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Simultaneous assessment of gastric emptying and secretion in rats by a novel computed tomography-based method

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Jordi J, Verrey F, Lutz TA. Simultaneous assessment of gastric emptying and secretion in rats by a novel computed tomography-based method. Am J Physiol Gastrointest Liver Physiol 306: G173–G182, 2014. First published November 21, 2013; doi:10.1152/ajpgi.00230.2013.—Gastric emptying and gastric secretion are two major physiological functions of the stomach. The assessment of these functions in small animals is challenging; no method currently available allows the simultaneous measurement of both functions, and methods used are lethal or invasive and often limited by spatial, temporal, or quantitative resolution. Here, we report the establishment and validation of a quantitative noninvasive high-throughput computed tomography-based method to measure simultaneously gastric emptying and secretion in rats in vivo. The imaging strategy enables one to visualize stomach anatomy and to quantify stomach volume and stomach contrast agent content. The method was validated by comparing the results to classical lethal methods (stomach phenol red content and stomach wet weight). Additionally, we showed that the use of a mild anesthetic does not interfere with normal gastric function, thereby enabling high-resolution temporal studies within single animals. These combined advantages were applied to reevaluate the impact of cholecystokinin (CCK), histamine, and oral glucose solutions on gastric function with high temporal resolution. CCK inhibited gastric emptying completely for 20 min, leading to the accumulation of gastric juice in the stomach. The CCK antagonist devazepide blocked this effect. Histamine stimulated both gastric secretion and delayed emptying. Oral glucose solution emptied at a fixed rate of 24–31 cal/min and stimulated gastric secretion. These results confirm previous observations and add volumetric changes as a new dimension. As computed tomography scanners become broadly available, this method is an excellent approach to measure the combined gastric functional readout and to reduce the number of animals used.

gastric emptying; gastric secretion; computed tomography; cholecystokinin; histamine; glucose

GASTROINTESTINAL (GI) transit, nutrient digestion, and absorption are highly controlled functions. Each of them is to some extent autonomously regulated by neuronal and endocrine mechanisms but also controlled by the central nervous system (4). Based on the multifactorial control mechanism, it is not surprising that impaired GI function is associated with a broad spectrum of pathologies (diabetes types 1/2, Parkinson’s, etc.) (12, 20, 24). Because of the multiple involved signals, GI function needs to be studied in vivo, ideally with noninvasive methods.

The present study focuses on two main functions of the stomach, i.e., gastric secretion and gastric emptying. Gastric secretion participates in nutrient digestion, but acid secretion is also an important component of the body’s defense mechanisms. For instance, patients with decreased gastric secretion are more prone to enteric infections by Helicobacter pylori, Escherichia coli or Vibrio cholera (17, 24). The rate of gastric emptying determines nutrient release into the small intestine, which is particularly important to maintain postprandial nutrient homeostasis. Our understanding of stomach functions evolved rather slowly because of the lack of suitable in vivo methods applicable to high-throughput studies in rodents.

One major challenge in measuring stomach function, i.e., secretion and emptying, is to assess both functions simultaneously. Gastric emptying is typically assessed by measuring the clearance of a dye from the stomach, whereas gastric secretion is assessed by changes in stomach volume relative to the emptied volume (reviewed in Refs. 7 and 25). Only MRI can assess both functions simultaneously in humans, whereas methods for rats are currently not available (23). The smaller size of rodents and the high speed of gastric function are key technical hurdles for in vivo imaging strategies.

Gastric emptying is classically assessed by comparing the residual gastric dye content (e.g., radioactive compounds or phenol red) between treatment groups at specific time points (10, 14). This method requires stomach excision and therefore is lethal for the experimental animal. A nonlethal alternative is the paracetamol absorption test, where the appearance of paracetamol in the blood stream serves as an indirect measure of gastric emptying (18). Following the same logic, breath tests (13C acetate) measure the appearance of heavy 13C in the exhalation of animals (5). Both methods are indirect and are limited by the fact that unchanged metabolism and absorption kinetics across experimental groups have to be assumed. Currently, the most promising avenue might be the use of in vivo imaging strategies including ultrasound, positron-emission-tomography, or bioluminescence, among others (1, 2, 13, 22).

