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Time-course of recovery of gastric emptying and motility in rats with experimental spinal cord injury

Emily Qualls-Creekmore, Melissa Tong, and Gregory M. Holmes

Neurotrauma and Nutrition Laboratory, Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808

Abstract

We have shown recently that spinal cord injury (SCI) decreases basal gastric contractions 3 days after injury. In the present study we used the [¹³C] octanoic acid breath test as well as gastric strain gauges with the aim to investigate the time-course of recovery from post-injury gastric stasis in rats that underwent experimental SCI at the level of the third thoracic (T3) vertebra. Following verification of the [¹³C]-breath test sensitivity in uninjured rats, we conducted our experiments in rats that underwent T3- spinal contusion injury (T3-CI), T3- spinal transection (T3-TX) or laminectomy (control) surgery at three days, one, three or six weeks post-injury.

Our data show that compared to rats that underwent laminectomy, rats that received SCI showed a significant reduction in the cumulative percent [¹³C] recovery. Although more marked in T3-TX rats, the delayed gastric emptying in T3-CI and T3-TX rats was comparable in the 3 days-three weeks period post-injury. At six weeks post-injury, the gastric emptying in T3-CI rats recovered to baseline values. Conversely animals in the T3-TX group still show a significantly reduced gastric emptying. Interestingly, the almost complete functional recovery observed in T3- CI rats using the [¹³C]-breath test was not reflected by analysis of spontaneous gastric contractions after SCI.

These data indicate that T3-SCI produces a significant reduction in gastric emptying independent of injury severity (T3-CI vs. T3-TX) that persists for at least 3 weeks after injury. However, 6 weeks post-injury T3-CI, but not T3-TX, rats begin to demonstrate functional recovery of gastric emptying.

Keywords

¹³C-breath test; gastric stasis; gastrointestinal motility; gastrointestinal transit; spinal cord contusion; spinal cord transection; vagal reflexes

The most well recognized consequence of spinal cord injury (SCI) consists in the partial-to-complete loss of motor and sensory functions. However, it has also been known that severe gastrointestinal (GI) consequences occur in SCI patients. Dysphagia, gastrointestinal stasis, gastric hypersecretion, gastric dilation, nausea and emesis are observed immediately after SCI 1–2 and may persist for years after the initial injury 3–11.

The acute failure of proper gastric function is a major cause of post-SCI morbidity and its persistence may affect the patients' long-term quality of life and nutritional status 12. Post-SCI gastric disturbances require aggressive nutritional supplementation through enteric feeding tubes 13, parenteral feeding or, occasionally, percutaneous gastrostomy 14. Severe

and prolonged gastric stasis is often accompanied by an elevated risk of vomiting and pulmonary aspiration. These co-morbidities often necessitate intensive management of the airway, and limit the appropriateness of some enteral feeding strategies 1–15.

We have reported recently that both spontaneous gastric contractions and the esophageal-gastric relaxation reflex are significantly diminished in animals three days after contusion injury at the third thoracic (T3) segment 16. Our data further indicate that changes in the sensitivity of vago-vagal reflexes may be responsible for post-SCI gastroparesis since the reductions in spontaneous gastric contractions, and esophageal-gastric reflex relaxations, were not ameliorated by celiac sympathectomy in T3 SCI rats. Other experimental SCI models have investigated the effects on gastric and duodenal emptying of liquid test meals following SCI at spinal level T9 as well as at levels rostral to T4. These reports showed inhibition of gastric emptying and duodenal transit 17–20.

It has to be kept in mind, however, that different mechanisms regulate gastric emptying of liquids or solids 21–22. As such, experimental designs must specifically address the inherent differences in the transpyloric flow of liquid or solid meals. Surprisingly, the effects of SCI on gastric emptying of solid meals have not been tested. Furthermore, traditional gastric emptying measures rely upon the use of phenol red techniques and post-mortem recovery of gastric contents 21–23, thus precluding the repeated testing of animals and the understanding of the recovery processes that may be occurring following SCI.

