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Permalink

<https://escholarship.org/uc/item/66w5c177>

Journal

Journal of Zoo and Wildlife Medicine, 47(1)

ISSN

1042-7260

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Publication Date

2016-03-01

DOI

10.1638/2014-0187.1

Peer reviewed

INTRAPERITONEAL DEXTROSE ADMINISTRATION AS AN ALTERNATIVE EMERGENCY TREATMENT FOR HYPOGLYCEMIC YEARLING CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Abstract: The Marine Mammal Center (TMMC) cares for malnourished California sea lion (CSL) (*Zalophus californianus*) pups and yearlings every year. Hypoglycemia is a common consequence of malnutrition in young CSLs. Administering dextrose during a hypoglycemic crisis is vital to recovery. Traditional veterinary approaches to treat hypoglycemia pose therapeutic challenges in otariids, as vascular access and catheter maintenance can be difficult. The current approach to a hypoglycemic episode at TMMC is to administer dextrose intravenously (IV) by medically trained personnel. Intraperitoneal (IP) dextrose administration is an attractive alternative to IV administration because volunteer staff with basic training can administer treatment instead of waiting for trained staff to treat. This study compares the effects of IV, IP, and no dextrose administration on serum glucose and insulin in clinically healthy, euglycemic CSL yearlings. Three groups of animals, consisting of five sea lions each, were treated with 500 mg/kg dextrose using one of the following routes: IV, IP, or no dextrose (control). A jugular catheter was placed, and blood samples were collected at times 0, 5, 15, 30, 60, 120, 180, and 240 min after dextrose administration. IV dextrose administration resulted in an increase of serum glucose concentrations from a baseline level of approximately 150 mg/dl to a peak of approximately 350 mg/dl. The resulting hyperglycemia persisted for approximately 2 hr and was associated with an attenuated plasma insulin response compared with most terrestrial mammals. Intraperitoneal dextrose administration resulted in increases of serum glucose to approximately 200 mg/dl, which gradually declined to baseline by 2 hr after dextrose administration. These data suggest that the initial treatment of a hypoglycemic crisis in young malnourished CSLs can be accomplished with IP dextrose, thus enabling minimally trained volunteer staff to respond immediately to a crisis. Further studies are needed to determine the most appropriate long-term treatment.

Key words: California sea lion, dextrose, hypoglycemia, intraperitoneal, marine mammal, and *Zalophus californianus*.

INTRODUCTION

Marine mammal rehabilitation centers along the west coast of the United States care for hundreds of malnourished California sea lion (CSL) (*Zalophus californianus*) yearlings every

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year, and the stranding rate has increased over the last few years (The Marine Mammal Center [TMMC], pers. comm.).⁶ One of the common sequelae to malnutrition in young CSLs is hypoglycemia. Clinical signs of hypoglycemia range from lethargy to seizures. Administering dextrose during a hypoglycemic crisis is vital to recovery. Premature removal of dextrose supplementation can cause a recurrence of severe hypoglycemia and is often followed by death from hypoglycemia.¹⁷ Traditional veterinary approaches to hypoglycemia treatment involve the use of intravenous (IV) or oral dextrose supplementation; however, otariids pose therapeutic challenges, as vascular access and catheter maintenance can be difficult.¹¹ Further, neither pinniped milk nor their prey contain simple sugars. Therefore, adverse side effects such as diarrhea can often accompany oral dextrose treatment.^{4,7} Historical treatment protocols for hypoglycemia at TMMC in Sausalito,

Table 1. Description of the three study groups of healthy yearling California sea lions.

Treatment group	Treatments	Number animals
1	No dextrose	5
2	2.5 ml/kg of 20% dextrose administered intraperitoneally	5
3	2 ml/kg 25% dextrose administered intravenously	5

California, USA, are to administer an IV bolus of 50% dextrose via the subclavian vein. At TMMC, this vein is only used when the animal is unconscious, as this vein is accessed with the animal in ventral-dorsal recumbency. Animals typically regain consciousness after the dextrose bolus, but within hours end up in another hypoglycemic crisis.

