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Original research article

Endoscopic luminal impedance planimetry of the lower oesophageal sphincter and pylorus in experimental pigs: a pilot study

Methods and initial experience

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Abstract

Background/Aims: The functional lumen imaging probe (FLIP) relies on the principle of impedance planimetry that enables direct measurement of intraluminal pressure, cross-sectional areas, and wall biomechanical properties. The aim of our pilot project was to introduce this method to assess function of the lower oesophageal sphincter and pyloric muscle in experimental pigs.

Methods: All measurements were accomplished in one session in six adult female pigs (mean weight 34.2 ± 3.6 kg), using the EndoFLIP 1.0 System with EndoFLIP catheters. Five major parameters were evaluated: balloon pressure (mm Hg), estimated diameter (mm), cross-sectional area (mm^2), distensibility (mm^2/mm Hg), and zone compliance (mm^3/mm Hg).

Results: In total, 180 readings were successfully accomplished. Most of the measured values were nearing lower average figures for the lower oesophageal sphincter, and upper average figures for the pylorus in healthy humans. The porcine pyloric sphincter is composed of the *Torus pyloricus*. It serves as a study "gatekeeper" between the stomach and D1 duodenum, thus explaining higher pyloric readings. There was a clear trend for increasing values of CSA (cross-sectional area), diameter, and balloon pressure with increased filling balloon volumes. However, the sphincter distensibility did not change with increasing filling volumes, either for the lower oesophageal sphincter or pylorus.

Conclusion: Endoscopic functional luminal planimetry in experimental pigs is feasible, both for the lower oesophageal sphincter and the pylorus. This is an important starting point for future experimental endoscopic trials and pharmacology studies.

Keywords: Endoscopic luminal impedance planimetry; Experimental pigs; Lower oesophageal sphincter; Pylorus

Highlights:

- Investigation of the motility function of the lower oesophageal sphincter and pylorus by means of endoscopic functional luminal impedance planimetry (FLIP) is a new method in the experimental setting.
- FLIP enables the direct measurement of luminal pressure (mm Hg). The remaining parameters are finished by calculation: intraluminal diameter (mm), cross-sectional area (mm^2), distensibility defined as cross-sectional area divided by balloon distending pressure (mm^2/mm Hg), and zone compliance (mm^3/mm Hg).
- FLIP in experimental pigs is feasible, both for the lower oesophageal sphincter and the pylorus.
- Most of the measured values were nearing lower average figures for the lower oesophageal sphincter and upper average figures for the pylorus in healthy humans.

Note:

The study was presented in part, as an abstract and e-poster, at the ESGE Days (European Society of Gastrointestinal Endoscopy Conference), Berlin, April 25–27, 2024.

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Introduction

The functional lumen imaging probe (FLIP) relies on the principle of impedance planimetry that enables the direct measurement of intraluminal cross-sectional areas, allowing the assessment of intraluminal diameters, lumen geometric profiles, and wall biomechanical properties (Clarke et al., 2020). Electrical impedance refers to the opposition to alternating current presented by the combined effect of resistance and reactance in a closed circuit. Planimetrics is the study of plane measurements, including distances and areas (Clarke et al., 2020; Wu et al., 2020). Since the early 2000s, impedance planimetry has been introduced into clinical practice, principally allowing assessment of any sphincter function/dysfunction (Dogan and Mittal, 2006; McMahon et al., 2004, 2005; Mittal et al., 2005; Pandolfino et al., 2002, 2005; Shaker et al., 2004).

Several comprehensive reviews have been published recently (Ata-Lawenko and Lee, 2017; Bredenoord et al., 2022; Chen et al., 2019; Desprez et al., 2020; Donnan and Pandolfino, 2020; Hirano et al., 2017; Lottrup et al., 2015; Soliman and Gourcerol, 2023; Vackova et al., 2023). The functional lumen imaging probe is applied mostly to diseases of the oesophagus (achalasia, dysphagia, gastro-oesophageal junction outflow obstruction, eosinophilic oesophagitis, gastro-oesophageal reflux disease), pyloric distensibility (gastroparesis), and ano-rectal disorders (faecal incontinence) (Clarke et al., 2020). It may also have a potential to assess the biomechanical properties of strictures, either clinical (Crohn's disease) or experimental (personal experience).