While scintigraphic visualization of gastric emptying overcomes the limitations of test meal composition and the collection of only one data point per animal, it is hampered by the need to restrain or anesthetize the test animal. Conversely, non-invasive breath tests for gastric emptying that utilize [^{13}C]- isotope-enriched substrates have been validated in a number of species including humans and rodents 24–28. The use of this technique permits safe, repeatable, measurement of gastric emptying in the unanesthetized, freely-moving animal.

In the present study, after verifying the sensitivity of our noninvasive [^{13}C]-breath test for indirect measurements of gastric emptying of a solid meal in rats, we aimed to determine the temporal recovery of gastric emptying in rats during the first six weeks following contusion SCI or complete spinal cord transection. Finally, we aimed at determining the effects of contusion SCI or complete spinal transection on the basal level of contractions of the gastric corpus over the same six week period.

Materials and Methods

All procedures were performed according to National Institutes of Health guidelines for the care and use of animals in research and were approved by the Institutional Animal Care and Use Committee at the Pennington Biomedical Research Center.

All experiments were conducted on male Wistar rats ($n = 7$ for bethanechol/atropine [^{13}C] study, $n = 36$ for SCI [^{13}C] study and $n = 45$ for gastric contraction studies; Harlan, Indianapolis, IN). Rats were ≥ 8 weeks of age at the start of the experiment and were double housed in a room maintained at 21–24°C and a 12:12-h light-dark cycle with food and water provided *ad libitum*. After surgical manipulation, rats were moved to individual cages and remained single-housed throughout the study. We randomly assigned rats to receive either a spinal cord transection or spinal cord contusion centered at T2-T3 for both the [^{13}C]-breath test or gastric contraction studies. The remaining rats were selected to serve as surgical controls. We recorded body weights prior to surgery to ensure that no significant weight difference existed between groups.

Surgical Procedures and Animal Care

Rats were anaesthetized with Nembutal (60mg kg^{-1} , ip, Abbott Laboratories, Chicago, Ill.), and were supplemented as necessary to maintain areflexia. All animals were administered ophthalmic ointment to both eyes, buprenorphine (0.1mg kg^{-1} , ip, Reckitt Benckiser Pharmaceuticals Inc., Richmond VA) to alleviate post-operative pain, and antibiotics (Baytril, 2.5 mg/ml concentration @ 1ml kg^{-1} s.q., Bayer, Shawnee Mission KS) to reduce post-surgical infection prior to any surgical manipulation.

We performed spinal cord transections or contusions at the T2-T3 levels of the vertebral column as described previously 12, 16. Briefly, the T2-T3 spinal cord is exposed through a midline incision over the vertebral column, the overlying muscles are detached from the vertebrae and the T2 spinous process is removed with fine tipped rongeurs. Spinal transection (T3-TX) is performed with microscissors and subpial aspiration at the rostral most T3 spinal segment. For spinal contusion (T3-CI), the spinal cord is exposed as above and rats are placed in the clamps of the Infinite Horizon controlled impact device (Precision Systems and Instrumentation, LLC, Lexington, KY) whereby a rapid 300 kDyne displacement of the cord and overlying dura is performed. Procedures for the control animals are the same as for spinal injury except that the spinal cord and surrounding dura mater are not disturbed following laminectomy. Upon completing the surgical procedure, the muscle tissue overlying the lesion site are closed in anatomical layers with Dexon II suture and the skin closed with 9mm wound clips. Animals are administered warmed supplemental fluids (5 cc lactated Ringer's solution) and placed in an incubation chamber maintained at 37°C until the effects of anesthesia have subsided.

Chronic care of both control and injured animals utilized procedures that have been described previously 29. Briefly, post-operatively, animals are kept in a warm environment and receive subcutaneous supplemental fluids (5–10 cc lactated Ringer's solution), analgesics (carprofen, 5mg kg^{-1} IP, Pfizer Animal Health, Lititz, PA) once daily for 3 d and antibiotics (Baytril, 2.5 mg kg^{-1}) twice daily for 5 d after surgery.

Body weight and food intake is recorded each morning and bladder expression and cleaning of the hindquarters is performed at least twice daily in animals with SCI until the return of spontaneous voiding. The ventrum of control animals is inspected daily without need for manual compression of the bladder. Following the return of spontaneous voiding in SCI rats, all animals are inspected only once daily after weighing.

