The contribution of glucose and glutamine to energy metabolism in newborn pig enterocytes

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The aim of this work was to investigate glucose (2 mmol/L) and glutamine (2 mmol/L) metabolism in metabolically active enterocytes isolated from newborn pigs, as well as the contribution of these substrates to enterocyte energy metabolism. At birth or in 2-day-old suckling animals, the capacity of these cells to utilize glutamine was high (2.5 to 3.8 nmol/min. 10^6 cells), and the major products from glutamine were ammonia, glutamate, CO_2, and aspartate. Glucose decreased glutamine utilization by 25% but did not affect glutamine oxidation. The capacity of enterocytes to utilize glucose increased threefold between birth and 2 days of age (0.9 to 3.2 nmol/min. 10^6 cells). At birth, glutamine strongly reduced glucose utilization and oxidation, whereas in intestinal cells from 2-day-old suckling pigs only glucose oxidation was modified by glutamine. Oxygen consumption increased twofold in enterocytes isolated from suckling animals. Estimating potential ATP production from glutamine and glucose metabolism indicated that the complete oxidation of both substrates could contribute to 50% of total ATP turnover at both stages. In contrast, the contribution of aerobic glycolysis to ATP generation was low at birth and increased more than threefold in intestinal cells from 2-day-old suckling pigs. (J. Nutr. Biochem. 5:284–290, 1994.)

Keywords: intestine; energy; glucose; glutamine; neonate; pig

Introduction

In most mammals, birth is associated with important modifications of various physiological functions and dramatic changes in nutrition and environment.[1] The successful adaptation of neonates to these changes requires important modifications of substrate metabolism, including the metabolism of glucose and fatty acids, in various tissues.[1] Furthermore, during the perinatal period, the rates of protein turnover and accretion are very high, in contrast to the rates of amino acid catabolism, which are profoundly reduced.[2,3]

Among the organs, the gastrointestinal tract undergoes an active growth and a functional adaptation after birth.[4,5] In pigs fed normally immediately after birth, the small intestinal weight has been shown to increase by 60%, and that of the jejunal mucosa by 88% during the first day of suckling.[6,7] This rapid growth correlates with considerable variation in cellular structure[8] and apical transport of hexoses.[9]

In the adult small intestine, glucose, glutamine, and ketone bodies represent the major oxidative substrates.[10–12] In the suckling rat, glutamine oxidation was found to be high on intestinal tissue slices.[13] In contrast, glucose and 3-hydroxybutyrate oxidation are very low before weaning.[13,14] However, the nature and contribution of substrates to intestinal energy metabolism just after birth remains unknown.

Previous work on isolated enterocytes from neonatal pigs indicated a high capacity for glutamine utilization immediately after birth, which remained high during the suckling period.[15,16] Moreover the capacity for glucose utilization, which was low at birth, increased threefold in 2-day-old suckling animals.[16,17] The aim of the present study was (1) to know whether the emergence of a high capacity for glucose utilization could be modified by the presence of glutamine and (2) to assess the relative contribution of both substrates to the energy metabolism of intestinal cells.

Methods and materials

Chemicals

Fatty-acid–free albumin (bovine serum albumin, fraction V), hyaluronidase (EC.3.2.1.35), dithiothreitol, HEPES, methylbenzetho-

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nium hydroxide, D-glucose, L-glutamine, and β-D(+)glucose were purchased from Sigma Chemical (St Louis, MO USA). Perchloric acid, pyruvate, and Tris buffer were obtained from Merck (Darmstadt, Germany). EDTA and all inorganic products were from Prolabo (Paris, France). All enzymes and coenzymes used for enzymatic assays were from Boehringer (Meylan, France). D-[U-^{14}C]glucose and L-[U-^{14}C]glutamine were purchased from Amersham (Les Ulis, France). The scintillation cocktail was obtained from Pharmacia (St Quentin-en-Yvelines, France).

