Quantitative aspects of glucose and glutamine metabolism by intestinal cells

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Abstract

Gut fuel utilisation has several unique features. Arterial and luminal fuels provide nutrition for the enterocyte, the former being of more importance. This factor, and the heterogeneity of cell types within the gut makes it difficult to define its fuel utilisation. Metabolic control logic suggests that modulation of the maximal activity of any pathway resides in those enzymes that operate *in vivo* at rates far below their maximal capacity and that catalyse non-equilibrium reactions. On this basis, although enterocyte hexokinase activity is much higher than in other 'glycolytic' cells (for example, brain), potentially high rates of glucose utilisation are modulated by substrate cycling of glucose 6-phosphate back to glucose through glucose 6-phosphatase. Glutamine metabolism proceeds by glutaminase to produce glutamate, which may then be transaminated (aspartate-aminotransferase and alanine-amino transferase) to produce α -ketoglutarate, alanine, and aspartate. The end products of glutamine metabolism by incubated gut preparations *in vitro* (mainly alanine), suggests that enterocytes, not immune cells, are responsible for most gut glutamine metabolism. High flux rates of glucose and glutamine metabolism in the enterocyte may result from the need for *de novo* synthesis of purines and pyrimidines and ribose sugars for nucleic acid synthesis. Sepsis reduces rates of glucose and glutamine metabolism, perhaps to preserve the increased consumption of these fuels by activated lymphocytes and macrophages in the gut wall.

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In this paper, aspects of fuel utilisation by the small intestine are compared with those in other tissues, in an attempt to raise important questions concerning the nutrition of the gut. Firstly, the interrelation between glucose and glutamine utilisation by the intestine is discussed. The role of enterocytes as contributors to the whole small intestinal glutamine metabolism has been established from the metabolic characteristics of enterocytes compared with those of other cells that constitute the intestinal mucosa (for example, lymphocytes). In addition, a hypothesis for the regulation of glucose metabolism in the intestine and its possible significance for the whole animal is put forward. Secondly, the effects of sepsis on intestinal metabolism are briefly discussed.

Fuel utilisation in the intestine

Fuels are required to provide energy for the cell. According to conditions, various fuels (glucose, triglycerides, fatty acids, ketone bodies, and amino acids) are available both in the bloodstream and in the intestinal lumen. As fuels can be supplied to the intestine from the vascular bed or from the lumen, the physiological significance of the substrate will be different. In general, substrates absorbed from the lumen are eventually released into the blood (as parent compound or metabolites) and distributed to other tissues. In contrast, the utilisation of substrates from blood is probably followed by an extensive metabolism in the intestine. Even if substrates from the lumen can be used to provide energy, the intermittency of alimentation will still require an adequate vascular energy supply, especially between meals. Therefore a distinction must be made between the terms 'substrate uptake' and 'substrate utilisation'. The first refers to the disappearance of the substrate from the medium (for example, vascular or luminal perfusates), whereas the second refers to the difference between the amount of substrate absorbed – that is, from the lumen – by the intestine and that which is released on the other side – that is, into the blood. This difference is not always made clear – possibly because it is not easy to distinguish between the two.¹ Two important questions, therefore, are which fuels are used by the intestine, and whether the pattern of fuel utilisation or the rate of utilisation, or both depend on the site of uptake of the fuel? Unfortunately, available data do not permit complete answers to these questions, but it is nevertheless necessary to understand how enzyme activities in the test tube can provide useful information about fuel utilisation *in vivo*.