Experimental studies on impedance planimetry assessing the function of sphincters throughout the digestive tract are at present sparse (Arroyo Vázquez et al., 2018; Gonzalez et al., 2019; O'Dea and Siersema, 2013; Perretta et al., 2010, 2011; Rivera Bermudez et al., 2015; Ullal et al., 2022). Nevertheless, experimental pigs have been frequently used in various preclinical studies as their gastrointestinal physiology is similar to that of humans (Gonzalez et al., 2015; Kararli, 1995; Suenderhauf and Parrott, 2013). The aim of this pilot project was to introduce endoscopic functional luminal impedance planimetry (EndoFLIP) in experimental pigs for the purpose of assessment of function of the lower oesophageal sphincter and pyloric muscle.

Materials and methods

Animals

Six experimental female adult pigs (*Sus scrofa* f. *domestica*, hybrids of Czech White and Landrace breeds; 3-month-old; mean weight 34.2 ± 3.6 kg (median 36.0 kg)) were enrolled into the study. The animals were purchased from a certified breeder (Stepanek, Dolni Redice, Czech Republic; SHR MUHO 2050/2008/41). The pigs were housed in an accredited animal laboratory (Military Faculty of Medicine, Hradec Králové). During a two-week acclimatization, all animals were fed with a standard assorted A1 food (Ryhos, Nový Rychnov, Czech Republic) in equal amounts twice a day, and had free access to drinking water.

Design of the study

All experiments commenced in the morning on overnight fasting animals under general anaesthesia. Drugs used for induction of anaesthesia were medetomidine 0.1 mg/kg *i.m.*, butorphanol 0.3 mg/kg *i.m.*, and midazolam 0.3 mg/kg *i.m.* Subsequent general anaesthesia was maintained by *i.v.* propofol (repeated one-ml boluses of 20 mg, in total less than 10 ml per animal within one hour). Pigs were intubated (received an endotracheal cannula). They were breathing spontaneously, without any need for artificial ventilation. Vital signs were secured by pulse oximetry. There were no adverse events in the course of the experiments.

Both oesophageal and pyloric impedance planimetry measurements were accomplished in one session in all pigs, using the EndoFLIP 1.0 System (Medtronic, Minneapolis, MN, USA) with EndoFLIP catheters EF-325N (length of measuring zone 80 mm; 16 paired impedance planimetry sensors). Just one catheter was used for both pyloric and oesophageal measurements in a single animal. EndoFLIP catheters were purchased from Imedex, Hradec Kralove, Czech Republic. Catheters were introduced into the porcine stomach and duodenum endoscopically by means of a 20-mm snare (diameter 2.3 mm; Micro-Tech Endoscopy, Ann Arbor, MI, USA), Fig. 1). A video-gastroscope GIF-Q180 (Olympus Optical Co, Tokyo, Japan) was used, dedicated for animal experiments only. Pyloric measurements were accomplished under the gastroscopic control of a correct catheter position; the snare was left in the duodenum. Both endoscope and snare were withdrawn before subsequent impedance planimetry of the gastro-oesophageal junction.

Fig. 1. Catheter (open arrow) was introduced into the porcine stomach and duodenum endoscopically by means of a 20-mm snare (closed arrow)

Endoscopic functional luminal imaging probe panometry

First, an upper gastrointestinal endoscopy was performed to identify the distance of the lower oesophageal sphincter and pylorus (*Torus pyloricus*), and to decompress the stomach. Afterwards, the impedance catheter was calibrated (pressure-zeroed to atmospheric pressure) and introduced to the stomach and duodenum.

