Gastrointestinal Motility: Kinematic Properties of Segmentation Contraction

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ABSTRACT

The kinematic processes of the small intestine play integral roles in overseeing the digestion and transportation of food throughout the gastrointestinal tract. Specifically responsible for governing the flow and digestion of chyme along the gastrointestinal tract are the two fundamental patterns of motility, propulsion and mixing [1]. While peristalsis is the principle muscle contraction for propulsive motility, segmentation contractions are responsible for mixing and chopping the chyme [2]. Previous studies on the contraction kinematics of the small intestine have provided evidence that contraction frequencies alter in response to different types of food. We propose that the pattern of segmental contraction varies in response to the different compositions of intraluminal contents as well. As the composition of chyme alters and it becomes less dense, segmental contraction frequency increases as a response. Our *in vivo* observational technique conducted on the small intestine of male Sprague-Dawley rats is much less invasive than previous studies, thus allowing us to better quantify the kinematic properties of the small intestine, such as frequency and amplitude of contraction. Understanding and comparing essential patterns of motility across patients can improve medical diagnostics as well as the manufacturing of food supplements and pharmaceutical medications.

INTRODUCTION

The small intestine is comprised of stringently organized patterns of motility that allow the organ to govern the transportation and absorption of nutrients, water, supplements, and pharmaceutical medications [3]. Made up of an autonomous intrinsic nerve complex, the small intestine has the capability to regulate its motility patterns in response to mechanical and chemical stimuli from cells within the gut wall [1]. Every year, billions of dollars are spent on the diagnoses and treatment of gastrointestinal infections, malnutrition, and a wide variety of other health issues; yet, despite the integral role motility patterns play in digestive function, our knowledge on the subject remains fairly fragmented and incomplete [4]. A better understanding of the underlying biomechanical mechanisms in the small intestine should provide the foundation necessary for the development of drastically improved models of gastrointestinal motility. Not only will these models immensely aid in the correct assessment and diagnosis of gastrointestinal abnormalities, but they will also serve as a guide for postoperative management and treatment of patients. The pharmaceutical industry, for instance, has begun to invest heavily in researching the physical processes of digestion in order to develop more efficient pharmaceutical drugs treatments. Furthermore, a greater comprehension of the physiology of gastrointestinal motility may aid food technologists in the engineering of highly nutritious foods, fortified with essential vitamins, that could counteract the world problem of malnutrition, a condition which claims the lives of an estimated 3.1 million children each year [5].

Present in the small intestine are many factors that drive the flow and digestion of chyme, a mixture of partially digested food and gastric juices that empties into the small intestine from the stomach [1]. The fundamental patterns of motility have historically been divided up into two main categories: propulsion and mixing. As the gastrointestinal tract does not have a central pumping organ, it relies greatly on the contraction of smooth muscles to propel chyme through the bowl and mix in digestive enzymes to aid with absorption and digestion. The principle propulsive motility, peristalsis, involves propagating rings of longitudinal muscles contractions that push the chyme distally through the small intestine [4]. Segmentation contractions, on the other hand, serve as the

predominate form of mixing motility in the small intestine. Unlike peristalsis, segmentation consists of rings of circular muscle contractions that can push the chyme in both the proximal and distal directions. Not only does this slow the propulsion of chyme through the intestine, but this also allows for greater mixing of chyme with digestive enzymes and increased contact of chyme with the nutrient-absorbing epithelial cells of the lumen [2].

Previous studies suggest that the frequency of peristalsis contractions decrease distally along the small intestine. The peristalsis propulsion rate in the proximal small intestine is theorized to be faster than that in the distal small intestine because it allows for greater amounts of stomach contents to enter the small intestine by quickly spreading the chyme over a large area. Slowing of peristalsis in the distal portion of the small intestine therefore would allow for more absorption to occur. [6]. The exact behavior of the contractile nature of segmentation, however, has not been as thoroughly investigated. Based on the frequency gradient of peristalsis, one would think that segmentation contraction frequency would be highest in the distal portion of the small intestine so as to maximize absorption of the slower moving chyme. Walter Alvarez was the first person to characterize the rhythmic contractions of segmentation and measure its frequency gradients in isolated pieces of small intestine. Alvarez's studies indicate that segmentation contractions instead seems to follow the same decreasing frequency gradient as seen in peristalsis [7]. Due to the high degree of invasiveness associated with Alvarez's studies, however, the validity of the results is highly questionable because it is not likely to be very representative of natural segmentation motility in an *in vivo* animal model. A less invasive study is necessary for the proper characterization of segmentation contraction in the small intestine.

Additionally, further work is needed regarding the effect of the chyme density on gastrointestinal motility. It is possible that the walls of the small intestine may react to the density and perhaps even the chemical and nutritional composition of the chyme. This has been supported by several studies, which show how the contraction frequencies alter in response to different types of food. If the gut truly had the ability to respond to sensory information by controlling both the spatial and temporal patterns of muscle contraction, a network of intrinsic control mechanisms in the wall of the small intestine would be required [3]. Jackie Wood's studies on the electrical activity of myenteric neurons in the 1970s backs up the existence of such a network, which he terms the "gut brain" [1]. Wood claims that the gut wall's complex decision-making ability is independent of the central nervous system. As the chyme moves down the small intestine, it is further mixed with digestive enzymes that break it up and alter its composition. As the composition of chyme decreases in density as it moves through the small intestine, segmental contraction frequency may increase as a response.