Measurement of gastric emptying using [^{13}C]-octanoic acid breath test

The solid meal used in this study is a prepared pancake to which the rats are adapted in the days prior to testing. The day of the experiment, rats are fasted overnight with unlimited access to water, and then are placed into a square plexiglass chamber (7 liter capacity). The chamber is continually flushed with air scrubbed for CO_2 at a flow speed of 0.65 l min^{-1} ; this flow rate is adequate in keeping chamber CO_2 levels below 1%. The chamber is lined with a small amount of sterilized corncob bedding. After two baseline air measurements are collected, rats receive 1g of pancake containing $5\mu\text{l}$ [^{13}C]-octanoic acid. During testing, all rats consumed the pancake fully within a window of 1–5 min. Air containing the exhaled breath is collected and analyzed to determine the ^{13}C -to- ^{12}C carbon dioxide ratio using the nondispersive Infra Red Isotope Analyzer (IRIS; Wagner Analysen Technik, Bremen, Germany). Air samples are initially collected at 5 min intervals for the first 1h of testing; subsequent air samples are taken at 15 min intervals for a total testing time of 360 min.

Sensitivity of [^{13}C]-octanoic acid breath test

We performed [^{13}C]-octanoic acid breath tests in surgically naïve rats accustomed to the breath test chambers. Each rat was administered the [^{13}C]-octanoic acid breath test 20 min following the administration of the muscarinic agonist bethanechol (5 mg kg⁻¹ ip) to enhance gastric motility, the muscarinic antagonist, atropine (7.5 mg kg⁻¹ ip) to reduce motility or control injections of saline. We randomized drug order across animals with each test separated by a minimum of 5 d. To confirm the gastric retention of the solid test meal in SCI rats, we fasted 3 d post-operative T3-TX and control rats, and administered the full test meal (1g pancake containing [^{13}C]-octanoic acid). The animals were rapidly euthanized by exsanguination 60 min after consuming the meal, the stomach exposed through a midline incision and the cardia and pylorus clamped with hemostats. The stomach was removed and the gastric contents were retrieved, dried and weighed.

Gastrointestinal studies

We performed gastric contraction studies as described recently 30. Briefly, rats were deeply anesthetized with thiobutabarbital (Inactin®; Sigma, St. Louis, MO; 100–150 mg/kg IP), anesthetized, intubated with a tracheal catheter, a laparotomy was performed to expose the gastric surface and we sutured a 6 × 8mm encapsulated miniature (2mm²) strain gauge (RB Products, Minneapolis, MN) in alignment with the circular smooth muscle in the gastric corpus. The abdominal incision and the wound margins were closed with suture. The strain gauge signal was amplified (QuantaMetrics EXP CLSG-2, Newton, PA) and recorded on a Grass polygraph (Model 79, Quincy, MA) or a PC using Axotape software (Axon Instruments, Union City, CA). Motility signals were low-pass filtered at 3 Hz to reduce respiratory artifact.

Anesthetized animals are maintained at 37±1°C using a feedback-controlled warming pad. Following one hour of stabilization, a 10 min period of gastric contractions were recorded prior to any experimental manipulation.

Data collection and analysis

We assessed the extent of contusion injury relating to locomotor function by scoring the range and frequency of hindlimb joint movement and coordination with the widely used Basso, Beattie, Bresnahan (BBB) locomotor rating scale 31, where 0=no hindlimb movement and 21=normal locomotion. We also calculated the mean energy intake (MEI) to assess the daily average of kilocalories consumed per 100g of body weight for each 1 wk period of monitored feeding. Gastric half-emptying time ($T_{1/2}$) was determined by calculating the time to reach 50% recovery of the administered [^{13}C] substrate. Animals failing to reach 50% recovery were assigned the maximum 6 h test time. We quantified spontaneous gastric contractions by calculating the motility index from raw polygraph traces as described previously 16, 32, 33.