Use of maximum activities of enzymes as quantitative indices of maximum flux through metabolic pathways

The theory underlying the use of maximal enzyme activities to show the maximum capacity for fluxes in biochemical systems has been described elsewhere.¹ Enzymes can be classified as to whether they catalyse reactions far removed from equilibrium (non-equilibrium) or near equilibrium. The advantage of monitoring a near equilibrium reaction in a metabolic pathway *in vivo* is that the reaction may be very sensitive to small changes in concentrations of cosubstrate or coproduct. Consequently, large changes in flux can be transmitted through such a reaction without any requirement for complex regulatory

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properties. In general, the activity of the enzyme can be measured comparatively easily in crude tissue extracts; the convenience of this assay has, unfortunately, been used by some investigators as the sole criterion for selecting an enzyme to study the maximum flux through a metabolic pathway. This cannot be done. Metabolic control logic tells us that these enzymes cannot be used as quantitative indices of flux¹ but despite this they are still being used even in the 1990s!

Enzymes that catalyse non-equilibrium reactions in a metabolic pathway provide directionality in that pathway and are usually subject to allosteric control. Indeed, the control mechanisms may be complex. Thus, knowledge of such control mechanisms must be available before a satisfactory assay for measurement of the maximum activity can be developed; hence, knowledge of metabolic control is necessary to permit maximum enzyme activity to be assayed adequately in tissue extracts.

There are at least two conditions that must be satisfied before enzyme activity can be used to provide quantitative information about maximum rates of fuel utilisation in vivo. Firstly, it is necessary to establish the reactions in the pathway that catalyse non-equilibrium reactions (see above). Secondly, it is necessary to show experimentally that the maximum activities of such enzymes in vitro can be used to show quantitatively the maximum flux through the pathway.

Systematic studies in the 1970s examined the maximum activities of key enzymes of carbohydrate and fat metabolism in muscle. Information was gleaned on the types of fuel used by different muscles and their maximum contribution to energy formation to support contractile activity. This information permitted a systematic and comprehensive analysis to be made of the fuels used by different muscles from different animals across the animal kingdom.^{1,2}

More recently, a similar approach has been applied to lymphocytes, macrophages, and endothelial cells. These studies have provided,

TABLE II Maximal flux through glycolysis from glucose and maximal activities of hexokinase in various tissues

Preparation	Glycolytic flux	Hexokinase flux
Enterocytes	602	9.8
Lymphocytes	37.2	28.2
Murine macrophage	1050	10.4
Colonocytes	414	8.2

for the first time, evidence that such cells can use glutamine or long chain fatty acids, or both, for energy formation and, indeed, that these fuels could be quantitatively more important than glucose.³⁻⁵ (It was previously believed that glucose was the main, if not the only, fuel to be used by lymphocytes.) In addition, such studies have provided quantitative information on the rates of energy production from different fuels for macrophages in culture.⁶ This 'maximal enzyme activity approach' has been used for the small intestine.

Glucose metabolism in the small intestine

Hexokinase activity in the mucosa of the small intestine is lower than that of other glycolytic enzymes (Table I). Its activity is, however, more than 10-fold greater than the reported maximal flux of glucose through glycolysis (Table II). This suggests either that the study conditions were not conducive to glycolysis or that the flux through this enzyme is normally under considerable inhibitory control within the enterocyte (see below).

If it is accepted that hexokinase activity points to the maximum flux through glycolysis from glucose in the enterocytes, (as is the case in muscle and brain), it is clear that the intestine's maximal capacity to use glucose is greater than that of the brain, and yet glucose is known to be an obligatory and important fuel for the brain (Table III). This is of considerable importance, because the hexokinase activity in the intestinal cells is sufficient to use almost all glucose consumed in a normal rat diet (assuming 60% of the energy of the diet is carbohydrate).

A mechanism must, therefore, exist to decrease the rate of glucose utilisation within the enterocyte. One possible mechanism is the activity of glucose-6-phosphatase; its maximum activity is similar to that of hexokinase in the fed state (Table I). This mechanism would require that both enzyme activities occur in the same cell and that both enzymes are simultaneously catalytically active. This would then provide an example of a substrate cycle (the glucose/glucose-6-phosphate cycle), which occurs when a non-equilibrium reaction proceeds in both the forward and reverse directions of the pathway simultaneously. This cycle is considered to play an important part in the control of glucose utilisation by the liver.² The cycle would demand a considerable expenditure of energy, which might account, in part, for some of the thermic effect of food. The intriguing question is, however, why a substrate cycle should restrict glucose utilisation in this tissue. The answer is not known.

