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Magnetic resonance imaging as a non-invasive tool to assess gastric emptying in mice

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Abstract

Background: Methods to study gastric emptying in rodents are time consuming or terminal, preventing repetitive assessment in the same animal. Magnetic resonance imaging (MRI) is a non-invasive technique increasingly used to investigate gastrointestinal function devoid of these shortcomings. Here, we evaluated MRI to measure gastric emptying in control animals and in two different models of gastroparesis.

Methods: Mice were scanned using a 9.4 Tesla MR scanner. Gastric volume was measured by delineating the stomach lumen area. Control mice were scanned every 30 min after ingestion of a 0.2 g meal and stomach volume was quantified. The ability of MRI to detect delayed gastric emptying was evaluated in models of morphine-induced gastroparesis and streptozotocin-induced diabetes.

Key Results: Magnetic resonance imaging reproducibly detected increased gastric volume following ingestion of a standard meal and progressively decreased with a half emptying time of 59 ± 5 min. Morphine significantly increased gastric volume measured at $t = 120$ min (saline: 20 ± 2 vs morphine: 34 ± 5 mm^3; $n = 8$–10; $p < 0.001$) and increased half emptying time using the breath test (saline: 85 ± 22 vs morphine: 161 ± 46 min; $n = 10$; $p < 0.001$). In diabetic mice, gastric volume assessed by MRI at $t = 60$ min (control: 23 ± 2 mm^3; $n = 14$ vs diabetic: 26 ± 5 mm^3; $n = 18$; $p = 0.014$) but not at $t = 120$ min (control: 21 ± 3 mm^3; $n = 13$ vs diabetic: 18 ± 5 mm^3; $n = 18$; $p = 0.115$) was significantly increased compared to nondiabetic mice.

Conclusions and Inferences: Our data indicate that MRI is a reliable and reproducible tool to assess gastric emptying in mice and represents a useful technique to study gastroparesis in disease models or for evaluation of pharmacological compounds.

KEYWORDS
diabetes mellitus, experimental, gastric emptying, gastroparesis, magnetic resonance imaging, mice

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1 | INTRODUCTION

Gastroparesis is a clinical condition characterized by delayed emptying of the stomach, in the absence of mechanical obstruction.[1] Gastroparesis may be idiopathic or arise as a consequence of diabetes mellitus, neurological disorders or surgical/pharmacological interventions.[1,2] Its prevalence in females is reported to be 38/100,000 compared to 10/100,000 in men.[3] However, this disorder is often not correctly diagnosed, potentially due to unawareness of the condition or confusion with other pathologies such as functional dyspepsia.[4] According to one study, it is estimated that gastroparesis may occur in up to 1.8% of the general population,[5] therefore having a much higher impact on the community than previously considered.

The pathogenesis of gastroparesis is most likely multifactorial, including alterations in the innervation, either in the extrinsic innervation (vagus nerve) and/or the Enteric Nervous System (ENS), the network of intestinal pacemakers or interstitial cells of Cajal (ICC), and changes in fibroblast-like cells and smooth muscle cells.[6,7] Disruption of one or more of these players impairs gastric function, leading to dyspeptic complaints that can have a severe impact on quality of life.[1,8]

To improve insight in the pathophysiological mechanisms of gastroparesis, it is key to have access to techniques that accurately and reproducibly assess gastric emptying in preclinical models. In most studies, animals are sacrificed at fixed timepoints after oral administration of radioactively labeled material,[9] glass beads,[10] or a dye such as phenol red[11] and the remaining gastric content is measured. However, such terminal experiments can only assess one time point per animal. In contrast, non-invasive techniques such as pinhole scintigraphy[12] or breath tests[13] can be performed multiple times in the same animal allowing the construction of emptying curves. In rodents, the stable isotope breath test is considered the gold standard for the assessment of gastric emptying. This test consists in the administration of a carbon-labeled solid meal with [^{13}C]octanoic acid, which allows to trace gastric emptying via detection of 13C-labeled CO_2 in the exhaled breath. This technique is, however, highly time consuming, induces high levels of stress on the animals, is low-throughput, and is only an indirect measurement of gastric emptying. Hence, there is an urgent need for an alternative technique to accurately assess gastric emptying in preclinical models in order to better study the mechanisms underlying gastroparesis.

