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The Effects of Fasting and General Anesthesia on Serum Chemistries in KCG Miniature Pigs

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Investigators are obligated to optimize the perioperative care of experimental animals, but little is known about the effects of anesthesia and surgery on serum chemistries in KCG pigs. The objective of this study was to examine the influence of fasting and surgery under general anesthesia on 27 serum chemistries in KCG miniature pigs to improve management. Crossbred KCG minipigs were used at a mean of 12.3 mo of age (range, 8.6 to 14.9) and 33.4 kg of body weight (range, 24.0 to 40.2). Serum chemistries were evaluated at the start and end of a 24 h fasting period in fasted animals (n = 6). No significant differences were observed between the starting and postfasting studies. Partial hemilaminectomy of the lumbar spine was carried out in 2 groups of animals. Those given sevoflurane anesthesia (n = 7) had significant decreases in serum albumin, potassium, inorganic phosphorus, γ-glutamyltransferase peptidase, cholinesterase, and glucose postoperatively compared with preoperative values. Animals given isoflurane (n = 7) anesthesia had significantly decreased total protein, albumin, triglyceride, phospholipids, sodium, potassium, calcium, alanine aminotransferase, alkaline phoshatase and glucose after surgery compared with levels before surgery. In a separate experiment (n = 7), serum glucose and insulin also decreased during the postoperative period after isoflurane anesthesia. These results demonstrate that select serum electrolytes, glucose, and insulin of KCG miniature pigs are altered after general anesthesia. Investigators must be aware of the effects of anesthetic agents on experimental animals to provide optimal care and for interpretation of experimental data.

Abbreviations: KCG, Kogata Chinese–Clawn–Gottingen crossbred minipigs; KX, ketamine–xylazine.

Pigs have been widely used in medical research, either as a general medium-sized model that is larger than a rodent[27,31] or as models in specific medical applications,[7,25,34,44] in light of their anatomic and physiologic characteristics. Swine have gained prominence in medical research for endoscopic and laparoscopic surgery[11,12,28] as well as for medical training[8,13,14,18,36,41,42] and education as a replacement for other animal species of similar size. A wide range of baseline physiologic and detailed biochemical information regarding various breeds of swine used today is available.[10,38] Detailed serum biochemistry data are available for Yucatan miniature pigs, including data for animals at various ages.[30,33]

In Japan, because of recent concerns about the welfare and care of stray dogs, which were used in medical experiments commonly in the past, the use of pigs to replace or complement dogs has become more widespread. Because domestic pigs are obtained easily and are relatively inexpensive, most institutions have used domestic pigs more often than miniature pigs as subjects in experiments. However, because of their small size, miniature pigs are easier to handle and cheaper to maintain compared with larger animals.[3] Compared with the vast number of domestic pigs raised for food production, only a few miniature pigs are produced annually for use as experimental animals.[39] Limited information is available currently regarding the effects of anesthesia and surgery on the serum chemistries of Kogata Chinese–Clawn–Gottingen crossbred (KCG) miniature pigs, which are being used increasingly in medical education and research in Japan.[16,23,29,40] Performing survival experiments in minipigs demands optimal pre- and postoperative care, requiring a detailed knowledge of the biochemical effects of anesthesia and surgery on the animals.

Ketamine is a dissociative anesthetic that is ideal for inducing short periods of restraint and is commonly used in pigs. Because ketamine alone may not provide sufficient muscle relaxation, it is often administered in combination with other agents.[37] Ketamine and xylazine are often used in tandem to achieve a balanced state of anesthesia, muscle relaxation, and analgesia during short-term procedures in pigs.[5,26] By virtue of their minimal influence on hemodynamics and low incidence of complications, inhalation anesthetics such as isoflurane provide an alternative to ketamine–xylazine (KX) anesthesia in swine research.

In our experience, the use of isoflurane or sevoflurane after KX in fasted swine allows stable sedation and anesthesia that can be maintained as long as needed. The objective of this study was to examine the influence of fasting and surgery under general anesthesia on the serum chemistries in KCG miniature pigs to optimize their management during the perioperative period and improve overall postoperative care.