The presence of a cycle in the enterocytes would account for the findings of several

TABLE I Effects of endotoxin treatment on activities of enzymes of glucose metabolism

Enzyme	Conditions	Enzyme activities	
		$\mu\text{mol/min}^{-1}/\text{g}^{-1}$ fresh wt	$\text{nmol/min}^{-1}/\text{mg}^{-1}$ protein
Hexokinase	Fed	11.0	65.3
	Starved	8.4*	48.9*
	Septic	6.2*	47.5*
Glucose-6-phosphatase	Fed	9.9	63.1
	Starved	15.1*	90.3*
	Sepsis	9.0	69.6
Phosphofructokinase	Fed	12.3	72.7
	Starved	9.9	57.3*
	Septic	8.0*	57.7*
Lactate dehydrogenase	Fed	102	580
	Starved	95.6	560
	Septic	81.7	585
Pyruvate kinase	Fed	23.2	122.7
	Starved	18.1*	101.3
	Septic	13.5*	96.2
Pyruvate dehydrogenase	Fed	0.28	1.62
	Starved	0.19*	1.18*
	Sepsis	0.39*	2.81*
Pyruvate carboxylase	Fed	0.41	2.2
	Starved	0.43	2.6
	Sepsis	0.26	2.0

Results are presented as means. The temperature of assay was 25°C except for glucose-6-phosphatase (30°C). The statistical difference (Student's *t* test) of the difference in enzyme activities in the intestine of septic animals – with that of fed animals is shown by * (*p* < 0.05).

TABLE III Maximal capacity for glucose utilisation in various rat tissues

Tissues	Hexokinase activity	
	$\mu\text{mol/min/g}$	$\mu\text{mol/min/rat}$
Intestine	11.0	33
Brain	8.7	21.2
Kidney	2.1	4.2

workers that only a very small proportion of the glucose taken up from the lumen of the intestine is metabolised despite the very high hexokinase activity in these cells.^{7,8} Similar low rates of glucose utilisation have been shown for humans.⁹

These findings also suggest that glucose is not normally an important fuel for energy generation in the intestine. The fact that isolated incubated enterocytes or the perfused intestine, however, can take up and use glucose at a high rate does suggest that, under some conditions, glucose may become an important fuel. Unfortunately, the way in which the activities of hexokinase and glucose 6-phosphatase are controlled in the intestine is not known.

Hypothesis for the control of glucose metabolism during starvation

Starvation decreases the glucose metabolism rate in tissues, in several of which an important mechanism for the regulation of glucose utilisation is the glucose/fatty acids/ketone body cycle.² Fatty acid or ketone body oxidation, or both, raises the intracellular concentration of citrate and acetyl CoA, and this inhibits phosphofructokinase and pyruvate dehydrogenase, respectively. This is, however, probably not the mechanism in the small intestine because the rate of glucose utilisation by the vascularly perfused intestine *in vitro* is unchanged by the utilisation of ketone bodies.¹⁰ Furthermore, the concentrations of ATP, citrate, and glucose-6-phosphate in the intestinal mucosa are not increased during starvation, so these metabolites probably control the glycolytic flux. Hence, an alternative mechanism for the regulation of glucose metabolism in the intestine has to be found. Hexokinase and glucose-6-phosphatase are present in the intestine of fed rats at similar activities, but in the starved state, hexokinase activity decreases, whereas glucose-6-phosphatase

TABLE V Rates of utilisation of glutamine by various *in vitro* cell preparations of the rat and mouse

Type of cell	Rate of utilisation ($\mu\text{mol/h/g dry wt}$)
Rat enterocytes	220
Rat colonocytes	730
Rat lymphocytes	331
Rat thymocytes	160
Murine macrophages	186

activity increases. In addition, in starvation, the concentration of glucose-6-phosphate in the intestine is considerably decreased. This suggests that the control of glycolysis occurs at an earlier step than that catalysed by phosphofructokinase, that is, at the step of glucose phosphorylation by the glucose/glucose-6-phosphate cycle. The existence of such a cycle in the small intestine has not, however, been investigated in any detail.