Alternative techniques used in humans include single-photon emission computed tomography (SPECT) or magnetic resonance imaging (MRI) to measure changes in gastric volume.[14,15] In particular, MRI is a safe, non-invasive imaging technique that can differentiate between different types of soft tissue and between gas, solids, and liquids across different organs. MRI has been successful in distinguishing gastric content from the stomach wall in humans, allowing the assessment of gastric emptying.[16–18] Here, we developed a simple and robust MRI protocol to quantify pre- and postprandial gastric volume in mice, allowing the detection of gastric emptying without the need to sacrifice animals and without the use of contrast agents. Based on our data, we propose MRI as a robust, time-effective and reproducible tool for the non-invasive evaluation of gastric emptying in rodents.

KEY POINTS

- MRI can be used to determine changes in gastric volume in mice.
- MRI successfully detects delayed emptying in models of morphine-induced gastroparesis and streptozotocin-induced diabetes.
- The sensitivity of MRI in detecting delayed gastric emptying is comparable to the gold standard for the assessment of gastric emptying in rodents, the stable isotope breath test.

2 | MATERIALS AND METHODS

2.1 | Animals

All experimental procedures were performed in accordance with the European Community Council of Animal Care guidelines and approved by the Animal Care and Animal Experiments Committee of the Medical Faculty of the KU Leuven (P214/2018). Male C57/BL6J (12 weeks old) were purchased from Janvier (Mayenne, France). Mice were maintained under a 12 h/12 h dark/light cycle, at a temperature of 20–22°C (45–70% humidity), provided with food and water ad libitum.

2.2 | Fasting and feeding prior to assessment of gastric emptying

Mice were fasted overnight in cages without bedding, with free access to water. Animals were transferred to fresh cages and single-housed 2 h before the start of the experiment to limit coprophagy and were kept single housed during the entire experimental procedure. Any fecal pellets deposited prior to the scanning period were immediately removed. To assess gastric emptying, mice were fed a standardized meal containing 0.20 ± 0.01 g of scrambled cooked egg yolk and standard mouse chow carbon-labeled with 2.5 µmol [^{13}C] octanoic acid. To ensure habituation to the experimental conditions, animals were trained prior to gastric emptying studies. Training consisted in fasting the mice overnight followed by the administration of the standardized test meal. This was repeated twice with 1 week between training sessions. Meal size was limited in order to guarantee that the full portion was ingested by all mice in ≤5 min, to minimize the impact of feeding time in the time course of gastric emptying.

2.3 | Experimental protocol for MRI studies

MRI scans were performed at baseline (fasted) and at 0 min, 30 min, 60 min, 90 min, 120 min, and 150 min after meal ingestion. Prior to each scan, animals were anesthetized by inhalation of isoflurane-oxygen solution (4% for induction, 1%–1.5% for maintenance) and were kept anesthetized during scanning time. Different timepoints were assessed on separate days to exclude the effects of repetitive anesthesia on gastric emptying. Water bottles were removed 15 min prior to scanning to minimize variability in gastric volume. Following the procedure, mice were allowed to recover and given free access to food and water.

