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Metabolic cooperation between intestine and liver. Implications in relation to fat and glycogen synthesis

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The cooperation among organs is a well known phenomenon in mammalian metabolism. Examples abound, such as the Cori and alanine cycles that transfer carbon and amino groups between muscle and liver and the complementary distribution, in the same two organs, of enzymes involved in branched-chain amino acid breakdown (1,2). On the same token, the intestine is not only a permeability barrier for the products of digestion, but also processes and sorts some of them before their transfer to the blood stream or lymph. For instance, citrulline formation from glutamate and related amino acids in the intestine provides the precursor for renal arginine synthesis (3,4). In relation to fatty acids, while those with long-chains are converted to acylglycerides and released in chylomicrons to lymph, the short ones are delivered to the liver via the portal blood.

In spite of the well known capability of intestinal mucosa to perform aerobic glycolysis (5,6), it has not been clearly established to what extent glucose is broken down to lactate during absorption. Considering that the intestinal mucosa possesses the whole complement of glycolytic enzymes (7), it is likely that the metabolic machinery would compete with the basolateral transport system (GLUT 2) for absorbed glucose (8,9). Traditionally, it has been considered that the lactate produced by several intestinal preparations is an artifact caused by a poor oxygenation of the tissue (10,11). Due to that, in very few biochemistry textbooks it is recognized that lactate is a major product released by the intestine during the normal course of glucose assimilation (12,13).

Nonetheless, several reports point to the fact that lactate is the main product of carbohydrate digestion, comprising from 50-75% of absorbed glucose (14,15). This poses an interesting case of metabolic cooperation between intestine and liver. The latter readily uses lactate as a precursor for glycogen and fat synthesis (16,17), while takes up less than 20% of the glucose which crosses the liver bed (18). These findings support the theory of the «Indirect pathway of glycogen synthesis» (19,20) according to which, up to 70% of glycogen synthesis in the liver arises from gluconeogenic precursors such as lactate.

An unsolved question regarding this theory is where the initial breakdown of glucose to lactate, or another 3-carbon intermediate, takes place. Several suggestions have been put forward such as skeletal muscle, intestine and the liver itself (21). The major aim of my work, over the past few years, has been to examine the small intestine's contribution to the initial metabolism of glucose during absorption. The utilization of the intestinal metabolite output for liver anabolism has also been considered.

Carmona et al. (22) measured the time-course changes in glucose and lactate levels in the portal blood of 18 hours starved rats which were refed a sucrose diet for 8 hours. Portal glucose increased from 5 to 12 mM after 1 hour, while lactate went from 1 to 4 mM during the same period. Glucose and lactate levels remained relatively constant for up to 8 hours, and were lower in rats killed 14 hours after the beginning of the feeding period. As evidenced by the change in lactate/glucose ratio, the increase in lactate doubled that of glucose during the first two hours of refeeding and was 50% higher during the next six hours.

Considering that lactate clearance from blood is accomplished mainly by the liver, and appears to be more effective than that for glucose (18,23), the observed differences in portal levels may underestimate the extent of intestinal lactate output as compared to that of glucose, suggesting that intestinal lactate production *in vivo* may account for most of the absorbed glucose.

The longitudinal distribution of glucose uptake activity along the small intestine is presented in Figure 1. (^{14}C)-glucose uptake was measured using intestinal sleeves (24), 1 cm in length, obtained from young rats fed diets containing either 60% glucose (plus 13% starch) or 73% starch. In the former group, glucose uptake peaked in the proximal jejunum, 22 cm away from pylorus, decreasing thereafter towards the distal ileum. For the rats fed starch the peak was observed in the distal jejunum (52 cm away from pylorus). Although glucose uptake in the proximal side of the intestine was significantly higher for the rats fed glucose, this difference disappeared towards the distal jejunum and ileum. Displacement of glucose uptake towards the distal section of the small intestine, in the starch fed rats, reflects the fact that this polymer must be digested to simple sugars. In contrast, glucose is ready for absorption following stomach emptying.

The metabolic fate of absorbed glucose was studied using either the everted sac technique (25) or an *in vitro* perfusion system of everted intestinal segments. The former is simple but suffers of many limitations, while the later resembles in many respects the behavior of sophisticated *in vivo* perfusion systems (11,14,26). In these experiments, the effects of diet composition, oxygen supply and substrate addition on intestinal glucose transactions (uptake, transport and metabolism) were investigated.