Filling volumes of 20, 30, and 40 ml were used for both the gastro-oesophageal junction and pylorus. Filling was done in a graded approach, with 60 seconds allowed at each fill level before readings (in triplicates). Sample rate was 10 Hz. All measurements were completed at an optimal catheter position (Figs 2 and 3).

Five major parameters were evaluated for each filling volume: balloon pressure (mm Hg), estimated diameter (mm), cross-sectional area (mm^2), distensibility defined as cross-sectional area divided by balloon distending pressure (mm²/mm Hg), and zone compliance defined as the change in volume over a 2cm-long segment spanning five electrodes, centred around the gastro-oesophageal junction (mm³/mm Hg). All five parameters were automatically assessed by the EndoFLIP system itself.

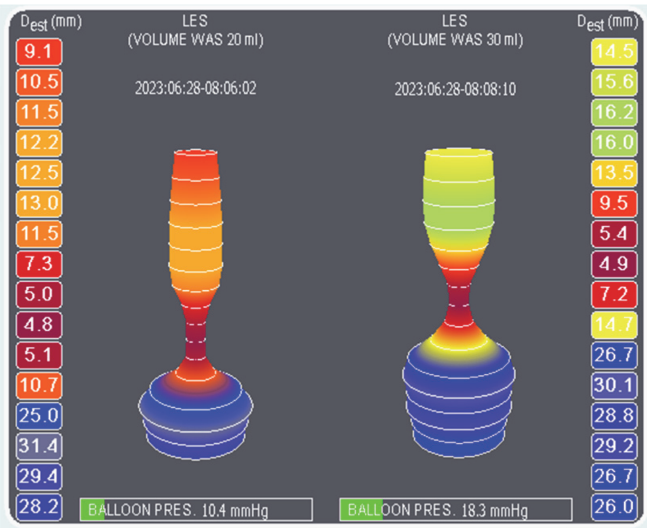

Fig. 2. Impedance planimetry of the lower oesophageal sphincter

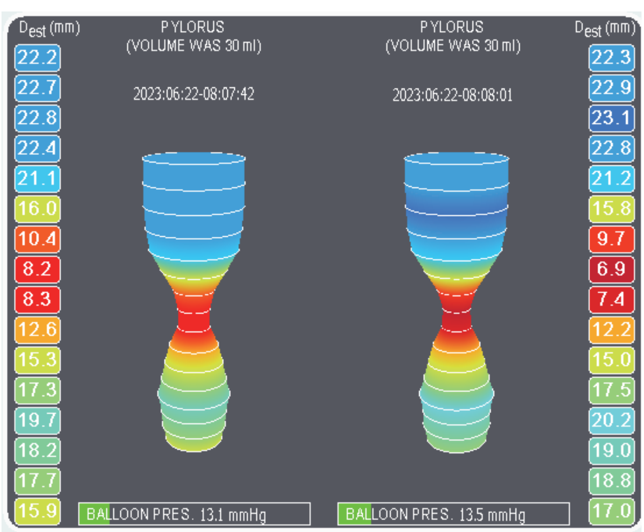

Fig. 3. Impedance planimetry of the pylorus (*Torus pyloricus*)

Statistics

All data were tested statistically by means of SigmaStat software (Version 3.1, Jandel Corp, Erkrath, Germany). Distribution of data was assessed by Kolmogorov–Smirnov test, and Shapiro–Wilko test was used for evaluation of the normality of sampled data. Descriptive statistics, unpaired *t*-test (for normal distribution), and Mann–Whitney rank sum test (for non-normal distribution) were used to treat variables.

Ethics

The Project was approved by the Institutional Review Board of the Animal Care Committee (Protocol Number MO 652499/2023-2994). The study was conducted in accordance with the policy for experimental studies (Tveden-Nyborg et al., 2018). Animals were held and treated in conformity with the European Convention for the Protection of Vertebrate Animals (Council of Europe, 1986). All ethical rules were strictly observed.

Results

Technical aspects

Impedance planimetry measurements were feasible. In total, 180 readings were successfully accomplished.