Animals

Newborn pigs of the Large White breed (Institut National de la Recherche Agronomique, Jouy-en-Josas, France) were used. In this strain, normal delivery occurs during the night of day 114 to 115. As precise timing of birth was desired, parturition was induced by injecting pregnant sows on day 113 of pregnancy with a prostaglandin analog (cloprostenol, 10 μg/kg body weight; Bellon, Neuilly, France.). With this technique, newborn pigs were delivered on the morning of day 114, i.e., a few hours before the normal time of delivery. Newborn pigs (0-day-old animals) were taken prior to the first suckling and enterocytes were isolated within 30 min of birth. Suckling piglets were left with their mother for 2 days in the maternity building. Thereafter, they were withdrawn from their mother and placed under a heating lamp 2 hours before enterocyte isolation. This lag period was chosen to allow a sufficient degree of jejunum emptying.

Enterocyte isolation

Piglets were anesthetized by intraperitoneal injection of thiopental (Nesdonal; Rhone Merieux, France; 25 mg/kg body weight in saline), and the jejuno-ileum was promptly excised. Villus enterocytes were isolated according to Blachier,[18] using an adaptation of a method previously described.[19] Briefly, the intestinal lumen was flushed with a solution of NaCl (155 mmol/L) and perfused (35 to 42 mL/min) for 20 min at 37°C with a Ca^{2+} and Mg^{2+} free Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 mmol/L HEPES, 5 mmol/L EDTA, 1 mmol/L dithiothreitol, and 2.5 g/L albumin, and equilibrated against a mixture of O_2-CO_2 (19:1, vol/vol). Afterwards, the intestine was gently squeezed along its length, and the luminal fluid was collected and centrifuged (150 g, 3 min). The cell fraction was subjected to an additional 15 min incubation (100 oscillations/min, 37° C and continuous gassing with O_2-CO_2, 19:1; vol/vol) using the same buffer without EDTA but containing 1.3 mmol/L $CaCl_2$, 2 mmol/L $MgCl_2$, and 0.1 g/L hyaluronidase to dissociate cell clumps. Thereafter, isolated cells were washed three times (150 g, 3 min) in an oxygenated Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 mmol/L HEPES, 1.3 mmol/L $CaCl_2$, 2 mmol/L $MgCl_2$, and 10 g/L albumin (incubation buffer).

Whatever the age of the animals, cell viability, assessed by determining the percentage of total lactate dehydrogenase (EC 1.1.1.27) activity recovered in the cell pellet, was higher than 95% before cell incubation, and higher than 85% after a 30-min incubation in the presence of glucose (2 mmol/L) or glutamine (2 mmol/L).

Incubation conditions and assay of metabolites

Incubations were carried out in 25-mL polycarbonate Erlenmeyer flasks (Nalge Company, Rochester, NY USA) containing 1 mL of cell suspension (15 to 20 × 10^6 cells) in a final volume of 4 mL incubation buffer. The flasks were gassed with O_2-CO_2 (19:1, vol/vol), sealed, and incubated in a shaking water bath in the absence or in the presence of 2 mmol/L glucose and/or 2 mmol/L glutamine (100 oscillations/min, 30 min, 37° C). Incubations were stopped by adding 0.5 mL of ice-cold perchloric acid (30% vol/vol), and metabolites were assayed in the neutralized, deproteinized supernatant by specific enzymatic methods,[20] using a UVIKON 860 recording spectrophotometer (KONTRON, Les Ulis, France). The maximal activities of alanine aminotransferase (EC 2.6.1.2.) and aspartate aminotransferase (EC 2.6.1.1.) were determined according to Sugden[21] in homogenates of enterocytes isolated from 0-day- and 2-day-old suckling piglets.

Respiratory measurements

Carbon dioxide production was determined by measuring $^{14}CO_2$ released during incubation with ^{14}C-labeled substrates (1.7 to 3.4 mBq/mmol). After the incubation had been stopped with perchloric acid, $^{14}CO_2$ was trapped with methylbenzethonium hydroxide (90 min shaking at 100 oscillations/min, room temperature), and counted in a liquid scintillation counter (LKB-Pharmacia, St Quentin-en-Yvelines, France). Blank CO_2 production rates were obtained from incubation flasks containing ^{14}C-labeled substrates without any cell suspension added.