The objective of this study is to investigate a nonvenous method of dextrose administration that can be used by nonmedical staff with minimal training to treat young CSLs during a hypoglycemic emergency and to compare with IV dextrose administration, which is only given by trained medical staff. To achieve this, serum glucose and insulin levels were compared in healthy euglycemic yearling CSLs following IV or intraperitoneal (IP) dextrose administration or no dextrose administration (control).

MATERIALS AND METHODS

This study was conducted from July 2011 through August 2012 at TMMC and was authorized by a Stranding Agreement with the National Marine Fisheries Service, under the approval of The Marine Mammal Center's Institutional Care and Use Committee No. 2011:1 and Marine Mammal Protection Act Permit No. 932-1905/MA-009526. TMMC is a private, nonprofit organization located at Fort Cronkhite, Sausalito, California, USA, that is licensed to recover, rehabilitate, and release marine mammals. While undergoing rehabilitation at TMMC, sea lions are housed outdoors and are given access to closed-system, ozone-disinfected, salt water pools.

Fifteen, 1-yr-old CSLs, six females and nine males, were used in the study. The animals weighed between 22 and 37 kg, and their standard lengths were between 81 and 124 cm. The sea lions underwent rehabilitation following stranding due to trauma, abscesses, pneumonia, and/or malnutrition. On admission to TMMC, the animals are given a physical examination, and blood

is drawn and used for serum chemistry, using a Wasserman chemistry analyzer (Alfa Wassermann Diagnostic Technologies, West Caldwell, New Jersey 07006, USA) for testing and a complete blood cell count (CBC) using a Vet ABC machine (SCIL Animal Care Company, Gurnee, Illinois 60031, USA) for testing. Medical treatment is then administered depending on the results of the blood work and physical. The animals used were successfully rehabilitated over the course of about 6 wk. Animals were included in the study based on age classification (yearling) and clinical health status. Sea lions were deemed clinically healthy if they competed for food and haul-out space with conspecifics, were at or above normal weight, exhibited normal behavior, and had clinical resolution of disease. As part of normal veterinary practices at TMMC, each animal received a physical examination, serum chemistry, using a Wasserman chemistry analyzer (Alfa Wassermann Diagnostic Technologies), and CBC using a Vet ABC machine (SCIL Animal Care Company) prior to release. To decrease the amount of stress on each animal, the physical examination and blood work were completed within the first 30 min of the study period for each animal. If the blood work or physical examination were abnormal, the animal was removed from the study and continued rehabilitation.

The animals were each randomly assigned to one of three treatment groups: IV dextrose, IP dextrose, or a group with no dextrose (control) for comparison (Table 1). These groups were chosen because IP was determined to be a method of dextrose delivery that nonveterinary personnel could administer with minimal training, compared with IV, which is a method of dextrose delivery in which only highly trained personnel can administer during a hypoglycemic event (TMMC, pers. obs.). A dose of 500 mg/kg was chosen based on small animal emergency hypoglycemia treatment guidelines, which advise dextrose to be given as a slow IV bolus.¹¹ The 20% concentration of dextrose given IP was a higher concentration than what has been previously published in pinnipeds because the authors wanted to use a more concentrated solution to decrease the volume of fluid that needed to be administered. The 20% dextrose solution was chosen because it was used with little side effects in large animal medicine to rescue hypoglycemic neonatal lambs, *ovis aries*, in a field situation.⁹

All animals in the study were fasted for 12 hr overnight and then placed in a dry pen with no

access to water. Each animal was examined physically prior to anesthesia. The animals were physically restrained to facilitate anesthesia mask placement and then induced utilizing 5% isoflurane (Henry Schein Animal Health, Dublin, Ohio 43017, USA) in oxygen. The animals were then intubated and maintained at an average of 1.4% isoflurane in oxygen. Heart rate, end-tidal CO₂, respiratory rate, and body temperature were monitored throughout the procedure. Heart rate was an average of 102 beats/min and end-tidal CO₂ averaged 54 mm Hg for all the animals in all the groups. All animals spontaneously ventilated for the duration of the anesthesia period.