Glutamine utilisation

The first reaction seen in glutamine degradation is catalysed by a phosphate dependent glutaminase (a mitochondrial enzyme hereafter referred to as glutaminase). Glutaminase's activity in the intestine is higher than that in most other tissues (Table IV).

Glutamine could also be degraded to glutamate by a reaction catalysed by a glutamine aminotransferase, but this enzyme has much lower activity than that of glutaminase in the intestine, suggesting that most glutamine is converted by glutaminase.

The activity of glutamate dehydrogenase in intestinal mucosa is low compared with that found in the kidney; this, together with the comparatively high aspartate and alanine aminotransferase activities, suggests that glutamate is metabolised by an aminotransferase rather than by glutamate dehydrogenase. Indeed, the important end products of glutamine metabolism in all intestinal preparations are alanine and CO₂. Such high decarboxylation rates of glutamine may occur if 2-oxoglutarate is first converted to pyruvate, if pyruvate is then converted to acetyl CoA, and subsequently decarboxylated by the reactions of the Krebs' cycle.

The intestinal mucosa contains various cell types (for example, enterocytes and immune cells). As immune cells (lymphocytes and macrophages) are known to use glutamine at a high rate (see below), how do enterocytes, lymphocytes, and macrophages contribute to the high rate of glutamine uptake by the small intestine? Unfortunately, the exact proportion of lymphocytes and enterocytes in the intestinal mucosa is not yet firmly established. As much as 25% of the mucosal mass may be lymphoid tissue, and 80% of the intestinal epithelium may be comprised of enterocytes. Hence, it can be assumed that enterocytes contribute most to the 75% of the mucosal mass that is non-lymphoid. The rate of glutamine utilisation by isolated lymphocytes is similar to that of enterocytes (Table V).⁵ Hence, the whole intestinal glutamine metabolism could be attributed to that of the enterocytes plus lymphocytes.

TABLE IV Effects of sepsis on glutaminase activity in rat tissue

Tissue	Nutritional state	Enzyme activities	
		$\mu\text{mol/min}^{-1}/\text{g fresh wt}$	$\text{nmol/min}^{-1}/\text{mg protein}$
Intestinal mucosa	Fed	12.9	81.0
	Starved	9.4*	59.3*
	Septic	7.1*	52.1*
Mesenteric lymph node	Fed	3.3	18.9
	Starved	4.4	23.8*
	Septic	5.1*	28.2*
Kidney	Fed	27.4	172
	Starved	25.7	163
	Septic	29.5	188
Heart	Fed	0.95	4.7
	Starved	0.7	3.6*
	Septic	0.69*	3.2*

Results are presented as means. The temperature of assay was 37°C. The statistical difference (Student's *t* test) between septic, starved, and fed rats is shown by * (*p* < 0.05).

An important difference between metabolism of glutamine by isolated enterocytes and that by lymphocytes is the nitrogen containing end products of metabolism. Isolated preparations of enterocytes produce alanine, whereas lymphocytes produce aspartate. This suggests that the enterocytes rather than immune cells are responsible for much of the glutamine metabolism in isolated preparations of the intestinal mucosa.