Oxygen consumption was measured polarographically with an oxygraph (Gilson Medical Electronics model 5/6 H, Middelton, WI USA) equipped with a 2-mL water-jacketed chamber maintained at 37° C and a Clark oxygen electrode. One milliliter of cells (20 to 35 × 10^6 cells) suspended in the oxygenated incubation buffer was preincubated for at least 10 min with or without substrates in a final volume of 2 mL of the same incubation buffer. An aliquot of preincubation medium containing 7 to 8 × 10^6 cells was then transferred to the stirred chamber in the absence or in the presence of 2 mmol/L glucose and/or 2 mmol/L glutamine, and O_2 consumption was measured for 3 to 5 min. Oxygen consumption rates were found to be constant for at least 40 min (data not shown). Thus, metabolite production and O_2 consumption were performed in the same conditions of density (3.5 to 5 × 10^6 cells/mL) and substrate concentration (2 mmol/L). Blank O_2 consumption rates determined without cells were run before and after measurements of cell O_2 consumption. Oxygraph calibration was performed under identical conditions using increasing amounts of freshly dissolved β-D-glucose (12 to 60 nmol) in the presence of glucose oxidase (800 U/mL).

Calculations

Rates of glucose and glutamine utilization were calculated from the net amounts of substrates that disappeared from the incubation medium. For both substrates, rates of metabolite production were calculated from the net amounts of metabolites generated.

To evaluate the maximal contribution of a given substrate to ATP synthesis, it must be assumed that all the NADH formed during its metabolism is used in the respiratory chain to form ATP. Potential ATP production from glucose or glutamine was calculated on the basis of measured end-products, as previously reported.[22-24] It is assumed that 1 mol of pyruvate or 1 mol of lactate formed in glycolysis yields 4 and 1 mol of ATP, respectively. The complete oxidation of glucose was obtained by subtracting CO_2 production in the pentose cycle pathway from total $^{14}CO_2$ produced. CO_2 production through the pentose cycle pathway was estimated according to Larrabee;[25] it represented 12 ± 1% (n = 3) and 15 ± 1% (n = 4) of glucose utilization at 0 and 2 days, respectively. This percentage was found not to be modified by the presence of glutamine. Each mol of glucose oxidized yields 38 mol ATP. The complete oxidation of glutamine was obtained by subtracting CO_2 production associated with aspartate generation from total $^{14}CO_2$ production. The formation of 1 mol aspartate from glutamine yields 9 mol ATP and the complete oxidation of glutamine to CO_2 yields 24 mol ATP/mol glutamine. Moreover, glutamate produced from glutamine could be deaminated to α-ketoglutarate and ammonia, producing 1 NADH (i.e., 3 ATP) via glutamate dehydrogenase (GLDH) reaction. ATP formed in this pathway is calculated as indicated: [(Glutamine utilized − glutamate, aspartate, alanine produced) × 3].

In addition, the oxidative fluxes of glucose and glutamine were compared with carbon or nitrogen balances established between the amount of substrate that disappeared and the amount of metabolites produced after deduction of endogenous values. It must be emphasized that such balances represent upper limits for complete oxidation fluxes.[22]

The rate of ATP generation in isolated enterocytes was estimated from O_2 consumption (6 mol ATP per mol of O_2).

Presentation of results and statistical analysis

All results are expressed as nanomoles per minute and 10^6 viable cells, the viability being taken at the onset of incubation. The values shown are the means ± SEM for the number of cell preparations indicated. For each cell preparation, data are based on duplicate measurements. Comparison between cells isolated from 0-day- versus 2-day-old piglets was done using unpaired Student's *t* test; paired Student's *t* test was used to compare the effects of the various conditions on the same cell preparations.

