

JOURNAL OF ANIMAL SCIENCE

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J Anim Sci 2009.87:2013-2019.

doi: 10.2527/jas.2008-1423 originally published online Feb 27, 2009;

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://jas.fass.org/cgi/content/full/87/6/2013>



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Technical note: An improved surgical model for the long-term studies of kinetics and quantification of nutrient absorption in swine^{1,2}

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ABSTRACT: An improved technique to study kinetics and quantitative absorption of nutrients in pigs is described. Three female pigs (35 kg of BW) were surgically modified with catheters in the hepatic portal vein and carotid artery and an ultrasonic flow probe around the portal vein. Catheter placement and patency was secured using distal modifications (rings and holes) and nonabsorbable suture. Catheters and flow probe cable were tunneled subcutaneously after exteriorization for further protection. Fibrosis and adhesions in the body cavity were minimized by avoiding excessive manipulation and drying of viscera. Pigs were supported during recovery by intravenous fluid therapy of AA and electrolytes until regular feeding resumed. Catheters were flushed daily with heparinized saline (200 IU/L). After 10 d, pigs were fed a diet based on wheat and soybean meal for 6 consecutive 7-d periods. On d 7, blood was

collected postprandially every 15 min from -15 to 60 min, 30 to 240 min, 60 to 480 min, and 120 to 720 min. Blood flow was measured simultaneously. Plasma was analyzed for glucose, and net glucose absorption was calculated from plasma portal-arterial differences \times plasma flow [blood flow \times (1 - hematocrit)]. The specific improvements for long-term use of this model are distal modifications of the catheters, postoperative treatment using parental nutrition and gut motility drug, prevention of infection of body cavity by further tunneling of catheters and blood flow probe cable, and use of ultrasonic blood flow probes and meter. Blood flow measurements using an ultrasonic blood flow probe was not changed after 52 d compared with 10 d post-surgery, indicating the reliability of this model. This catheterized pig model, thus, will allow the long-term study of the kinetics of nutrient absorption.

Key words: absorption, catheterization, kinetics, pig, portal vein

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J. Anim. Sci. 2009. 87:2013–2019
doi:10.2527/jas.2008-1423

INTRODUCTION

Dietary nutrient characteristics affect the kinetics of nutrient absorption, gastrointestinal and pancreatic hormone responses, and nutrient metabolism. Catheterization of the portal vein and carotid artery and simultaneous installation of a blood flow probe in the pig (Rerat et al., 1980) is an excellent model to study the kinetics of absorption. The difference in nutrient concentration between blood in the portal vein and carotid artery is multiplied with simultaneously measured blood flow through the portal vein. The net nutrient flux into the portal vein and absorption kinetics has

been measured for carbohydrates (Rerat et al., 1984), AA (Caine et al., 1999), urea, and ammonia (Van Leeuwen et al., 1995).

Major constraints for successful application of the model are complicated surgical procedures, postoperative management, catheter maintenance, and accurate blood flow measurements. Surgical procedures have been modified in individual laboratories to accommodate surgical capabilities, material availability, and model application (e.g., Bajjalieh et al., 1981; Yen and Killefer, 1987; Van Leeuwen et al., 1995; Ten Have et al., 1996). Emergence of ultrasonic, instead of electromagnetic flow probes, improved blood flow measurements. However, in the last decade, the model was used sparsely (Bach Knudsen et al., 2000, 2005; Reverter et al., 2000; Lambert et al., 2002; Yen et al., 2004; Le Floc'h and Sève, 2005), in part due to the constraints. Therefore, a need exists to describe the current advances in the model and thereby maximize use in nutrition studies.

The objectives of the present study were to improve the method for catheterization of the portal vein and

¹S. Hooda is supported by an Alberta Ingenuity PhD Student Scholarship.

²The authors acknowledge N. Le Floc'h for her invaluable help in the initial major steps of the technique set-up in the laboratory of J. J. Matte.

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Received August 21, 2008.

Accepted February 23, 2009.

carotid artery, postoperative management, and establishment of blood flow measurements using an ultrasonic flow meter and to test the effectiveness of this model for nutrient absorption for long-term studies in swine.

