glycogen breakdown may be a source of some of the glucose appearance in the diabetic state.<sup>1</sup> Another intriguing finding is that the dilution of glucose specific activity in starved animals is abolished by insulin infusion.<sup>1</sup> This is

difficult to interpret with regard to gluconeogenesis, but it would be in agreement with a rapid inhibition of glycogenolysis if glycogen were the source of the unlabeled glucose. Interestingly, Hahn and Wei-Ning<sup>10</sup> reported incorporation of lactate carbon into glucose in neonatal rat intestine in vitro, but also documented considerable intestinal glycogen stores after weaning. Therefore, it will be important to quantify glycogen metabolism in the small intestine in both diabetic and non-diabetic conditions. Similarly, labeled precursor experiments with post-absorptive animals (where no gluconeogenesis is expected) have not been reported. If such animals showed incorporation of glutamine carbon into glucose, then the glucose label dilution or the precursor incorporation data would contradict each other, raising doubts about their validity.

Since the tracer data are subject to very large variations and involve multiplication of very small differences by large values (especially since intestinal blood flow rates double in diabetic animals), it is difficult to draw definitive conclusions about the gluconeogenic role of the small intestine. There is, however, additional evidence for the concept of intestinal gluconeogenesis. The changes in enzyme activities—G6Pase and PEPCK increase with starvation and diabetes—are in the correct direction, and although PC decreases with starvation and diabetes,<sup>3</sup> this also fits, since there is no need for PC if glutamine were the major substrate (Figure 1). In addition, when calculations for gluconeogenesis and lactate production (assuming two lactates per glucose) are made on the basis of carbon balance, the rates are similar to those obtained with tracers.<sup>1</sup> In this regard, the estimates of gluconeogenesis are probably low, since some of the lactate would be derived from glutamine.<sup>20</sup>

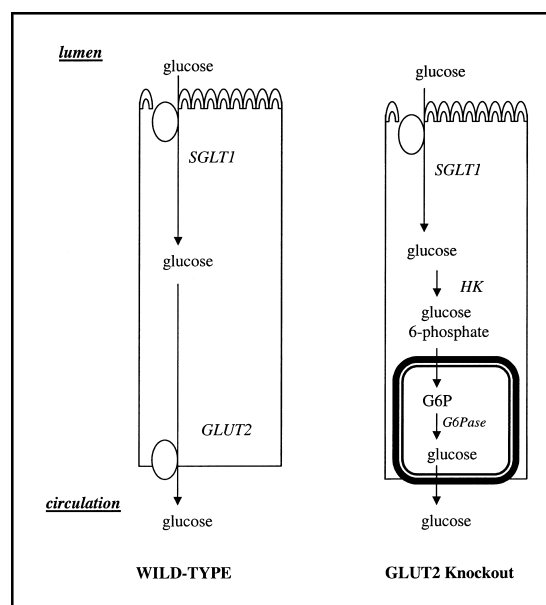
Thus, although none of the data are unequivocal, the fact that they all tend to point in the same direction is probably the best evidence to suggest that the small intestine may be a site of gluconeogenesis. A number of key experiments could potentially provide more defini-

tive evidence. The conversion of glutamine carbon to three-carbon compounds (pyruvate, lactate, alanine) for complete oxidation requires an enzyme capable of taking a four-carbon unit (malate, oxaloacetate, aspartate) from the citric acid cycle and converting it to a three-carbon compound (pyruvate or PEP).<sup>2,4,20-22</sup> Numerous experiments have ruled out PEPCK for this purpose, since the level of activity is low in adult animals and 3-mercaptopicolinate (3MPA), a reasonably specific inhibitor of PEPCK, has little effect on metabolism.<sup>20-22</sup> Thus, it would be a very important experiment to determine if 3-MPA blocks the isotopic dilution of glucose across mesenteric drained viscera.

Similarly, although there is evidence that glutamine carbon is incorporated into glucose across the intestine in starvation and diabetes, it would be important to show that this does not occur in the post-absorptive state, where glucose tracer dilution does not indicate active gluconeogenesis. In addition, although intestinal glutaminase activity increases during diabetes,<sup>3,16,17,23</sup> this is due to an increase in size of the organ, not to a change in glutaminase per cell. Furthermore, despite this increase, there is strong evidence that the intestine shuts down glutamine utilization under such conditions.<sup>16,17,23,24</sup> Such findings emphasize that changes in enzyme activity are often adaptive in nature and are not always accompanied by changes in flux. Therefore, changes seen in intestinal PEPCK and G6Pase with starvation and diabetes may be of no metabolic significance.

In summary, the evidence for intestinal gluconeogenesis in adult animals is tentative at best. The induction of key enzymes during starvation and diabetes, together with some evidence of isotope dilution, make it a possibility in the adult, but it seems more likely to be of significance in the neonate. The low level of enzymes raises the question of insufficient enzymatic capacity, but non-gluconeogenic purposes for gluconeogenic enzymes may also explain the presence of some of the enzymes. For example, PEPCK may be involved in glyceroneogenesis,<sup>6</sup> and PC plays an important role in lipid metabolism. However, if the intestine is capable of releasing free glucose from glycogen, this would require (and explain) the presence of G6Pase (Figure 1).

Another, novel function of the enzyme in the intestine has come to light from experiments with the intestinal GLUT2 knockout mouse.<sup>25</sup> In wild-type animals, dietary carbohydrate is absorbed into the body as glucose via the SGLT1 (sodium-linked glucose transporter) on the brush border of the enterocyte, and then passes across the basolateral membrane into the blood via the facilitative GLUT 2 transporter (Figure 2). In the GLUT 2 knockout mouse, however, glucose absorption appears to occur without problems and without up-regulation of other glucose transporters. When investigated, this glu-



**Figure 2.** Role of glucose-6-phosphatase in glucose absorption in the intestinal GLUT2 knockout mouse.<sup>25</sup> In wild-type animals, glucose from the intestinal lumen enters the enterocyte via the SGLT1 (sodium-linked glucose transporter 1) on the brush border and exits to the circulation on the GLUT2 facilitative glucose transporter. In the absence of GLUT2 (GLUT2 knockout) the glucose is phosphorylated by hexokinase (HK) and then enters the endoplasmic reticulum (on a specific G6P transporter), where it is hydrolyzed by glucose-6-phosphatase (G6Pase), with the free glucose being released to the circulation.

cose transport was found to require phosphorylation of the glucose with subsequent transport into the endoplasmic reticulum, followed by dephosphorylation by G6Pase and glucose release out of the cell (as normally occurs in hepatic gluconeogenesis and glycogenolysis). Clearly, this mechanism did not evolve to deal with GLUT2-deficient conditions, but these findings do raise the possibility that intestinal G6Pase plays a role in physiological glucose absorption.

Intestinal gluconeogenesis is far from proven. The best argument for its existence is the “preponderance of the evidence,” but the very large variations seen preclude any more definitive claims. Finally, it should be noted that even if the small intestine does produce new glucose, it is simultaneously utilizing glucose and thus is not, in a net sense, a source of glucose for other tissues.

## NOTE ADDED IN PROOF

After the preparation of this article, a further publication<sup>26</sup> showed changes in PEPCK and G6Pase expression in rat small intestine during starvation and refeeding, but G6Pase activity and protein levels did not always change in the same direction, and gluconeogenic flux was not



measured. In addition, stable isotope studies showing a lack of intestinal gluconeogenesis in the piglet were reported at a recent symposium.<sup>27</sup>

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