Results

Substrate utilization and oxidation

As previously shown,[15] the capacity of newborn pig enterocytes to use glucose increased more than three times between birth and 2 days of suckling *(Tables 1 and 2)*. Glucose conversion into CO_2 accounted for 23% of glucose utilization at birth, and only 9% after 2 days of suckling. Glycolysis was the major metabolic pathway responsible for glucose metabolism in these cells. Lactate, pyruvate, and alanine production accounted for 63 to 68% of glucose disappearance at both stages.

Tables 1 and 2Glutamine utilization was already high at birth and increased by 54% after 2 days of suckling *(Tables 1 and 2)*. Whatever the age of the animals, glutamine conversion into CO_2 accounted for 20% of the substrate utilization. There was a net generation of ammonia, glutamate, aspartate, and to a lesser extent alanine from glutamine. Aspartate production was much higher than alanine production and accounted for 24 to 30% of glutamate metabolized. Whatever the stage, the maximal activity of aspartate amino transferase was much higher than that of alanine amino transferase: 28.1 ± 5.8 (n = 3) versus 1.0 ± 0.2 (n = 3) nmol/min 10^6 cells.

When enterocytes were incubated with both substrates, the capacity for glutamine utilization or ammonia and glutamate generation decreased slightly at both stages, whereas the capacity for glutamine oxidation remained high *(Tables 1 and 2)*. In the presence of glutamine, the capacity to produce lactate and pyruvate from glucose was decreased only in enterocytes isolated at birth *(Tables 1 and 2)*. However, glucose oxidation was significantly reduced at both stages.

Potential ATP production from glutamine and glucose

The calculated fluxes of glucose or glutamine completely oxidized were compatible with the carbon and nitrogen balances reported in *Tables 1 and 2*. At birth, the complete oxidation of glucose accounted for 72% of total ATP production of intestinal cells incubated with glucose alone *(Table 3)*. Likewise, the complete oxidation of glutamine provided 63% of total ATP production of enterocytes incubated with glutamine alone *(Table 3)*. In the presence of both substrates, ATP production from glucose and glutamine complete oxidation accounted for 65% of ATP production, the majority being provided by glutamine.

After 2 days of suckling, potential ATP production from either glucose or glutamine increased 1.5-fold to twofold as compared with prior stage. In the presence of glucose alone, glucose oxidation accounted for 45% of total ATP production. The complete oxidation of glutamine provided 70% of total ATP production. In the presence of both substrates, ATP production from substrate oxidation accounted for 54% of total ATP production, the majority again being provided by glutamine. However, the contribution of aerobic glycolysis to total

Table 1 Glucose and glutamine metabolism in enterocytes isolated from 0-day-old pigs

Substrate(s)	none	Glucose (2 mmol/L)	Glutamine (2 mmol/L)	Glucose + Glutamine (2 mmol/L)(2 mmol/L)
		nmol/(min.10^6 viable cells)		
Substrate(s) utilization				
Glucose*	—	0.89 ± 0.17 (5)	—	0.40 ± 0.11[a] (5)
Glutamine†	—	—	2.50 ± 0.32 (5)	1.85 ± 0.11[b] (5)
Metabolite generation				
Lactate	0.28 ± 0.08 (6)	1.11 ± 0.14 (5)	0.21 ± 0.05 (5)	0.49 ± 0.07[a] (5)
Pyruvate	0.10 ± 0.01 (6)	0.36 ± 0.06 (5)	0.22 ± 0.07 (5)	0.28 ± 0.02[a] (5)
Alanine	0.03 ± 0.01 (6)	0.06 ± 0.01 (5)	0.12 ± 0.03 (5)	0.17 ± 0.04[a] (5)
Ammonia	0.08 ± 0.02 (5)	—	2.52 ± 0.35 (5)	2.25 ± 0.35[b] (5)
Glutamate	0.03 ± 0.01 (5)	—	0.98 ± 0.17 (5)	0.83 ± 0.13[b] (5)
Aspartate	ND	ND	0.37 ± 0.01 (4)	0.38 ± 0.02 (4)
Glucose converted into $^{14}CO_2$	—	0.21 ± 0.03 (5)	—	0.07 ± 0.01[a] (5)
Glutamine converted into $^{14}CO_2$	—	—	0.51 ± 0.06 (5)	0.52 ± 0.06 (5)

Cells were incubated (30 min, 37° C) without substrate or in the presence of glucose, glutamine, or both substrates. Results are means ± SEM for the number of separate experiments indicated in parentheses.