MATERIALS AND METHODS

The animal use protocol was approved by the Animal Care Committee of the University of Alberta, in accordance with the guidelines of the Canadian Council on Animal Care (CCAC, 1993).

Animals and Diets

The animal experiment was conducted at the Swine Research and Technology Centre at the University of Alberta (Edmonton, Alberta, Canada). Three female pigs (35 to 40 kg of BW) were moved to pens 1 wk before surgery and handled daily to habituate them to close human contact for better postoperative management and blood sampling. Pigs were fed at 0800 and 2000 h a diet based on wheat and soybean meal along with water containing electrolytes (Gatorade, Quaker Oats, Chicago, IL). Pigs were fasted for 12 h before surgery but had free access to water.

Catheter Preparation and Ultrasonic Blood Flow Meter

The portal and arterial catheters were prepared using 1-m-long polyvinyl tubing (inner diameter, 1.02 mm; outer diameter, 1.78 mm; Saint-Gobain Performance Plastics, Cleveland, OH) with an 18-gauge blunt needle as an adaptor. For the portal vein catheter, two 2-mm rings of tubing (inner diameter, 1.27 mm; outer diameter, 2.29 mm) were installed 1 cm apart by stretching the rings using forceps, with the first ring 2.5 cm from the catheter tip (Figure 1). Two small holes were cut in the sides of the catheter between the tip and first ring with small scissors to have an alternate route of blood collection. In preliminary work (data not shown), postmortem examination of pigs of nonpatent catheters occasionally revealed a catheter tip placed against the wall of the portal vein. For the carotid artery catheter, one 2-mm ring was installed 10 cm from the tip of the catheter. Rings were glued with Loctite 406 (Loctite Corp., Rocky Hill, CT), and edges were made smooth with a file after complete drying. Both catheters were flushed and dried for 48 h with tridodecylmethylammonium complex heparinate (TDMAC-heparin, Poly-science Inc., Warrington, PA) as an anticoagulant. Pouches to secure the exteriorized catheters were constructed from 10-cm-wide strips of generic adhesive bandage and hook-and-loop fasteners. The dimensions of arterial and portal pouches were 10 × 22 cm and 12 × 30 cm, respectively.

To measure blood flow, an ultrasonic flow meter (model TS 206, Transonic Systems, Ithaca, NY) with 14-mm

flow probes (S series, back cable exit, U reflectors) was used. Windaq data acquisition software (Dataq Instruments, Akron, OH) was used to measure pulsatile blood flow continuously by computer. Data were transferred from the flow meter to the computer with a generic Bayonet Neill-Concelman cable connector. The 10-min average flow rate at each collection time point was used for calculations of quantitative absorption.

Surgery

Preparation. Pigs were sedated first with an intramuscular injection of 12 mg of Ketalean/kg of BW (Ketamine HCl, Biomedica MTC, Cambridge, Ontario, Canada), 2 mg of Rompun/kg of BW (Xylazine, Bayer Cross, Toronto, Ontario, Canada), and 0.05 mg of atropine/kg of BW (Atropine sulfate, Rafter, Calgary, Alberta, Canada). After 30 min, general anesthesia was induced using 4% isoflurane (Isoflo, Abbott Laboratories Ltd., Saint-Laurent, Québec, Canada) provided using a face mask. Then, an endotracheal tube was inserted and connected to a closed-circuit anesthetic apparatus to maintain general anesthesia with isoflurane during surgery. A catheter was inserted in the ear vein for continuous intravenous infusion of 1,500 mL of Ringer lactate (solution of NaCl, Na lactate, KCl, and CaCl₂; Hospira, Montreal, Quebec, Canada) during surgery. Before surgery, 0.2 mg of Metacam/kg of BW (Boehringer Ingelheim, Burlington, Ontario, Canada) was administered subcutaneously. The pig was placed in dorsal recumbency position. The skin at the mid line and right side was shaved and prepared aseptically with Betadine (7.5% povidone-iodine, Purdue Pharma, Pickering, Ontario, Canada).