However, so far, their use remains limited because of difficulties with time or spatial resolution, throughput, or quantitative measures. Nevertheless they are critical components in the efforts to reduce lethal and invasive animal experiments and thereby important in context of the current 3R (replacement, refine, reduce) goals anchored in most national guidelines on...
animal welfare. Apart from these technical limitations, these imaging strategies are also unable to assess gastric secretion, as they cannot detect changes in stomach volume. Gastric secretion is often assessed by aspiration of gastric contents with an implanted fistula (15). Here, the experimental success depends strongly on fistula positioning because it determines localization and extent of aspiration. Apart from its invasive nature, surgery may also interfere with normal gastric function. A noninvasive alternative is the use of a pH-sensitive radio telemetry capsule (7) or the measurement of blood parameters to assess indirectly changes in the alkaline tide (10). In humans, MRI was applied to measure gastric secretion, but to our knowledge MRI was so far not applied to study gastric secretion in rodents (23). Overall, there are numerous methods to measure gastric emptying and secretion [recent reviews (7, 25)]; however, none can measure both stomach functions simultaneously, and methods used are lethal or invasive and often limited by spatial, temporal, or quantitative resolution.

Advancements in small animal X-ray technology led to a reduction of the scanning times for computed tomography (CT) to a few seconds while the image quality increased markedly in spatial resolution (8). Hence, X-ray exposure was markedly reduced, enabling several hundred scans per animal before reaching critical cumulative X-ray doses (>4 Gy for rats). A disadvantage of X-ray-based techniques is the poor absorption of radiation in soft tissue, such as the stomach epithelium and intestinal content. This can be compensated by the use of contrast agents, which are standard in clinical settings to detect anatomical changes in vessel structure. We envisioned that a contrast agent-based CT strategy could be applied to visualize the dynamic changes in stomach volume and contrast agent content to quantify stomach function in vivo.

Here, we report the establishment and validation of a quantitative noninvasive high-throughput computed tomography-based method to measure simultaneously gastric emptying and secretion in rats in vivo. The contrast agent-based strategy enables visualization and quantification of the total stomach volume and the residual contrast agent with high temporal and anatomical resolution. The method was extensively validated using known modulators of gastric emptying and secretion.

MATERIALS AND METHODS

**Animals.** Male Wistar rats (Janvier, Le Genest, France) were group housed [room temperature 21 ± 1°C, artificial 12-h:12-h light/dark cycle, water ad libitum, rat chow ad libitum (3433 Kliba; Nafag, Kaiseraugst, Switzerland)]. Body weight increased throughout the study by 3–4 g/day. All procedures for rat handling and experimental interventions were according to the Swiss Animal Welfare legislation and approved by the Kantonales Veterinäramt Zürich.

**Contrast agent.** Sodium diatrizoate hydrate (SDH; Sigma-Aldrich, Buchs, Switzerland) was solubilized in tap water and adjusted to pH 7.2, if not stated differently. A liquid hydrosoluble contrast agent was selected to enable accurate stomach volume detection. We selected SDH instead of Meglumine diatrizoate because SDH generated a stronger contrast and is less expensive. Both agents are used in clinics (gastrografin), and side effects are at most mild and transitory (FDA rev.10/11, NDC 0270-0445-40).

**In vitro quantification of SDH concentration and liquid volume by micro-CT.** Different solutions were prepared in vitro, and of each solution one CT image was acquired. First, SDH was diluted to different concentrations (50, 100, 200, 300, 400, and 500 mg/ml) with tap water. Each of these solutions (120 µl) was used for imaging in a 1.5-ml tube. Second, 125 mg of SDH was dissolved in 0.5, 0.6, 0.7, 0.9, 1.3, and 2.1 ml tap water in a 1.5-ml tube (concentrations of 250, 208, 179, 139, 96, and 60 mg/ml). The entire volume was imaged using the CT scanner. Third, 200 mg SDH were dissolved in 1 ml of tap water, pH adjusted to 3, 5, 7, 9, 11 with HCl or NaOH, respectively, and one CT image was acquired of each solution in a 1.5-ml tube.

**In vivo quantification of SDH concentration and liquid volume by micro-CT.** Food deprivation was used to ensure similar initial stomach contents between animals. Four-hour food-deprived rats were anesthetized with isoflurane (5%), gavaged with different solutions, and immediately scanned by CT under isoflurane anesthesia. In the first set of experiments, 2 ml of solutions were orally applied with different SDH concentrations (2 ml/animal; 30, 50, 70, 100, 150, 200 mg/ml; 60, 100, 140, 200, 300, 400 mg/animal). In the second set of experiments 200 mg SDH was applied in different volumes of tap water (1.0, 1.1, 1.2, 1.4, 1.8, 2.5, 3.0 ml/animal; 200, 181, 167, 143, 111, 80, 67 mg/ml; 200 mg/animal).