Oesophagus

All five major parameters were successfully measured in all animals. There was a statistically significant difference for pressure of the lower oesophageal sphincter between fillings 20 ml and 40 ml ($p = 0.029$). Other results are summarised in Table 1 and displayed in Figs 4 and 5. No outliers were omitted.

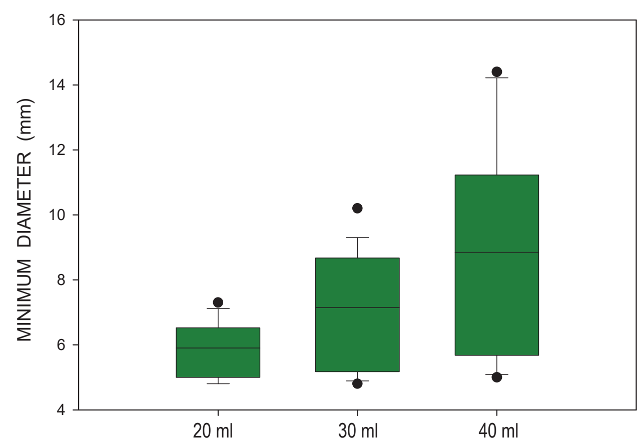

Fig. 4. Endoscopic functional luminal imaging probe in experimental pigs. Lower oesophageal sphincter. Estimated diameter. Median, interquartile range, standard deviation and outliers. There were statistically significant differences between fillings 20 ml and 30 ml ($p = 0.031$), 20 ml and 40 ml ($p = 0.002$), 30 ml and 40 ml ($p = 0.046$).

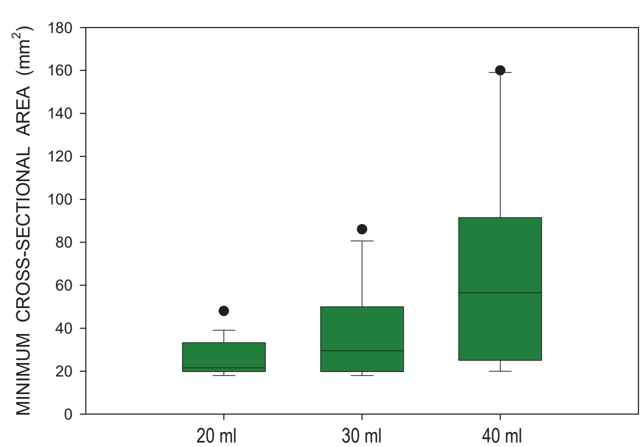

Fig. 5. Endoscopic functional luminal imaging probe in experimental pigs. Lower oesophageal sphincter. Minimum cross-sectional area. Median, interquartile range, standard deviation and outliers. There were statistically significant differences between fillings 20 ml and 40 ml ($p < 0.001$), 30 ml and 40 ml ($p = 0.024$).

Table 1. Endoscopic functional luminal imaging probe panometry of the lower oesophageal sphincter and pylorus in six experimental pigs