Portal Vein Catheter and Flow Probe. The pig was placed on a surgery table equipped with a recirculating warm water pad and covered with disposable surgical drape (Jorgensen Laboratory Inc., Loveland, CO). An incision was cut in the skin from the base of the sternum down the midline of the abdomen to the umbilicus. After opening the muscle layers and peritoneum, 4 towel clamps (2 on each side) were installed in the muscle layer and attached to the side of the surgery table using stainless-steel chains to open the surgery site. Sterile surgical towels (45.7 × 45.7 cm; Dukal Corp., Hauppauge, NY) soaked in warm normal saline (0.9% NaCl solution) were used to retract the stomach, spleen, and intestines away from the surgical site. A V-shaped purse-string suture with 22-mm curved needle (Vicryl, Polyglactin 910; Ethicon Inc., Somerville, NJ) was installed in the wall of the portal vein 2.5 cm posterior to the bifurcation of the portal vein into the liver (Figure 2). A 14-gauge needle with stopper was used to create an opening into the portal vein in the middle of the purse-string suture. The needle was removed, and the bleeding was stopped by applying pressure with gauze pads. The catheter was inserted into the portal vein using a wire guide (Cook Canada Inc., Stouffville, Ontario, Canada) until the first ring of the catheter

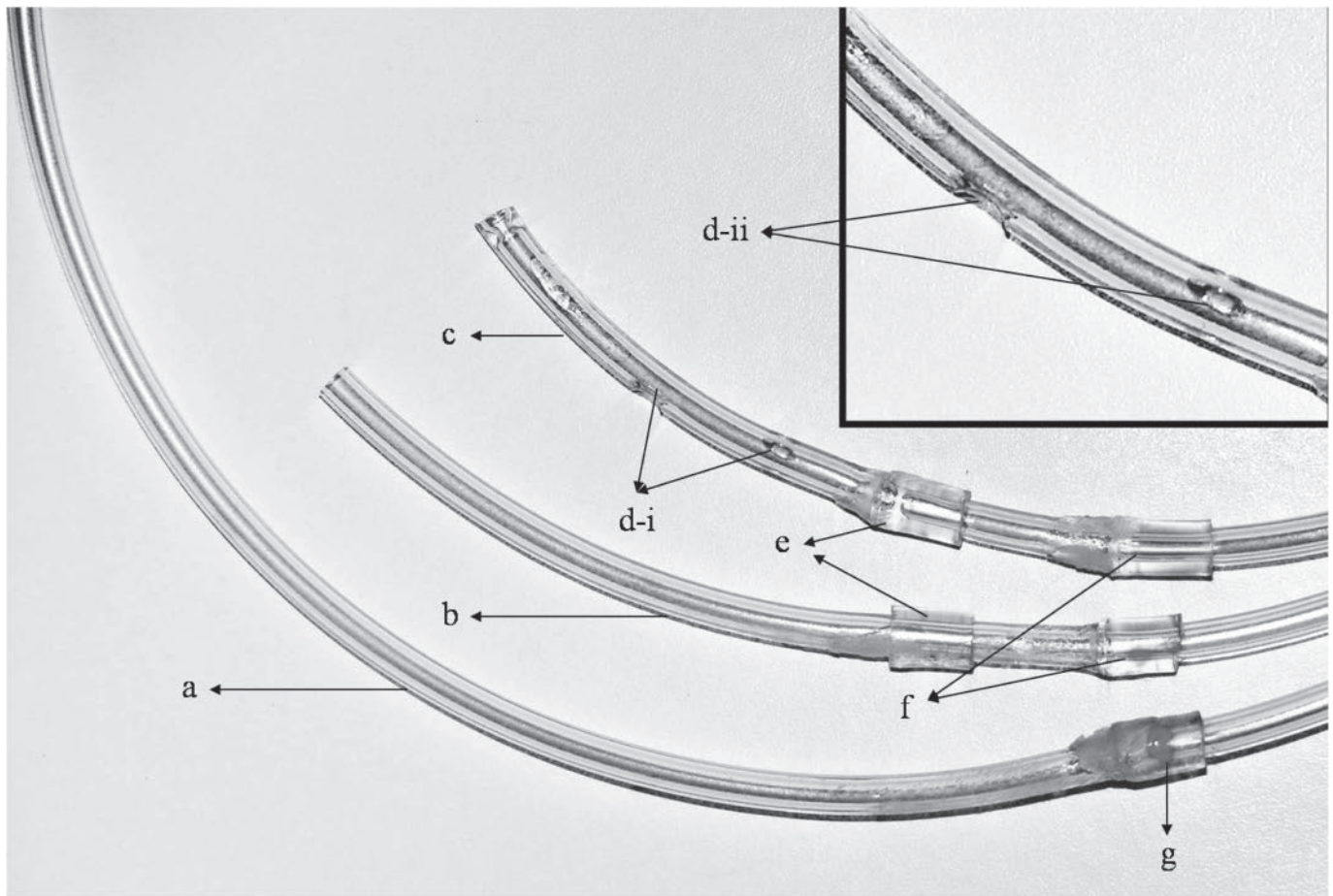


Figure 1. Carotid artery with unmodified and modified (with holes) portal vein catheters. (a) carotid artery catheter; (b) unmodified portal vein catheter; (c) modified portal vein catheter; (d-i) holes of the portal vein catheter; (d-ii) enlarged view of holes of portal vein catheter; (e) inner rings of portal vein catheter; (f) outer rings of portal vein catheter; and (g) ring of carotid artery catheter.

was inserted into the sheath covering the portal vein. Then the suture was closed using 4 square knots. The catheter was secured to the sheath using a nonabsorbable suture (Prolene 4-0, polypropylene, Ethicon Inc.) with 6 square knots around the second ring. Catheter patency and position were checked after withdrawing the wire guide and flushing with 200 IU/mL of heparin saline solution. The site of catheterization