Using techniques described previously 16, animals were sacrificed at the conclusion of testing by transcardial perfusion with ice cold isotonic phosphate buffered saline followed by 4% paraformaldehyde. The spinal cord lesion site was removed and cryoprotected before cutting frontally at 40 µm thickness (Microm HM560, Richard-Allan Scientific, Kalamazoo MI). Tissues were stained with luxol fast blue to show myelinated fibers. Luxol fast blue-stained myelin in injured tissue was then expressed as a percent of the total cross-sectional area as would be predicted for intact tissue.

Data are presented as mean ± standard error. Differences between BBB locomotor score, mean energy intake (MEI), cumulative percent recovery (of the administered [^{13}C] dose), latency to the peak in fractional dose h⁻¹ (T_{max}), gastric half-emptying time ($T_{1/2}$), and basal

gastric motility for the subject groups were evaluated by a one-way ANOVA using SPSS for Windows (SPSS Inc, Chicago, IL), followed by the Tukey A *post-hoc* test. Significance was set at $p < 0.05$.

Results

Sensitivity of [^{13}C]-breath test

We verified the sensitivity of the [^{13}C]-octanoic acid breath test by measuring gastric emptying in intact male Wistar rats ($n=7$) following administration of saline, bethanechol (5 mg kg $^{-1}$ ip), or atropine (7.5 mg kg $^{-1}$ ip) 20 minutes prior to receiving the [^{13}C]-octanoic acid containing solid test meal.

The gastric half emptying time ($T_{1/2}$) following saline treatment was 119 ± 12 min, bethanechol decreased the gastric emptying time to 78 ± 6 min ($p < 0.05$; Fig. 1), while atropine slowed gastric emptying to 201 ± 28 min ($p < 0.05$). Similarly, the latency to the peak in fractional dose/h (T_{max}) was 65 ± 8 min in controls, and it was shortened in the bethanechol-treated group and prolonged after atropine treatment (bethanechol: 39 ± 6 min; atropine: 83 ± 5 min, $p < 0.05$). The cumulative percent recovery of the administered [^{13}C]-octanoic acid dose was not different between groups 6 hrs after the beginning of the test (saline $90 \pm 10\%$; bethanechol $87 \pm 13\%$; atropine $83 \pm 10\%$ recovery; $p > 0.05$). These data indicate that the [^{13}C]-octanoic acid breath test was sensitive to experimental alterations in the rate of gastric emptying.

Histological and behavioral assessment of SCI

The extent of the intact total tissue area after SCI lesion was verified prior to further data analysis in the study as described previously 16. In control animals the total tissue area was $5.2 \pm 0.4 \text{ mm}^2$, in T3-CI the total tissue area was $1.2 \pm 0.3 \text{ mm}^2$ (i.e. 23% of control) while no intact tissue was left in the T3-TX rats. Therefore, all contusion injured and transected animals were included in data analysis.

Three days after the surgery, locomotion scores were 21 ± 0 for control animals, 1.6 ± 0.4 for T3-CI and 0.2 ± 0.2 for T3-TX rats ($p < 0.05$). At 6 wk post-injury, T3-CI rats showed greater hind-limb recovery of function compared to T3-TX rats (10.1 ± 0.7 and 4.0 ± 0.5 , respectively; $p < 0.05$).

These data are comparable to the chronic deficits reported previously 12, 34 and indicate the effectiveness of our surgical procedures.

Effect of spinal cord contusion and transection on voluntary feeding

The mean energy intake (MEI) in the first week after surgery for control animals was $35.3 \pm 0.9 \text{ kcal} \cdot \text{day}^{-1} \cdot 100 \text{ g body weight}^{-1}$, while the MEI of T3-CI and T3-TX animals was lower than controls (T3-CI: 22.4 ± 0.7 ; T3-TX: 23.2 ± 1.2 ; $p < 0.05$). By the second week after injury, the MEI was similar across groups. Beginning at 5 wk after injury, T3-TX animals began to display an elevation in MEI over that of T3-CI animals (Control: 29.3 ± 0.5 ; T3-CI: 30.5 ± 0.7 ; T3-TX: 32.5 ± 0.8 , $p < 0.05$). The elevation in MEI continued throughout the remainder of the experiment at which time T3-TX rats had greater energy intake than control and T3-CI rats (Control: 27.5 ± 0.5 ; T3-CI: 28.1 ± 0.8 ; T3-TX: 31.5 ± 1.0 ; $p < 0.05$).