Once the animals were under anesthesia, they were placed in left lateral recumbency to access the right side of the neck. Each animal's neck was shaved along the right jugular furrow to access the right jugular vein. Alcohol was placed on the skin to facilitate ultrasound use. A portable GE Logiq E Vet ultrasound machine (GE Corporate, Fairfield, Connecticut 06828, USA) utilizing a 12-MHz linear probe was used to guide placement of the sampling catheter. Once the jugular vein was identified, the skin was aseptically prepared using a 4% chlorhexidine gluconate (Henry Schein Animal Health) and 70% isopropyl alcohol rinse (First Priority, Elgin, Illinois 60123, USA). An 18-ga, 2.75-inch angiocath needle (MILA 1606, MILA International, Erlanger, Kentucky 41018, USA) was placed into the right jugular vein to introduce the guide wire (MILA 1606, MILA International). The needle was then removed while the guide wire stayed in place in the jugular vein. A 16-ga, 5-French 6-inch long single lumen catheter (MILA 1606, MILA International) was then introduced into the jugular vein over the guide wire. Once the catheter was in place, the guide wire was removed. The catheter was secured using a Secur-a-cath® device (Interrad Medical, Plymouth, Minnesota 55441, USA) and one to three skin sutures using a 3-0 polydioxanone suture. A heparinized saline solution (100 U heparin/ml solution) was flushed through the sampling catheter to maintain patency.

The first blood sample taken from the jugular catheter was used for a time 0 sample for the study, and a CBC and serum chemistry panel were performed utilizing the machines described above to ensure that blood parameters were within normal limits.⁶ After the time 0 blood sample was collected and the CBC and serum chemistry results were deemed within normal limits, the dextrose was administered IV or IP or the animal was given no dextrose. For the IP injection, the

skin and hair were prepped with 20% betadine spray (Henry Schein Animal Health), saturating the area. A site 1 inch to the right of the umbilicus was chosen for the injection because there are generally only small intestinal loops in this area, which should push away from the needle when it is inserted. A 22-ga, 1-inch needle was used and inserted into the body wall to the hub, and then the dextrose solution was administered slowly. If the CBC and serum chemistry results were not considered normal, the catheter was removed, and the animal was placed back into rehabilitation.

Blood was collected using the sampling catheter 5, 15, and 30 min after dextrose treatment and placed into serum separator tubes. The serum separator tubes were immediately centrifuged after clot formation at 768 g for 10 min. The animals were recovered from anesthesia after the 30-min blood sample was collected. The animals were then manually restrained to obtain blood, via the catheter, at the 60-, 120-, 180-, and 240-min time points. The blood was placed into serum separator tubes and processed as explained above. After the 240-min blood sample was collected, the sampling catheter was removed, and firm pressure was applied to the catheter site for 5 min or until bleeding stopped. Triple antibiotic ointment was then applied to the skin wound. No catheter site complications were encountered.

Serum collected was analyzed immediately for blood glucose using a Wasserman chemistry analyzer (Alfa Wassermann Diagnostic Technologies), and an aliquot was banked at -80°C until analysis for insulin levels was performed at the University of California at Davis Veterinary School Molecular Biosciences laboratory. Insulin concentrations were measured by radioimmunoassay (RIA; EMD Millipore, Billerica, Massachusetts 01821, USA). A human insulin RIA was used for this study, and insulin concentrations in sea lion serum samples diluted in parallel as expected, suggesting good cross-reactivity of sea lion insulin measured with this assay. The inter-assay coefficient of variation was 8.9%, and the intra-assay coefficient of variation was 4.3%.

Due to the low sample size, generalized linear models followed by Tukey multiple comparison tests were used to compare differences between groups for the preadministration glucose and insulin levels between groups, using treatment as a dependent variable and animal as a random factor.⁸ Statistical analysis of repeated-measures data was performed using R (lme, version 3.1.0, 2014, R Foundation for Statistical Computing,