was lavaged repeatedly with warm normal saline. Subsequently, upstream from the catheter near the lymph nodes the connective tissue around the vein was dissected free to create clear space around the vein. The flow probe was installed and immersed into normal saline to check the probe function. The flow probe was exteriorized by cutting a hole in the right flank at the base of the vertebral ribs. The catheter was exteriorized using a tunneling rod 3 cm caudal from the probe cable. Both flow probe cable and catheter were wrapped in sterile surgical towel to maintain sterility until further tunneling. The abdominal cavity was filled with warm normal saline and closed by suturing separately the peritoneum, muscle layer, and subcutaneous tissue using a continuous suture (PDS II, Polydioxanone, Ethicon Inc.) and the skin using a single interrupted suture (Novafil, Covidien, Mansfield, MA).

Carotid Artery Catheter. For the arterial catheter, the skin was incised on the right side of the neck, close to the trachea. The carotid artery was dissected free, and damage to the vagus nerve was avoided. A square knot was tied around the artery with vicryl suture toward cranial side to stop blood flow to the head. A loose loop of suture was inserted beneath the artery toward the heart and lifted to stop blood flow to the site of catheterization. The artery wall was dissected and cut with small iris scissors and the catheter was inserted gently 10 cm (to reach the vessel junction between carotid and subclavian arteries) until the ring passed the suture. The catheter was secured by tying 4 square knots with vicryl. An additional suture was made with the nonabsorbable suture (Prolene, polypropylene, Ethicon Inc.) around the catheter using 6 square knots to ensure secure placement. The catheter was tunneled dorso-caudally beneath the skin and exteriorized from the right side to the nape using a tunneling rod, and the incision was closed by suturing the skin using a simple interrupted suture.

External Protection. The blood flow probe cable was tunneled subcutaneously 10 cm dorsally. The portal vein catheter was tunneled subcutaneously dorsally to exit near the midline in the mid lumbar region. The

