Intestinal Oxygen Uptake and Glucose Metabolism During Nutrient Absorption in the Pig (43821)

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Abstract. **Intestinal transport of nutrients coincides with their partial catabolism in the gut. The aim of the present study was to measure intestinal oxygen consumption and nutrient metabolism after a meal or during a short fast. Nutrient and oxygen balances across the small intestine were measured in conscious 50 kg (live wt) pigs. Jejunal enterocytes were also isolated from 1-hr postprandial, postabsorptive, or 3-day fasted pigs, in order to evaluate their capacities to metabolize 5 m*M* glucose and 2 m*M* glutamine. Whatever the nutritional state, intestinal oxygen consumption was high, since 26 ± 2% (n = 6) of the oxygen arterial supply was extracted by the small intestine. Furthermore, the consumption of a mixed meal induced a rapid and transient rise in oxygen consumption. In the postabsorptive state, the intestinal uptake of glucose (0.31 ± 0.08 mmole/min, n = 6) was twice higher than that of glutamine. The role of glucose as a fuel was also evidenced after a 3-day fast. During nutrient absorption, glutamine was highly utilized, and lactate was produced. The capacity of enterocytes isolated from fed pigs to metabolize glucose was dramatically reduced, as was 6-phosphofructo 1-kinase activity. In contrast, intestinal muscle presented a high glycolytic capacity from glucose, suggesting that the main site of intestinal lactate production during nutrient absorption would be the muscular rather than the epithelial layer.**

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The consumption of a mixed meal in humans as well as in animal models is generally accompanied by an increased splanchnic blood flow and energy expenditure (1–3). Among splanchnic tissues, the gastrointestinal tract has one of the highest oxygen (O_2) demand, amounting to 20%–25% of the whole body O_2 consumption (4). An increase in either intestinal blood flow or O_2 extraction can account for the enhanced intestinal O_2 uptake observed after a meal (5, 6).

Glutamine and to a lesser extent ketone bodies are widely taken up by intestinal tissues and represent the major oxidative substrates of the small intestine in fed or postabsorptive conditions (7, 8). Indeed, the essential role of the intestine in overall glutamine uptake and metabolism has been reported in various physiological or pathological situations (9). This is also supported by the high capacity for glutamine utilization found in isolated enterocytes from humans or rats (10, 11). The small intestine is also a potential site of glucose uptake, the extent of which depends on the nutritional status of the animals and on the methodology used. *In vitro* measurements using intestinal mucosal scrapings or isolated enterocytes have pointed to a high capacity for glucose metabolism and lactate production (11–13), particularly in fed animals (14). However, *in vivo* observations are conflicting, as luminal glucose has been reported to be absorbed intact into the portal vein (15) or partly metabolized into lactate (16–18). In contrast, intestinal glucose uptake seems to be high in rats taken in the postabsorptive state (7, 19).

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In order to reconcile these divergent results, we have combined *in vivo* and *in vitro* measurements on a pig model. The latter is increasingly used in biomedical research, including for gastrointestinal studies (20). Moreover, its size makes it suitable both for chronic catheterization and intestinal cell isolation. Metabolic studies have been performed in the basal state, following a meal, and after a 3-day period of fasting. In addition, information on the site of intestinal lactate production after a meal intake is also provided.

Materials and Methods

Experimental Animals. Experiments were performed on a total of 25 female pigs (mean age: 20 ± 1 weeks; mean live weight: 53 ± 3 kg) of the Large White breed (Institut National de la Recherche Agronomique, Domaine de La Minière, 78280 Guyancourt). During the whole experiment and beginning 2 weeks before surgery or intestinal cell isolation, animals were kept in restraining cages and fed twice daily 800 g of a semisynthetic diet (content in g/kg: casein, 178; purified corn starch, 516; sucrose, 65; soyabean-oil, 150; purified cellulose, 50; mineral and vitamin supplement, 41), mixed with 1.6 liter of water.

Surgical Procedure and *in Vivo* Study. Under general anaesthesia induced by halothane (Fluothan, Pitman-Moore, France), six pigs were surgically implanted with polyvinyl chloride catheters (i.d.: 1.27 mm, o.d.: 2.29 mm; Tygon Norton, Cleveland, OH) in the portal vein and the right carotid artery and with a portal blood flow probe according to a technique previously described (21). Carotid artery cannulation, allowing to sample blood from the thoracic aorta, has been reported to be the preferred technique for obtaining systemic arterial blood in the pig (22).