[a], significantly different from glucose alone ($P < 0.05$); [b], significantly different from glutamine alone ($P < 0.05$) as analyzed by paired *t* test; ND, non detectable.

*Carbon balances (nmol/min.10^6 cells; see Methods and materials for calculation) were 0.33 for glucose alone and 0.19 in the presence of glutamine.

†Carbon balances were 0.97 for glutamine alone and 0.74 in the presence of glucose. Nitrogen balances were 1.15 for glutamine alone and 0.26 in the presence of glucose.

Table 2 Glucose and glutamine metabolism in enterocytes isolated from 2-day-old pigs

Substrate(s)	none	Glucose (2 mmol/L)	Glutamine (2 mmol/L)	Glucose + Glutamine (2 mmol/L)(2 mmol/L)
		nmol/(min.10^6 viable cells)		
Substrate(s) utilization				
Glucose*	—	3.23 ± 0.43 (6)	—	2.97 ± 0.50[a] (6)
Glutamine†	—	—	3.85 ± 0.07 (6)	2.95 ± 0.09[c] (6)
Metabolite generation				
Lactate	0.46 ± 0.07 (6)	3.50 ± 0.44 (6)	0.55 ± 0.10 (6)	3.33 ± 0.49 (6)
Pyruvate	0.31 ± 0.06 (6)	1.46 ± 0.20 (6)	0.42 ± 0.07 (6)	1.52 ± 0.22 (6)
Alanine	0.21 ± 0.04 (7)	0.36 ± 0.05 (6)	0.49 ± 0.08 (6)	0.67 ± 0.09[ab] (6)
Ammonia	0.50 ± 0.09 (6)	—	4.06 ± 0.14 (6)	3.38 ± 0.11[c] (6)
Glutamate	0.06 ± 0.01 (6)	—	1.61 ± 0.10 (6)	1.32 ± 0.10[c] (6)
Aspartate	ND	ND	0.68 ± 0.07 (4)	0.47 ± 0.06[b] (6)
Glucose converted into $^{14}CO_2$	—	0.30 ± 0.04 (6)	—	0.18 ± 0.03[a] (6)
Glutamine converted into $^{14}CO_2$	—	—	0.83 ± 0.09 (6)	0.72 ± 0.08[b] (6)

Cells were incubated for 30 min at 37° C without substrate or in the presence of glucose, glutamine or both substrates. Results are means ± SEM for the number of separate experiments indicated in parentheses.
[a], ($P < 0.01$) significantly different from glucose alone; [b], ($P < 0.05$); [c], ($P < 0.001$) significantly different from glutamine alone as analyzed by paired *t* test; ND, non detectable.
*Carbon balances (nmol/min.10^6 cells; see Methods and materials for calculation) were 1.06 for glucose alone and 0.82 in the presence of glutamine.
†Carbon balances were 1.14 for glutamine alone and 0.94 in the presence of glucose. Nitrogen balances were 1.63 for glutamine alone and 1.01 in the presence of glucose.

Table 3 Potential ATP production from glucose and glutamine in enterocytes isolated from newborn pigs