Figure 2 compares the glucose transactions in everted sacs from chow fed rats of different body weights. Although sugar uptake decreased with the increase in average body weight, glucose transport to the serosal side was more severely affected, decreasing in almost 80%. The difference between the amount of glucose absorbed and that transported to the interior of the sacs is accounted for by lactate output and hexose retention within the tissue phase (8). Partition of absorbed glucose between transport and metabolism was, apparently, influenced by the type of diet and the age of donor animals. These factors altered the width of the intestinal wall and, apparently, the oxygen availability to the tissue.

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FIGURE 1
Distribution of glucose uptake activity along the small intestine. (14C)-glucose uptake was measured in 1 cm intestinal sleeves obtained at the indicated distances from pylorus from rats fed purified diets based on either glucose or starch. Incubations (4 min) were performed in Krebs-Ringer buffer (pH 7.4) continuously gassed with a 95:5 oxygen-CO_2 mixture.

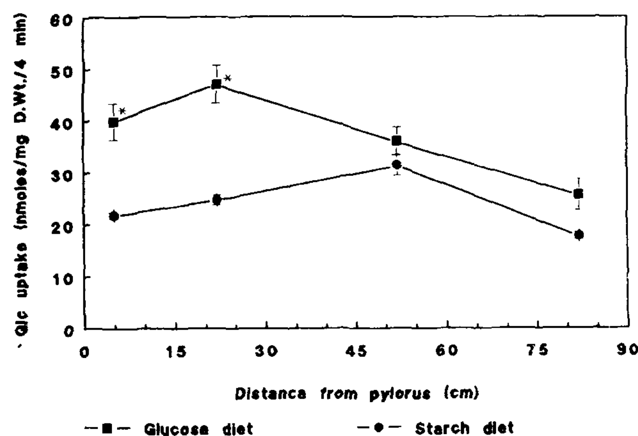
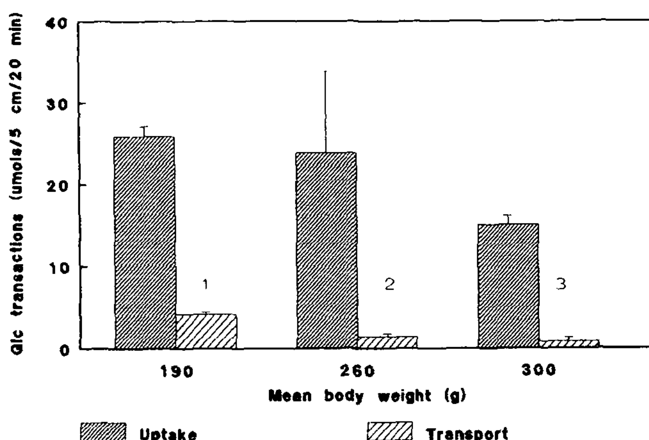


FIGURE 2
Glucose uptake and transport in everted intestinal sacs. Donor rats, of different body weights, were fed a commercial diet. Glucose transactions were measured as described before (25). Data were taken from reference 25 (1) and unpublished observations of A.I. Laurentín (2) and A. Nemeth (3).



Although the everted sac technique is simple and produces reliable results, it has some limitations related with the serosal compartment, such as its reduced volume, poor oxygenation and the impossibility to be sampled during the incubation period. These shortcomings were solved with an *in vitro* perfusion preparation which, while remaining simple, represents a better approximation to the conditions prevailing *in vivo* (27).

Briefly, an everted intestinal segment, 10 cm in length, is fixed through canulas to a plastic chamber filled with 100 ml of Krebs-Ringer buffer (pH 7.4) (luminal solution) continuously bubbled with a 95% $O_2$5% CO_2 mixture. The serosal fluid, also continuously gassed with oxygen, is perfused through the segment at a rate of 1 ml.min^{-1} and collected in 3 ml fractions (Figure 3). After a 9 min equilibration period, glucose (10 mM final) is added to the luminal solution and the perfusion is continued for up to 1 hour. At the end, the intestinal segment is rinsed, and its dry weight determined. Results are expressed as nmoles of glucose transported or metabolized.mg dry weight^{-1}.

FIGURE 3
The everted segments were affixed to the incubation chamber through cannulas (Insyte 18G/2 in; Deseret Medical Inc., Sandy, Utah, USA). Luminal and serosal fluids were Krebs-Ringer buffer (pH 7.4) continuously gassed with a 95:5 oxygen-CO_2 mixture, unless otherwise indicated. In all experiments segments were obtained from intestinal sections located between 24 and 34.5 cm away from pylorus.