Experiments began 8 to 10 days after the surgical procedure when the animals had reached a normal feed intake level. After a fasting period of 20–24 hr (postabsorptive state), pigs were fed 800 g of the experimental diet. Portal blood flow rate was recorded continuously and blood was sampled from 30 min before the meal until 6 hr after the meal to determine glucose, lactate, glutamine, alanine, total ketone body, and O_2 concentrations in the blood, and plasma insulin concentration. This experimental procedure was repeated twice again: first, 5 days later (feed intake level: 200 g), then 10 days later (feed intake level: 800 g). Finally, the last test meal was followed by a 3-day fast period, at the end of which blood parameters were measured as explicated above.

Cell Isolation and Incubation Procedure. The remaining 19 pigs were also used in three different conditions: (i) 1 hour after the meal intake (n = 7); (ii) in the postabsorptive state (n = 5); (iii) after a 3-day fast (n = 7). They were anaesthetized with halothane and villus enterocytes were isolated from the proximal jejunum as recently described (23), using an adaptation of a method previously reported (13). The degree of enterocyte removal was assessed by following sucrase activity (as a marker of villus enterocytes) in the pool of isolated enterocytes versus in the remaining muscular tissues. Isolated enterocytes retained most of the jejunal sucrase activity expressed on the wet weight basis (90% ± 2%, n = 9), suggesting an effective partition between absorptive cells and muscular layers of the intestine during the cell isolation procedure.

Isolated cells were resuspended in a Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 10 m*M* Hepes, 1.3 m*M* $CaCl_2$, 2 m*M* $MgCl_2$, and 1% fatty acid-free albumin (incubation buffer), under continuous gassing (O_2/CO_2, 19:1, v/v). The cell density of the final suspension was assessed by counting an aliquot in a Malassez hemocytometer. Starting from the cell suspension, cell smears were prepared and stained with Alcian blue, periodic acid Schiff, hematoxylin, and picroindigo carmin, in order to visualize absorbing cells and goblet cells. The final suspension consisted in 96% ± 1% (n = 9) of absorbing enterocytes whatever the nutritional state of the animals. Cell viability was checked by following the percentage of total lactate dehydrogenase activity (LDH, EC 1.1.1.27) released in the extracellular medium. The mean viability was 90% ± 2% (n = 17) at the onset of incubation and 84% ± 2% (n = 17) after a 30-min incubation, whatever the nutritional status of the animals.

Incubations were carried out in 25-ml polycarbonate Erlenmeyer flasks (Nalge Company, Rochester, NY) containing 1 ml of the cell suspension (30 ± 4 × 10^6 cells, n = 17) in a final volume of 4 ml incubation buffer, unless otherwise stated. The flasks were gassed with O_2/CO_2 (19:1, v/v), sealed, and incubated in a shaking water-bath (100 oscillations/min, 30 min, 37°C) in the absence or in the presence of 5 m*M* glucose and 2 m*M* glutamine. Incubations were stopped by adding 0.125 ml of ice-cold perchloric acid (30% v/v) per ml incubation buffer; and lactate, pyruvate, glutamine, glutamate, aspartate, ammonia, and alanine were assayed in the neutralized, deproteinized supernatant.

Carbon dioxide production was determined by measuring $^{14}CO_2$ released during incubation with [^{14}C]labeled substrates (1.7–3.4 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 (Pharmacia, St Quentin-en-Yvelines, France).

O_2 consumption was measured polarographically with an oxygraph (Gilson Medical Electronics model 5/6H, Middelton, WI) equipped with a 2-ml water-jacketed chamber maintained at 37°C and a Clark O_2 electrode. After preincubation with or without sub-

strates in the oxygenated incubation buffer for at least 10 min, enterocytes were transferred to the stirred chamber in order to measure O_2 consumption during 3–5 min in the conditions of cell density and substrate concentration described above.

Glucose utilization was determined from [5-^{3}H] glucose (5 m*M*, 3–6 MBq/mmol) detritiation when the hexose was the sole exogenous substrate. ^3H_2O production and recovery were measured as described previously (24).The rates of lactate and pyruvate produced from 5 m*M* glucose were also measured in isolated enterocytes and in the remaining muscular tissues (100 mg wet wt/flask), after a 30-min incubation and compared on a dry weight basis.