Parameter	Filling volume 20 ml mean ± standard deviation median interquartile range	Filling volume 30 ml mean ± standard deviation median interquartile range	Filling volume 40 ml mean ± standard deviation median interquartile range
Oesophagus Balloon pressure (mm Hg)	18.7 ± 20.3 10.4 6.6–22.1	28.8 ± 10.2 20.4 18.4–33.9	44.9 ± 28.9 36.3 24.2–56.2
Oesophagus Diameter (mm)	5.9 ± 0.9 5.9 5.0–6.5	7.1 ± 1.8 7.2 5.2–8.6	9.0 ± 3.2 8.9 5.7–10.9
Oesophagus Cross-sectional area (mm^2)	25.8 ± 8.7 21.5 20.0–33.0	38.4 ± 22.7 29.5 20.0–50.0	66.4 ± 44.7 56.5 25.0–91.0
Oesophagus Distensibility (mm^2/mm Hg)	1.7 ± 1.5 1.6 0.3–1.9	1.5 ± 1.5 1.0 0.3–2. 0	2.1 ± 2.8 1.2 0.3–2.1
Oesophagus Zone compliance (mm^3/mm Hg)	70.2 ± 63.1 50.0 11.3–135.8	64.7 ± 50.3 55.0 12.0–125.3	28.8 ± 32.8 11.6 4.7–54.5
Pylorus Balloon pressure (mm Hg)	15.9 ± 13.8 11.0 10.5–17.6	20.9 ± 17.3 14.8 13.1–23.6	28.6 ± 17.0 25.6 17.1–31.3
Pylorus Diameter (mm)	5.9 ± 1.4 5.3 5.0–6.0	6.9 ± 1.3 6.7 5.9–8.1	9.2 ± 3.2 8.2 6.5–12.0
Pylorus Cross-sectional area (mm^2)	28.5 ± 14.1 22.0 20.0–28.0	38.5 ± 14.4 35.0 27.3–51.0	73.8 ± 52.2 52.5 29.0–118.0
Pylorus Distensibility (mm^2/mm Hg)	2.4 ± 1.9 1.6 0.3–1.9	2.0 ± 1.1 1.8 1.0–2.9	2.1 ± 1.3 1.6 0.8–3.2
Pylorus Zone compliance (mm^3/mm Hg)	86.1 ± 48.5 67.0 47.0–119.3	72.1 ± 41.7 72.6 40.0–99.7	34.2 ± 24.8 24.0 20.7–47.6

Pylorus

All five major parameters were successfully measured in all animals. There was a statistically significant difference for pressure of the pylorus between fillings 20 ml and 40 ml ($p = 0.006$). Other results are summarised in Table 1 and displayed in Figs 6 and 7. No outliers were omitted.

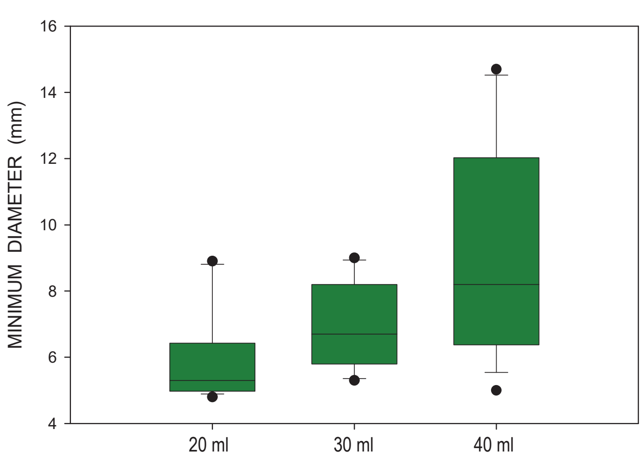

Fig. 6. Endoscopic functional luminal imaging probe in experimental pigs. Pylorus. Estimated diameter. Median, interquartile range, standard deviation and outliers. There were statistically significant differences between fillings 20 ml and 30 ml ($p = 0.007$), 20 ml and 40 ml ($p < 0.001$), 30 ml and 40 ml ($p = 0.047$).

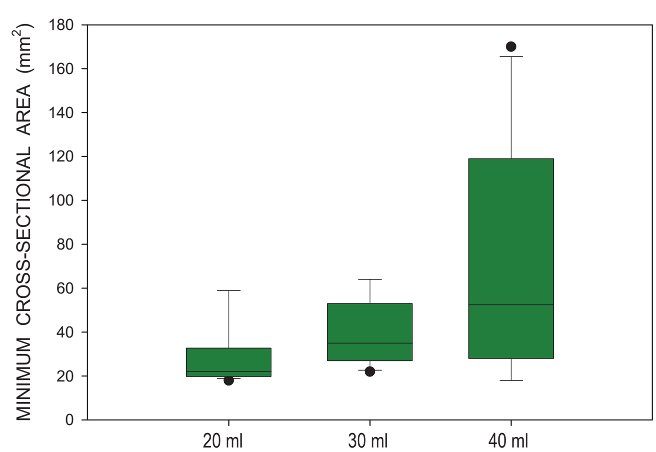

Fig. 7. Endoscopic functional luminal imaging probe in experimental pigs. Pylorus. Minimum cross-sectional area. Median, interquartile range, standard deviation and outliers. There were statistically significant differences between fillings 20 ml and 30 ml ($p = 0.006$), 20 ml and 40 ml ($p = 0.002$).