	(nmol/min.10^6 viable cells)						
	ATP from glucose			ATP from glutamine			
	gc to pyr lac and ala	gc to CO_2	Total	gn to Aspartate	gn to CO_2	gn to αKG + NH_4^+	Total
0-day-old pig							
Glucose	2.8 ± 0.2	7.4 ± 1.1	10.2 ± 1.3 (5)	—	—	—	—
Glutamine	—	—	—	3.3 ± 0.1	10.6 ± 1.7	2.9 ± 0.6	16.8 ± 0.9 (5)
Glucose + Glutamine	1.8 ± 0.2	2.3 ± 0.3	4.1 ± 0.3[a] (5)	3.5 ± 0.1	10.6 ± 1.7	1.4 ± 0.1	15.7 ± 0.7 (5)
2-day-old pig							
Glucose	10.8 ± 1.4	8.7 ± 1.1	19.5 ± 2.5 (6)	—	—	—	—
Glutamine	—	—	—	6.1 ± 0.6	17.1 ± 2.7	3.3 ± 0.7	24.5 ± 2.2 (6)
Glucose + Glutamine	10.9 ± 1.2	4.6 ± 0.80	15.5 ± 2.0[b] (6)	4.2 ± 0.6	15.2 ± 1.8	2.0 ± 1.0	20.9 ± 1.4[c] (6)

Potential ATP production from glucose (2 mmol/L) or glutamine (2 mmol/L) was calculated as described in Methods and materials section using values given in *Tables 1 and 2*. Data are means ± SEM for the number of separate experiments given in parentheses.
Total ATP production values were compared using paired *t* test.
[a], $P < 0.05$; [b], $P < 0.005$ significantly different from glucose alone; [c], $P < 0.05$ significantly different from glutamine alone.
gc, glucose; gn, glutamine; pyr, pyruvate; lac, lactate; ala, alanine.

ATP production was more than threefold higher in suckling animals than that estimated in 0-day-old ones. Indeed, ATP derived from lactate, pyruvate, and alanine production amounted to 30% of total ATP production in cells incubated with both substrates *(Table 3)*, and this was higher than ATP derived from glucose oxidation (13%).

Oxygen consumption

At birth, the basal rate of O_2 consumption in enterocytes, i.e., without exogenous fuel, was already high. The addition of glucose or glutamine increased by 65 and 95%, respectively, the rate of O_2 consumption *(Table 4)*. Addition of both substrates further enhanced the rate of O_2 consumption. After 2 days of suckling, the basal rate of O_2 consumption by intestinal cells was twice that measured at birth ($P < 0.001$). Nevertheless, adding glucose or glutamine increased the rate of O_2 consumption by 41 and 64%, respectively. Again, adding both substrates further enhanced the rate of O_2 consumption by enterocytes.

Discussion

In the present work, the contribution of glucose and glutamine to energy metabolism of intestinal cells has been investigated in the newborn pig. The pig is increasingly used as a model in biomedical research, including gastrointestinal studies.[26,27] For the first time, metabolic data were obtained in isolated metabolically active intestinal cells from neonates, represent-

Table 4 Oxygen consumption in enterocytes isolated from 0- or 2-day-old pigs

Substrate(s)	none	Glucose (2 mmol/L)	Glutamine (2 mmol/L)	Glucose + Glutamine (2 mmol/L)(2 mmol/L)
		nmol/(min.10^6 viable cells)		
0-day-old pig O_2 consumption	2.0 ± 0.2[a]	3.3 ± 0.2[b]	3.9 ± 0.1[c]	4.3 ± 0.1[d]
2-day-old pig O_2 consumption	3.9 ± 0.3[a]	5.5 ± 0.6[b]	6.4 ± 0.7[c]	7.1 ± 0.8[c]

Oxygen consumption was measured polarographically as described in Methods and materials. Data are expressed as means ± SEM (n = 7 for both groups). Within a row, values bearing different letters differ significantly (paired Student's t test; $P < 0.05$).

ing a well-defined population of villus absorptive cells without contamination by crypt cells.[16] Although exposure of the cell surface to nutrients at the same concentration could be considered as a nonphysiological situation, the present data provide information on the metabolic capacities of the intestinal cells just after birth.