Materials and Methods

After review and approval by the Judging Committee of Experimental Animal Ethics of Jichi Medical University, noncastrated male KCG minipigs (*Sus scrofa var. domesticus*) were used at a mean age of 12.3 mo (range, 8.6 to 14.9 mo) and mean body weight of 33.4 kg (range, 24.0 to 40.2 kg). The KCG minipigs were produced by interbreeding Kogata Chinese, Clawn, and Gottingen minipigs at the National Livestock Breeding Center, Ibaraki Station, Independent Administration Institution of Japan (NLBC), since 1991. These pigs are a closed herd and are not commercially available at this time.[40] Sexual maturity in these

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Table 1. Serum chemistry values (mean ± SE) in KCG minipigs before and after a 24-h fast

	Before	After
Total protein, g/dL	8.1 ± 0.34	8.2 ± 0.81
Albumin, g/dL	4.1 ± 0.33	4.4 ± 0.23
Albumin:globulin ratio	1.1 ± 0.15	1.2 ± 0.12
Total bilirubin, mg/dL	0.05 ± 0.009	0.05 ± 0.005
Direct bilirubin, mg/dL	0.10 ± 0.048	0.08 ± 0.031
Indirect bilirubin, mg/dL	0.02 ± 0.015	0.01 ± 0.007
Triglycerides, mg/dL	20 ± 3.7	34 ± 6.9
Phospholipids, mg/dL	67 ± 7.3	59 ± 6.1
Free fatty acid, μEq/L	280 ± 73.1	294 ± 71.0
Total cholesterol, mg/dL	65 ± 2.4	65 ± 3.5
Cholesterol ester, mg/dL	50 ± 1.5	48 ± 2.7
Free cholesterol, mg/dL	15 ± 0.9	17 ± 2.1
Cholesterol esterase, %	77 ± 0.8	75 ± 2.5
BUN, mg/dL	18 ± 2.5	19 ± 2.3
Creatinine, mg/dL	1.29 ± 0.213	1.10 ± 0.153
Sodium, mEq/L	143 ± 2.2	144 ± 3.5
Chloride, mEq/L	102 ± 2.0	101 ± 1.2
Potassium, mEq/L	5.7 ± 0.38	7.5 ± 1.72
Calcium, mg/dL	10.2 ± 0.15	9.8 ± 0.55
Phosphate, mg/dL	7.3 ± 0.56	8.4 ± 0.71
Asparatate aminotransferase, IU/L	100 ± 52.2	388 ± 317.4
Alanine aminotransferase, IU/L	50 ± 7.3	78 ± 20.7
Lactate dehydrogenase, IU/L	577 ± 162.7	2739 ± 2141.1
Alkaline phosphatase, IU/L	450 ± 115.2	597 ± 187.9
γ-glutamyltransferase peptidase, IU/L	55 ± 7.1	67 ± 8.9
Cholinesterase, IU/L	6 ± 0.8	8 ± 1.1
Glucose, mg/dL	73 ± 2.7	68 ± 3.4

animals typically occurs at 5 to 6 mo of age. All animal care was consistent with the laws of Japan.

Animals were bred in confinement housing facilities and were specific pathogen-free for foot-and-mouth disease, hog cholera, swine vesicular disease, African swine fever, Aujesky disease, transmissable gastroenteritis virus, porcine epidemic diarrhea virus, porcine reproductive and respiratory syndrome virus, *Mycoplasma pneumoniae*, *Erysipelothrix rhusiopathiae*, and hepatitis E virus. All animals were provided with adequate individual pens measuring 1 × 1.5 m in size with a bedding space (0.5 × 1 m) of covered rubber. The pens were temperature- (18-23C) and humidity (40-60%) -controlled with a natural light:dark cycle (lights on, 0730 to 1800). Pigs each were fed 600 g of feed (Kyoken Ultrabreed 72 Ibaragi, JA East Japan, and Gunma, Japan) once daily.

