

Liquid Gastric Emptying in the Pig: Effect of Concentration of Inhaled Isoflurane

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An animal model of gastric emptying may have use in the study of gastric physiology and pharmacoscintigraphy. The pig has anatomy and physiology similar to that of humans. Our aim was to develop a model of gastric emptying in the pig. It was not possible to perform this study in conscious pigs; therefore, an anesthetic model was developed. **Methods:** Fifteen studies were performed on 4 pigs (age, 2–6 mo; weight, 20–100 kg). After acclimatization and training, pigs were fasted overnight before the study. Pigs were anesthetized using inhaled isoflurane without the use of injected premedication agents. An orogastric tube was inserted for the administration of a liquid meal, which consisted of ^{99m}Tc-diethylenetriaminepentaacetic acid either in water (nonnutrient) or with dextrose (nutrient meal). The pig was laterally positioned to enable right lateral dynamic acquisition to be performed. Anesthesia was maintained at 2% ± 0.5% isoflurane in 4 studies and 0.8% ± 0.5% in 11 studies (4 nutrient, 7 nonnutrient). **Results:** With 2% ± 0.5% isoflurane, there was delayed gastric emptying with a mean 50% emptying time (±SEM) of 141 ± 14 min. With 0.8% ± 0.5% isoflurane, the liquid meal emptied in an exponential manner similar to that of humans, with mean 50% emptying times (±SEM) of 30 ± 7 min (nutrient) and 31 ± 4 min (nonnutrient). **Conclusion:** The results indicate that high-dose anesthesia inhibits gastric emptying, but with low-dose anesthesia a useful pig model of liquid gastric emptying can be developed.

Key Words: pig; gastric emptying; anesthesia; scintigraphy

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Gastric scintigraphy of a radiolabeled meal is well established as the gold standard noninvasive method of studying gastric emptying in humans (1–3). The technique has been invaluable in establishing normal gastric physiology (4). It has also proved very useful in the assessment of gastric motility in a wide range of diseases such as diabetes

mellitus, scleroderma, and AIDS as well as after surgery (5–8). In humans the gastric emptying rates of solids and liquids differ significantly. The nonnutrient liquid emptying rate from the stomach is rapid and exponential, whereas solids empty at a relatively constant rate determined by the nutrient content of the food and small intestinal feedback mechanisms (2). The lag phase is the initial period, during which emptying of the solid meal is delayed. The delay is a finite time determined by the nature of the solid meal. Semisolid meals and nutrient liquids empty in an intermediate pattern.

Gastric emptying can play an important role in the bioavailability and pharmacokinetic profile of oral drug preparations. Evaluation of the in vivo performance of oral drugs is frequently carried out in healthy volunteers; however, multiple studies in humans with multiple experimental dosage forms can be costly and time-consuming and, in the case of multiple scintigraphic studies of gastric emptying, involve repeated doses of radiation (9).

The development of a reproducible animal model of gastric emptying to access physiology or pharmacologic effects on gastrointestinal motility or the pharmacokinetics of new oral drug forms has significant potential benefits. Many animal studies of gastric emptying have been described using rats, guinea pigs, rainbow trout, ponies, dogs, and pigs (10–14). Few involve scintigraphy (11) and most depend on acetaminophen absorption, manometry or gastric and duodenal aspiration, and sampling. The anatomy of the human stomach and the pig stomach is similar (15), and the pig has been used as an animal model of gastric motility with duodenal cannulation to measure gastric outflow of liquids (16). This has limitations because it involves prior surgical cannulation in the animal. A conscious noninvasive scintigraphic model would be ideal (17). Despite a long period spent acclimatizing pigs to reduce anxiety and the use of a special cage and harness, we were unsuccessful in maintaining the animal's cooperation for sufficient time.