Glutamine metabolism

The essential role of the small intestine as regards glutamine uptake and metabolism in the body has been reported in various physiological or pathological situations.[10,28,29] This is also supported by the high capacity for glutamine utilization found in isolated enterocytes from adult rat, chicken, or human.[11,12,30] The present data confirm this metabolic feature for enterocytes isolated from newborn pigs. At both stages, even in the presence of glucose, intestinal cells exhibit a high capacity for glutamine utilization that is in the range of those previously reported in adult enterocytes.[11,12,30] The net ammonia production matched the glutamine disappearance, suggesting that ammonia production through the glutamate dehydrogenase reaction was limited. Glutamate generated through the glutaminase reaction was in part metabolized, predominantly by transamination to aspartate and preferentially to alanine. Even in the presence of glucose that was generating additional pyruvate, alanine production remained modest. This can be explained by the very low alanine aminotransferase activity found in these cells. Among intestinal tissues studied for a number of species,[31] only the rat small intestine displays a high alanine aminotransferase activity as compared with that of aspartate aminotransferase. Such a metabolic fate of glutamine, giving rise to glutamate, aspartate, and ammonia, is also found in colonic cells[32,33] and in rapidly dividing cells such as lymphocytes.[34]

Although glutamine could be used in other pathways leading to purine or pyrimidine synthesis,[10,35,36] a relatively high amount of glutamine was converted into CO_2. Indeed, whatever the age of the animals, the rate of glutamine oxidation amounted to 18% of glutamine disappearance, which is twofold higher than that measured in adult rat,[37] or in weaned pig enterocytes.[16] This agrees well with previous data showing a high glutamine rate of oxidation by intestinal tissue slices in late suckling rats.[13,38,39] In turn, complete oxidation of glutamine represented the main source of ATP in intestinal cells incubated with glutamine.

Glucose metabolism

The small intestine is a potential site of glucose metabolism and lactate production, their relative importance depending on the nutritional status of the animals[40] or the methodology used. In vitro measurements using intestinal mucosal scrapings or isolated enterocytes indicated a high capacity for lactate production from glucose.[11,12,19,30,41,42]

In newborn pig enterocytes, glycolysis did represent the predominant metabolic pathway for glucose, because lactate, pyruvate, and alanine production accounted for almost 70% of glucose disappearance. Moreover, the emergence of a high glycolytic capacity in enterocytes isolated from 2-day-old suckling animals[16] was confirmed and observed even in the presence of glutamine. As suggested for intestinal cells[11,43] or for rapidly dividing cells from other types,[35,44] a high rate of glycolysis could be a mechanism for conserving glucose as C3 units, which would subsequently be available for hepatic gluconeogenesis. In addition, glycolysis could also provide pyruvate needed for glutamate transamination[11] or precursors for phospholipid synthesis. Regarding energy metabolism, even though glycolysis contribution increased between 0 and 2 days of age, glucose oxidation remained the major source of ATP production in cells incubated with glucose alone. However, when expressed as a fraction of glucose disappearance, glucose conversion into CO_2 dropped from 20% to less than 10% after 2 days of suckling. This was not modified by the presence of glutamine. In intestinal tissue slices from suckling rat, the rate of glucose oxidation to CO_2 represents 2 to 5% of the rate of glycolysis.[45]

O_2 consumption

In previous studies, O_2 consumption of isolated enterocytes was measured to test viability rather than to evaluate energy metabolism. In this work, O_2 consumption measured in piglet enterocytes was used to estimate total ATP turnover. Indeed, as extramitochondrial ATP production is low in the intestine,[46] O_2 consumption, which is restricted to mitochondria, must reflect total ATP production rate. Because ATP balance has been shown to be maintained in viable cells,[12,16,19] measuring O_2 consumption indifferently evaluates the synthesis or the degradation rate of ATP and therefore total ATP turnover rate. Furthermore, as recently underlined,[47] measuring O_2 consumption allows estimation of the contribution of exogenous versus endogenous metabolism to total ATP production.