2.4 | Scan acquisition

The anesthetized animal was placed in an MRI-compatible mouse cradle with ear and tooth bars to restrict head motion. Respiratory rate was monitored throughout the entire scan (SAII), and isoflurane levels were adjusted to maintain a respiratory rate of 40–80 min^{-1}. Animal body temperature was maintained at 36.5–37.5°C. MR images were acquired using a Bruker Biospec 9.4 Tesla small animal MR scanner (Bruker BioSpin) with a horizontal bore of 20 cm and equipped with actively shielded gradients (600 mT/m). Data were acquired using a quadrature radio-frequency resonator (transmit/receive, inner diameter 7.2 cm, Bruker Biospin). After the acquisition of a localizer scan, 2D T_2-weighted rapid acquisition with relaxation enhancement (RARE) MR images were acquired in coronal orientation. For the coronal slices, the following parameters were used in order to capture the entire animal: 2 slice packages of 10 coronal slices of 1 mm thickness with a 1 mm gap placed interleaved and providing a coverage of 20 mm with no gap, effective TE: 30 ms, TR: 2000 ms, RARE factor: 8, 2 averages, FOV: 60 × 30 mm, in plane resolution 0.15 mm. MR images were acquired with prospective respiration gating (SAII) to minimize motion artifacts. All images were processed using the Paravision 6.0 software (Bruker BioSpin) and were converted to DICOM for further analysis in ImageJ software (National Institute of Mental Health).

2.5 | Image analysis

The acquired MR images were analyzed using the freely available ImageJ software (National Institute of Mental Health). The whole stack of 2D images was studied to detect the sections in which the stomach was observed. Regions of interest (ROI) were manually drawn in the 2D image slices encompassing the area of the stomach lumen in the correspondent slices (Figure 1). Gastric volume was then calculated for each animal following this formula based on Cavalieri's principle: $V = t \cdot \Sigma a$, where V accounts for gastric volume, t for the slice thickness and **a** for the area of all cross-sections of the stomach.[19] Area under the curve (AUC) of the collected data was calculated applying the trapezoidal rule using R software (R Core Team: R Foundation for Statistical Computing) and half emptying time ($T^{1/2}$) was derived from interpolation of the time in which 50% of the AUC was obtained.

2.6 | Pharmacological studies

Morphine, an opioid receptor agonist, is known to increase gastric relaxation and pyloric tone, resulting in a delay in gastric emptying.[20] To assess the capacity of MRI to detect alterations in gastric emptying, morphine hydrochloride (Laboratories STEROP NV) was injected subcutaneously (5 mg kg^{-1}) immediately after meal ingestion. Matched controls received a subcutaneous injection of vehicle (saline). Mice were then scanned 120 min after feeding.

2.7 | 13C Octanoic acid breath test

Gastric emptying was assessed using the standard [^{13}C]octanoic acid breath test as previously described.[13] Briefly, after overnight fasting with free access to water, mice were placed in a chamber (130 ml) flushed with CO_2-free air. After a baseline reading, animals were fed the previously described standardized meal containing 2.5 µmol [^{13}C]octanoic acid. Immediately after meal ingestion (<5 min), mice were treated with either morphine (5 mg kg^{-1}) or

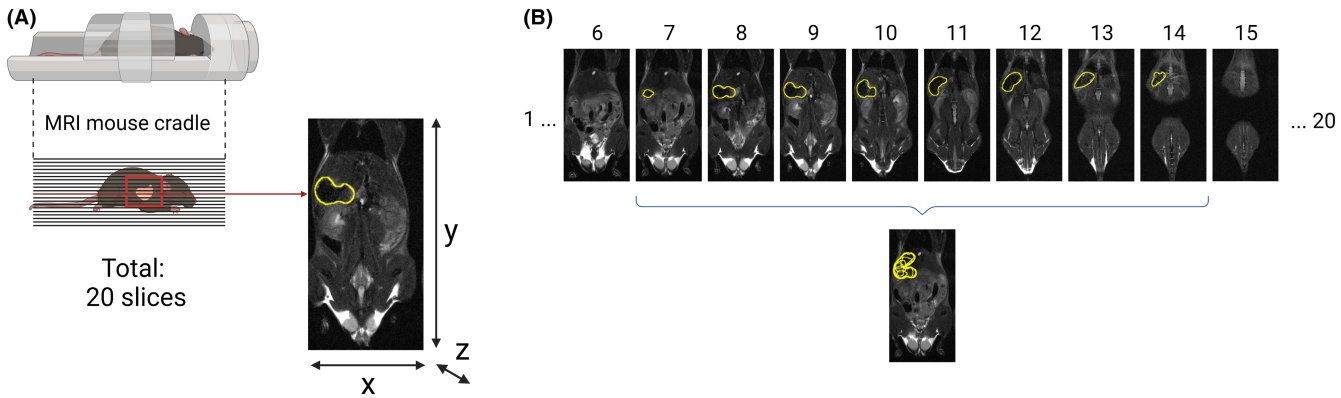

FIGURE 1 (A) Schematic representation of the experimental set-up and scan acquisition. (B) Representative T2-weighted MR image in coronal orientation. Yellow lines indicate delineation of the region of interest (ROI)