These data indicate that SCI groups were ingesting lesser quantities of food in the first week though they were not significantly different from each other with regard to overall energy intake. While both SCI groups quickly regained normal energy intake by 2 wk post-injury,

animals with the most severe SCI (T3-TX) developed a consistent hyperphagia 4 wk after injury.

Six weeks after spinal cord injury there is a partial recovery of the delayed gastric emptying in T3-CI rats

Post-mortem recovery of the gastric contents of overnight fasted rats 1 h after consuming the identical test meal used in the [^{13}C]-octanoic acid breath test revealed that T3-TX rats emptied a lower percentage of test meal (Control: $90\pm 3\%$, $N=5$; T3-TX: $74\pm 5\%$, $N=5$; $p<0.05$). These data indicate that a greater portion of the solid test meal is retained in the stomach of animals with SCI and provides a basis for interpreting our [^{13}C]-octanoic acid breath test data.

Three days after surgery, the cumulative percent recovery of $^{13}\text{CO}_2$ in control rats was $70\pm 5\%$ ($N=7$). In T3-CI and T3-TX animals, there was reduction in the cumulative percent recovery of the [^{13}C]-octanoic acid, i.e. $51\pm 4\%$ and $30\pm 3\%$, respectively; $n=6$ for both groups; $p<0.05$ vs control, $p>0.05$ vs T3-TX; Fig. 2). The $^{13}\text{CO}_2$ cumulative percent recovery remained significantly lower in both SCI groups throughout the experiment. In fact, six weeks after the surgery control rats had a $65\pm 5\%$ $^{13}\text{CO}_2$ cumulative recovery, while T3-CI recovered $47\pm 8\%$ and T3-TX rats recovered $49\pm 2\%$ ($p<0.05$ vs control; $p>0.05$ vs T3-TX; Fig. 2).

Three days after injury, the maximum concentration of $^{13}\text{CO}_2$ during the breath test (peak fractional dose/h) was the same across groups (Control: 31 ± 4 ; T3-CI: 27 ± 2 ; T3-TX: 26 ± 3 min; $p>0.05$), suggesting similar absorption and respiratory excretion of [^{13}C]-labeled substrate after SCI. The latency to the peak in fractional dose/h (T_{max}), however, was shortened in the T3-TX group (Control: 89 ± 5 ; T3-CI: 85 ± 5 ; T3-TX: 53 ± 4 min; $p<0.05$, Fig. 2). At 1 week and continuing to three weeks after SCI, T_{max} was shorter than control in the T3-CI ($p<0.05$ vs control) and the T3-TX group (3 wk, Control: 101 ± 11 ; T3-CI: 94 ± 4 ; T3-TX: 82 ± 7 min; $p<0.05$ vs control, $p<0.05$ vs T3-CI; Fig. 2). Differences in T_{max} were not significant at six weeks.

The gastric half emptying time ($T_{1/2}$) at 3d was delayed in both groups of SCI rats. (Control: 150 ± 16 min; T3-CI: 277 ± 35 ; T3-TX: 360 ± 0 min; $p<0.05$; Fig. 3). The delay in $T_{1/2}$ continued for both SCI groups through 3 wk (Control: 201 ± 18 min; T3-CI: 327 ± 28 ; T3-TX: 325 ± 25 min; $p<0.05$ vs control, Fig. 3). At 6 wk after injury, the $T_{1/2}$ remained significantly delayed only in the T3-TX group (Control: 226 ± 33 ; T3-CI: 286 ± 31 ; T3-TX: 348 ± 11 min, $p<0.05$, Fig. 3).

These data indicate that SCI at the T3 level of the spinal cord produces a significant reduction in gastric emptying of a solid meal independent of injury severity (T3-CI vs. T3-TX). The reduction in gastric emptying continued in animals with complete SCI (T3-TX) through at least 6 wk, while animals with contusion injury appear to begin to display some degree of functional recovery of gastric emptying after 3 wk recovery.