Discussion

In this study, we have elaborated the protocol of endoscopic luminal impedance planimetry in experimental pigs and provided pilot normative values for both the oesophagus and pylorus. The impedance planimetry measurements were feasible in all animals. Most of the measured values were nearing lower average figures for the lower oesophageal sphincter and upper average figures for the pylorus in healthy humans (Ata-Lawenko and Lee, 2017; Clarke et al., 2020; Bredenoord et al., 2022; Chen et al., 2019; Desprez et al., 2020; Donnan and Pandolfino, 2020; Hirano et al., 2017; Lottrup et al., 2015; Soliman and Gourcerol, 2023; Vackova et al., 2023). The porcine pyloric sphincter is composed of the *Torus pyloricus*. It serves as a sturdy "gatekeeper" between the stomach and D1 duodenum, thus explaining higher pyloric readings.

Impedance planimetry measurement is an emerging diagnostic method which is currently being tested in several indications in clinical practice. It may help to diagnose oesophageal motility disorders, such as achalasia, and may possibly decrease the need for or replace high resolution manometry (Carlson et al., 2019). Oesophageal distensibility measurement may also be used to assess the completeness of oesophageal myotomy in patients with achalasia. In the pylorus, it is currently the only method enabling assessment of pyloric function in patients with gastroparesis and functional dyspepsia. However, normal values have not been generally accepted and an examination protocol has not been unified throughout the world. EndoFLIP may provide interesting information about pyloric and oesophageal function or dysfunction in health and disease and may also be used to study the effect of different drugs or techniques on pyloric and oesophageal distensibility (Carlson et al., 2019; Jagtap et al., 2020; Popescu et al., 2023; Regan et al., 2013; Zheng et al., 2022). It seems to be useful to establish an experimental method for an EndoFLIP measurement.

As in humans, there was a clear trend for increasing values of CSA, diameter, and balloon pressure with increased filling balloon volumes in our study. However, the sphincter distensibility did not change with increasing filling volumes, either for the lower oesophageal sphincter or the pylorus. The *Torus pyloricus* serves as a muscular "gatekeeper" between the porcine stomach and D1 duodenum. According to our previous endoscopic experience, the porcine stomach has almost always contained remnants of food, even after a long fasting period. The *Torus pyloricus* contributes to this fact. Introduction of any endoscopic accessories into the duodenum may sometimes be demanding. In addition, there is a short length of the lesser curvature between the porcine gastric cardia and pylorus with an acute angle (van Hees, 2022). Because of this shape, it is necessary to ensure the proper catheter position endoscopically during the entire pylorus measurement. Nevertheless, all our pyloric measurements were successful. Even in humans, balloon position within the pylorus significantly affects impedance planimetry measurements (Yim et al., 2023).