vehicle (saline) subcutaneously. Exhaled breath was collected in order to assess the amount of [^{13}C] labeled octanoic acid using an Infra-Red Isotope Analyzer (IT IS: Wagner Analysen Technik, Bremen, Germany). Samples were collected every 5 min for 60 min and then every 15 min up to 300 min. The data for $^{13}CO_2$ enrichment in the breath vs time were fit by a nonlinear regression model expressed as $y = at^b e^{-ct}$, where y is the percentage of [^{13}C] recovered in breath per hour (t), and a, b, and c are regression parameters estimated for each breath vs. time curve. The half emptying time ($T^{1/2}$) was calculated from a numerical integration procedure using an inverse-gamma function.

2.8 | Streptozotocin-induced diabetes

To induce diabetes, five consecutive daily injections of streptozotocin (STZ) (50 mg kg^{-1} body weight: Sigma-Aldrich) were administered in 1-week-old male C57/BL6J mice, as previously described.[21] Animals were considered diabetic after two consecutive non-fasting tail-vein blood glucose concentrations of ≥250 mg dl^{-1}, measured by a OneTouch Verio glucometer (LifeScan). Blood glucose was monitored twice weekly along the course of the experiment. Mice were scanned 12 and 14 weeks after diabetes induction at 60 and 120 min after feeding.

2.9 | Statistical analysis

Statistical analysis was performed using Prism 9 (GraphPad Software). Data are represented as median ± IQR and analyzed with the ordinary one-way ANOVA with Tukey's multiple comparisons test or the two-tailed unpaired t-test. A p value of <0.05 was considered statistically significant.

3 | RESULTS

3.1 | Gastric volume reconstruction and assessment of gastric emptying

Gastric volume was determined before (fasting) and at 30 min intervals after meal ingestion up to 150 min (Figure 2A,B). We observed that, when assessing the same animal in 30 min intervals allowing short periods of recovery from anesthesia in between, gastric volume hardly decreased resulting in almost similar volumes at 150 min as immediately after meal intake (data not shown). Hence, each time point was measured on a separate day in animals that received a single acute dose of anesthesia 5 min prior to scanning.

Fasting volume measured 10 ± 3 mm^3 (n = 9). After meal ingestion, gastric content increased up to 34 ± 3 mm^3 (n = 9) and slowly decreased in time with a faster emptying during the first 30 min, followed by a phase of plateau between 60 and 90 min (Figure 2B). Propulsion of gastric contents resumed from t = 90 min onwards. Area under the curve (AUC) for the collected data at the different timepoints was determined, and half emptying was derived from interpolation of the time in which 50% of the AUC was obtained. The mean half-emptying time of the administrated solid meal was of 59 ± 5 min (n = 8).

To assess reproducibility of the technique, gastric volume was evaluated on two different days at the timepoints t = 60 min and t = 120 min in the same animal (Figure 2C), showing comparable volumes (23 ± 1 mm^3 scan 1 at t = 60 versus 23 ± 2 mm^3 scan 2 at t = 60; n = 5; p = 0.859 and 21 ± 1 mm^3 scan 1 at t = 120 versus 21 ± 2 mm^3 scan 2 at t = 120; n = 4; p = 0.609).

3.2 | Detection of morphine-induced gastroparesis

As we aimed to apply our MRI protocol to assess gastroparesis, mice were treated with morphine, an opioid agonist known to delay gastric emptying,[20] and results obtained via MRI were compared with the gold standard, that is, the [^{13}C]octanoic acid breath test.