Glutamine utilisation in lymphocytes (and macrophages) provides not only energy but also metabolic intermediates necessary for biosynthetic pathways. The very high rate of glutaminolysis in lymphocytes (and macrophages), however, may provide optimal conditions for controlling the rate of biosynthetic processes at crucial times. This permits increased macromolecular synthesis at precise times during the cell cycle, such as when lymphocytes respond to an immunological challenge.^{11 12} Enterocytes isolated in this work are not believed to be dividing, nor secreting any products (as macrophages would be). The question arises, therefore, why enterocytes use glutamine at such a high rate. Perhaps it provides energy, which might be required, for example, for active transport or for maintaining the integrity of the intestinal wall. Thus, intestinal utilisation of glutamine may 'spare' glucose metabolism. Glucose absorbed luminally by the enterocytes, then, is not metabolised to any large extent, but is released into the blood to maintain the blood glucose concentration available to other tissues (for example brain).^{13 14} In this way, the intestine could be considered to play an important part in regulation of the blood glucose concentration. This could be of considerable importance in a number of conditions, but especially in diabetes mellitus. This may be an important area for new research.

Effects of sepsis

Intestinal fuel utilisation may be changed by starvation, injury, surgery, and sepsis. Sepsis (by endotoxin injection) affects both glucose and glutamine metabolism.¹⁵

GLUCOSE METABOLISM

The maximal *in vitro* activities of some enzymes participating in glucose metabolism were measured in intestinal mucosal extracts. Sepsis decreased the maximal activities of all the glycolytic enzymes; this reduction of activities was quantitatively similar to that of starvation. One important difference is that, in contrast with starvation, sepsis had no effect on the maximal activity of glucose-6-phosphatase (Table I). This is of considerable interest in sepsis, as the activity of pyruvate dehydrogenase was increased by 73% compared with the normal (fed) state, and by 138% compared with the starved state.

The decrease in the maximal activities of the glycolytic enzymes suggests a reduced ability of the small intestine to utilise glucose in

response to sepsis, as has been shown in incubated enterocytes that decreased their rate of glucose utilisation by 26%. The proportion of glucose that is metabolised to lactate, pyruvate, and alanine is, however, unchanged in sepsis.

Although rates of glucose utilisation by isolated enterocytes are decreased in response to sepsis, rates of pyruvate decarboxylation are increased. Hence, 'efficiency' of energy generation from glucose or glutamine may improve under septic conditions.

GLUTAMINE METABOLISM

Intestinal glutaminase activity decreases in sepsis, whereas that in lymphocytes increases (Table IV). It is possible that decreased glutaminase intestinal activity and glutamine uptake together ensure adequate glutamine supply for the immune cells, whose number and activity would be increased under sepsis. Under these conditions, however, hexokinase activity in the intestine also decreased, but the rate of ¹⁴CO₂ production from [¹⁴CU]-glucose was increased compared with that in the fed state. Hence, the decreased rate of glutamine utilisation could instead 'allow' more glucose to be oxidised to provide energy. These increased rates of ¹⁴CO₂ production from the radiolabelled substrates (glucose and glutamine) in sepsis were paralleled by increased pyruvate dehydrogenase and oxoglutarate dehydrogenase activities. The mechanism by which these changes occur is unknown but it suggests that investigation of the control of pyruvate metabolism in enterocyte mitochondria may be a fruitful line of research.

Rates of glutamine utilisation and rates of glutamate and alanine production by isolated enterocytes are decreased in response to sepsis.¹⁶ These changes, together with the decrease in glutaminase activity, support the view that sepsis may decrease the rate of glutamine utilisation by the small intestine *in vivo*. This change in the rate of glutamine utilisation in sepsis is in contrast with that seen in thermally injured rats which increase the rate of glutamine utilisation.¹⁷ This increased rate may subsequently decrease glucose utilisation locally to make more glucose available for repair of the burn wound, which requires high rates of glucose utilisation.