Blood was sampled from the superior vena cava of each animal. Serum chemistries measured (Fuji Dri-chem 7000v, Fujifilm, Tokyo, Japan) included total protein, serum albumin, albumin:globulin ratio, total bilirubin, direct and indirect bilirubin, triglycerides, phospholipid, free fatty acid, total cholesterol, cholesterol ester, free cholesterol, cholesterol esterase, BUN, creatinine, sodium, chloride, potassium, calcium, phosphate, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase, γ-glutamyltransferase peptidase, cholinesterase, and glucose. Serum chemistries were evaluated in 4 separate experiments.

Experiment 1 involved 6 KCGs and examined the influence of fasting on serum chemistries. Animals received normal feed until the beginning of a 24 h fast (water provided ad libitum). Blood samples for serum chemistries were drawn just before and immediately after the fast. These animals were not sedated, and surgery was not performed on these animals after the fasting period.

Experiment 2 used 7 KCGs and examined the influence of sevoflurane on serum chemistries, whereas experiment 3 used 7 KCGs and examined the influence of isoflurane on chemistry values. In both of these experiments, animals received normal feed until 24 h preoperatively, when serum chemistries were evaluated just before a 24 h fast (water provided ad libitum). After the fast, all animals underwent KX sedation, followed by inhalation anesthesia and hemilaminectomy. Blood was sampled again at 90 min after the start of surgery. A single animal in experiment 2 required a second (half-dose) injection of sedatives to achieve the desired clinical effect.

Experiment 4 used 7 KCGs and examined the influence of isoflurane on blood glucose and insulin levels by using a 2-step sandwich enzyme immunoassay (LS Agent Insulin Kit; Eiken Chemical, Tokyo, Japan) after the preoperative fast and again 90 min after the start of surgery. All animals underwent a hemilaminectomy as described below.

Minipigs undergoing surgery were sedated by intramuscular injection of ketamine hydrochloride (10 mg/kg; Ketalar 50, Sankyo, Tokyo, Japan) and xylazine (2 mg/kg; Celactor, Sankyo) after pretreatment with atropine sulfate (0.03 mg/kg; Atropine, Tanabe, Osaka, Japan). Subsequently, the inhalant anesthetics sevoflurane (Sevofrane, Maruishi, Osaka, Japan) and isoflurane (Isiflu, Dainipon-sumitomo, Osaka, Japan) were used with maintenance at a 2% flow rate after orotracheal intubation.

The operation included skin incision and partial hemilaminectomy of the lumbar spine followed by intraepidural injection of

Table 2. Effect of sevoflurane anesthesia on serum chemistry values (mean± SEM) in KCG minipigs

	Before surgery	After surgery
Total protein, g/dL	7.9 ± 0.34	7.3 ± 0.05
Albumin, g/dL	4.4 ± 0.21	3.9 ± 0.11[a]
Albumin:globulin ratio	1.3 ± 0.10	1.2 ± 0.05
Total bilirubin, mg/dL	0.13 ± 0.072	0.09 ± 0.009
Direct bilirubin, mg/dL	0.11 ± 0.055	0.08 ± 0.009
Indirect bilirubin, mg/dL	0.02 ± 0.018	0.01 ± 0.004
Triglyceride, mg/dL	24 ± 6.2	18 ± 3.7
Phospholipid, mg/dL	67 ± 7.3	57 ± 6.5
Free fatty acid, μEq/L	322 ± 73.5	234 ± 81.7
Total cholesterol, mg/dL	64 ± 2.2	63 ± 5.5
Cholesterol ester, mg/dL	49 ± 1.3	49 ± 4.4
Free cholesterol, mg/dL	15 ± 0.9	14 ± 1.1
Cholesterol esterase, %	77 ± 0.8	78 ± 0.5
BUN, mg/dL	16 ± 1.5	14 ± 2.3
Creatinine, mg/dL	1.09 ± 0.113	0.96 ± 0.124
Sodium, mEq/L	145 ± 1.9	139 ± 1.0
Chloride, mEq/L	103 ± 1.3	99 ± 1.2
Potassium, mEq/L	5.8 ± 0.45	4.0 ± 0.14[a]
Calcium, mg/dL	10.2 ± 0.17	10.2 ± 0.27
Phosphate, mg/dL	7.6 ± 0.55	5.4 ± 0.24[a]
Asparatate aminotransferase, IU/L	114 ± 61.3	59 ± 12.0
Alanine aminotransferase, IU/L	55 ± 6.3	39 ± 5.4
Lactate dehydrogenase, IU/L	630 ± 188.3	474 ± 42.3
Alkaline phosphatase, IU/L	485 ± 134.4	357 ± 128.2
γ-Glutamyltransferase peptidase, IU/L	60 ± 5.6	44 ± 3.0[a]
Cholinesterase, IU/L	7 ± 0.7	4 ± 0.2[a]
Glucose, mg/dL	70 ± 1.2	55 ± 4.5[a]