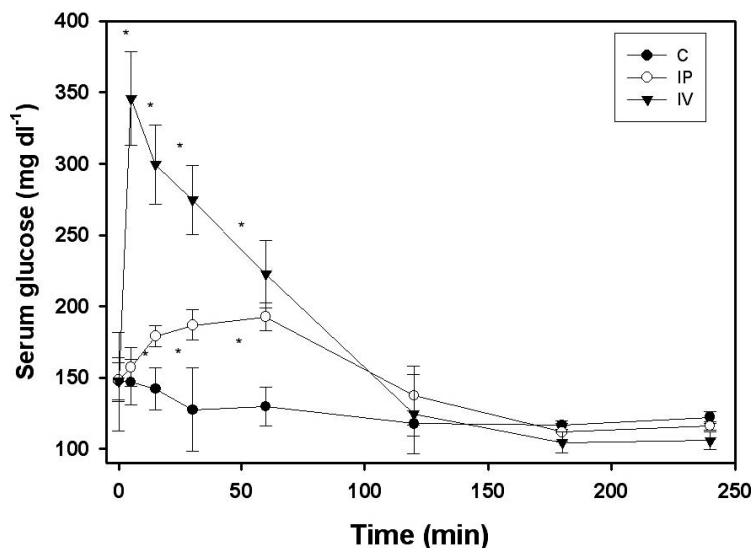


Figure 1. Serum glucose levels (mg/dl) in yearling California sea lions for control (C) animals or following administration of 500 mg/kg dextrose either intraperitoneally or intravenously.

Vienna, Austria 1300). The duration the blood glucose remained significantly above 135 mg/dl was determined using a *t*-test of the average (\pm SD) blood glucose levels.

RESULTS

Serum glucose concentrations

Baseline fasting serum glucose concentrations for all euglycemic sea lions averaged approximately 150 mg/dl (Fig. 1). There were no statistical differences in baseline fasting serum glucose between groups. Serum glucose tended to decrease over time in animals that received no dextrose. Animals that received IV dextrose exhibited the largest increases of serum glucose, increasing from approximately 150 mg/dl to a mean peak of approximately 350 mg/dl. Thereafter, serum glucose levels declined steadily, but remained over 200 mg/dl at 60 min and then returned close to baseline by 120 min after administration. In sea lions that received intraperitoneal dextrose, serum glucose concentrations increased gradually, from a baseline of approximately 150 mg/dl, to 200 mg/dl 60 min after dextrose administration. The serum glucose then declined and reached baseline at 120 min. The maximum average blood glucose values after dextrose administrations were significantly higher after IV injection (346 ± 33 mg/dl at 5 min) compared with either IP (193 ± 10 mg/dl at 60 min) or no dextrose (148 ± 13 mg/dl at time 0). The peak serum glucose levels were delayed for IP

injection compared with IV injection, whereas there was no increase in the blood glucose levels in the control animals (Fig. 1).

Serum insulin responses

Mean fasting serum insulin concentrations were between 5 and 10 μ U/ml in the three groups of animals. Serum insulin did not increase and tended to decline slightly in the control group (Fig. 2). Insulin levels increased rapidly within 5 min after IV dextrose administration and peaked at 18 μ U/ml at 60 min after dextrose administration (Fig. 2). Serum insulin increased slowly after IP dextrose administration, doubling from 5 to 10 μ U/ml at 15 min, increasing to a peak mean of 15 μ U/ml at 60 min, and then declining thereafter. When comparing the three groups, the insulin levels appeared to increase only when serum glucose was >160 mg/dl (Fig. 3).

DISCUSSION

In this study, changes in circulating serum glucose concentrations following no dextrose administration versus IV and IP dextrose administration in euglycemic yearling CSLs indicate that successful initial treatment of hypoglycemic sea lions can be achieved with both IP and IV dextrose administration. Although IV dextrose administration resulted in a faster increase in blood glucose, IP dextrose increased blood glucose above baseline (100–130 mg/dl) by the 15-min time point and had the highest increase by 60