Changes in the rate of glutamine utilisation by the small intestine in sepsis may permit more glutamine to be available to the cells participating in the immune process, because the glutamine requirement of those cells will probably increase during sepsis. Fluctuations in intestinal glutamine utilisation may provide precise control of some biosynthetic pathways in immune cells.^{11 12} Sepsis is also characterised by a decreased rate of glucose utilisation by the intestine, and as both high rates of glutaminolysis and glycolysis are required by cells of the immune system, blood glucose may also be directed preferentially to these cells. The question arises as to why the intestine, paradoxically, has a reduced ability to utilise glutamine during sepsis despite the rich lymphoid population of the mucosa. It is

possible that overall glutamine utilisation in the intestine during sepsis is the net result of a decreased rate of glutamine utilisation by enterocytes, which exceeds the increased rate of utilisation by the immune cells of the intestine.

- 1 Newsholme EA, Crabtree B, Newsholme P. Use of enzyme activities as indices of maximum rates of fuel utilization. *Ciba Found Symp* 1980; **73**: 245–58.
- 2 Newsholme EA, Leech AR. *Biochemistry for the medical sciences* New York: John Wiley, 1983.
- 3 Ardawi MSM, Newsholme EA. Metabolism in lymphocytes and its importance to the immune response. *Essays Biochem* 1985; **21**: 1–44.
- 4 Ardawi MSM, Newsholme EA. Maximum activities of some enzymes of glycolysis, the tricarboxylic acid cycle and glutamine utilization pathways in lymphocytes of the rat. *Biochem J* 1982; **208**: 743–9.
- 5 Ardawi MSM, Newsholme EA. Glutamine metabolism in lymphocytes of the rat. *Biochem J* 1983; **212**: 835–42.
- 6 Newsholme P, Newsholme EA. Rates of utilization of glucose, glutamine and oleate and formation of end-products by mouse peritoneal macrophages in culture. *Biochem J* 1989; **261**: 211–8.
- 7 Windmueller HG, Spaeth AE. Respiratory fuels and nitrogen metabolism in vivo in small intestine rats. *J Biol Chem* 1980; **255**: 107–22.
- 8 Rich-Denson C, Kimure RE. Evidence in vivo that most of the intraluminally absorbed glucose is absorbed intact into the portal vein and not metabolized to lactate. *Biochem J* 1988; **254**: 931–4.
- 9 Bjorkman O, Eriksson LS, Nyberg B, Wahren J. Gut exchange of glucose and lactate in basal state and after oral glucose ingestion in post-operative patients. *Diabetes* 1990; **39**: 747–51.
- 10 Hanson PJ, Parsons DS. Factors affecting the utilization of ketone bodies and other substrates by rat jejunum: effects of fasting and of diabetes. *J Physiol (London)* 1976; **278**: 55.
- 11 Newsholme EA, Crabtree B, Ardawi MSM. The role of high rates of glycolysis and glutamine utilization in rapidly-dividing cells. *Biosci Rep* 1985; **4**: 393–400.
- 12 Szondy Z, Newsholme EA. The effect of glutamine concentration on the activity of carbamoyl-phosphate synthase II and on the incorporation of [³H]-thymidine into DNA in rat mesenteric lymphocytes stimulated by phytohaemagglutinin. *Biochem J* 1989; **261**: 979–83.
- 13 Fernandez Lopez JA, Casado J, Argiles JM, Alemany M. In the rat intestinal lymph carries a significant amount of ingested glucose into the bloodstream. *Arch Int Physiol Biochim Biophys* 1992; **100**: 231–6.
- 14 Fernandez Lopez JA, Casado J, Argiles JM, Alemany M. Intestinal handling of a glucose gavage by the rat. *Molecular Cellular Biochem* 1992; **113**: 43–53.
- 15 Parry-Billings M. *Studies on glutamine metabolism in muscle*. D Phil [Thesis], Oxford: Oxford University, 1989.
- 16 Carrié A-L. *Studies on glucose and glutamine metabolism in cells of the small intestine* [Thesis]. Oxford: Oxford University, 1989.
- 17 Ardawi MSM, Newsholme EA. Maximal activities of glutaminase and some enzymes of glycolysis and ketone body utilization and rates of utilization of glutamine glucose and ketone bodies by intestinal mucosa after burn injury. *Burns* 1987; **13**: 438–44.