Chronic spinal cord injury reduces spontaneous gastric contractions

Using a separate group of control and SCI animals to measure spontaneous gastric contractions, we observed no significant difference in the motility index of control animals at 3 d, 3 wk or 6 wk after surgery ($n=5$ /timepoint) and these data were collapsed across groups.

At 3 d after surgery, spontaneous gastric contractions were significantly lower in both groups of SCI rats compared to controls as well as between contusion and transection SCI (Control: 67.3 ± 8.9 ; T3-CI: 27.8 ± 3.9 ; T3-TX: 8.0 ± 1.6 ; $n=15, 5, 5$ respectively, motility

index, $p < 0.05$; Fig. 4A&B). Gastric contractions of animals tested 3 wk after injury, remained significantly lower in both SCI groups (Control: 67.3 ± 8.9 ; T3-CI: 31.8 ± 10.6 ; T3-TX: 28.4 ± 9.9 ; $p < 0.05$; Fig. 4A&B) and there was no longer a difference between injury groups ($p > 0.05$). Gastric contractions remained significantly lower in animals tested 6 wk after surgery (Control: 67.3 ± 8.9 ; T3-CI: 30.4 ± 6.0 ; T3-TX: 14.8 ± 3.5 ; $n = 15, 5, 5$, motility index, $p < 0.05$; Fig. 4A&B). These data indicate that T3-TX produces a significant reduction in spontaneous gastric contractions that persists for a greater period of time than what is observed for gastric emptying after the [^{13}C]-breath test.

Discussion

In the present study we 1) pharmacologically demonstrated the sensitivity of the [^{13}C]-breath test for our measurement of gastric emptying; 2) demonstrate that high thoracic (T3) SCI impairs gastric emptying beginning 3 d after injury, regardless of injury model (transection vs. contusion); 3) demonstrated that complete SCI (spinal transection) impairs gastric emptying as long as 6 wk after injury while spinal contusion injury appears to demonstrate recovery of function; and 4) demonstrate prolonged gastric dysmotility in animals with transection and contusion SCI. Our data suggest that SCI leads to long-lasting derangements in gastric motility and emptying with only partial recovery of function 6 wk after injury.

Our conclusions are based upon the following lines of evidence. Our pharmacological data validates the sensitivity of our ability to use the [^{13}C]-breath test to detect changes in gastric emptying within the same rat as evidenced by bethanechol or atropine challenge. The [^{13}C]-breath test is well established in animal and human models 24, 26–28, 35, 36. Repeated measurements of gastric emptying within the same SCI animal underscores the utility of the [^{13}C]-breath test over post-mortem recovery assays. Furthermore, the [^{13}C]-breath test is equally suited for the analysis of gastric emptying of solids or liquids in our model of SCI, a shortcoming inherent to the phenol-red technique.

Selective alterations in small bowel transit, ileal fluid and electrolyte transport have been reported after SCI 37, 38. Enhanced intestinointestinal inhibitory reflexes have been proposed as one factor leading to reduced gastric emptying 39. The mechanisms responsible for reduced small bowel transit have not been completely identified and altered enteric reflexes, vago-vagal preponderance, and GI peptide release may all contribute to reduce gastric emptying. Our studies would not discern reduced small bowel transit, since absorption of [^{13}C]-octanoate occurs upon entry into the duodenum 24. Conversely, altered absorption of more complex molecules may also occur after SCI and limit the utility of the [^{13}C]-breath test. However, the maximum concentration of $^{13}\text{CO}_2$ was similar across groups over the course of our tests. This suggests that SCI did not substantially alter duodenal absorption nor hepatic oxidation of [^{13}C]-octanoate, which is a short-chain fatty acid. We propose that the acceleration of T_{max} may reflect the rapid duodenal entry of a liquid fraction containing [^{13}C]-octanoic acid from the test meal. Indeed, gastric recovery of a liquid test meal after SCI was increased while duodenal recovery of dye was either the same as control 17 or increased 20. In both studies, recovery of dye was reduced in the jejunal and ileal segments of the small intestine which suggests an early entry of liquid content into the duodenum that occurred in conjunction with diminished intestinal transit.