Only a few experimental projects on endoscopic functional luminal planimetry have been published so far (Arroyo Vázquez et al., 2018; Gonzalez et al., 2019; O'Dea and Siersema, 2013; Perretta et al., 2010, 2011; Rivera Bermudez et al., 2015; Ullal et al., 2022). These partial studies did not provide normal values on the gastro-oesophageal junction and pylorus impedance planimetry in full complexity. Perretta et al. (2010) investigated preoperative and postoperative oesophageal manometry and planimetry in experimental pigs before and after open and endoscopic oesophageal myotomy. Postoperative manometry demonstrated a drop in mean lower oesophageal sphincter pressure in both groups (by 69% and 50%, respectively). Postoperative planimetry measurement confirmed increased distensibility after myotomy without any distensibility difference between open and endoscopic myotomy (Perretta et al., 2011). O'Dea and Siersema (2013) performed impedance planimetry in two animals securing progressive balloon dilation of the gastro-oesophageal junction. Rivera Bermudez et al. (2015) developed a porcine model of benign oesophageal stricture using impedance planimetry for better evaluation of oesophageal stents. Arroyo Vázquez et al. (2018) explored pyloric dynamics in experimental pigs under different conditions (balloon distension, administration of prokinetics, food stimulation). In all pigs studied, both the average minimum cross-sectional area/diameter and the average pyloric pressure were seen to increase as the catheter volume expanded. This implies that, as expected, the general trend was that the pylorus opens more with an increased filling volume in the catheter (Arroyo Vázquez et al., 2018), however, its distensibility did not change. We found a similar trend in our current study, too. Gonzalez et al. (2019) used porcine impedance planimetry to measure diameter, cross sectional area, distensibility, and compliance of the gastro-oesophageal junction before and after a hybrid laparoscopic and endoscopic placement of a gastric port (TAGSS – trans-abdominal gastric surgical system) and subsequent fundoplication. They found higher preoperative values of particular parameters compared with our measurement. These values decreased after the procedure (Gonzalez et al., 2019). Endoscopic luminal impedance planimetry was also suggested as a feasible method to investigate swallowing disorders and gastro-oesophageal reflux in dogs (Ullal et al., 2022).

We adopted filling volumes according to human studies in children (20–40 ml) (Popescu et al., 2023), as the approximate body weight of animals were roughly comparable. We did not use a 50 ml volume because of luminal diameter of the porcine oesophagus. Nevertheless, three-month-old pigs are adult animals. Maturity/adulthood are based on their fertility. Size and lumen of the porcine oesophagus are similar to that of children. However, function of the oesophagus and stomach of young adult pigs is similar (e.g., oesophageal manometry) or even the same (e.g., electrogastrography) as in adult humans. That is why porcine experimental models are advantageous for various preclinical experimental studies (Gonzalez et al., 2015; Kararli, 1995; Suenderhauf and Parrott, 2013).

We are aware of possible limits of our current study. All measurements were carried out in female pigs. According to our previous experimental study on porcine oesophageal manometry (Tachecí et al., 2015), peristaltic wave pressure was significantly higher in female animals compared to male pigs. There was also a clear and distinct trend in other parameters in favour of female gender (higher baseline pressure of the lower oesophageal sphincter, longer duration of relaxation and longer duration of peristaltic wave) (Tachecí et al., 2015). A similar gender-related difference in oesophageal motility has been reported in healthy humans, too (Dantas et al., 1998, 2009). Among other differences, women had longer duration of oesophageal contraction in the distal oesophageal body (Dantas et al., 2009). The explanation for the results observed may lie in anatomic and/or hormonal differences between the genders (Dantas et al., 1998). Since we found that baseline values of oesophageal or pyloric distensibility may be subject to significant inter-/intra-individual variability, it is advisable to use study designs in preclinical research in which each animal would represent its own actual control. This would allow comparison of the real effect of any experimental intervention

(Bures et al., 2015, 2020, 2021a, b, 2023; Tsianou et al., 2023). Nevertheless, a total of six animals is a sufficient number to obtain relevant data in an experimental setting.

The strength of our project is that it involves the first systematic measurements of all major FLIP parameters in an experimental setting. We used a single catheter for both pyloric and oesophageal measurements. We omitted ketamine, an NMDA-receptor antagonist, for induction of anaesthesia. In our previous study, we found that ketamine, even in a single intramuscular dose, affected myoelectric function of the porcine stomach (Bures et al., 2022). There were neither apnoea nor any other side effects of propofol administration throughout the experiments.

Conclusion

Endoscopic functional luminal planimetry in experimental pigs is feasible, both for the lower oesophageal sphincter and the pylorus. This is an important starting point for future experimental endoscopic trials (including bariatric endoscopy) and pharmacology or toxicology studies.

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Ethical aspects and conflict of interest
The authors have no conflict of interest to declare.

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