Even in the absence of exogenous substrates, O_2 consump-

tion was high in newborn pig enterocytes. Such high rates of O_2 uptake are in agreement with those reported for adult rat, chicken, or human enterocytes.[11,12,30] Such a metabolic activity must be supported by endogenous substrates that are not removed during cell preparation. These endogenous fuels have not been identified precisely, although an endogenous production of metabolites such as lactate, pyruvate, and alanine has been detected. At birth, one of these fuels could be glycogen, which is found to be present in the enterocytes.[16] In 2-day-old piglets, the endogenous metabolism could also be supported by oxidizing fatty acids or amino acids from colostrum.[48]

Whether in the absence or presence of exogenous substrates, O_2 consumption rates were enhanced after 2 days of suckling, suggesting that the energy requirement was considerably increased in these cells, probably in relation to the active growth and functional development of the small intestine. Similarly, intestinal O_2 uptake has been shown to increase in the newborn pig after feeding.[49,50]

The stimulation of O_2 consumption by glucose ranged from 40 to 60% in piglet enterocytes, whereas there was 100% stimulation in human, rat, or chicken adult enterocytes. The enhancement of O_2 consumption by glucose in intestinal cells contrasts with the Crabtree effect observed in proliferating cells such as thymocytes.[47] The effect of glutamine was more marked in piglet enterocytes as compared with rat or chicken enterocytes, but remained smaller than in human enterocytes.[11,12,30] Similar effects of glucose and glutamine have been reported previously for segments of stripped jejunum from 1- to 2-wk-old piglets,[51] except for a slightly lower stimulation by glutamine in the latter experiments.

Glucose and glutamine contributions to energy metabolism

The contribution of glucose or glutamine to energy metabolism can be obtained by comparing ATP production from these substrates *(Table 3)* to total ATP turnover rate derived from O_2 consumption *(Table 4)*.

At birth, endogenous fuels support 48% of the ATP turnover of cells incubated with glucose and 28% of that of cells incubated with glutamine. Thus, it is suggested that exogenous glutamine can spare to a large extent ATP production from endogenous metabolism. After 2 days of suckling, glucose contribution increased and almost reached that of glutamine (53 versus 64%). Whatever the stage, incubating the cells with glucose and glutamine together *(Table 5)* resulted in the largest sparing of endogenous fuels: 49% at 0 days and 73% at 2 days. When enterocytes isolated at birth were incubated with both substrates, there was a dramatic fall of glucose contribution to the ATP turnover rate, whereas glutamine contribution was only slightly affected. At 2 days, such an effect was detected but to a lesser degree. Thus, it is suggested that glutamine can also exert a sparing effect on glucose oxidative metabolism.

Whatever the stage, ATP production from glucose and glutamine oxidation *(Table 5)* accounted for approximately 50% of total ATP turnover, showing that villus enterocytes derive most of their energy from oxidative metabolism. Indeed, the glycolytic contribution to total ATP turnover remained modest in these cells, even though it increased considerably between 0 and 2 days. This is in contrast to other tissues, such as the neonatal heart,[52] or with some rapidly dividing cells[24,47] in which glycolysis is responsible for most of ATP production.

In conclusion, the present work allowed us to characterize glucose and glutamine use in viable, metabolically active enterocytes isolated from newborn pigs. Our data confirm that these cells derive most of their energy from oxidative metabolism, the majority being supported by glutamine. In addition, glutamine can exert a sparing effect by reducing glucose contribution to total ATP turnover. Even in the presence of glutamine, a high capacity for glucose utilization in the glycolytic pathway emerges after 2 days of suckling. Thus, we conclude that this glycolytic activity, which does not primarily serve energetic purposes, could be responsible for generating specific metabolites in the suckling neonate.

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Table 5 Relative contributions of glucose and glutamine to ATP turnover in newborn pig enterocytes

Age (d)	0-day-old		2-day-old	
Substrates	none	gc + gn	none	gc + gn
Total ATP turnover (nmol/min.10^6 cells)	12.2 ± 1.0	26.0 ± 1.0	23.5 ± 1.9	42.5 ± 4.8
% ATP supported by				
Glucose metabolism	—	16	—	36
oxidation		9		11
glycolysis		7		25
Glutamine metabolism	—	60	—	49
oxidation		41		36
endogenous fuels	100	24	100	15

gc, glucose (2 mmol/L); gn, glutamine (2 mmol/L).

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