The diaphragm, intercostal and abdominal muscles all contribute to respiration. Therefore, some degree of respiratory compromise follows any level of SCI 40. In the rat, intercostal motoneurons originate in the cervical and thoracic cord (C6-T13) 41. Therefore, special attention must be paid to pulmonary sufficiency when utilizing the [^{13}C]-breath test. Respiratory compromise, such as diminished end tidal volume, after SCI would predict a

reduction in $^{13}\text{CO}_2$ expression throughout the breath test. However, our SCI animals have CO_2 levels ($\text{mmol/h}\cdot\text{cm}^2$) virtually identical to controls when at rest (data not shown) in addition to the similar peak levels of $^{13}\text{CO}_2$ during the test. We propose that cervical and thoracic intercostal motoneurons above the lesion center, and which are active during steady-state respiration⁴¹, combined with respiratory patterns mediated through pulmonary vagal afferents⁴² may adequately compensate for any respiratory deficit that would limit the utility of the [^{13}C]-breath test after SCI.

Inhibitory duodenal feedback mechanisms to the proximal stomach may limit further gastric emptying (reviewed in 43, 44) after SCI, leading to the rapid attenuation of exhaled $^{13}\text{CO}_2$ that we observed. For example, release of cholecystokinin (CCK) from small intestine endocrine cells activates inhibitory gastric feedback circuits through peripheral endings of vagal afferents (45–47) and neurons within the brainstem (30, 48–50). We did not monitor the post-prandial activation of gastric inhibitory mechanisms in our SCI rats, thus, the mechanism for the observed decline in $^{13}\text{CO}_2$ levels requires further experimentation.

We have previously observed the gastric retention of chow following an overnight fast 3 d after SCI but not control surgery 16. Our present study expands upon these findings since a significantly greater amount of the test meal remained at 60 min in T3-TX than control rats. These observations support our proposal that the profound reduction in cumulative [^{13}C] recovery in SCI rats reflects retention of the solid meal within the 6 h sampling period. These data, and the reduced gastric emptying of awake rats reported in other studies 17, 20, indicates that gastric emptying is impaired in SCI animals up to 3 wk after contusion injury and beyond 6 wk after more profound transection SCI.

The entirety of the chronic deficits after SCI demonstrates varying degrees of functional recovery that are proportional to injury severity. Our data show that gastric contractions were significantly diminished 3- to 6-wk following SCI in a manner that is consistent with our previous findings 16. We propose that the profoundly reduced gastric contractions observed in the anesthetized SCI rats reflects one component ultimately leading to the functional gastrointestinal deficits observed in the [^{13}C]-breath test. However, we interpret the reductions in gastric contractions and emptying with caution since our strain gauge measurements do not provide insight to the functional changes in gastric tone, gastric contractile patterns, duodenal feedback, pyloric tone and antropyloric coordination following SCI. Unlike the dominant vagal control of the stomach, pyloric function involves the integrated actions of vagal, splanchnic intra- and extramural mechanisms 43. The recovery of gastric emptying after contusion SCI remains to be elucidated but may likely involve plasticity of vagal and enteric neurocircuitry.

Based upon anatomical evidence of spinosolitary neurocircuitry 51–53, we have proposed that high thoracic SCI interrupts ascending spinosolitary input to brainstem vagal nuclei thereby altering the sensitivity of vagal reflex function 16. However, delayed gastric emptying after SCI may also involve intrinsic changes within the gastrointestinal tract. The gastrointestinal expression of neuronal nitric oxide synthase (nNOS) is down-regulated post-SCI 20. Inhibitory neurotransmission by nitric oxide (NO, which is produced by nNOS) mediates gastrointestinal relaxation 54, 55, and the reduction of endogenous NO may, in turn, lead to reduced motility rates.