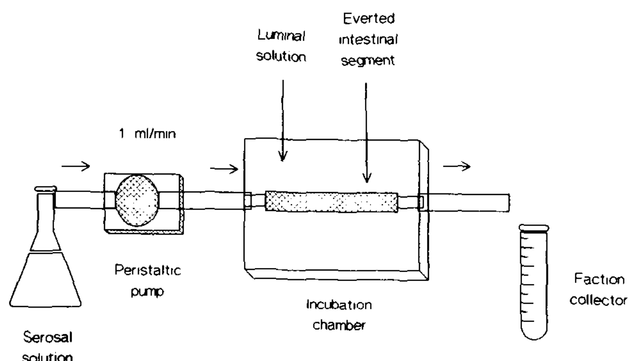


Figure 4 compares glucose (A) and lactate (B) output to the serosal fluid by intestinal segments perfused under ample oxygen supply. Segments were obtained from the proximal jejunum of rats fed glucose, starch or a commercial diet. After glucose addition to the luminal medium, sugar transport to the serosal solution followed a sigmoidal curve, reaching a plato after a lag phase that lasted between 5-7 min. A similar pattern was reported for the uptake of galactose in intestinal segments perfused *in vivo* [24]. It proves that the *in vitro* preparation, while being more simple, retained the same behavior as the *in vivo* one, in spite of the interruption in blood flow through the tissue.

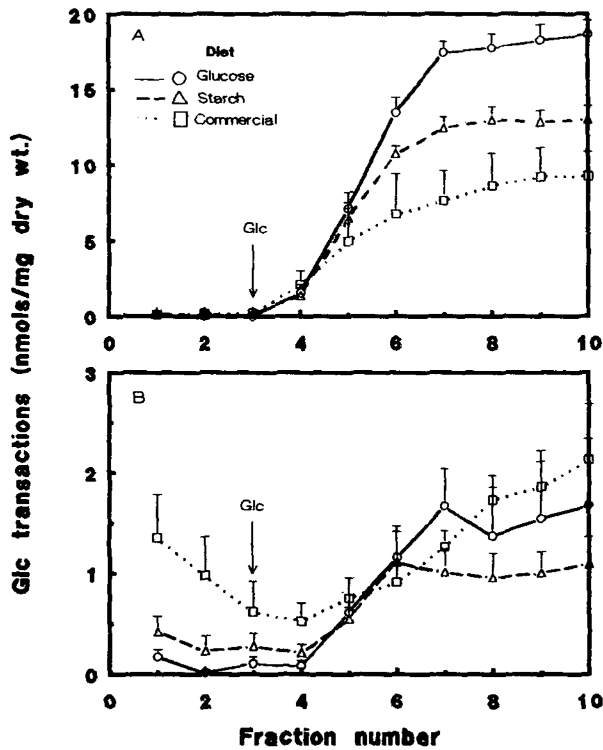
It was found (Figure 4A) that segments from rats fed glucose transported twice as much sugar than those from the animals fed chow. For the starch fed rats, glucose appearance in the serosal fluid laid between those from the other two groups. Glucose output in the starch and commercial groups was statistically different from that in the glucose fed animals.

Lactate output to the serosal fluid is shown in Figure 4B. From the begining of perfusion, lactate was detected in the serosal perfusate, particularly in segments from chow fed rats. Apparently, it represents the drainage of lactate accumulated in the tissue before perfusion was started. After glucose addition to the luminal chamber, lactate release increased following a similar trend to that of glucose transport, but lagging behind it. Lactate output reached a plato after fraction 6 in

segments from the glucose and starch groups. In those from the rats fed the commercial diet the plato was not reached even after 10 fractions (30 min).

FIGURE 4

Glucose transactions in perfused intestinal segments. Donor animales were fed either a purified diet (based on glucose or starch) or a commercial rat chow. Perfusion was performed as indicated in the text. Glucose translocation (A) and lactatate output to the serosal fluid (B) were measured. Mean dry weight of segments were 51.6±4.18, 51.7±4.19 and 68.3±5.12 mg/10 cm for those obtained from rats fed glucose, starch or chow, respectively.