**Fig. 1.** In vitro calibration of sodium diatrizoate hydrate (SDH) concentration and solution volume measurement by micro-computed tomography (CT).

**A:** CT measurement of in vitro signal intensity and volume of solutions with different SDH concentrations but equal volume (120 µl). Linear regression accounts for all data until 200 mg/ml. **B:** in vitro signal intensity and volume of solutions with different volumes but equal SDH content (125 mg). **C:** in vitro signal intensity of solutions with equal volume and SDH content but at different pH. Means ± SE, n = 3.
In vivo calibration of SDH concentration and solution volume measurement by micro-CT. A: workflow of in vivo experiments starting with image acquisition. The stomach was segmented (solid line) and object volume and mean intensity extracted. This object was subject to 3D volume rendering for image presentation. B: in vivo signal intensity and volume recorded from the stomach immediately after oral application of different SDH concentrations with equal gavage volumes (2 ml). C: in vivo signal intensity and volume recorded from the stomach immediately after oral application of different volumes with equal SDH content (200 mg/ml). Means ± SE, n = 4–5.

**mCT validation against the classical phenol red method.** Four-hour food-deprived rats received an intraperitoneal (IP) injection of cholecystokinin (CCK)-8 (4 µg/kg; Bachem, Bubendorf, Switzerland) or saline (vehicle), immediately followed by 2-ml gavage of 200 mg/ml SDH and 0.75 mg/ml phenol red (Sigma). Animals were returned to their home cages but did not have access to water or food. Twenty minutes after application, animals were euthanized using pentobarbital (IP, 100 mg/kg; Virbac, Glattbrugg, Switzerland) in combination with isoflurane (5%) for quicker induction. Immediately after anesthesia induction, CT images were acquired and the stomach excised. The stomach wet weight was measured and the residual stomach phenol red content extracted and quantified spectrophotometrically at 560 nm as described before (10).

**Gastric function following oral glucose application.** Four-hour food-deprived rats received an IP injection of devazepide (1 mg/kg, Sigma) or vehicle (10% DMSO 10% Tween 80 in 0.9% NaCl, all Sigma). After 10 min, rats received an IP injection of CCK-8 (4 µg/kg) and saline and Zoletil (IP, 20 mg/kg). In the second experiment, 4-h food-deprived rats received an IP injection of histamine (25 mg/kg, Sigma) or vehicle (10% DMSO 10% Tween 80 in 0.9% NaCl, all Sigma) and Zoletil (IP, 20 mg/kg). In both experiments, eyes were covered with Vitamin A ointment after anesthesia induction; then rats were gavaged with 2 ml of SDH (200 mg/ml) and subsequently imaged immediately and then every 5 min. Animals were kept on a heat plate during the experiment to limit heat loss.

**Solid emptying.** A nutrient suspension was prepared by mixing 1.5 ml SDH (267 mg/ml) with 0.75 g of rat chow. The rat chow was reduced in size by a mixer. This nutrient suspension had a paste-like consistency. Animals were treated with cholecystokinin (CCK) (IP, 4 µg/kg) and received a 2 ml oral SDH (200 mg/ml) and phenol red (0.75 mg/ml) application. Stomach volume (C) and SDH content (D) was measured 20 min postapplication by CT. The same stomachs were then excised, and their wet weight (A) and phenol red content (B) was assessed. Means ± SE, n = 5, unpaired 2-tailed Student’s t-test, ***p < 0.001.
consistency and was administered by using a large gavage tool (3.0-mm diameter). Four-hour food-deprived rats received an IP injection of Zoletil (IP, 20 mg/kg). Eyes were covered with Vitamin A ointment after anesthesia induction, and then rats were gavaged with this nutrient suspension and subsequently imaged immediately and then every 15 min. Animals were kept on a heat plate during the experiment to limit heat loss.

Image acquisition. Images were acquired using Quantum FX micro-CT (PerkinElmer, Waltham, MA). Rats were placed in the CT in a prone position under isoflurane or Zoletil anesthesia as described for each experiment separately. The animal position was adjusted so that the area of image acquisition was centered on the stomach in all three dimensions. The field of view for image acquisition was 73 mm. Respiratory gating was used to reduce motion artifacts. The radiation dose was 26 mGy per scan, enabling a voxel size of 148 μm³. Scan time was 34 s per scan.