Potential methods of reducing the mobility of the pig are parenteral sedation or inhaled anesthesia. These agents may

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potentially impact upon gastric emptying. Opioid drugs have been shown to delay gastric emptying (18–20). Propofol may not have as marked an effect. A recent study measuring gastric emptying indirectly using acetaminophen absorption showed no significant difference when anesthesia was maintained with enflurane, an agent closely related to isoflurane used in our experiment (21). Desflurane, isoflurane, or sevoflurane are the anesthetics of choice for use in swine. These newer agents have significantly less deleterious effects on the myocardium than other available agents. Halothane is associated with severe depressant effects upon the myocardium and cardiac arrhythmias, whereas enflurane is associated with seizures episodes in susceptible animals (22). Our aims were to develop a reproducible scintigraphic model of gastric emptying of a liquid meal in the pig, with as little pharmacologic intervention as possible, and with this emptying show a time course similar to that seen in the human.

MATERIALS AND METHODS

Fifteen studies were performed on 4 female Southern White pigs (age, 2–6 mo; weight, 20–100 kg). Animals were obtained from Adelaide University's research farm and were housed at the Institute of Medical and Veterinary Science (IMVS), Animal Research Facility, Gilles Plains, South Australia. Each animal was housed individually. Each pen conformed to guidelines established for space allowance, and the wire flooring was covered with open slatted rubber matting to prevent foot lesions or joint inflammation. Pigs were maintained on a standard diet of commercially available pellets not containing any medication (Barastoc Stock Feeds, Murray Bridge, South Australia). Water was allowed ad libitum. The studies were carried out with approval of the Animal Ethics Committee of the IMVS. Before any scintigraphic studies were performed, the animals were acclimatized to the housing environment and to the room and equipment that were to be used in the measurement of gastric emptying. Considerable time was spent developing a trusting relationship with the main investigators. This included provision of environmental enrichment devices such as balls and other toys as well as regular handling and reward-based training systems.

Animals were fasted from food overnight, with ad libitum access to water maintained. The pig was brought into a purpose-built scanning room, which included an operating table and anesthetic machine with CO₂, respiratory, and pulse rate monitor, and access to a ventilator if required. The studies were performed during isoflurane anesthesia without any premedication. Induction of anesthesia was achieved through a mask using an inhaled concentration of 5% isoflurane with supplemental oxygen. After induction, the pigs were intubated endotracheally. An orogastric tube was then inserted for introduction of the test meal. The isoflurane concentration was then gradually reduced, with equilibrium reached before the gastric emptying study. The orogastric tube was removed immediately after instillation of the radiopharmaceutical meal combination. All animals studied were monitored for end-tidal volume CO₂, respiratory rate, and pulse rate throughout the anesthetic procedure. They were imaged lying on their left side.

^{99m}Tc-Diethylenetriaminepentaacetic acid (DTPA) (28 ± 2

MBq) in 200 mL water was administered for the nonnutrient liquid studies. For the nutrient liquid studies, ^{99m}Tc-DTPA (28 ± 2 MBq) in 200 mL glucose solution was administered at a concentration of 85 g/L.

Scintigraphy was performed using a mobile (low-energy mobile) gamma camera (Searle Nucleonics, Erlangen, Germany) connected to a Micro-Delta computer (Siemens, Erlangen, Germany) for data acquisition. The pig's abdomen was imaged from the right lateral position, and a dynamic study was performed; sixty 2-min frames were acquired over a 120-min period. Four studies were performed with the pigs anesthetized and maintained on a dose of 2% ± 0.5% isoflurane and oxygen mixture after ingestion of the nonnutrient liquid meal. Seven studies were performed while the pigs were maintained on a lower concentration (i.e., 0.8% ± 0.5% isoflurane) after administration of the nonnutrient liquid meal. Four studies were performed using the low-dose isoflurane (0.8% ± 0.5%) and nutrient liquid meal. When using the low-dose isoflurane, the dose was titrated to maintain the pig at the lightest possible level of anesthesia that would allow a dynamic study without movement artifact. Maintenance of the eyelash response, jaw tone, and pedal and swallow reflexes was observed in all pigs receiving lower doses of anesthesia. For all animals in this study group, the use of physical restraint devices was required. Because of the rapid growth rate of pigs and associated logistic problems, it was not possible to work in a strictly paired study situation.

The acquired data were subsequently transferred to disk and converted to Starcam (General Electric Medical Systems, Milwaukee, WI) format for processing.

Time-activity curves were calculated using a region of interest around the stomach, with starting counts normalized to 100%. The mean retention in the stomach at each time point was calculated, and the composite curve was fitted to an exponential curve by least-squares regression. Half-clearance times were calculated and, for each group, were compared using the Student *t* test (Excel 2000; Microsoft, Seattle, WA).