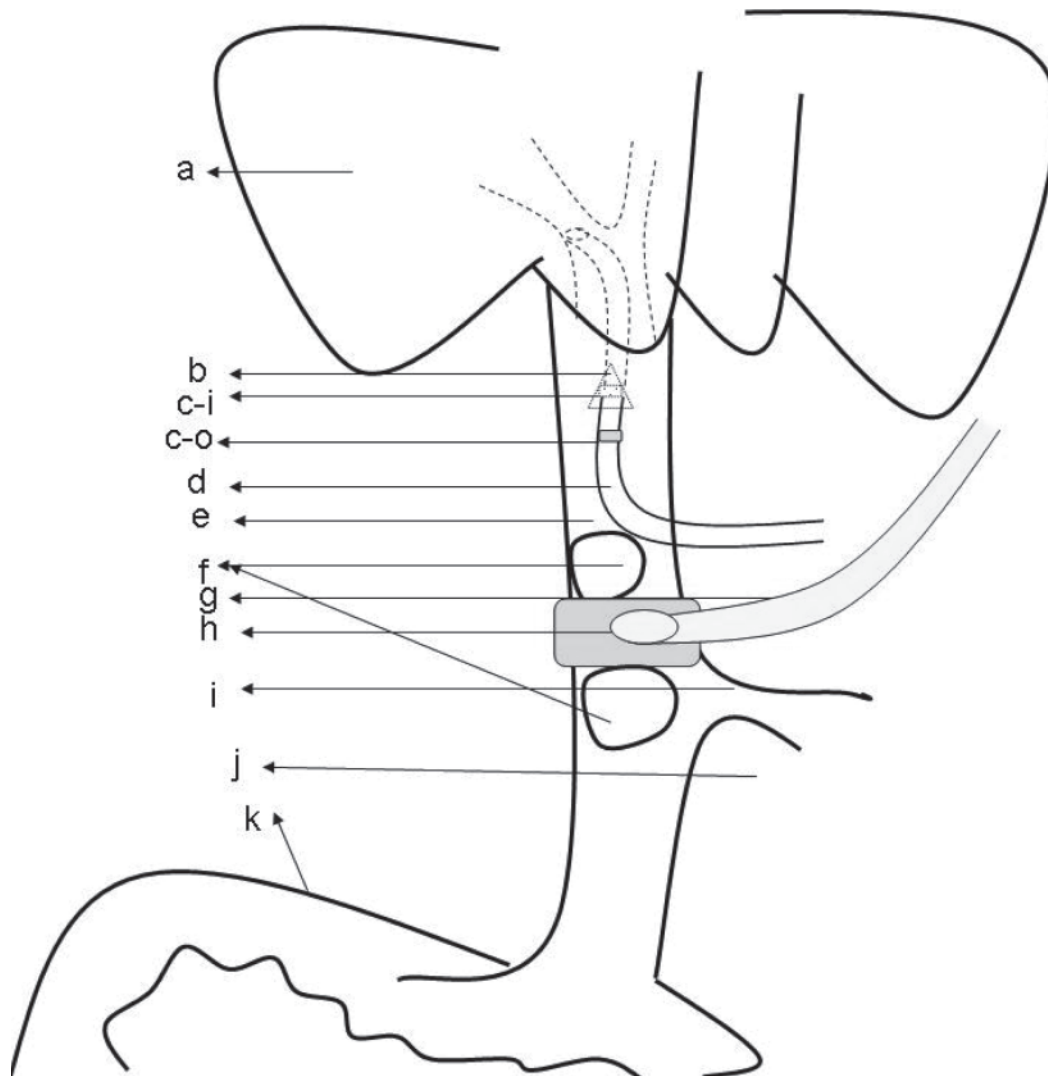


Figure 2. Schematic of portal vein catheter and probe placement (adapted from Ten Have et al., 1996): (a) liver; (b) V-shaped purse-string suture; (c-i) ring of the portal vein catheter inside the portal vein; (c-o) ring of the portal vein catheter outside the portal vein; (d) portal vein catheter; (e) portal vein; (f) lymph nodes; (g) probe cable; (h) probe body; (i) gastro-splenic vein; (j) pancreas; and (k) intestine.

probe cable and portal catheter were secured together in a pouch. The arterial catheter was also tunneled dorsally and caudally to exit just behind the shoulders and was secured in a pouch.