Image analysis. Images were analyzed using Caliper Analyze 11.0 (Analyze Direct, Kansas City, KS). All images were treated equally, i.e., no changes to contrast or intensity were applied or any filter to enhance image quality. All registered images contained the entire stomach as depicted in Fig. 2A. To quantify the stomach volume and mean signal intensity in the 3D space, this information had to be extracted from each dataset. Therefore, the original data set was subjected to a semiautomated object extraction algorithm, which is part of the software package. Briefly, a seed point was selected within the object of interest, which, in our case, was the contrast agent within the stomach; a threshold was set to the intensity 2,000 based on the in vitro experiments (see Fig. 1A). The algorithm automatically assigned every voxel connected to the seed point above the threshold to the object. The extracted object was visually inspected by a treatment-blinded investigator. If the algorithm had falsely assigned voxels within the esophagus or the small intestine to the object, they were manually removed. Subsequently, object volume and mean intensity were extracted. The displayed stomach images are 3D-volume renderings of representative stomachs for the specified condition. For the mathematical analysis of the results, see Equations. Equations. The equations used in this study are listed below.

\[
\text{Stomach mean signal intensity} = \text{measured variable}
\]

\[
\text{Total stomach volume} = x_{\text{min}} = \text{measured variable}
\]

\[
\text{SDH concentration} = x_{\text{min}} = (\text{stomach mean signal intensity} \times 1867)/18.57 \text{ (see Fig. 1A)}
\]

\[
\text{Stomach SDH content} = x_{\text{min}} = (\text{SDH concentration} \times x_{\text{min}}) + 15/0.63 \text{ (see Fig. 2A)}
\]

\[
\text{SDH volume} = x_{\text{min}} = 2 \text{ ml} \times \text{Stomach SDH content} = x_{\text{min}}/400 \text{ mg (we apply 400 mg SDH in 2 ml)}
\]

\[
\text{Nonadministered stomach volume} = x_{\text{min}} = \text{total stomach volume} - \text{SDH volume} = x_{\text{min}}
\]

Statistics. Rats were randomly allocated to treatment groups, and the order of application was randomized. Results are presented as means ± SE. The data were analyzed using GraphPad Prism 5.0. Statistical significance between the means was tested using unpaired Student’s t-test or two-way ANOVA and a Bonferroni posttest as appropriate. Differences were considered significant at \( P < 0.05 \).

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Fig. 4. Comparison of stomach function without anesthesia or under continuous Zoletil anesthesia. The effect of different anesthetics on stomach SDH content (A), volume (B), and secretion (C) was analyzed after oral SDH application. The single acute anesthesia group is composed of animals acutely anesthetized with isoflurane to acquire 1 image \( (n = 6) \) at 6 time points; total = 54 animals) after a given anesthesia-free time. In contrast, the animals of the Zoletil groups were kept under anesthesia and imaged every 5 min as indicated by the connecting lines \( (n = 6) \). D: representative 3D volume renderings of stomachs are shown. Means ± SE, no statistical significant differences were found.
RESULTS

In vitro quantification of SDH concentration and liquid volume by micro-CT. To test whether SDH can be detected quantitatively, we diluted SDH to different concentrations and quantified the signal intensity and the liquid volume with the CT method. In vitro SDH concentrations correlated in a linear manner with signal intensity until 200 mg/ml (Fig. 1A). At higher concentrations, the signal intensity tended to plateau, but volume detection remained accurate. To test whether the CT method can detect volume changes, we measured different liquid volumes containing equal amounts of SDH with the CT method. Volume changes showed an excellent linear correlation without a loss in signal intensity in vitro (Fig. 1B). To exclude the fact that pH, such as a low pH in the stomach, affected SDH quantification, we measured SDH signal intensity at different pH values in vitro. pH did not affect signal intensity down to pH 3 (Fig. 1C). However, SDH precipitated more acid pH.