[a] Significantly ($P < 0.05$) different from preoperative value.

Freund adjuvant (as part of another approved study). Surgery was performed with animals in the prone position and lasted approximately 2 h in each case. Blood was sampled from the anterior vena cava of unanesthetized pigs in the orthostatic position or of prone anesthetized pigs.

All minipigs in experiments 2, 3, and 4 were monitored carefully until emergence from anesthesia. Emergence was defined as the initiation of spontaneous breathing and movement of the extremities. Animals received routine postoperative care, including physiologic monitoring and routine postoperative analgesia. No arrhythmias appeared on cardiac monitors during the early postoperative period.

Data were analyzed by using paired *t* tests using Excel software (Microsoft Corporation, Redmond WA, USA).

Results

Serum chemistry values of KCG minipigs obtained just before and after a 24-h fast did not differ (experiment 1; Table 1), although the standard errors (SEs) for aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase were quite large. Review of these data revealed that a single animal yielded values that were significantly different from those of the others in that group. Omitting values from this animal did not change the results regarding the lack of statistically significant changes after fasting.

KCG minipigs that underwent hemilaminectomy under sevoflurane anesthesia had significantly ($P < 0.05$) decreased albumin, potassium, phosphate, γ-glutamyltransferase peptidase, cholinesterase, and glucose after surgery compared with preoperative values (experiment 2; Table 2). Again, the SEs for aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase were quite large due to the results for a single animal in the group; omitting values from this animal did not change the overall results.

When isoflurane was used as a general anesthetic for minipigs undergoing hemilaminectomy, postoperative serum chemistries were significantly ($P < 0.05$) decreased for total protein, albumin, triglyceride, phospholipids, sodium, potassium, calcium, asparatate aminotransferase, alanine aminotransferase, and glucose compared with preoperative values (experiment 3; Table 3). Similarly, immunoassay revealed that serum glucose and insulin levels after surgery (58 ± 5.3 mg/dL and 0.3 ± 0 μIU/mL, respectively) were significantly ($P < 0.01$) lower than before surgery (79 ± 7.0 mg/dL and 4.66 ± 0.990 μIU/mL, respectively).

In these studies with minipigs, the duration (mean ± SD) of anesthesia until emergence was 22 ± 13.3 min (range, 13 to 49 min). Time to emergence did not differ between isoflurane and sevoflurane.

Discussion

The use of experimental animals in medical research and teaching requires that adequate support systems exist for their optimal care. When the use of animals is limited to nonsurvival experiments, such as for teaching new surgical techniques, the care required is quite limited. However, it is essential to charac-

Table 3. Effect of isoflurane anesthesia on serum chemistry values (mean ± SEM) in KCG minipigs