Assay of Enzyme Activities. Hexokinase (EC 2.7.1.1.), 6-phosphofructo-1-kinase (EC 2.7.1.1.1.), and pyruvate kinase (EC 2.7.1.40.) activities were determined, as previously described (25), on freshly isolated cells. Cells were pelleted (150*g*, 3 min), frozen in liquid nitrogen and stored at − 80°C before analysis. The cell pellets were resuspended in 3–5 volumes of the appropriate extraction buffer (25) at 4°C and homogenized at 0°C, using an ultrasonic cell disruptor (Sonifier B15, Branson, Soest, The Netherlands). The final volume of the assay mixture was 2.0 ml. Enzyme activities were measured by monitoring the change in A_{340} at 30°C.

Analytical Methods. Metabolites were assayed by specific enzymatic methods (26). Insulin was determined by radioimmunoassay as previously reported (20). Blood content of O_2 was analyzed with an automated blood gas analyzer (ABL_3, Radiometer, Copenhagen, Denmark). Before calibration with porcine blood parameters, O_2 partial pressure corresponding to 50% hemoglobin saturation was first determined in arterial and portal venous blood (32.5 ± 0.2 mm Hg, n = 33).

Chemicals. Bovine serum albumin (fraction V, fatty acid free), Hepes, methylbenzethonium hydroxide, D-glucose, and L-glutamine were obtained from Sigma Chemical Co. (St Louis, MO). Perchloric acid and Tris buffer were obtained from Merck (Darmstadt, Germany). All inorganic products were from Prolabo (Paris, France). All enzymes and coenzymes used for enzymatic assays were from Boehringer (Meylan, France). [U-^{14}C] glucose, [5-^{3}H] glucose, [U-^{14}C] glutamine, and [^{3}H] water were purchased from Amersham (Les Ulis, France). The radiochemical purity of all isotopes used was higher than 98%.

Calculations and Statistical Analysis. All values shown are the means ± SEM for the number of separate experiments indicated. In *in vivo* studies, the net appearance or disappearance of nutrients and metabolites (intestinal balance) were calculated, according to the Fick principle, as the difference between portal and arterial concentrations multiplied by the flow rate in the portal vein. Two separate experiments were performed with the higher feed intake level (800 g). Since data obtained from both studies were quite similar, the values given correspond to the average. To estimate the maximum contributions of glucose, glutamine, and total ketone bodies to O_2 consumption by the gastrointestinal tissues in the postabsorptive and fasting state, metabolic quotients have been calculated by the ratio substrate uptake/O_2 uptake, multiplied by the number of O_2 moles required for complete oxidation of 1 mole of this substrate into CO_2. These constant values are 6 for glucose, 5 for glutamine, 4 for acetoacetate, and 4.5 for β-hydroxybutyrate.

For *in vitro* studies, all results are expressed as nmole/min and 10^6 viable cells, the viability being taken at the onset of incubation. For a single cell preparation, the data are based on duplicate or triplicate measurements. Rates of metabolite production correspond to the net amounts of metabolites generated. Blank values obtained from incubation flasks without any cell suspension added were subtracted to calculate the rates of glucose detritiation and substrate oxidation. To evaluate the maximal contribution of glucose or glutamine to ATP synthesis, potential ATP production from either substrate has been calculated on the basis of measured end products as was done previously (27). The rate of ATP generation in isolated enterocytes was estimated from the oxygen consumption (6 ATP/O_2). Statistical analysis was performed using the Student's paired or unpaired t test when appropriate.

Results

Portal Blood Flow and Intestinal Oxygen Uptake. As compared with the basal level, the portal blood flow (Table I) remained stable from 15 min to 6 hr after feeding (mean value: 1.99 ± 0.02 liter/min, n = 6; coefficient of variation during the period: 10% ± 1%). Nevertheless, during meal consumption (from 0 to 10 min), the portal blood flow decreased to 1.85 ± 0.09 liter/min and then increased again to reach a value similar to the basal level after 15 min. In addition, the mean blood flow remained stable after a 72-hr fast (Table I).

As indicated in Table I, arterial oxygen concentration was constant whatever the nutritional state. In the postabsorptive state or in fasting condition, a high intestinal oxygen uptake prevailed since the intestinal oxygen extraction represented 26% ± 2% (n = 6) of arterial oxygen supply. After the meal intake, intestinal oxygen uptake remained high and further increased at 30 and 60 min only (Table I). This result also observed when pigs were fed at a reduced level (200 g, data not shown) was explained by an enhanced intestinal oxygen extraction at both times (31% ± 2% of arterial oxygen supply).

Table I. Effect of Nutritional Status on Portal Blood Flow, Arterial Concentrations, and Intestinal Balance of Glucose, Lactate, Glutamine, Alanine, and Oxygen in the Pig

Nutritional conditions	Basal	Time after feeding (min)						Fasting state (72 hr)
		15	30	60	120	180	360	
Portal blood flow rate (liter/min)	2.04 ± 0.10	2.03 ± 0.13	2.01 ± 0.10	2.00 ± 0.08	2.08 ± 0.09	1.94 ± 0.10	1.83 ± 0.07	2.01 ± 0.17
Oxygen								
arterial concentration (mmol/liter)	6.27 ± 0.20	6.56 ± 0.17	6.39 ± 0.19	6.27 ± 0.22	6.06 ± 0.25	5.92 ± 0.21	6.20 ± 0.29	6.25 ± 0.40
intestinal balance (mmol/min)	−3.18 ± 0.27	−3.22 ± 0.15	−3.97 ± 0.19[a]	−4.24 ± 0.11[a]	−3.60 ± 0.42	−3.43 ± 0.28	−3.66 ± 0.37	−3.12 ± 0.38
Glucose								
arterial concentration (mmol/liter)	3.91 ± 0.16	4.91 ± 0.12[a]	5.47 ± 0.25[a]	5.29 ± 0.25[a]	4.97 ± 0.17[a]	5.19 ± 0.19[a]	5.18 ± 0.21[a]	3.97 ± 0.19
intestinal balance (mmol/min)	−0.31 ± 0.08	1.53 ± 0.32[a]	3.69 ± 0.47[a]	3.81 ± 0.21[a]	3.21 ± 0.54[a]	2.09 ± 0.49[a]	2.01 ± 0.17[a]	−0.32 ± 0.13
Lactate								
arterial concentration (mmol/liter)	0.79 ± 0.04	0.93 ± 0.06	1.33 ± 0.08[a]	1.93 ± 0.12[a]	1.33 ± 0.08[a]	1.14 ± 0.08	1.14 ± 0.19	0.94 ± 0.21
intestinal balance (mmol/min)	−0.03 ± 0.14	0.57 ± 0.07[a]	1.19 ± 0.21[a]	0.67 ± 0.15[a]	0.78 ± 0.11[a]	0.40 ± 0.09[a]	−0.14 ± 0.22	0.04 ± 0.02
Glutamine								
arterial concentration (μmol/liter)	382 ± 18	400 ± 25	470 ± 27[a]	429 ± 29	449 ± 30	477 ± 37[a]	554 ± 61[a]	359 ± 50
intestinal balance (μmol/min)	−153 ± 10	−43 ± 17	−73 ± 23	−76 ± 13	−121 ± 39	−136 ± 45	−92 ± 47	−104 ± 15
Alanine								
arterial concentration (μmol/liter)	255 ± 24	325 ± 24[a]	430 ± 31[a]	534 ± 34[a]	481 ± 31[a]	480 ± 36[a]	422 ± 10[a]	132 ± 15[a]
intestinal balance (μmol/min)	48 ± 8	128 ± 14[a]	292 ± 34[a]	249 ± 32[a]	283 ± 52[a]	195 ± 19[a]	156 ± 47[a]	35 ± 14

Note. Values are means ± SEM (for six animals). Each individual value represents the average of two separate experiments. The basal state corresponds to a fasting period of 20–24 hr. In each experiment, pigs were fed 800 g of an experimental diet described in Materials and Methods. Intestinal balances were calculated as follows: portal vein − carotid artery difference (mmol/liter or μmol/liter) × portal blood flow (liter/min).

[a] Statistically different from basal values ($P < 0.05$, paired Student's t test).

Intestinal Nutrient and Metabolite Balances. In the postabsorptive state, a significant intestinal uptake of glucose and glutamine was detected, together with a sizeable alanine release (Table I). However, intestinal lactate output was negligible; in addition, there was no intestinal ketone body uptake (data not shown). During a longer period of fasting (72 hr), arterial glucose and glutamine concentrations were kept constant. Arterial ketone body concentrations slowly increased (0.41 ± 0.04 mM, $n = 6$ vs 0.20 ± 0.02 mM, $n = 6$, in the basal state) while arterial alanine concentration significantly decreased (Table I). Significant intestinal uptakes of glucose, glutamine (Table I), acetoacetate (44 ± 7 μmol/min, $n = 6$), and β-hydroxybutyrate (65 ± 11 μmol/min, $n = 6$) were evidenced. Concomitantly, intestinal alanine and lactate output were modest.

After the meal intake, arterial concentrations of glucose, lactate, glutamine, and alanine significantly increased (Table I). Glucose and alanine were rapidly transported into the portal vein since the peaks of glucose and alanine absorption occurred within 30 min after feeding. However, glucose and alanine absorption remained high even 6 hr after feeding. In contrast, the intestinal balance was negative for glutamine throughout the experiment. Glucose absorption was associated with a high lactate output particularly during the first 3 hr after feeding.

To know whether lactate release from the intestine was related to glucose absorption, we have compared the intestinal balances of glucose and lactate for 1 or 3 hr after a meal given at a standard (800 g), or reduced (200 g) level (Fig. 1). During the first postprandial hour, the amount of glucose absorbed and that of lactate released did not differ according to the feed intake level. During the first 3 hr after feeding, the amount of glucose absorbed was strongly enhanced, although to a lesser extent when pigs were fed at a reduced level.

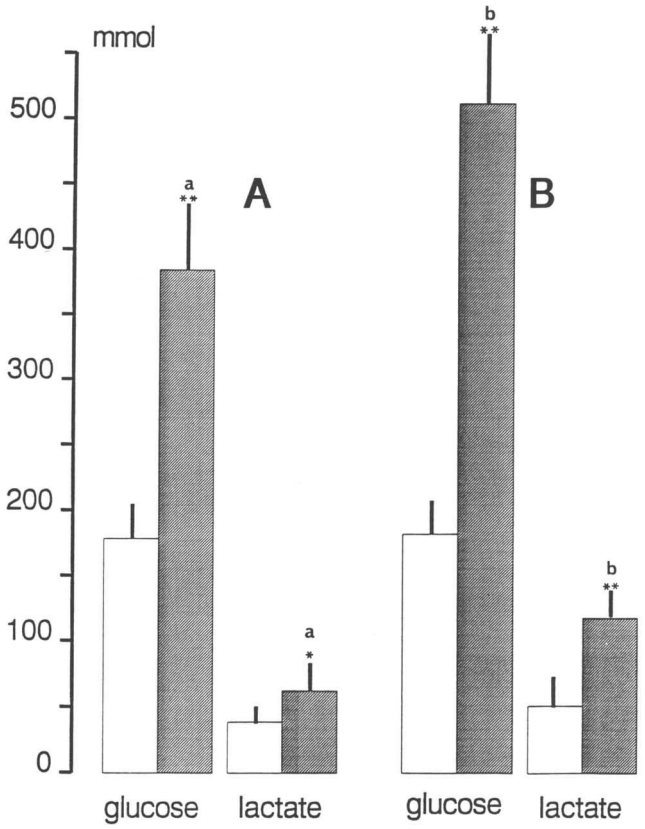

Figure 1. Effect of the feed intake level on glucose and lactate intestinal balances. Intestinal balances were measured in the same pigs within 1 hr (□) or 3 hr (▨) after a meal of 200 (A) or 800 g (B) of the experimental diet. Values are means ± SEM for six animals. Significant differences between 1 and 3 hr are shown by asterisks (*$P < 0.05$, **$P < 0.01$; paired Student's t test). Significant differences between intake levels are shown by different letters ($P < 0.05$, paired Student's t test).

Nevertheless, the increase of lactate release during this period was much lower than that measured for glucose absorption.

Arterial Insulin Concentration. Arterial insulin concentration was 7 ± 1 μU/ml (n = 6) at the basal state. It rose within 30 min after the meal to reach a peak level of 48 ± 20 μU/ml (n = 6) after a 200-g meal and of 135 ± 40 μU/ml (n = 6) after a 800-g meal. From then, it rapidly declined within 90 min after feeding (17 ± 3 μU/ml at 200 g and 24 ± 5 μU/ml at 800 g), and then went back progressively to the basal level.