In vivo quantification of SDH concentration and liquid volume by micro-CT. To test SDH quantification and volume detection in vivo, we performed similar experiments as described above for in vitro measurements. Briefly, 4-h fasted rats were anesthetized, and different concentrations or volumes of SDH were applied orally. Animals were immediately imaged by CT and stomach total volume and signal intensity assessed (Fig. 2A). Image resolution enables one to distinguish the anatomical difference of the stomach wall between the antrum and the body (Fig. 2A and Supplemental Movie S1; supplemental material for this article is available online at the American Journal of Physiology Gastrointestinal and Liver Physiology website). Applied SDH concentration and stomach signal intensity correlated linearly (y = 0.63 ± 0.02 − 15 ± 5, R² = 0.99) (Fig. 2B). Stomach volume detection, which is based on the presence of SDH, was accurate when the stomach SDH content was larger than 100 mg (Fig. 2B). When different volumes containing the same amount of SDH were given by gavage, the applied volumes correlated linearly with the measured stomach volume (y = 0.9 ± 0.1 + 0.8 ± 0.2, R² = 0.96) (Fig. 2C). Importantly, the stomach was not completely empty after 4-h fasting, and there was a residual gastric volume of 0.8 ± 0.2 cm³, here termed nonadministered stomach volume. Hence, in vitro and in vivo SDH concentration and volume changes were accurately detected by the applied CT method. Therefore, all following experiments were conducted with a

Fig. 5. Effect of CCK application on stomach function measured by micro-CT. Animals were treated with CCK (IP, 4 μg/kg), and the effect of CCK antagonized applying devazepide (IP, 1 mg/kg). Stomach SDH content (A, D), volume (B, E), and secretion (C, F) were recorded over time in the same animal after oral SDH application. The data were separated into 2 panels to improve visibility, but the data were collected in 1 experiment and the statistical analysis includes all groups. G: representative 3D volume renderings of stomachs are shown. Means ± SE, n = 5, unpaired 2-way ANOVA, Bonferroni posttest; *P < 0.05, **P < 0.01, ***P < 0.001.
fixed oral administration volume of 2 ml and a SDH concentration of 200 mg/ml, i.e., 400 mg/animal, which are experimental conditions in which signal intensity and stomach SDH content correlate linearly. When no or little (<100 mg) SDH was present in the stomach, volume detection was inaccurate. Hence, the method is amenable to measure gastric emptying by changes in gastric SDH content from 400 to 100 mg, reflecting 75% emptying.

**mCT validation against the classical phenol red method.** Next, we validated the CT method by comparing it to classical lethal methods (10, 14). Gastric emptying was inhibited in half of the animals by pretreating them with CCK (4 μg/kg IP), and after 20 min the extent of gastric emptying was quantified by different methods. Stomach wet weight, total stomach volume, phenol red, and SDH content were increased in the CCK-treated animals compared with saline control (Fig. 3). Importantly, the methods measuring stomach content showed similar relative increases after CCK administration.

**Stomach function under anesthesia.** A major advantage of in vivo imaging is the capability to assess the same phenomena in the same animal repeatedly. CT imaging requires an immobilized animal, but certain anesthetics such as isoflurane were shown to delay gastric emptying (26). Here, we compared stomach SDH content, total volume, and nonadministered volume in animals treated with a light anesthetic (Zoletil) to nonanesthetized animals. All animals received an oral SDH gavage. After application, the nonanesthetized animals were returned to their home cage and not exposed to anesthesia. After a specific time window, one CT image was acquired of these animals (here, termed single acute anesthesia). Zoletil-treated animals were under constant anesthesia; hence sequential imaging of the same animal over time was possible. Zoletil was administered IP and SC to test whether the exposure of the GI tract to the anesthetic altered gastric function. Under all conditions, stomach SDH content, total volume, and the nonadministered volume decreased similarly over time (Fig. 4). Of note, the single acute anesthesia group had a higher variability resulting from intraindividual differences in gastric function. This demonstrates that Zoletil-anesthetized animals have similar gastric function as nonanesthetized animals and therefore bear the advantage to allow repeated measures of stomach content over time in the same animal. Importantly, the gastric emptying curve was similar to the exponentially emptying of noncaloric meals observed by MRI in humans or phenol red in rats (14, 23). This indicates that SDH did not interfere with normal gastric emptying.

**Pharmacological modulation of gastric emptying and secretion by CCK and histamine.** To further validate our method, we pharmacologically altered gastric function by applying CCK or histamine. Their impact was monitored at a high temporal resolution. CCK was previously reported to delay gastric emptying, and devazepide is a known CCK antagonist (14, 21). Indeed, CCK (4 μg/kg IP) completely inhibited stomach SDH release for 20 min (Fig. 5A). Additionally, CCK induced an increase of the stomach total and nonadministered volume, presumably due to enhanced secretion (Fig. 5, B and C).