	Before surgery	After surgery
Total protein, g/dL	8.0 ± 0.07	7.0 ± 0.08[a]
Albumin, g/dL	4.1 ± 0.16	3.6 ± 0.13[a]
Albumin:globulin ratio	1.1 ± 0.08	1.1 ± 0.08
Total bilirubin, mg/dL	0.03 ± 0.014	0.06 ± 0.008
Direct bilirubin, mg/dL	0.03 ± 0.013	0.05 ± 0.006
Indirect bilirubin, mg/dL	0.00 ± 0.003	0.01 ± 0.003
Triglyceride, mg/dL	35 ± 3.4	15 ± 1.9[a]
Phospholipid, mg/dL	75 ± 7.1	63 ± 3.2[a]
Free fatty acid, μEq/L	179 ± 101.0	175 ± 29.5
Total cholesterol, mg/dL	65 ± 7.4	58 ± 3.8
Cholesterol ester, mg/dL	50 ± 5.8	45 ± 3.3
Free cholesterol, mg/dL	15 ± 1.6	13 ± 0.6
Cholesterol esterase, %	77 ± 0.7	77 ± 0.6
BUN, mg/dL	16 ± 1.8	15 ± 1.6
Creatinine, mg/dL	1.19 ± 0.056	1.16 ± 0.053
Sodium, mEq/L	146 ± 1.2	139 ± 0.6[a]
Chloride, mEq/L	101 ± 0.6	101 ± 0.5
Potassium, mEq/L	5.6 ± 0.46	3.9 ± 0.09[a]
Calcium, mg/dL	10.6 ± 0.26	9.9 ± 0.25[a]
Phosphate, mg/dL	6.5 ± 0.18	6.2 ± 0.21
Asparatate aminotransferase, IU/L	27 ± 5.1	39 ± 6.3
Alanine aminotransferase, IU/L	42 ± 1.3	36 ± 2.2[a]
Lactate dehydrogenase, IU/L	376 ± 24.1	366 ± 29.8
Alkaline phosphatase, IU/L	396 ± 50.3	275 ± 24.4[a]
γ-Glutamyltransferase peptidase, IU/L	49 ± 4.3	45 ± 3.6
Cholinesterase, IU/L	5 ± 0.3	4 ± 0.3
Glucose, mg/dL	74 ± 2.8	63 ± 3.3[a]

[a] Significantly ($P < 0.05$) different from preoperative value.

terize the effects of surgery in KCG minipigs to optimize their postoperative care in response to their increasing use in survival experiments. The data we obtained during the current studies are an index of normal values for minipigs in the perioperative period. To our knowledge, this article is the first report on the serum levels of these 27 serum chemistry analytes in KCG miniature pigs undergoing general anesthesia and surgery.

Fasting alone did not affect the serum chemistries of 8.6- to 14.9-mo-old KCG minipigs (experiment 1). In the current studies, chemistries evaluated at 24 h after the last feed defined the preoperative period and were used as the control values. The effect of fasting on serum biochemistry values in Yucatan miniature pigs has been reported.[30] These investigators found results similar to those reported here, with no significant effect of fasting. The effect of fasting on immune function has been evaluated in swine, and although investigators found a transient decrease in the number of neutrophils, no effect on the response of lymphocytes to selected mitogens was apparent.[2]

Most parameters had a tendency to decrease under anesthesia with sevoflurane or isoflurane. Although the observed hypoglycemia may have been due to anesthesia, serum levels should be monitored carefully during the postoperative period. Serum potassium and phosphate under sevoflurane anesthesia and sodium, potassium, and calcium under isoflurane anesthesia were lower than during the preoperative period. Despite the relative hypokalemia, routine postoperative cardiac monitoring did not reveal any arrhythmias. Detailed information about the effects of isoflurane on physiologic and serum biochemistry values in neonatal piglets has been reported.[8]

In addition, serum glucose levels were lower under both gaseous anesthetics than during the preoperative period. We determined the insulin levels associated with glucose metabolism under isoflurane anesthesia (experiment 4). Animals receiving isoflurane anesthesia had lower blood glucose and insulin levels compared with those during the preoperative period.

Because we used volatile anesthetics after pretreatment with KX, we needed to consider its possible influence on serum chemistries in KCG miniature pigs. Several previous reports indicate such effects in several species. Used alone, ketamine does not alter plasma insulin or glucose concentration in collared peccaries[17] or African green monkeys.[4] Ketamine-associated hypoinsulinemia and hyperglycemia in cats[20] and baboons[24] are generally mild. However, as an α-adrenergic receptor agonist, xylazine exerts profound effects on carbohydrate metabolism by reducing circulating insulin concentrations in cattle.[1,20,22] The effects associated with KX treatment are consistent with previous reports of decreased circulating insulin concentration in response to narcotic doses of xylazine in several species.[1,20,22,43] Although a significant reduction in immunoreactive insulin concentration has been reported in KX-anesthetized pigs, plasma glucose concentrations remained unchanged in the current study.[1,20,22] In horses, the hypoinsulinemic effect of the KX combination roughly approximates that of xylazine alone.[43] Evidently the KX combination and xylazine both suppress insulin release in these animals.