In a first series of experiments, gastric emptying at t = 120 min was assessed using MRI. Morphine or saline was administered immediately after feeding (Figure 3A). At t = 120 min, gastric volume in the morphine-treated group was significantly increased compared to the vehicle treated group (saline: 20 ± 2 mm^3; n = 8 vs morphine: 34 ± 5 mm^3; n = 10; p < 0.001) (Figure 3B). Of the 10 mice treated with morphine, all animals showed delayed gastric emptying using the threshold of 23 mm^3, as determined by the 95th percentile of the values obtained in the saline-treated mice at the same timepoint.

In a second series of experiments, the effect of morphine on gastric emptying was measured using the standard [^{13}C]octanoic acid breath test following ingestion of the same standard meal of 0.2 ± 0.01 g egg yolk containing 2.5 µmol [^{13}C]octanoic acid. The cutoff of normality for gastric emptying was 115 min, as determined by the 95th percentile of the values obtained in the saline-treated mice. As shown in Figure 3C, morphine treatment resulted in a significantly increased half emptying time ($T^{1/2}$ 85 ± 22 min saline and 161 ± 46 min morphine-treated; n = 10; p < 0.001). However, not all mice could be classified as delayed using the aforementioned threshold of 115 min. Gastric emptying was still within normal limits in two out of 10 mice, suggesting that breath testing may be less sensitive to detect delayed emptying.

3.3 | Detection of diabetic gastroparesis

In a next series of experiments, gastric emptying was determined using MRI in a model of diabetic gastroparesis. Diabetes was induced in 8-week-old C57BL/6J by injection of five consecutive doses of 50 mg kg^{-1} STZ once every 24 h, as previously described.[21] Diabetic gastroparesis is expected to develop around

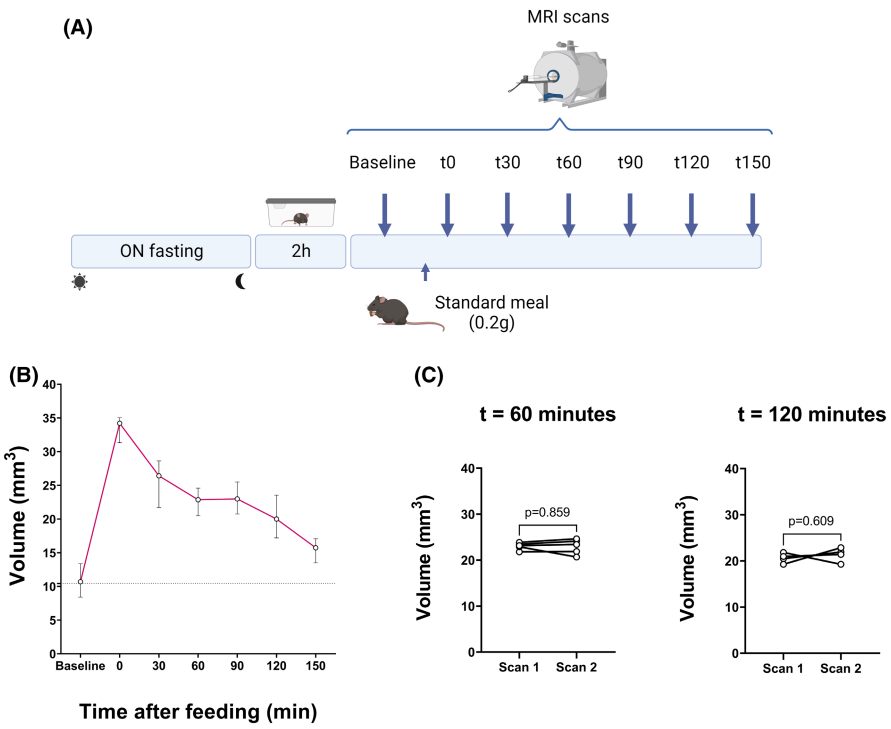

FIGURE 2 (A) Schematic representation of the experimental protocol to determine gastric emptying used by MRI scans. Each time point was measured on different days after feeding the standard meal. (B) Gastric emptying curve of control animals after ingestion of a 0.2 g standardized meal. Data are expressed as gastric volume in mm^3 ($n = 8–9$). Data were analyzed with ANOVA followed by Tukey's multiple comparisons and shown as median ± IQR. (C) Gastric volume assessed on two different study days at $t = 60$ min and $t = 120$ min after meal ingestion. Data were analyzed by paired t-test

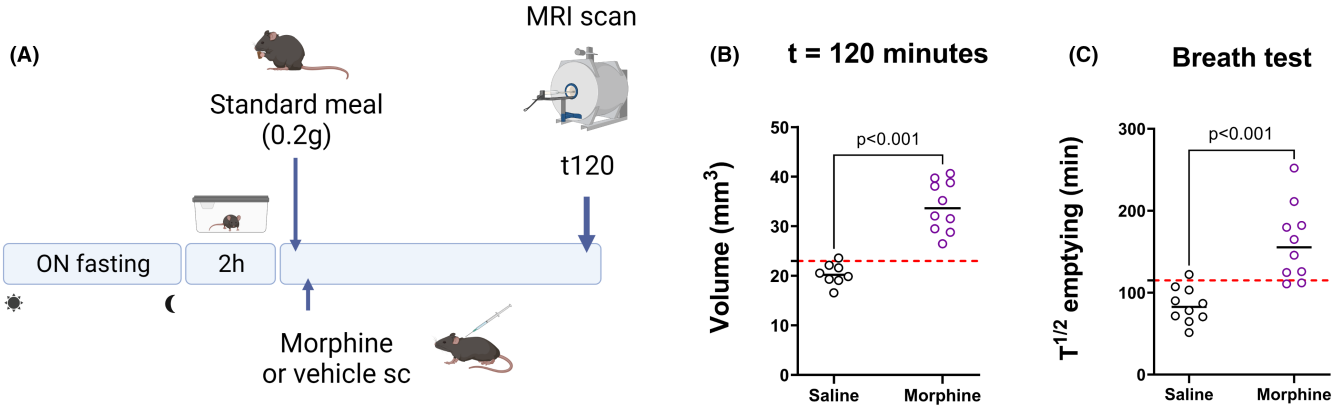

FIGURE 3 Detection of morphine-induced gastroparesis. (A) Schematic representation of the scanning protocol used to assess morphine-induced delayed gastric emptying using MRI. (B) Gastric volume assessed 120 min after food ingestion in morphine-treated mice and saline-treated controls. Volume is expressed in mm^3 ($n = 8–10$). (C) Half emptying time ($T^{1/2}$) of saline-treated mice and morphine-treated mice as assessed via the [^{13}C]octanoic acid breath test. Half emptying time is expressed in min ($n = 10$). Data were analyzed by unpaired t-test and shown as median

6 weeks after diabetes onset.[22] In order to investigate which time-point would be suitable to detect diabetic gastroparesis, mice that became diabetic were scanned at $t = 60$ min and $t = 120$ min, respectively. Mean values of gastric volume were increased in the diabetic group at $t = 60$ min (control: $23 \pm 2\,mm^3$; $n = 14$ vs diabetic: $26 \pm 5\,mm^3$; $n = 18$; $p = 0.014$). Eleven out of 18 mice in the diabetic group showed gastric volumes above the 95th percentile of $25\,mm^3$, as determined from the data obtained from control animals at $t = 60$ min (Figure 4A), with a more pronounced delay in four animals. In contrast, mean gastric volume did not differ between groups at $t = 120$ min (control: $21 \pm 3\,mm^3$; $n = 13$ vs diabetic: $18 \pm 5\,mm^3$; $n = 18$; $p = 0.115$). Gastric volume was outside the range of normality in only one out of 18 mice in the diabetic group (Figure 4B). Of interest, four diabetic mice showed evidence of increased gastric emptying. These data indicate that assessing gastric volume at $t = 60$ min is more suited to detect a delay in gastric emptying in this diabetes model.