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**Fig. 6.** Effect of histamine application on stomach function measured by micro-CT. Animals were treated with histamine (IP, 25 mg/kg). Stomach SDH content (A), volume (B), and secretion (C) were recorded over time in the same animal after oral SDH application. D: representative 3D volume renderings of stomachs are shown. Means ± SE, n = 4–5, unpaired 2-way ANOVA, Bonferroni posttest; *P < 0.05, **P < 0.01.
Devazepide (1 mg/kg IP) completely antagonized the effect of CCK but alone did not differ from vehicle control. Next, we pharmacologically induced gastric secretion by administering histamine (25 mg/kg IP) (3). Histamine injection delayed SDH and total stomach volume decrease (Fig. 6, A and B). Importantly, nonadministered volume increased after 20 min, indicating gastric secretion (Fig. 6C). Together, these results show that the CT method can detect the expected pharmacological effects of CCK and histamine on gastric emptying and secretion with an excellent temporal resolution.

Oral glucose empties at a rate of 24–31 cal/min. We evaluated whether physiologically induced meal-born signals can modulate gastric function. Most prominently, orally applied glucose solutions were shown previously to calibrate gastric emptying to a fixed rate of 30–45 cal/min in rats (16). To test these phenomena, we orally administered 0.2 and 0.4 g/ml glucose, respectively, in combination with the contrast agent and monitored gastric function by CT over time. Indeed, glucose delayed the release of SDH content and induced an increase of total and nonadministered stomach volume (Fig. 7, A–C). When energy (in kJ) emptied from the stomach was plotted over time, a linear regression could be fitted, which suggests a linear gastric emptying rate of 24–31 cal/min (Fig. 7D; linear regressions are not statistically different). This highlights that our CT method can detect and reproduce the expected effect of oral glucose on gastric emptying.

Fig. 7. Effect of glucose application on stomach function measured by micro-CT. Animals received different oral glucose solutions including SDH. Stomach SDH content (A), volume (B), and secretion (C) was recorded over time in the same animal after application. kJ emptied from the stomach calculated based on the emptied SDH content are plotted over time in D and a linear regression fitted (no statistical differences were found between the 2 curves). E: representative 3D volume renderings of stomachs are shown. Means ± SE, n = 4, unpaired 2-way ANOVA, Bonferroni posttest; *P < 0.05, **P < 0.01, ***P < 0.001.
Solid emptying. We also tested whether our method is able to measure gastric function with a semisolid nutrient suspension to mimic better the physiological situation and the ingestion of a solid meal. Therefore, we administered a nutrient suspension, which contained food particles of maximal 3-mm size mixed with the contrast agent (Fig. 8D), and monitored gastric function by CT over time. Due to its semisolid state, the nutrient suspension did not enable us to visualize the full stomach anatomy (Fig. 8E). Consequently, the readout of total stomach volume was imprecise and did not allow the measurement of gastric secretion. Nevertheless, the release of contrast agent from the stomach enabled the measurement of gastric emptying (Fig. 8A), which followed the expected time course. We therefore believe that, even though most studies reported in this paper were performed with liquid solutions rather than solid diets, the results are probably also valid under different experimental conditions.

**DISCUSSION**

Gastric emptying and secretion are two key functions of the stomach. The current methodology does not allow the assessment of both simultaneously in small mammals like rats in vivo (7, 25). Here, we demonstrate that, based on CT, it is possible to accurately measure the volume and concentration of SDH, a liquid contrast agent, in vitro but also in the stomach of living animals (Figs. 1 and 2). Gastric emptying therefore can be quantified by measuring changes of the absolute SDH content in the stomach over time, and gastric secretion can be calculated based on stomach SDH concentration and volume. Changes of the total stomach volume depend on the volumes emptied, secreted, or ingested within the specified time frame. The ingested volume can be neglected when animals have no access to food or drinking water during the study. The emptied volume can be calculated based on the changes in the total SDH content. Therefore, the remaining volume reflects the volume that has not been administered, which can therefore be approximated as gastric secretion. This assumes proper and rapid mixing of the contrast agent with the secreted volume, emphasizing the importance of the liquid and hydrophilic nature of the contrast agent. Therefore, the use of a liquid contrast agent is required to quantify both stomach functions simultaneously. Similar strategies were already previously ap-