We also considered the influence of isoflurane after treatment with KX. Isoflurane-associated hypoinsulinemia is generally mild and occurs in dogs,[19] rat islet cell cultures,[6] and Yucatan

minipigs.[16] Sevoflurane anesthesia reportedly has a rapidly reversible inhibitory effect on basal and glucose-stimulated insulin secretion.[35] Our result is in agreement with previous reports, which showed that the insulin concentration decreases under anesthesia.[6,16,19,35] However, previous reports do not agree with our result that blood glucose was lower under anesthesia in miniature pigs. Seven hormones synergize with regulation of blood glucose for maintenance of homeostasis.[32] The glucogenic hormones are glucagon, catecholamine (adrenaline and noradrenaline), glucocorticoid (principally cortisone), thyrotrophic hormone, and growth hormone. By contrast, insulin is the only hormone that decreases blood glucose.

Emergence from anesthesia is prolonged by intraoperative hypothermia, which also reduces anesthetic excretion from the liver and kidney. Therefore, delayed emergence from general anesthesia has been attributed to a mechanical response.[15] In our preliminary study, miniature pigs under general anesthesia underwent surgery on the operating table with a thermal blanket adjusted to 39 °C, yet their body temperature (mean ± SE) fell from about 39 °C to 35.2 ± 0.32 °C during the immediate postoperative period. Intraoperative hypothermia therefore may affect the time required for emergence from anesthesia. In the current study, the duration between stopping inhalation anesthesia and emergence was quite variable (range, 13 to 49 min). Anesthesia with isoflurane or sevoflurane likely influences the electrolyte and blood glucose levels of miniature pigs. Further studies are needed to determine whether the variability in time for emergence can be decreased by infusion of electrolytes and glucose during the postoperative period.

Further studies are needed to identify the mechanism of hypoglycemia under anesthesia in miniature pigs and to examine the effects during laparotomy and thoroacotomy. The results of the current study demonstrate that the blood electrolyte, glucose, and insulin levels of KCG miniature pigs after surgery are influenced by surgery under general anesthesia.