4 | DISCUSSION

Here, we developed a new protocol to assess gastric emptying in mice using MRI. We showed that this technique accurately measures gastric volume at different time points without the need to sacrifice animals. In two models of gastroparesis, that is, mice treated with morphine and a model of diabetic gastroparesis, we were able to

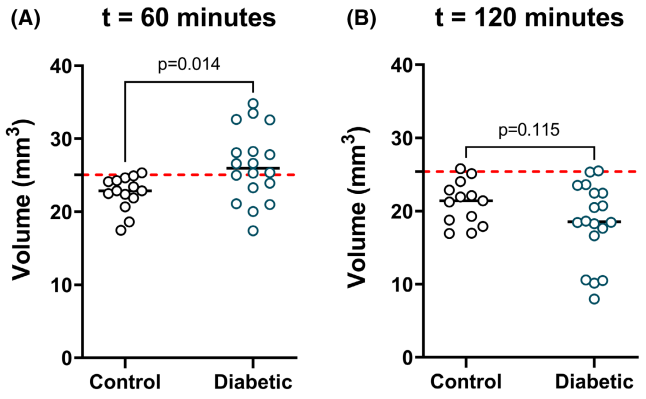

FIGURE 4 Detection of diabetic gastroparesis. (A) Gastric volume assessed 60 min after food ingestion in STZ-induced diabetic mice ($n = 18$) and controls ($n = 14$). (B) Gastric volume assessed 120 min after food ingestion in STZ-induced diabetic animals ($n = 18$) and controls ($n = 13$). Volume is expressed in mm^3. Data were analyzed by unpaired *t*-test and shown as median

detect delayed emptying. Based on these data, we propose MRI as a robust non-invasive tool for the repeated assessment of gastric emptying in mice.

Magnetic resonance imaging is a non-invasive imaging technique that can provide detailed images of internal organs with high resolution and high soft tissue contrast without the use of contrast agents, and is increasingly used to assess gastrointestinal function in humans.[23] Using paramagnetic agents mixed with a test meal, residual meal present in the stomach can be accurately measured and discriminated from gastric secretions and air. By plotting changes in volume against time, one can calculate half emptying time and determine whether patients are indeed suffering from gastroparesis. Here, using MRI we assessed gastric volume in mice before and after intake of a standard meal, without the need of contrast agents. To prevent movement artifacts during scanning, mice were briefly anesthetized using isoflurane. Although we previously demonstrated that this anesthetic did not affect gastric emptying of a radiolabeled meal when assessed 6 h after anesthesia,[12] previous studies show the impact of short-term isoflurane exposure on gastrointestinal transit, in which motility is reported to be affected up to 120 min post-anesthesia in rodents.[24] Indeed, we could observe how gastric emptying was almost completely inhibited when the animals were repeatedly exposed to isoflurane. Hence, mice were only scanned once a day to minimize the impact of anesthesia. Using this protocol, we could observe a time-dependent decrease in gastric volume. In contrast to human gastric emptying studies showing a linear emptying curve for solids, we observed an initial fast emptying phase followed by a period of approximately 30 min during which almost no emptying occurred. A similar behavior of the emptying curve was previously reported when administering a solid egg yolk based meal in mice.[25]

As we were not able to calculate half emptying time using the standard mathematical models due to the aberrant shape of the emptying curve, we determined the area under the curve (AUC) and the half emptying time was derived from interpolation of the time in which 50% of the AUC was obtained, as previously described.[26] Application of this approach yielded half emptying values between 52 and 67 min, in contrast with the 62–131 min obtained using the [^{13}C]octanoic acid breath test.[13] This difference could result from differences in methodology to assess gastric emptying and the algorithm used to calculate half emptying time. To further evaluate whether data obtained using our technique were reproducible, gastric volume was measured in the same animals at a 2-week interval. Both at 60 and 120 min after meal ingestion, we obtained comparable gastric volumes, indicating that this technique is well suited to evaluate gastric emptying in mice.