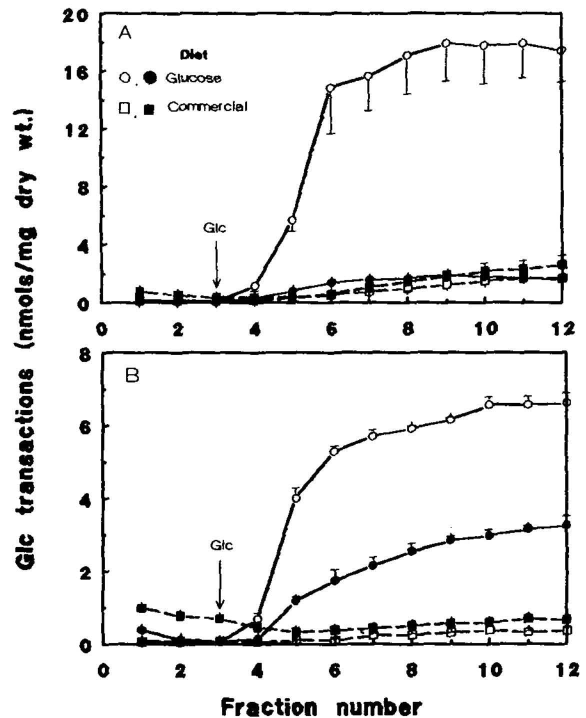
No statistical differences were found among the three groups in terms of the serosal lactate output. Nonetheless, the lactate/glucose ratio in this fluid was 3 times higher in chow fed animals as compared to those from the other two groups. This indicates that most of the absorbed glucose was being metabolized to lactate in the segments from rats which received the commercial diet.

Part of these changes could be attributed to the difference in thickness of the intestinal wall among the three groups. Although the actual widths were not measured, they could be indirectly estimated through the dry weight of segments of equal length (28). Mean dry weight of 10 cm segments from the rats fed glucose or starch was 51.6 ±4.18 and 51.7±4.19 mg, respectively, while that from the third group was 68.3±5.12 mg. The increase in width of the intestinal wall is a trophic response to the presence of dietary fiber and/or other components of rat chow in the lumen. This tissue proliferation may limit oxygen availability and, therefore, favor glycolysis.

Figure 5 compares the effect of partial oxygen deprivation on glucose transactions. Segments were obtained from rats fed either the glucose diet or chow plus a 5% glucose solution substituted for the drinking water. The intestinal wall of segments from the latter group was inordinately thick, with dry weights 2.5 times larger than those from the glucose group.

FIGURE 5

Effect of partial oxygen deprivation on intestinal glucose transactions. Donor animals were fed a glucose diet or a commercial rat chow. Animals from the latter group received 5% glucose in the drinking water. Segments were perfused under aerobic (A) and partially anaerobic (B) conditions. Mean dry weight of segments (mg/10 cm) from glucose fed rats were 42.57±6.29 (A) and 40.4±1.63 (B) (n=3). For the chow fed animals dry weights were 108.1±18.41 (n=4) (A) and 132.1±4.16 (n=3) (B). See Figure 4 for further details. ○, □ Glucose translocation. ●, ■ Lactate output to the serosal fluid.



When there was ample oxygen supply (Figure 5A), segments from rats fed the glucose diet translocated the sugar at a rate 8 times that of segments from the chow + glucose solution group. In this case, the lactate/glucose ratio fluctuated around 0.2, indicating that the largest proportion of the absorbed glucose was being translocated as such. In contrast, in the segments from rats that received chow + glucose solution, the lactate/glucose ratio stayed around 2.6. Therefore, in the latter group, with oxygen supply through the luminal and serosal sides, oxygen transfer to the cells may be limiting, causing a large activation of glycolysis.

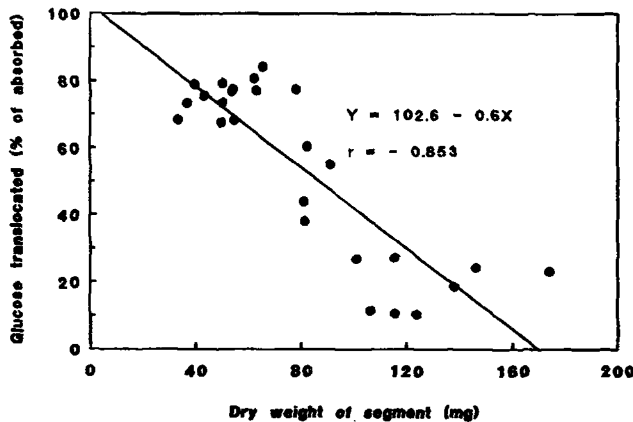
Under conditions of partial anoxia (Figure 5 B), glucose output of segments from the rats fed glucose was decreased, reaching a plato around 6 nmoles/mg dry weight. As expected, the amount of glucose metabolized to lactate and released to the serosal fluid doubled to 3 nmoles/mg dry weight per fraction. The demonstration of the «Pasteur effect» confirms the metabolic competence of these intestinal segments. Since the lactate/glucose ratio increased to almost 1, segments from the glucose group behaved as those from the chow + glucose solution group which were incubated with full oxygen supply.

The effect of partial oxygen deprivation was more drastic on the segments from the chow + glucose solution group. Glucose translocation fell to around 0.3 nmoles/mg dry weight per fraction, and the lactate/glucose ratio increased to 3.8. These results agree with those of Pritchard and Porteus (8) who observed that, under extreme anoxia, glucose uptake and translocation by everted sacs were severely decreased. Remarkably, translocation was more strongly reduced due to the routing of absorbed sugar towards lactate formation.

Figure 6 shows a scatter plot relating glucose transport to the serosal side and the dry weight of intestinal segments. A strong negative correlation between both parameters was found indicating that the change in thickness of the intestinal wall is the major factor explaining the variability of glucose translocation. Part of the changes in segment thickness could be caused by variations in the composition of the diet (28,29).

FIGURE 6

Correlation between glucose translocation to the serosal side and the dry weight of intestinal segments. Segments from the animals fed the different diets were perfused as indicated in the text. Sugar translocation was expressed as percentage of absorbed glucose.



Although the increase in lactate output appears to be an artifact of the *in vitro* perfusion system, due to an inadequate oxygen supply, this phenomenon also seems to occur during *in vivo* perfusion of thick segments (compare 11 and 14). It should be mention that, in these *in vivo* preparations, an important fraction of available oxygen is supplied through the luminal side of the intestine. Hyperoxygenation of the luminal compartment is a state far removed from the true *in vivo* conditions, where this compartment is anoxic.

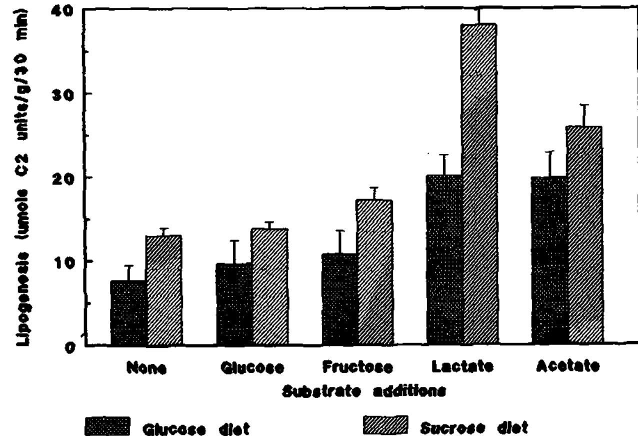
Results from *in vitro* (this paper) and *in vivo* (10,14) experiments suggest that the diversion of absorbed glucose towards lactate output may be a physiological process which could be inherent to the maturation of the intestinal tissue. Such a process could result from the interaction between diet composition and body weight.

Partition of glucose between transport and metabolism was determined in jejunal segments from rats fed the commercial diet, whose dry weights did not exceed 85 mg/10 cm. In segments incubated with (^{14}C)-glucose, 52% of the absorbed sugar was released intact to the serosal fluid and 42% was metabolized to lactate. The rest appeared as glycogen and CO_2 (results not shown). This results agree with those from *in vivo* experiments (22) and show that lactate makes most of the carbons derived from carbohydrate assimilation.

The extent of metabolic cooperation between intestine and liver is exemplified in Figure 7. Lactate was a better substrate for fatty acid synthesis in isolated hepatocytes than glucose, particularly in cells from rats fed sucrose which maximizes the hepatic lipogenic response (17).

FIGURE 7

Effect of various substrates on fatty acid synthesis in isolated hepatocytes. Donor rats were fed either a 60% glucose or sucrose diet. Lipogenesis was measured using $3H_2O$ as tracer. Modified from reference 17.



Results presented in this paper prove that, after absorption, dietary glucose is partitioned, within the epithelial cells, between translocation to portal blood and metabolism. The extent of partition is determined by the type of diet fed to the animals and their body weight. The close association between sugar translocation and lactate production indicates that both processes contribute to handle the bulk of absorbed glucose. The fate of absorbed glucose depends upon the relative activities of the basolateral glucose transporter (Glut 2) and the glycolytic machinery. If lactate output matches or exceeds glucose translocation, liver anabolism should be favored (17,19).

Therefore, the intestinal breakdown of glucose must be considered of utmost significance in terms of the handling of dietary carbohydrates and an important form of metabolic cooperation between intestine and liver.

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