References

1. **Abdel el Motal SM, Sharp GM.** 1985. Inhibition of glucose-induced insulin release by xylazine. Endocrinology **116:**2337–2340.
2. **Becker BA, Niwano Y, Johnson HD.** 1992. Physiologic and immune responses associated with 48-hour fast of pigs. Lab Anim Sci **42:**51–53.
3. **Bollen P, Ellegaard L.** 1997. The Gottingen minipig in pharmacology and toxicology. Pharmacol Toxicol **80:**3–4.
4. **Brady AG, Koritnik DR.** 1985. The effects of ketamine anesthesia on glucose clearance in African green monkeys. J Med Primatol **14:**99–107.
5. **Breese CE, Dodman ME.** 1984. Xylazine–ketamine–oxymorphone: an injectable anesthetic combination in swine. J Am Vet Med Assoc **184:**182–183.
6. **Desborough JP, Jones PM, Persaud P, Landon MJ, Howell SL.** 1993. Isoflurane inhibits insulin secretion from isolated rat pancreatic islets of Langerhans. Br J Anaesth **71:**873–876.
7. **Donias HW, Schwartz T, Tang DG, DeAnda A Jr, Tabaie HA, Boyd DW, Karamanoukian HL.** 2003. A porcine beating heart model for robotic coronary artery surgery. Heart Surg Forum **6:**249–253.
8. **Earp PP.** 2003. Percutaneous renal surgery—new model for learning and training. Int Braz J Urol **29:**151–154.
9. **Eisenhauer CL, Matsuda LS, Uyehara CF.** 1994. Normal physiologic values of neonatal pigs and the effects of isoflurane and pentobarbital anesthesia. Lab Anim Sci **44:**245–252.
10. **Green SA, Jenkins SJ, Clark PA.** 1982. A comparison of chemical and electrophoretic methods of serum protein determinations in clinically normal domestic animals of various ages. Cornell Vet **72:**416–426.
11. **Grunebaum LD, Rosen D, Krein HD, Keane WM, Curtis M, Tereschuk DA, Pribitkin EA.** 2005. Nerve monitoring and stimulation during endoscopic neck surgery in the pig. Laryngoscope **115:**712–716.
12. **Hamad MA, Mentges B, Buess G.** 2003. Laparoscopic sutured anastomosis of the bowel. Surg Endosc **17:**1840–1844.
13. **Hammond I, Taylor J, Obermair A, McMenamin P.** 2004. The anatomy of complications workshop: an educational strategy to improve the training and performance of fellows in gynecologic oncology. Gynecol Oncol **94:**769–773.
14. **Hanly EJ, Marohn MR, Bachman SL, Talamini MA, Hacker SO, Howard RS, Schenkman NS.** 2004. Multiservice laparoscopic surgical training using the daVinci surgical system. Am J Surg **187:**309–315.
15. **Heier T, Caldwell JE, Sessler DI, Miller RD.** 1991. Mild intraoperative hypothermia increases duration of action and spontaneous recovery of vecuronium blockade during nitrous oxide–isoflurane anesthesia in humans. Anesthesiology **74:**815–819.
16. **Heim KE, Morrell JS, Ronan AM, Taglifero AR.** 2002. Effects of ketamine–xylazine and isoflurane on insulin sensitivity in dehydroepiandrosterone sulfate-treated minipigs (*Sus scrofa domestica*). Comp Med **52:**233–237.
17. **Hellgren EC, Lochmiller RL, Amoss MS Jr, Grant WE.** 1985. Endocrine and metabolic responses of the collared peccary (*Tayassu tajacu*) to immobilization with ketamine hydrochloride. J Wildl Dis **21:**417–425.
18. **Hochberger J, Euler K, Naegel A, Hahn EG, Maiss J.** 2004. The compact Erlangen active simulator for interventional endoscopy: a prospective comparison in structured term-training courses on 'endoscopic hemostasis' for doctors and nurses to the Endo-Trainer model. Scand J Gastroenterol **39:**895–902.
19. **Horber FF, Krayer S, Miles J, Cryer P, Rehder K, Haymond MW.** 1990. Isoflurane and whole body leucine, glucose, and fatty acid metabolism in dogs. Anesthesiology **73:**82–92.
20. **Hsu WH, Hummel SK.** 1981. Xylazine-induced hyperglycemia in cattle: a possible involvement of alpha-adrenergic receptors regulating insulin release. Endocrinology **109:**825–829.
21. **Hsu WH, Hembrough FB.** 1982. Intravenous glucose tolerance test in cat: influenced by acetylpromazine, ketamine, morphine, thiopental, and xylazine. Am J Vet Res **43:**2060–2061.
22. **Kasuya E, Hodate K, Matsumoto M, Sakaguchi M, Hashizume T, Kanematsu S.** 1996. The effects of xylazine on plasma concentrations of growth hormone, insulin-like growth factor I, glucose, and insulin in calves. Endocr J **43:**145–149.
23. **Laber-Laird K, Smith A, Swindle MM, Colwell J.** 1992. Effects of isoflurane anesthesia on glucose tolerance and insulin secretion on Yucatan minipigs. Lab Anim Sci **42:**579–581.
24. **Lehmann R, Wagner JL, Fernandez LA, Bourgoignie JJ, Ricordi CC, Alejandro R, Kenyon NS.** 1997. Effects of ketamine sedation on glucose clearance, insulin secretion and counterregulatory hormone production in baboons (*Papio hamadryas*). J Med Primatol **26:**312–321.
25. **Martinez-Gonzalez JM, Cano-Sanchez J, Campo-Trapero J, Gonzalo-Lafuente JC, Diaz-Reganon J, Vazquez-Pineiro MT.** 2005. Evaluation of minipigs as an animal model for alveolar distraction. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **99:**11–16.
26. **Nishimura R, Sakaguchi M, Mochizuki M, Sasaki N, Takahashi H, Tamura H, Takeuchi A.** 1992. A balanced anesthesia with a combination of xylazine, ketamine, and butorphanol and its antagonism by yohimbin in pigs. J Vet Med Sci **54:**615–620.
27. **Nomori H, Imazu Y, Watanabe K, Ohtsuka T, Naruke T, Kobayashi T, Suemasu K.** 2005. Radiofrequency ablation of pulmonary tumors and normal lung tissue in swine and rabbits. Chest **127:**973–977.
28. **Oleynikov D, Rentschler M, Hadzialic A, Dumpert J, Platt SR, Farritor S.** 2005. Miniature robots can assist in laparoscopic cholecystectomy. Surg Endosc **19:**473–476.
29. **Otis CR, Wamhoff BR, Sturek M.** 2003. Hyperglycemia-induced insulin resistance in diabetic dyslipidemic Yucatan swine. Comp Med **53:**53–64.