In conclusion, our use of the [^{13}C]-octanoic acid breath test has facilitated the long term observation of gastrointestinal competence in the SCI rat. The [^{13}C]-breath test permitted us to gain temporal insights to the gastric emptying rates of SCI rats that would not be possible with traditional dye, or gastric content, recovery techniques that collect a single time point per animal. With the [^{13}C]-octanoic acid breath test technique, we identified a rapid

reduction in fractional [^{13}C] recovery that we infer represents the diminished emptying of the solid test meal. Understanding the mechanisms responsible for delayed gastric emptying may serve to explain the long term alterations in nutritional state observed in previous studies 12, as will identifying the consequences of altered fluid and electrolyte transport 37, impaired small bowel transit³⁸ and absorptive capacity of the GI tract. Such insights will be essential for the maintenance of proper energy balance appropriate to the neurotrauma patient. Considering the recent evidence that energy balance impacts functional recovery following partial SCI 56, it becomes clear that the nutritional, and gastrointestinal, status of the SCI patient is of therapeutic relevance.

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Figure 1.

Summary of $[^{13}\text{C}]$ -breath test data in animals administered saline, bethanechol and atropine. Animals received each drug in randomized order. **A.** Graphic representation of the cumulative percent recovery of $[^{13}\text{C}]$ for animals pretreated with either saline, the pro-motility muscarinic cholinergic agonist bethanechol (5mg kg^{-1} , ip) or the motility-reducing muscarinic cholinergic antagonist atropine (7.5mg kg^{-1} , ip). The final percent cumulative recovery of $[^{13}\text{C}]$ at the termination of the 6 h test was unaffected. **B.** Graphic representation of the gastric half emptying time ($T_{1/2}$). Compared to the $T_{1/2}$ of saline injected animals, bethanechol induced a significant acceleration of $T_{1/2}$ while in the same cohort of animals, atropine significantly delayed $T_{1/2}$. Data expressed as mean $\pm$ SEM, * = $p < 0.05$.

Figure 2.

Summary of [^{13}C]-breath test data in control and SCI animals using the 3 d post-surgery (3 DPO) test as a representative example. **A.** T3 SCI leads to a significant reduction in cumulative percent recovery of [^{13}C]. **B.** Graphic summary of the cumulative percent recovery of [^{13}C] for all test days through 6 wk post-surgery (6 WPO). Cumulative recovery of [^{13}C] was significantly reduced in both contusion and transection SCI groups (* = $p < 0.05$ vs. control, # = $p < 0.05$ vs. T3-CI). **C.** At 3 d post-surgery, the group averages of the fractional dose demonstrate an initial acceleration in the early phase of gastric emptying in animals with complete SCI (T3-TX). This early peak was followed by a rapid reduction in [^{13}C] values, suggesting retention of the test meal that led to the reduced cumulative recovery of [^{13}C]-substrate. **D.** Graphic summary of the peak fractional dose of [^{13}C] (T_{max}) for all test days. The early phase of gastric emptying was significantly accelerated by SCI through the first 3 wk after surgery (3 WPO; * = $p < 0.05$ vs. control, # = $p < 0.05$ vs. T3-CI).

Figure 3.

Summary of the gastric half emptying time reveals a significant reduction of gastric emptying after SCI in both groups of animals through the first 3 wk after surgery (3 WPO). Animals with the more severe complete spinal cord transection continued to display reduced gastric emptying throughout the entire 6 wk post-injury time period (6 WPO; * = $p < 0.05$ vs. control).

Figure 4.

A. Original polygraph records of gastric motility in a separate cohort of fasted experimental animals. Animals with both contusion and transection SCI (T3-CI and T3-TX, respectively) display a marked reduction in both the amplitude and periodicity of gastric motility at all three time points that coincide with reduced gastric emptying of a solid test meal, as measured by [^{13}C]-octanoic breath test. **B.** Graphic summary of the calculated motility index of animals tested at 3 d, 3 wk and 6 wk after surgery (3 DPO, 3 WPO * 6 WPO, respectively). Gastric motility was significantly reduced in all SCI groups compared to respective controls. Furthermore, the gastric motility of T3-TX animals was significantly lower than all other groups in the cohort of animals tested at 3 d after surgery (3 DPO; * = $p < 0.05$ vs. control, # = $p < 0.05$ vs. T3-CI).