Capacity for Glucose and Glutamine Metabolism in Isolated Enterocytes. The basal rate of O_2 consumption was similar in enterocytes isolated from fed or fasted pigs (Table II), and increased by 70% (fed group) and 115% (fasted group) after adding both glucose (5 mM) and glutamine (2 mM). When cells originating from either group were incubated with both substrates, a net production of lactate, pyruvate, alanine, ammonia, glutamate, and aspartate was detected (Table II). The net generation of ammonia and glutamate decreased significantly in enterocytes isolated from fasted pigs, while the capacity for glutamine oxidation was kept constant. This suggested that the capacity for glutamine utilization was lower in enterocytes isolated from fasted pigs, although the difference observed in glutamine disappearance from the incubation medium was not significant (0.7 ± 0.1 vs 0.9 ± 0.2 nmol/min/10^6 cells, n = 6). In contrast, lactate plus pyruvate generation increased threefold in enterocytes isolated from fasted pigs as compared with fed pigs (Table II). Similarly, glucose oxidation was slightly enhanced in enterocytes isolated from fasted pigs. These data suggest that the capacity for glucose utilization determined in the presence of glutamine was low in enterocytes isolated from fed pigs and profoundly increased during fasting. This was also confirmed by the rate of glucose utilization measured by [5-^{3}H] glucose detritiation (Fig. 2). It was low in fed pigs (0.10 ± 0.01 nmol/min/10^6 cells, n = 4) and increased 3- to 4-fold in postabsorptive and fasted pigs respectively.

To investigate further the changes observed in glucose metabolism according to the nutritional status of the animals, we have determined the maximum activity of some key enzymes of glycolysis in freshly isolated cells. Hexokinase (Fig. 2) and pyruvate kinase (data not shown) activities did not differ significantly among the different groups. In contrast, 6-phosphofructo 1-kinase activity gradually increased with fasting (Fig. 2).

Glycolytic Capacity in Intestinal Muscle Layer. The capacity for glycolysis was measured in the presence of 5 mM glucose in the intestinal muscle layer left after having removed the enterocytes. As shown in Figure 3, the rate of lactate plus pyruvate production was high in fed animals and dramatically decreased in postabsorptive or fasted pigs. As expressed on a dry weight basis, the rate of lactate plus pyruvate production from glucose in intestinal muscle from fed pigs was 4-fold higher than that measured in enterocytes. In contrast, in fasted pigs, the rate of lactate plus pyruvate production was twice lower in intestinal muscle than in enterocytes.

Discussion

The present study confirms that the gastrointestinal tract is a site of high O_2 uptake, even in the postabsorptive or fasting state. The consumption of a mixed meal induced a rapid and transient rise in O_2 consumption. Similarly to what has been reported in the dog (5), this was essentially explained by an increased O_2 extraction rate. Indeed, when intestinal O_2 uptake increased (i.e., 30 to 60 min after the meal) the portal blood flow was higher than that measured during the meal consumption, but it was similar to the

Table II. Glucose and Glutamine Metabolism in Enterocytes Isolated from Fed or Fasted Pigs

	Fed pigs		3-Day fasted pigs	
	No substrate(s)	Glucose + glutamine	No substrate(s)	Glucose + glutamine
O_2 consumption (nmol/min/[10^6 cells])	0.65 ± 0.10	1.12 ± 0.23	0.57 ± 0.15	1.23 ± 0.24
Pyruvate (nmol/min/[10^6 cells])	0.01 ± 0.01	0.05 ± 0.01	0.01 ± 0.01	0.34 ± 0.04[a]
Lactate (nmol/min/[10^6 cells])	0.05 ± 0.01	0.24 ± 0.03	0.02 ± 0.01	0.46 ± 0.07[a]
Ammonia (nmol/min/[10^6 cells])	0.02 ± 0.01	1.01 ± 0.06	0.04 ± 0.01	0.76 ± 0.13[a]
Glutamate (nmol/min/[10^6 cells])	0.01 ± 0.01	0.44 ± 0.04	0.01 ± 0.01	0.25 ± 0.03[a]
Aspartate (nmol/min/[10^6 cells])	0.01 ± 0.01	0.06 ± 0.02	0.01 ± 0.01	0.05 ± 0.02
Alanine (nmol/min/[10^6 cells])	0.02 ± 0.01	0.11 ± 0.01	0.02 ± 0.01	0.12 ± 0.01
Glucose oxidation (nmol/min/[10^6 cells])	—	0.03 ± 0.01	—	0.05 ± 0.01
Glutamine oxidation (nmol/min/[10^6 cells])	—	0.09 ± 0.01	—	0.08 ± 0.01

Note. Enterocytes were isolated from fed (1 hr after meal intake) or 3-day fasted pigs. O_2 consumption and metabolite production were measured on intestinal cells incubated (30 min, 37°C) in the absence or in the presence of 5 m*M* glucose and 2 m*M* glutamine. Substrate oxidation corresponds to the net conversion of [^{14}C]labeled substrate into $^{14}CO_2$. All values were significantly higher ($P < 0.05$; paired student's *t* test) in cells incubated in the presence versus absence of glucose + glutamine. Values are means ± SEM of six to seven separate experiments.