Postoperative Management

After surgery, the antibiotic Cefazolin-Na (25 mg/kg of BW, every 8 h; Cefazolin-Na, Novopharm Ltd., Toronto, Ontario, Canada), the analgesic Torbugesic (0.4 mg/kg of BW, every 6 h i.v.; Butorphanol tartrate, Wyeth Animal Health, Guelph, Ontario, Canada), and the analgesic/anti-inflammatory drug Meloxicam (0.1 mg/kg of BW, every 24 h orally; Metacam Oral Suspension, Boehringer Ingelheim, Burlington, Ontario, Canada) were given for 3 d. On the evening of the surgery day and the next morning 2 mL/kg of BW of a cocktail containing dextrose, electrolytes, AA, and B-complex vitamins (Aminolean, Vetoquinol N.-A. Inc., Lavaltrie, Quebec, Canada) was given intravenously via the portal vein catheter as a slow drip to provide parental nutri-

tion. To induce gut motility and reduce postoperative nausea, Metaclopramide HCl (10 mg intramuscularly, every 8 h; Sandoz Canada Inc., Boucherville, Québec, Canada) was given for 2 d. Pig body temperature, pain, and general health were monitored daily at 0800 and 1600 h.

The day after surgery, 100 g of feed was offered in the morning and evening. Feed allowance was gradually increased to 1 kg/d on d 5 postsurgery. The catheters were flushed aseptically daily with 200 IU of heparinized normal saline to maintain their patency. Sutures were removed using a 50% dose of Rompun and Ketalean 10 d after surgery. Pigs were wearing stretchable shirts to cover arterial and venous pouch to further secure catheters and probes.

Experimental Sampling, Measurement of Blood Flow, and Sample Analysis

Blood samples (10 mL each) were collected from 3 pigs 1 d every week in heparinized tubes (BD Canada,

Oakville, Ontario, Canada) from the carotid artery and portal vein. Blood was collected every 15 min from -15 to 60 min, then every 30 min to 240 min, then every 60 min to 480 min, and 600 and 720 min postprandially; blood flow was measured simultaneously. During measurements, the probe was attached to the flowmeter with a cable. Flow was recorded continuously at each collection for 10 min using Windaq software. After the collection, the catheter was flushed with 10 mL of 10 IU/mL of heparinized saline to prevent clotting and replace the fluid loss. Plasma was collected from blood after centrifugation at $3,000 \times g$ for 10 min at 4°C and then stored at -20°C. Plasma was analyzed for glucose (glucose oxidase kit; Diagnostics Chemicals Ltd., Charlottetown, Prince Edward Island, Canada). Net glucose absorption was calculated from plasma portal-arterial differences \times plasma flow (Rerat et al., 1980). Plasma flow rate was calculated from blood flow rate using the following equation: plasma flow = blood flow \times (1 - hematocrit). Blood collection and blood flow measurements were done weekly for consecutive 6 wk.

Calculations and Statistical Analysis

Net glucose absorption was calculated from plasma portal-arterial differences and plasma flow measurements using the formula $q = (C_p - C_a)F(dt)$ (Rerat et al., 1980). Cumulative net glucose absorption can be calculated subsequently using the formula

$$Q = \sum_{t_0}^{t_1} q.$$

In the formulas, q is the amount of nutrients absorbed within time period dt , C_p and C_a are the concentrations of nutrient in portal and arterial plasma, respectively, F is the plasma flow in the portal vein, and Q is the amount of nutrient absorbed production from time t_0 to t_1 . Blood flow rates of period 1 and 6 were compared using t -test. Plasma carotid and portal glucose and net glucose absorption were analyzed as repeated measures using the MIXED procedure (SAS Inst. Inc., Cary, NC).

RESULTS AND DISCUSSION

Surgery

The duration of surgeries averaged 5 h, despite anatomical variations inside the body cavity such as location and size of the portal vein and lymph nodes on the portal vein (Hecker, 1974). The dorsal recumbency position combined with a mid-line incision made the area of the portal vein accessible for catheterization and blood flow probe implantation. Use of wet surgical towels to keep the viscera away from the surgical site further facilitated access.