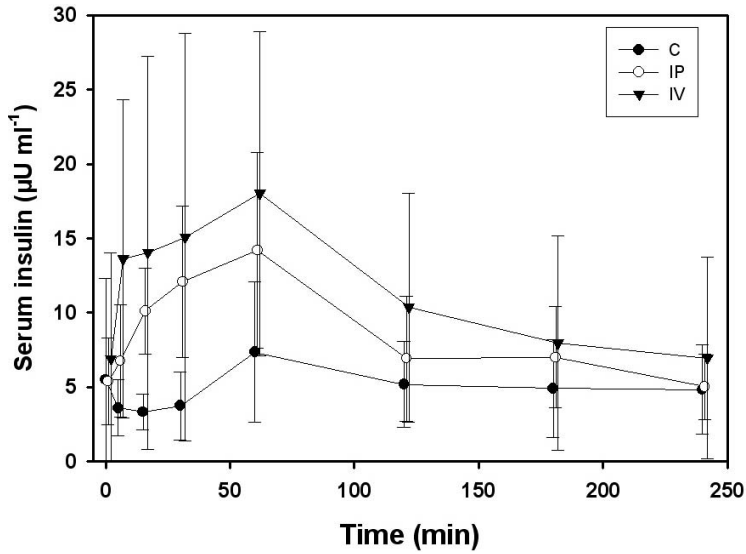


Figure 2. Serum insulin levels ($\mu\text{U}/\text{ml}$) in yearling California sea lions for control (C) animals or following administration of 500 mg/kg dextrose either intraperitoneally or intravenously.

min after administration. Blood glucose returned to baseline by 120 min after dextrose administration for both IP and IV groups; therefore, one treatment would most likely not last longer than 2 hr in hypoglycemic sea lions. This study was not intended to determine the optimal long-term management of hypoglycemia, but to provide preliminary information regarding the absorption of dextrose given IP versus IV, which, to the

authors' knowledge, has not been published before.

IP injections can pose problems due to the risk of accidental puncture of viscera and the risk of sepsis, especially with multiple needle sticks.³ In this study, dextrose at a 20% concentration was used to administer the highest concentration of dextrose in the lowest volume. The authors decided on this dose based on recommendations in large animal medicine for hypoglycemia treat-

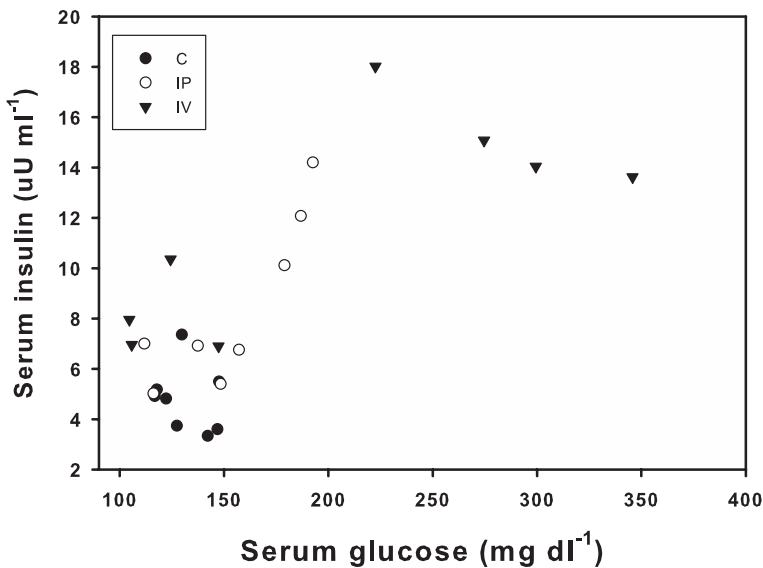


Figure 3. Serum insulin ($\mu\text{U}/\text{ml}$) versus glucose (mg/dl) levels in yearling California sea lions in control (C) and after administration of 500 mg/kg dextrose either intraperitoneally or intravenously.

ment of neonatal lambs in a field setting.⁹ In the short term, there were no signs of abdominal pain or discomfort following IP administration of dextrose. This study was unable to determine the potential long-term effects of IP dextrose administration in CSLs.

In all three groups, there was a statistically significant increase in insulin only after serum glucose concentrations increased to greater than 160 mg/dl, which is attributed to the increases of serum glucose stimulating β -cells in the pancreatic islets to release insulin. Increases of serum insulin concentrations measured in this study were substantially lower than what would have been expected in most terrestrial monogastric animals, such as dogs *Canis familiaris*.⁵ Furthermore, the stimulation to release insulin once the serum glucose concentrations increased above 160 mg/dl is also different from most terrestrial mammals, which are stimulated to produce insulin once concentrations reach >90 mg/dl.¹⁰ This may be due to the fact that marine mammals principally eat fish and other aquatic prey in the wild, which are high in protein and fat and low in carbohydrates. Pinnipeds are not exposed to simple carbohydrates during their adult lives (usually only glycogen in the liver and muscle of their prey), which means they have a lesser need for an immediate postprandial insulin response to help utilize ingested sugars.