Adequate images were obtained of all animals with the gastric fundus and antrum visible separately from the small bowel. Typical images are displayed (Fig. 1).

RESULTS

The 4 gastric emptying studies performed on pigs maintained on 2% ± 0.5% isoflurane revealed a mean half-clearance time (±SEM) of 141 ± 14 min.

With light anesthesia (0.8% ± 0.5% isoflurane), the animals maintained the eyelash response, pedal reflex, and swallow reflex. Scintigraphy showed early emptying of the liquid meal, with a mean half-clearance time (±SEM) of 31 ± 4 min for nonnutrient and 30 ± 7 min for nutrient. The rate of emptying followed an exponential pattern in all studies.

No significant difference was found in the half-clearance times of the nutrient and nonnutrient meals in the animals maintained on the low dose of isoflurane (*P* = 0.22), but the nutrient meal was significantly faster (*P* < 0.03) than that of the high isoflurane studies (Fig. 2). The difference between the high concentration anesthesia and nonnutrient meal did not reach statistical significance (*P* < 0.09).

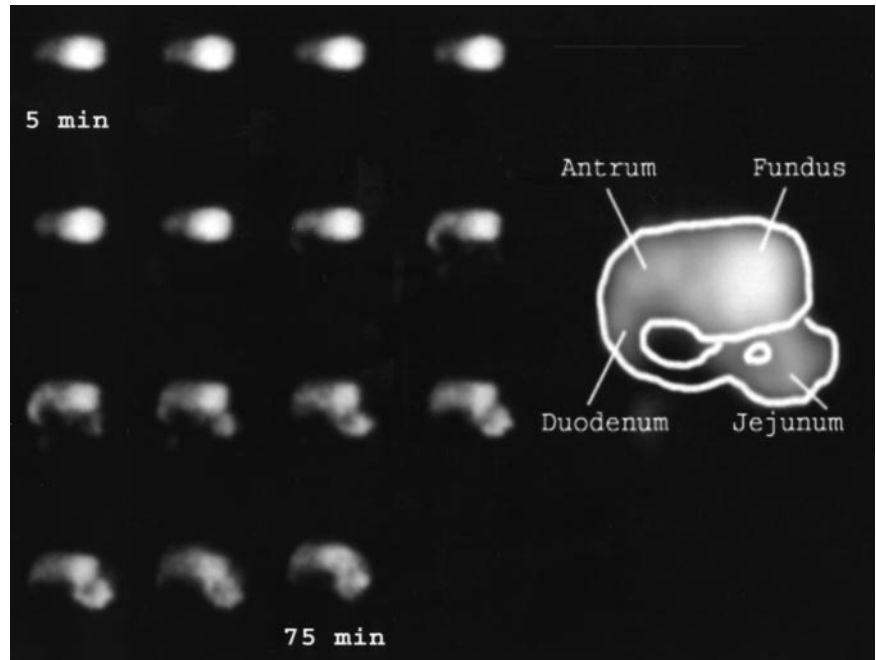


FIGURE 1. Serial images obtained in right lateral position showing gradual clearance of activity from fundus of stomach, through pylorus and duodenum, to small bowel.

DISCUSSION

These results describe a reproducible scintigraphic pig model of gastric emptying. The individual half-clearance times obtained with the nonnutrient and nutrient liquid meals at $0.8\% \pm 0.5\%$ isoflurane were similar, with little deviation from the mean values. These results followed an exponential curve similar to that found in human studies. The mean time for 50% emptying of the water in this lightly anesthetized pig model is marginally longer than that in humans. In the human, gastric emptying of a glucose-

containing meal is expected to show longer half-clearance times; however, we did not observe this in our porcine study.