The surgical approach using a mid-line incision was developed by Rerat et al. (1980). Another approach, the left lateral recumbency position, has been used (Bajjalieh et al., 1981; Yen and Killefer, 1987); however, the working field is smaller than with the present approach. The portal vein catheterization procedure using a purse string suture was developed by Rerat et al. (1980); however, in the present study a wire guide, instead of a modified needle, was used to guide the catheter into the vein. In swine and sheep, rings have been used previously to support suturing catheters outside the blood vessel to tissue (Mineo et al., 1991; Trottier et al., 1995); however, installation of the first of 2 rings inside the vein to secure catheter placement was a novel approach first described in the present study. Previously, portal catheters have been secured with a nylon mesh, glued to the catheter, and sutured to connective tissue (Yen and Killefer, 1987). Catheters with silicon rosettes may prevent postsurgical changes in catheter length (Van Leeuwen et al., 1995). The technique to install the flow probe was similar to that described previously for the external pudic artery in swine (Renaudeau et al., 2002) and ruminal arteries in sheep (Remond et al., 1993). The carotid artery catheterization procedure is a standard methodology (e.g., Rerat et al., 1980).

Recovery and Postoperative Outcome

Pigs did not have complications on the day of surgery and recovered from surgery without clinical signs of infection. After surgery, parental nutrition served as supply of essential AA, electrolytes, and energy to the intestine and pig until oral feeding resumed. Preoperative habituation of pigs allowed for postoperative management. Therapy with antibiotics for 3 d and sterile surgical techniques prevented postoperative infections. On d 2 after surgery, 1 pig started vomiting, refused to eat, and was lethargic; both catheters were functional. After gut motility medication treatment for 2 d, the pig recovered fully. The successful recovery postsurgery is likely due to factors during and after surgery (e.g., the use of sterile saline towels to hold viscera to prevent adhesions and infections inside the body cavity, subcutaneous tunneling of the probe cable to reduce tracking of skin contamination along the cable to prevent infection, parental nutrition, broad-spectrum antibiotics, and the gut motility drug). The 2 rings on the tip of the catheter to secure placement, use of anticoagulant coating (TDMAC-heparin), and regular flushing of catheters with heparinized saline maintained patency of the portal vein catheter for 52 d postsurgery, indicating that blood can be sampled long-term in catheterized swine (Trottier et al., 1995). The necessity of the extra holes in catheters cannot be demonstrated experimentally, and we have since completed studies successfully in our combined laboratories using portal catheters with and without the extra holes.

Table 1. Comparison of portal vein blood flow 10 and 52 d after surgery

Blood flow rate, L/min	Day after surgery		Pooled SEM	P-value
	10	52		
Preprandial ¹	1.11	1.16	0.16	0.79
Postprandial ²	1.23	1.30	0.16	0.67

¹Means of 3 observations of blood flow between 5 min before and 5 min after morning feeding.

²Means of 3 observations of flow between 5 min before and 5 min after 120 min of morning feeding.

Blood Flow Rate Measurements

Blood flow is the critical factor for quantitative nutrient absorption measurements and a major constraint for chronic studies. Blood flow rate has been measured using electromagnetic flow probes (Bajjalieh et al., 1981) or the indicator dilution method (Yen and Killefer, 1987). Some have simply used reported blood flow values (Van Leeuwen et al., 1995). In the present study, ultrasonic flow probes replaced old electromagnetic technology with the advantage that the probe is placed in alignment with the vessel and data can be collected immediately after implantation. The electromagnetic flow probe has major limitations for chronic studies as proliferation of fibrous tissues between the sensor of flow probe and vessel wall reduces its flow rate abnormally low after 13 to 28 d (Rerat et al., 1980).

The flow rate of portal vein at the time of feeding was 1.11 L/min at 10 d after surgery and 1.16 L/min at 52 d after surgery (Table 1). The average flow rate was 28.3 mL/(kg·min), similar to previous reports using ultrasonic blood flow probes (Bach Knudsen et al., 2000, 2005). Blood flow at the time of feeding and postprandially did not differ between 10 and 52 d after surgery (periods 1 and 6, respectively), indicating that

blood flow rate might remain constant within studies. Physiologically, blood flow normally plateaus after pigs reach 30 to 50 kg of BW and differences are, thus, abnormal and likely due to the measurement technique. A greater blood flow was measured with electromagnetic flow probes ranging from 38 to 55 mL/(kg·min) (Rerat et al., 1980, 1984; Simoes Nunes and Malmlof, 1992), a technology that is only reliable for 2 to 4 wk after surgery. The new ultrasonic probe has an advantage of taking measurements for longer periods after surgery (Ellis et al., 1995).