Weaned elephant seal (*Mirounga angustirostris*) pups challenged with an injection of dextrose IV lacked an insulin response all together, which is different from what was found in this study with CSL yearlings. This lack of response is likely due to an alternate regulation of blood glucose compared with most terrestrial mammals that utilize more rapid changes of insulin and glucagon to maintain circulating glucose homeostasis. Fasting elephant seals rely on the oxidation of their fat stores to meet much of their energy requirements for their peripheral tissues. This allows glucose to be directed toward glucose-dependent tissues such as red blood cells and the brain.^{2,15} The current study documented an insulin response to blood glucose concentrations >160 mg/dl, which may indicate that CSLs have more of a capacity to utilize glucose compared with elephant seals. This may extend into critical care of elephant seals in the rehabilitation setting that may not be able to respond to boluses of IV dextrose like the CSLs in this study did (TMMC, pers. obs.). More research is needed to investigate dextrose treatment for hypoglycemia in elephant seals.

The results observed in this study are different than what has been observed in bottlenose dolphins (*Tursiops truncatus*). In one study, bottlenose dolphins were challenged with oral dextrose, and a sustained hyperglycemia and insulinemia were seen for 10 hr after administration.^{13,14} Dolphins in this study also showed a sustained hyperglycemia after a protein meal of mackerel. Dolphins have a larger brain/body mass ratio compared with pinnipeds, and this sustained hyperglycemia may be attributed to the dolphin brain's high demand for glucose.^{13,14}

This study utilized normoglycemic, well-hydrated sea lions; therefore, there may be differences in the amount of absorption and time it takes for absorption in the peritoneal space and vascular space compared with sea lions experiencing hypoglycemia and dehydration. Currently at TMMC, a 20% dextrose IP treatment is being given to young CSLs during a hypoglycemic event, and this has successfully resuscitated animals within 5 min of administration (TMMC, pers. obs.). This is different compared with the data presented in the current study, which demonstrates a modest elevation in blood glucose 5 min following dextrose administration. Clinical response at only 5 min may be attributable to the animal only requiring a small increase in serum glucose to regain consciousness, but further studies are needed to investigate the effects of IV and IP dextrose therapy on clinically hypoglycemic animals. Further studies also need to investigate electrolyte status and serum glucose trends to evaluate the full biochemical picture.

Young CSLs that present to TMMC with malnutrition are placed on a feeding protocol, which consists of feeding via stomach gavage three times a day. First, the animals are gavaged an electrolyte formula without dextrose, and then they are transitioned over 3 days onto a formula that contains water, blended herring, and salmon oil (TMMC, pers. obs.). The sea lions are then offered freshly thawed herring and are switched to whole fish once they eat on their own. Refeeding syndrome occurs during judicial refeeding of severely malnourished humans. The hallmark signs are hypophosphatemia accompanied oftentimes with hypokalemia and/or hypomagnesemia with pathologic extracellular fluid. When feeding is recommenced following severe malnutrition, a surge of insulin is accompanied by a marked rise of metabolic rate and contributes to the electrolyte and vitamin imbalances.¹⁶ Refeeding syndrome has not been documented in pinnipeds but may be a factor in some animals

that do not respond to the traditional feeding plan described above. Furthermore, this study showed that sea lions appear to have an attenuated insulin response compared with terrestrial animals, which may prevent them from developing this syndrome. More research needs to be performed to truly understand whether refeeding syndrome exists for pinnipeds and how it may relate to hypoglycemia treatment.

Traditional treatment at TMMC for acute hypoglycemia in pups and malnourished yearling CSLs relied on IV administration of dextrose by trained veterinary staff. This study suggests that nonveterinary staff can administer IP dextrose and get similar results to IV dextrose administration. Further studies are needed to determine the clinical efficacy of this short-term treatment and to develop more effective modalities for long-term treatment. To the authors' knowledge, this is the first publication documenting serum glucose and insulin changes when yearling CSLs are challenged with dextrose IP and IV.

Acknowledgments: The authors are thankful for the hard work of the volunteers and staff at The Marine Mammal Center, especially Lauren Campbell, who was a major factor in the success of this study, and Dr. Sophie Denison, for taking time to assist with the catheter placement.

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Received for publication 26 September 2014