Although the anatomy and physiology of the pig stomach can be described as similar to that of the human, there are differences. In the human, the stretch-and-fill receptors play a role in gastric motility and emptying as do the glucose receptors. The pig may be more responsive to gut filling and stretching than the human. The main energy (kilojoules) sources in the pig diet come from cereal

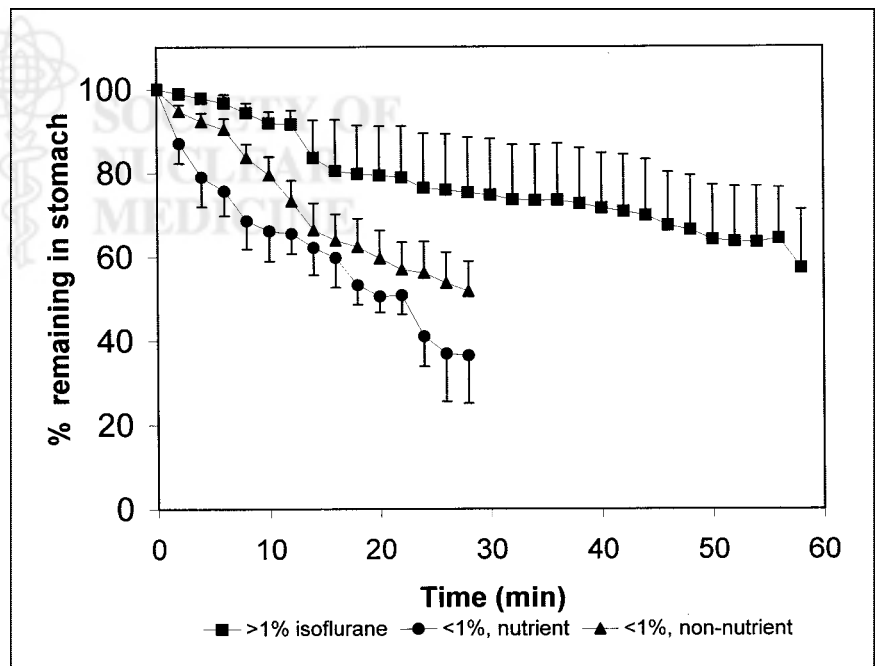


FIGURE 2. Time-activity curves of combined data (high isoflurane concentration, low isoflurane nutrient, and nonnutrient). Curves are plotted for time that data (mean \pm SEM) on all animals in each group were available.

grains and fats, with very little glucose present. The glucose receptors may be less responsive in the pig. In addition, there may be a subtle effect of anesthesia on the glucose receptors in the pig. Further studies focused on these hypotheses would provide useful information and aid in the refinement of the model. The results are similar to recently published data on gastric emptying of pellet preparations in the pig (23).

These results also confirm the effect of inhaled anesthetic agents in delaying gastric emptying. Earlier studies have shown a similar effect with opiates and sedatives used as premedication in combination with inhaled anesthetic agents, but most of these studies did not use scintigraphy (18,19). The results of the study confirmed that at 2% ± 0.5% isoflurane the delaying effect could be seen independent of premedication agents. The lower concentration of anesthesia would be insufficient to allow invasive surgery, but it is sufficient to allow scintigraphy to be performed and gastric emptying to be monitored.

Apart from the use of anesthesia there are other significant differences between these studies and conscious studies. The pigs were studied in the left lateral decubitus position and imaged from the right lateral position, whereas most human studies are performed upright in a more normal eating posture. Attenuation correction is also performed for human studies (3). In the pig the camera head was positioned laterally. Because the pig stomach is positioned more horizontally, and the bulk of the fundus tends to be more posterior than in the human, good quality images of the fundus, antrum, and duodenum were obtained. The relatively small differences in distance of these components from the camera made attenuation correction less important. The meal was introduced through an orogastric tube, but numerous studies in humans with feeding and manometric tubes have not shown these to have an effect on gastric emptying. The need to feed the pig through a tube may limit the consistency of meals that might be studied.

Despite the need for a dedicated facility with anesthetic equipment and a gamma camera along with the expertise needed, the scintigraphic study of gastric emptying has many advantages over the nonimaging indirect methods that are generally more invasive. The imaging time could be extended and studies of small and large bowel transit could also be performed. The data suggest that the pattern of gastric emptying of the liquid meal is similar in pigs and humans.

CONCLUSION

We have described an animal model that has the potential to allow the study of gastric physiology, the study of prokinetic drugs, and pharmacoscintigraphic studies of new drug formulations with associated absorption studies.

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