[a] Statistically different from values obtained from fed pigs ($P < 0.05$, unpaired Student's *t* test).

basal level measured before the meal. Such a feature had already been described (28).

Nutrient absorption was accompanied by a high rate of lactate production by the intestinal tissues. Nevertheless, the capacity of enterocytes isolated from fed pigs to generate lactate and pyruvate from glucose was dramatically reduced as compared with that measured in cells from postabsorptive animals. Thus, combined *in vivo* and *in vitro* observations suggest that the main site of intestinal lactate production during nutrient absorption would be the muscular part of the intestine.

Intestinal Glucose Metabolism in the Postabsorptive State. In the postabsorptive state, intestinal uptake of glucose determined in conscious pigs was twice higher than that of glutamine. Although a significant utilization of glucose by the gut has been previously reported (19, 29), this result is at variance with previous data obtained in rat jejunum segments (7). Thus, it is suggested that glutamine would be less es-

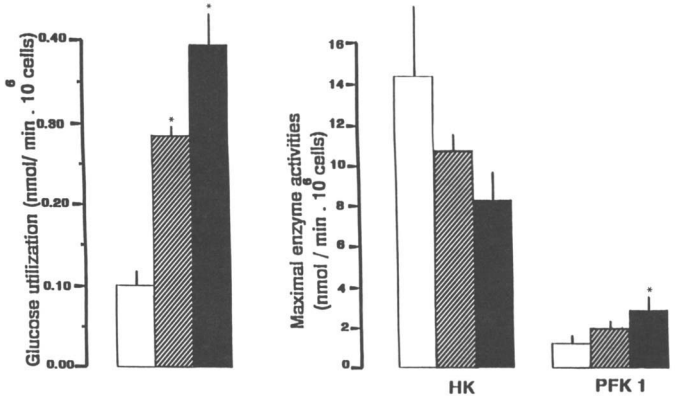

Figure 2. Effect of the nutritional status of the animals on glucose utilization and maximal activities of hexokinase and phosphofructokinase in pig isolated enterocytes. Intestinal cells were isolated from the jejunal part of the small intestine at various times after a 800-g meal: 1 hr(□), 20–24 hr (▨), or 72 hr (■). The cells were incubated for 30 min at 37°C in the presence of [5-^{3}H] glucose (5 m*M*). Glucose utilization was determined from the detritiation of [5-^{3}H] glucose. Hexokinase (HK) and 6-phosphofructo 1-kinase (PFK1) activities were determined at the onset of cell incubation, as described in Materials and Methods. For each condition, values are means ± SEM of five to six separate experiments. *Significantly different ($P < 0.05$, unpaired Student's *t* test) from values obtained in cells isolated from fed pigs.

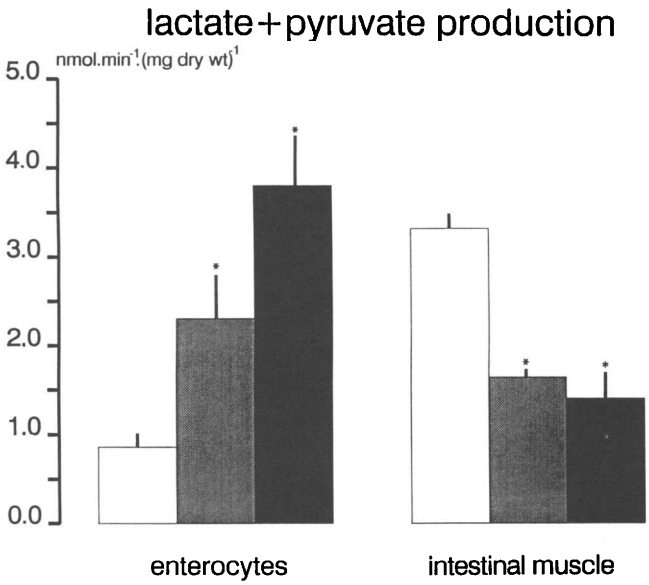

Figure 3. Effect of the nutritional status of the animals on lactate and pyruvate production from glucose (5 m*M*) in intestinal absorbing cells or intestinal muscle. The remaining muscular layer (20–30 mg dry wt) was obtained from pig jejunum after having removed the enterocytes and was incubated in the same conditions as isolated enterocytes (see Fig. 2). The rates of lactate and pyruvate production in enterocytes and intestinal muscle were compared on a dry weight basis at various times after feeding a 800-g meal: 1 hr (□), 20–24 hr (▨), or 72 hr (■). Values are means ± SEM of five to six experiments. *Significantly different ($P < 0.05$, unpaired Student's *t* test) from values obtained in pigs taken 1 hr after feeding.