30. **Parsons AH, Wells RE.** 1986. Serum biochemistry of healthy Yucatan miniature pigs. Lab Anim Sci **36:**428–430.
31. **Pemberton J, Li X, Karamlou T, Sandquist CA, Thiele K, Shen I, Ungerleidere RM, Kenny A, Sahn DJ.** 2005. The use of live three-dimensional Doppler echocardiography in the measurement of cardiac output: an in vivo animal study. J Am Coll Cardiol **45:**433–438.
32. **Pocock G, Richards CD.** 2004. Human physiology: the basis of medicine, 2nd ed, p 551. London: Oxford University Press.
33. **Rispat G, Slaoui M, Weber D, Salemink P, Berthoux C, Shrivastava R.** 1993. Hematological and plasma biochemical values for healthy Yucatan micropigs. Lab Anim **27:**368–373.
34. **Ryska M, Kieslichova E, Pantoflicek T, Ryska O, Zazula R, Skibova J.** 2004. A model of acute hepatic failure in a minipig from the surgical and anesthesia point of view. Rozhl Chir **83:**436–442.
35. **Saho S, Kadota Y, Sameshima T, Miyao J, Tsurumaru T, Yoshimura N.** 1997. The effects of sevoflurane anesthesia on insulin secretion and glucose metabolism in pigs. Anesth Analg **84:**1359–1365.
36. **Sanders CW, Sadoski M, Bramson R, Wiprud R, Van Walsum K.** 2004. Comparing the effects of physical practice and mental imagery rehearsal on learning basic surgical skills by medical students. Am J Obstet Gynecol **191:**1811–1814.
37. **Swindle MM, Smith AC, Hepburn BJS.** 1988. Swine as models in experimental surgery. J Invest Surg **1:**65–79.
38. **Swindle MM.** 2007. Swine in the laboratory: surgery, anesthesia, imaging, and experimental techniques, 2nd ed. Boca Raton (FL): CRC Press.
39. **Tanaka H, Yoshino H, Kobayashi E, Takahashi M, Okamoto H.** 2004. Molecular investigation of hepatitis E virus infection in domestic and miniature pigs used for medical experiments. Xenotransplantation **11:**503–510.
40. **Tanaka H, Kobayashi E.** 2006. Education and research using experimental pigs in a medical school. J Artif Organs **9:**136–143.
41. **Taylor JD, Hammond IG.** 2004. The freshly killed pig: a model for surgical training in gynaecological oncology. Aust N Z J Obstet Gynaecol **44:**146–148.
42. **Tokuhara KG, Boldt DW, Yamamoto LG.** 2004. Teaching suturing in a workshop setting: a comparison of several models. Hawaii Med J **63:**258–259.
43. **Tranquilli WJ, Thurmon JC, Neff-Davis CA, Davis LE, Benson G, Hoffman W, Lock TF.** 1984. Hyperglycemia and hypoinsulinemia during xylazine–ketamine anesthesia in thoroughbred horses. Am J Vet Res **45:**11–14.
44. **Zonta S, Lovisetto F, Lorenzo C, Abbiati F, Alessiani M, Dionigi P, Zonta A.** 2005. Ureteroneocystostomy in a swine model of kidney transplantation: a new technique. J Surg Res **124:**250–255.