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**Fig. 8. Solid emptying measured by micro CT.** Animals received a nutrient suspension containing contrast agent per oral gavage. Stomach SDH content (A), volume (B), and non-administered volume (C) were recorded over time in the same animals after application. The nutrient suspension had a paste-like consistency with solid nutrient particles of a maximal size of 3 mm. D: illustrates the nutrient suspension combined with the contrast agent in the left well and the food particle size in its solid state in the right well. E: representative 3D volume renderings of stomachs are shown. Means ± SE, n = 5.
plied for assessing gastric emptying and secretion based on MRI data sets obtained in humans (23). Furthermore, we show a good correlation between classic lethal methods and our CT approach as validation (Fig. 3).

Nonlethal in vivo methods provide the major advantage that a function can be measured in the same animal repeatedly over time. Here, we validated the use of a light anesthetic that does not modulate gastric function and can be used for high-temporal resolution studies (Fig. 4). The low radiation dose of 26 mGy per scan enables more than a hundred scans before reaching the cumulative radiation dose of 4 Gy per animal, which is considered severity grade 1 according to Swiss animal welfare regulations. Even though radiation dose and scan time are short, image resolution and quality are excellent, enabling us to distinguish anatomical landmarks such as the fundus and the antrum throughout a time-course study (see Fig. 4D or Supplemental Movie S1).

These technical advantages were applied to validate the method with pharmacological modulators of GI function and physiologically induced meal-born signals. Among them are several GI hormones, which contribute to proper GI function. CCK, for example, stimulates the relaxation of the proximal stomach, increases its reservoir function, and inhibits gastric emptying. This has been documented as delayed phenol red release upon CCK treatment that is antagonized by devazepide (14, 21). Our data confirmed this effect with high temporal resolution (Fig. 5). Interestingly, CCK inhibited stomach SDH release completely for 20 min, which might lead to the accumulation of basal gastric acid secretion. This would also explain the observed increase of total stomach volume and nonadministered volume. Alternatively, CCK might induce gastric secretion based on its structural relationship to gastrin (4). Histamine stimulates gastric secretion, but it can also delay gastric emptying by interacting with the H1 receptor (3, 6). Our data clearly support both effects and confirm its action to inhibit gastric emptying and to increase gastric secretion (Fig. 6). Endocrine and neuronal signals are physiologically induced by the ingested meal itself. Most prominently, gastric emptying is described to be modulated mainly by the caloric equivalent of the ingested meal. Glucose solutions were shown to empty at a fixed rate of 2–2.5 kcal/min in humans and at a rate of 30–45 cal/min in rats (9, 16). We confirmed this finding and additionally showed an increase in gastric secretion upon glucose ingestion potentially due to an insulin effect (Fig. 7).

In sum, we validated our method with a large number of well-characterized physiological modulators of gastric function. As animals mainly ingest solid meals, one can emphasize that focusing on a liquid contrast agent-based method is a nonphysiological simplification. We therefore showed that the CT method is able to measure the emptying of a semisolid nutrient suspension (Fig. 8). However, this approach truncates the major strength of this method of being capable of measuring gastric emptying and secretion simultaneously. Therefore, the use of a liquid homogenous contrast agent is recommended. From a physiological perspective, it is essential to consider that solid meals were shown to empty at a similar rate as liquids of comparable composition and energy density after a lag phase (27). This lag phase is required for gastric digestion, in which solids are broken down into a liquid-like suspension. This liquid-like suspension was shown to empty from the stomach, only when particle diameter was reduced to ~2 mm (19).

Therefore, it was not surprising that we did not observe such a lag phase, as our semisolid nutrient suspension does not contain such large particles (Fig. 8). In sum, gastric digestion is sensitive to the nature of the meal (solid vs. liquid), but the control of gastric emptying is considered similar for liquids and solids (11). The strategy used here, which was based on liquid emptying, therefore also seems to be reasonable from a physiological perspective.

Taken together, we developed and validated a method to simultaneously measure gastric secretion and emptying in rats in vivo based on noninvasive CT. This enables paired experiments and reduces future harmful animal experiments. Furthermore, we tested a light anesthetic strategy to increase temporal resolution of gastric experiments. As new CT scanners are getting broadly available, this method should be exploited for future efforts that may aim at measuring intestinal or colonic transit motility and volume.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES