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The Effect of Fasting on Rat Portal Venous and Aortic Blood Glucose, Lactate, Alanine, and Glutamine

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ABSTRACT. Using a chronically catheterized rat model, the effect of fasting on portal venous, aortic, and venous blood concentration minus aortic blood concentration ([PV-A]) blood glucose, lactate, alanine, and glutamine concentrations was determined. It has been postulated that the intestine is a source of lactate and alanine, precursors for glycogen synthesis, in the fed state. After 48 h of fasting portal venous glucose, lactate, and alanine blood concentrations decreased by 31, 28, and 41%, respectively. Portal venous glutamine concentration was not affected by fasting. A glucose [PV-A] was not found in either fed or fasted states. Whereas the lactate [PV-A] was not present in fed rats, it was negative in fasted rats. Alanine [PV-A] was positive in fed and fasted rats. The glutamine [PV-A] was negative in fed and fasted rats. These data indicate that portal venous concentrations of the gluconeogenic precursors, lactate and alanine, decrease in fasted rats. In fasted rats intestinal utilization of lactate increases as reflected by a negative [PV-A]. Fasting did not affect alanine production by the intestine or glutamine utilization. Despite these changes with fasting, we conclude that the intestine does not appear to be able to maintain portal venous blood concentrations of gluconeogenic precursors. (*Pediatr Res* 23: 241–244, 1988)

Abbreviation

[PV-A], portal venous blood concentration minus aortic blood concentration

Many low birth weight infants are nourished using parenteral methods. If the intestine is primarily an absorptive organ, this therapy may be appropriate. However, the small intestine performs several major metabolic functions including oxidation of glucose to lactate under aerobic conditions (1–3), oxidation of glutamine to CO_2 (3, 4), and conversion of lactate to alanine and CO_2 after infusions of lactate (3). These important intestinal metabolic functions release gluconeogenic precursors, alanine and lactate, into the portal venous effluent which perfuses the liver. Ramesy *et al.* (5) have reported elevated portal venous concentrations of glucose, alanine, and lactate in fed rats. Katz *et al.* (6) and Boyd *et al.* (7) have reported that gluconeogenic precursors, alanine (8) and lactate, are the primary precursors for hepatic glycogen synthesis during the fed state. Together, these data suggest that the small intestine may be a significant source of lactate and alanine for utilization by the liver. During fasting, intestinal glutamine oxidation to CO_2 and the subsequent production of alanine are not affected (3, 9) and so the intestine may be an important source of alanine.

These studies, which identified significant aerobic small intestinal metabolism of glucose to lactate and the oxidation of glutamine to CO_2 (1–4), were done under anesthesia and with bowel manipulation. Anesthesia can alter blood substrate concentrations as well as the metabolism of substrates (3). Ether, for example, increases blood glucose and lactate concentrations (10). Anesthesia also can decrease cardiac output which would decrease mesenteric blood flow and intestinal perfusion. Surgical manipulation can alter intestinal metabolism and perfusion. Metabolic studies involving the fetal lamb exemplify the importance of chronic catheterization in contrast to acute catheterization (11).

We set out to determine the effect of fasting on small intestinal metabolism and the resulting effect on portal venous blood substrate concentrations under physiologic conditions. We used a chronically catheterized rat model in which the portal vein and aorta remained patent for blood sampling. The portal venous and aortic blood concentrations of glucose, lactate, alanine, and glutamine were measured in the fasted and fed states. In the fasting state intraluminal substrates are unavailable to the intestine, so the portal venous to aortic blood concentration gradient reflects qualitatively the utilization or production of blood borne substrates by the intestine.

MATERIALS AND METHODS

Animals. All experiments were performed *in vivo* using adult male, albino, Sprague-Dawley rats (200–300 g body weight). These animals were obtained from Simonsen Labs (Gilroy, CA) and allowed free access to water and rat food. The adult rats were weighed and anesthetized with a 20 mg/kg ketamine injected intramuscularly followed by a 10 mg/kg pentobarbital injected intraperitoneally. Using aseptic technique, portal venous and aortic catheters were surgically placed as described previously (12). After surgery, animals were weighed daily and the catheters were flushed with 0.35 ml of normal saline containing 500 U/ml heparin and 2.5 mg/ml of ampicillin.

The effect of fasting on portal venous and aortic blood substrate concentrations. The effects of fasting on portal venous and aortic blood substrate concentrations were investigated in rats who were catheterized for more than 4 days. Portal venous and aortic blood (0.4 ml) samples were drawn and immediately placed in 0.4 ml of ice cold 1 M $HClO_4$. The protein precipitate was separated from the remaining solution by centrifugation and the supernatant was neutralized with 2 M potassium bicarbonate within 60 min of blood sampling. To determine blood substrate

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concentrations in small volumes of blood, we adapted spectrophotometric assays for glucose (13), lactate (14), alanine (15), and glutamine (16), to fluorometric assays. Blood samples were analyzed for substrate concentrations within 2 h. Because Aoki *et al.* (17) reported that red blood cells transport significant amounts of amino acids, we measured whole blood and not plasma concentrations of all substrates in order to determine portal venous and aortic blood concentrations differences ([PV-A]) as an indication of net flux of substrates across the gastrointestinal tract. A positive [PV-A] implies substrate production by the intestine.

Units for substrate concentrations are umol of substrate/g blood (mean ± SEM). Statistical significance was evaluated using the unpaired Student's *t* test with the Bonferroni correction for multiple *t* tests.

RESULTS

The chronically catheterized rat preparation. Three days after placement of the catheters, the rats had lost 10% of their preoperative weight. The weights remained constant for the next 4 days and then increased 1.4% of preoperative weight per day thereafter. Preoperative weight was regained 10.8 ± 0.7 days after surgery. Fifteen experiments were carried out in rats 4–9 days after catheter placement and 13 experiments were carried out in rats more than 10 days after catheter placement.

The effect of fasting on aortic and portal venous substrate concentrations in chronically catheterized rats. Glucose and Lactate. After 48 h of fasting, mean glucose concentrations decreased in portal venous and aortic blood by 32 and 26%, respectively ($p < 0.001$) (Fig. 1). A significant glucose [PV-A] was not found in fed or fasted catheterized rats.

Aortic lactate concentrations in catheterized rats did not change significantly after 48 h of fasting (Fig. 1). Mean portal venous lactate concentrations decreased from fed concentrations by 46% ($p < 0.001$). A significant lactate [PV-A] was not present in fed rats. The lactate [PV-A] of fasted rats was significantly negative (paired *t* test, $p < 0.012$).

Alanine. Mean aortic alanine and portal venous alanine concentrations of fasted catheterized rats were 42% ($p < 0.005$) and 47% ($p < 0.001$) less than concentrations in the fed state, respectively. The alanine [PV-A] was positive (paired *t* test, $p < 0.005$) in fed rats (Fig. 2). In fasted rats the alanine [PV-A] remained positive (paired *t* test, $p < 0.001$) but was one-half that in the fed state.

Glutamine. No significant change in mean aortic or portal venous glutamine concentrations were found between the fasted and fed states in chronically catheterized rats. Glutamine [PV-A] was negative in fed (paired *t* test, $p < 0.005$) and fasted (paired *t* test, $p < 0.001$) rats (Fig. 2). Fasting for 48 h did not alter the magnitude of the glutamine [PV-A].

DISCUSSION

During the fed state, the glucose [PV-A] in chronically catheterized rats varied from animal to animal. Because these animals were allowed unrestricted access to food, the variability in the glucose [PV-A] may reflect different amounts of rat food present in the intestine at the time of the study. In fed catheterized rats the portal venous lactate concentrations were not significantly greater than aortic concentrations indicating no significant lactate production by the intestine. This *in vivo* finding in a unanesthetized rat is consistant with data of Windmueller and Spaeth (3) who used an anesthetized *in situ* intestinal preparation. In contrast, Nicholls *et al.* (2) using a perfused intestinal model measured a significant amount of lactate production. Hanson and Parsons (1) determined that the amount of intestinal lactate production in a perfused model was dependent on the hematocrit of the perfusate. These studies indicate that presumed aerobic lactate production by intestine may actually be an artifact of the intestinal model. In our *in vivo* model without anesthesia, no significant lactate production was determined.

We found in our study that aortic glucose and lactate concentrations decreased after 48 h of fasting. This observation is consistent with the data of Ballard and Hanson (18). In addition to a decrease in blood glucose and lactate concentrations asso-

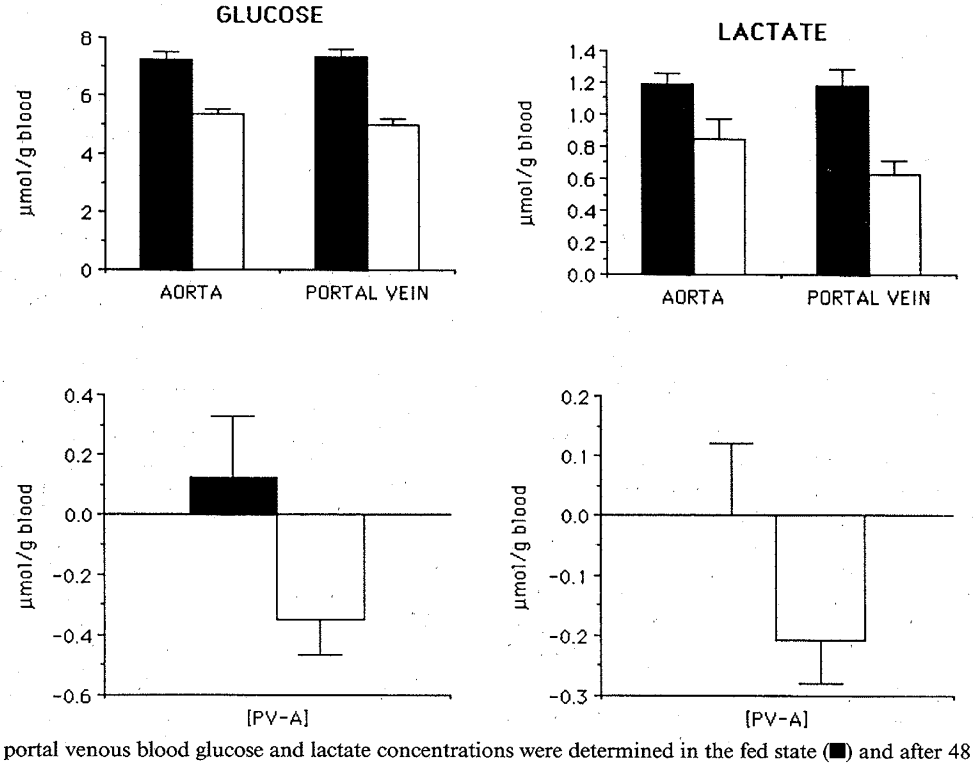

Fig. 1. Aortic and portal venous blood glucose and lactate concentrations were determined in the fed state (■) and after 48 h of fasting (□). The values are means ± SEM ($n = 11$).

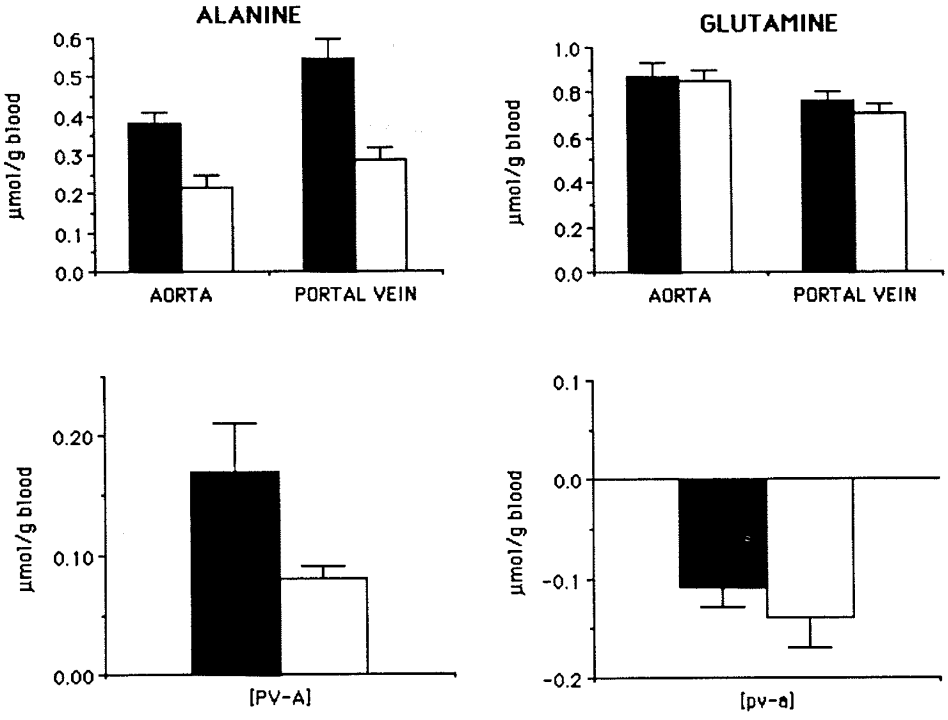

Fig. 2. Aortic and portal venous blood alanine and glutamine concentrations were determined in the fed state (■) and after 48 h of fasting (□). The values are means ± SEM (n = 8–11).