sential for the pig intestine than for the rat intestine. Indeed, in the present experiment, the maximal contributions of glucose and glutamine metabolism to O_2 consumption in the postabsorptive state were 60% and 25%, respectively. The prominent role of glucose as a fuel for the small intestine of the pig was also evidenced after a short fasting period (72 hr), since the maximal contribution of glucose to O_2 consumption (62%) remained at a high level while that of glutamine (17%) slowly declined. Moreover acetoacetate and β-hydroxybutyrate represented alternative fuel substrates (15% of the oxygen uptake) at this time despite a low blood ketone body concentration.

This important role of glucose during a 3-day fasting period is also supported by *in vitro* observations. The relative contributions of glucose and glutamine to energy metabolism can be obtained by comparing ATP production from these substrates with total ATP turnover rates derived from O_2 consumption (see Materials and Methods and Table II). Glucose metabolism in the presence of glutamine supported up to 47% of the ATP turnover, while the contribution of glutamine (i.e., 36% of the ATP turnover) seemed to be lower.

Intestinal Glucose Metabolism in the Postprandial State. Following a meal, as nutrient absorption takes place, it is not possible to appreciate *in vivo* the relative contribution of glucose and glutamine as fuel substrates. However, enterocytes isolated within 1 hr after feeding exhibited a very low capacity for glucose utilization and oxidation in the presence of glutamine. Indeed, the contribution of the hexose to energy metabolism represented only 20% of the ATP turnover, i.e., two to three times less than that of glutamine. *In vivo* observations also confirm that glutamine is highly utilized by the intestine for 6 hr following a meal intake, as glutamine concentration remained always lower in the portal vein than in the artery. Such a low contribution of glucose metabolism to intestinal energy demand agrees well with previous data obtained on jejunal segments from fed rats (8). Nevertheless, a very low capacity for glucose utilization in enterocytes isolated from fed pigs is somewhat surprising in view of data previously reported in rats (11, 14). Because the capacity for glucose utilization rapidly increased during a short fasting period, the discrepancy between the present data and those previously reported could arise from the difficulty of isolating enterocytes from postprandial rats. Our data suggest that a low maximal activity of 6-phosphofructo 1-kinase could be responsible for the low capacity of enterocytes taken from fed pigs to metabolize glucose. However, the maximal activity of the enzyme was still 10-fold higher than the glycolytic flux evaluated through [5-^{3}H] glucose detritiation (see Fig. 2). Such a discrepancy had been already noticed, as recently emphasized (30). It could be explained by changes in the cell content of 6-phosphofructo 1-kinase effectors (ATP, citrate or fructose 2,6-bisphosphate).

Site of Intestinal Lactate Production. Arterial blood lactate concentration progressively increased after a meal, which could be explained in part by intestinal lactate release. This result is in agreement with previous observations in conscious animals having received a glucose load (16–18, 31). However, in our conditions, we cannot exclude that a fraction of the lactate produced could originate from sources other than glucose in the diet. This intestinal lactate production was limited in time (1–2 hr after the meal), and did not last until the end of glucose absorption. Associated with an increase in oxygen consumption, this intestinal lactate production could result from the contractile activity of the intestinal smooth muscle, as previously suggested (32). The increase of glycemia and insulinemia could play a role in the enhancement of muscular glycolytic activity after the meal, although smooth muscle seems to be less sensitive to insulin than skeletal muscle (32). Such a potential regulation by hyperglycemia and hyperinsulinemia could explain the enhanced glucose utilization in the gut of fed dogs (33). In turn, the profound decrease in the circulating insulin concentration, associated with a limited muscular activity and a dramatic increase of plasma nonesterified fatty acid concentration (10- to 20-fold when compared with postprandial concentrations, data not shown) could explain the lower intestinal lactate production during the postabsorptive period.

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