In a next step, we studied the capacity of MRI to detect the impact of pharmacological interventions on gastric emptying or to assess delayed emptying in disease models. First, we tested if delayed gastric emptying induced by a pharmacological intervention could be detected to the same extent as with the gold standard, the [^{13}C]octanoic acid breath test. To this end, we used the delaying agent morphine, an opioid receptor agonist known to increase gastric relaxation and pyloric tone.[20] As expected, the average $T^{1/2}$ emptying time assessed by the breath test was significantly increased by morphine from 85 to 161 min, while the $T^{1/2}$ was outside the range of normality in eight out of 10 mice. Similarly, morphine significantly increased average gastric volume at $t = 120$ min measured with MRI but delayed gastric emptying was now detected in all 10 animals studied. Although the sample size was rather small, these data indicate that MRI is at least as sensitive as the breath test in detecting delayed gastric emptying.

To further evaluate the value of MRI in detecting gastroparesis, we studied gastric emptying in a mouse model of diabetic gastroparesis. Diabetes was induced by injection of five consecutive doses of streptozotocin, reported to cause a loss of insulin positive beta cells and to lead to the development of diabetes in around 70% of treated mice.[21,27] Abnormal blood glucose levels will eventually cause diabetic gastroparesis in around 30% of the diabetic mice, accompanied with an increased level of pro-inflammatory markers, reduction in inhibitory enteric neurons and damage to interstitial cells of Cajal networks.[22,28,29] Previous reports indicate that in animal models of STZ-induced diabetes, gastroparesis arises around 6 weeks after diabetes onset.[22] Therefore, animals were studied at weeks 12 and 14 after disease induction to allow enough time for gastroparesis to develop. Of note, 18 out of the 19 mice injected with streptozotocin developed diabetes, of which 61% had delayed gastric emptying at $t = 60$, as evidenced by a gastric volume above the 95th percentile of control animals. Of those, four mice had almost no emptying. Conversely, in previous studies using the [^{13}C]octanoic acid breath test, delayed emptying was detected in around 30% of diabetic animals.[28] Assessment of gastric volume using MRI at $t = 120$ min did, however, not show a significant increase in gastric volume in diabetic mice compared to controls. This finding contrasts with scintigraphic gastric emptying studies in patients suffering from diabetic gastroparesis. In humans, sensitivity to detect gastroparesis is higher at the 4 h than at the 2 h time point following ingestion of a radiolabeled meal.[30] We have no clear explanation for this discrepancy although differences in kinetics of gastric emptying, as indicated by

the shape of the gastric emptying curve, may potentially contribute. Of note, $t = 120$ min scans revealed four animals in the diabetic group displaying gastric volumes close to those recorded for empty stomachs, suggesting a more rapid gastric emptying. Acceleration in gastric emptying has been previously identified in a STZ-induced mouse model of diabetes at early stages of the disease.[31] There are no reports describing this phenomenon at later stages of diabetes in mice, yet reports in diabetic human patients describe accelerated gastric emptying times independently of the moment in which the disease was developed.[32] This highlights the importance of finding a reliable and precise method to further investigate the alterations in gastric function in animal models of diabetes, since the impact of the pathology on the upper gastrointestinal tract remains poorly understood. Nevertheless, based on our data, we propose to assess gastric volume using MRI at $t = 60$ min to detect delayed gastric emptying.

Overall, we developed a reproducible, robust, non-terminal approach that allows the *in vivo* monitoring of gastric emptying over time in the same animal, facilitating the study of gastroparesis in rodents. Accurately monitoring changes in gastric emptying will remain key to gain mechanistic insight behind altered gastric function in diabetic gastroparesis, with the ultimate goal to identify new targets for treatment.

AUTHOR CONTRIBUTIONS

MCP designed experiments, conducted experiments, and wrote the manuscript. MFV designed experiments. IA and WG provided excellent technical support throughout all the experiments. SA performed mathematical analysis. GM provided intellectual input. GB and UH led the project, and GB revised the manuscript.

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DISCLOSURE

The authors have no competing interests.

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