ciated with fasting, the lactate [PV-A] was negative after 48 h of fasting in chronically catheterized rats, suggesting active uptake and utilization of lactate by the intestine. Because intraluminal substrates are low in the fasted state, the negative lactate [PV-A] reflects intestinal utilization of vascular borne lactate. Most probably lactate is converted to pyruvate which is then converted to alanine by alanine aminotransferase. Windmueller and Spaeth (3) determined that 60% of lactate infused into the mesenteric vascular system was converted to alanine. The conversion of lactate to alanine, is supported by the observation that there is a positive alanine [PV-A] in the fasted rat at 48 h. It is possible that lactate can be used as an oxidative substrate by the small intestine. However, in experiments by Windmueller and Spaeth (3) and Hanson and Parsons (9), lactate does not appear to be a primary oxidative substrate in the postabsorptive intestine. Another possibility is the conversion of lactate to glucose through gluconeogenesis. However, Hahn and Wei-ning (19) have reported that gluconeogenesis in intestinal mucosa is low in adult rats.

Portal venous lactate concentrations decreased in chronically catheterized fasted rats. This decline in portal venous lactate concentration reflects the decrease in aortic lactate concentrations. In addition, in fasted chronically catheterized rats the lactate [PV-A] was negative suggesting lactate utilization by the small intestine, which would cause a decrease in portal venous concentration. These results suggest that in the fasting state there is a decrease availability of lactate as a precursor for gluconeogenesis.

It has been proposed that the small intestine is a significant source of alanine (20, 21). We measured a significantly positive alanine [PV-A] in both the fed and fasted states. The positive alanine [PV-A] in fasted rats must be secondary to net production of alanine by the small intestine and not related to absorption of intraluminal amino acids. The production of alanine from lactate infused into an intestinal artery has been reported by Windmueller and Spaeth (3). The significantly greater alanine [PV-A] in fed compared to fasted chronically catheterized rats may be secondary to the intraluminal source of alanine available in the fed state. This is supported by the decrease in PV alanine in fasted chronically catheterized rats compared to fed rats. The actual rate of production of alanine by the intestine has not been determined because we have not measured the rate of mesenteric blood flow.

Glutamine oxidation by the intestine involves the conversion of pyruvate to alanine via alanine aminotransferase and the conversion of glutamate to 2-oxoglutarate. We have determined that in both the fed and fasted states the glutamine [PV-A] is negative indicating a net utilization of glutamine by the small intestine. Our observations that fasting does not affect glutamine utilization by the intestine as indicated by the glutamine [PV-A] is consistent with the results reported by Hanson and Parsons (8) and Windmueller and Spaeth (3). It has been reported that glutaminase activity in rat mucosa is not affected by fasting (22, 23). The actual rate of utilization of glutamine has not been determined because we have not determined the rate of mesenteric blood flow.

In *in vitro* experiments, lactate, alanine, and galactose, gluconeogenic precursors, were the primary source of carbon in glycogen synthesis. These studies indicated that glucose is not incorporated into liver glycogen (5). In elegant studies, Newgard *et al.* (24) showed that in order for glucose carbon to be incorporated into liver glycogen, it must first be metabolized to pyruvate and then synthesized to glucose-6-phosphate by gluconeogenesis before glycogen synthesis. The intestine has been implicated as a significant organ for the production of lactate and alanine (7). Our study indicates that the intestine does produce alanine. However, we were not able to measure a positive lactate [PV-A] in either the fed or fasting states.

In summary, these *in vivo* studies in unanesthetized rat indicate that the small intestine is a source of alanine and a utilizer of glutamine. In addition, these studies indicate that gluconeogenic precursors, lactate and alanine, decrease in the portal vein with fasting. It is known that aerobic metabolism of glucose to lactate occurs in the fasting state. However, the lactate [PV-A] is negative in the fasting state and this suggests net intestinal utilization. The alanine [PV-A] remain positive during the fasting state suggesting intestinal production of alanine. Despite these changes with fasting, we conclude that the intestine does not appear to be able

to maintain portal venous blood concentrations of gluconeogenic precursors.

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