Quantitative Absorption Measurements

Baseline glucose in the portal vein and carotid artery was 5.5 mmol/L and increased to 10.1 mmol/L in the portal vein and 6.4 mmol/L in the carotid artery (Figure 3). The net glucose absorption from the intestine into the systemic blood circulation was -0.05 mmol/min before the meal and peaked at 3.39 mmol/min at 45 min postprandially. Although arterial and portal glucose concentrations and absorption kinetics cannot be compared with other studies because of differences in diets, the suitability of the present model has been demonstrated. The kinetics of glucose absorption affect

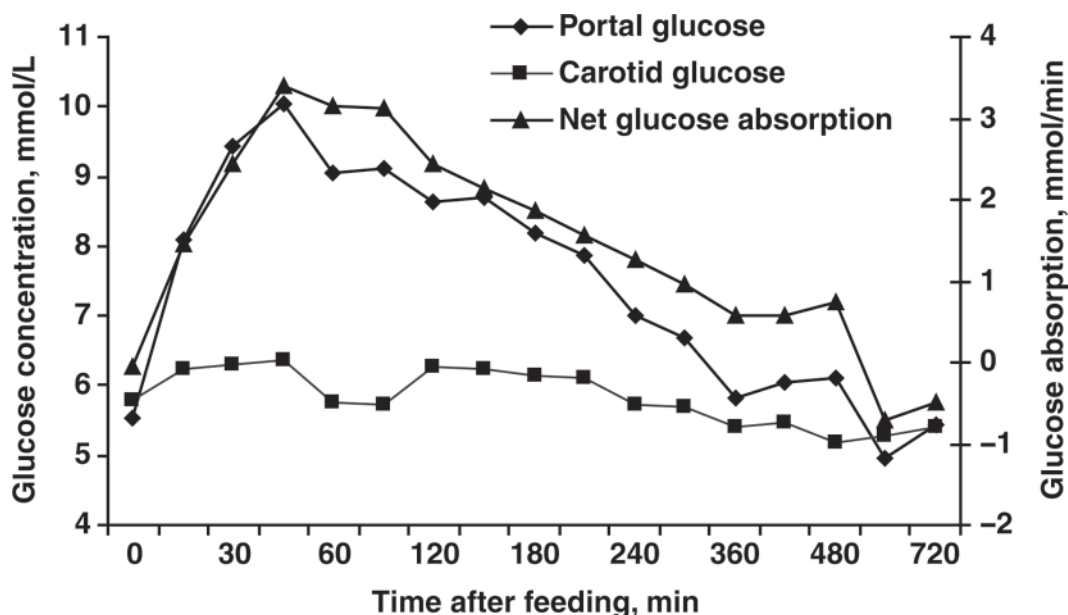


Figure 3. Portal and arterial plasma concentrations of glucose (mmol/L) and net glucose absorption rate (mmol/min) after feeding a diet based on wheat and soybean meal (based on 6 observations). For the 3 variables, there was a time effect ($P < 0.001$). The SEM was 0.36 for carotid glucose, 0.37 for portal glucose, and 0.43 for net glucose absorption.

glucose metabolism (Jenkins et al., 1995). In humans, effects of starch and fiber sources on glucose metabolism are normally studied using glucose measurements in peripheral blood. However, glucose homeostasis is tightly controlled by insulin and glucagon in the systemic circulation in normal human subjects. Furthermore, ethical and practical reasons limit experimental surgeries in human subjects. The present model in swine might, thus, be used as a model for nutrient absorption studies in humans.

The techniques to catheterize the portal vein and carotid artery and install a blood flow probe in the pig were described. Major improvements for long-term use of the model may include distal modifications of the catheters, specific postoperative treatment of parental nutrition and the gut motility drug, prevention of infection of body cavity by subcutaneous tunneling of catheter and blood flow probe cable, and use of ultrasonic blood flow probe and meter. The model can be used for several weeks after surgery to study effects of dietary ingredients on kinetics and total absorption of nutrients and hormones production and chronic metabolic studies.

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