In this study, we elucidate the *in vivo* kinematics of small intestinal segmentative and the contribution of chyme density on segmentation in rats. Our innovative *in vivo* observational technique is drastically less invasive than previous studies, and thereby better reflects gastrointestinal motility in the natural state of rats. We intend to quantify the density gradients throughout the small intestine, and compare that to the observed frequency of segmental contractions. Additionally, we will point out how the characteristics of segmental contraction, such as frequency and amplitude, vary in response to different motor requirements and different wall properties of the three small intestine sections – duodenum, jejunum, and ileum. Contrary to current published theories, we propose that the segmentation contraction frequency increases distally along the small intestine due to the lower density and slower propulsion of the chyme in the distal portion.

MATERIALS AND METHODS

Animal Preparation

Three male Sprague-Dawley rats weighing 250—350 g were used for these experiments. The rats were housed in an environmentally controlled, American Association for Accreditation of Laboratory Animal Care-approved reversed housing cycle vivarium. Each animal was not fasted before the experiments.

Surgery

Each rat was anesthetized with an intramuscular injection of fentanyl-droperidol (0.3 mL/kg) and diazepam (2.5 mg/kg). Supplemental doses of the anesthetic were given as needed. An abdominal midline incision was made to gain access to the gastrointestinal tract. A loop of the small intestine, ranging from 4—6 cm in length, was exteriorized through the incision and gently positioned and pinned in place onto a Petri dish coated in a dried Polydimethylsiloxane (PDMS) solution. The PDMS is a silicon-based organic polymer that was created using a 10:1 mixture of Sylgard 184 silicone elastomer base and Sylgard 184 curing agent, respectively. The exteriorized tissue was perfused with an albumin physiological salt solution (in mM: 145.00 NaCl, 4.7 KCl, 2.0 $CaCl_2$, 1.17 $MgSo_4$, 1.2 NaH_2PO_4, 5.0 dextrose, 2.0 sodium pyruvate, 0.02 EDTA, 3.0 MOPS, and 10 g/L bovine serum albumin). The solution was prewarmed to the rat's physiological body temperature of 37˚C, and the pH adjusted to the rat's physiological pH of 7.4. The temperature of the exteriorized tissue and the animal's core were maintained at 37˚C by placing a hot plate under the Petri dish.

Video recording

All of the experiments were digitized with the high speed video camera (Sony HDR-XR200). The Petri dish was placed under a light table to maximize contrast in the video footage. Starting at the proximal end of the small intestine, video was taken of each loop every 4—6 cm for rat 1 and rat 2. Additionally, video was taken of each loop in the reverse direction, starting at the distal ends of the small intestines of rat 2 and rat 3. Data were recorded in 30—60 s intervals, an interval long enough to acquire between 4—8 contractile cycles. For each rat, we recorded 6—17 successful sequences. After the completion of the experiment, the rats were euthanized with pentobarnital (120 mg/kg of body weight IP).

Video Analysis

After imaging the recording the specimen, the video sequences were analyzed through the program software, Tracker. The segmental contraction was tracked manually by marking a location on the wall of the small intestine and recording its coordinates over a 30—60 s interval. Each tracked point was determined by locating a location where segmental contraction was clearly observed. Data from each video analysis was further analyzed to determine the average amplitudes and frequencies of segmental contractions in each segment. The calculated contraction frequencies in all of the segments were plotted against the distance from the pylorus, the gastroduodenal junction where the stomach ends and the duodenum begins. The same was done with the calculated contraction amplitudes. The length of the small intestine was normalized for each rat by dividing each segment length by the total length of rat's small intestine.

Density measurement

To determine the density gradient of the small intestine, a reversed cycle rat was first euthanized in a carbon dioxide chamber. Once deceased, an abdominal incision was made and both the gastroduodenal junction and the ileocecal valve were tied up with suture thread to prevent gas from leaking out. The gastrointestinal tract was then removed and sections of the small intestine were tied off with suture thread every 5 - 7 cm. The entire gastrointestinal tract was then warmed to the rat's physiological core temperature by placing it in a beaker of 0.09% saline, maintained at 37˚C by an underlying hot place. A small rectangular container approximately was filled half way with 37˚C 0.09% saline, and then tarred on a scale. Each segment was then submerged into the saline of the small beaker and the weight was recorded. The saline was replaced after every two segments to maintain the temperature and to keep the saline fresh. Once all of the segments were submerged in the saline, the small intestine was cut into its specified segments. Each segment was then weighed on a scale. To determine the density of the saline at 37˚C, 0.05 mL of the warmed saline was weighed and the weight was then divided by the volume. The density of each small intestine segment was calculated using the following equation.

$$\rho_{segment} = \frac{m_{segment} * \rho_{saline}}{\Delta m}$$

Where ρ_{saline} is the calculated density of the saline, $m_{segment}$ is the weighed mass of each segment, and Δm is the change in mass of the saline after each segment of small intestine was submerged.

Elastic Modulus literature values

To determine the elastic modulus values of the three segments in the small intestine, the relation between circumferential stress and strain was analyzed in literature. The slope between each point in the circumferential stress-strain curve was determined and averaged for each segment of the small intestine. Each calculated average slope represents the elastic modulus (stress/strain) for each segment.

Thickness of the wall

The thickness gradient of the small intestinal wall was determined by first euthanizing a fasted rat in a carbon dioxide chamber. An abdominal incision was then made, and the gastrointestinal tract, from the stomach to the cecum, was exteriorized from the body. Starting at the proximal end of the small intestine near the pylorus, 10 cm segments were measured out, marked with a surgical pen, and then cut out into individual segments. A 5 mL syringe was used to flush each segment was flushed with a 0.09% saline solution. Once rinsed out, one end of each segment was tied off with suture thread. Using a new 5 mL syringe, the open end of each segment was then filled with microfil and then tied off with the suture thread. Once filled with microfil, the segments were scanned in a CT machine. Using the images provided by the CT, the program software, Tracker, was utilized to measure the intestinal wall thickness in each segment. The wall thickness measurements were then plotted against the normalized distance from pylorus.

FIGURES

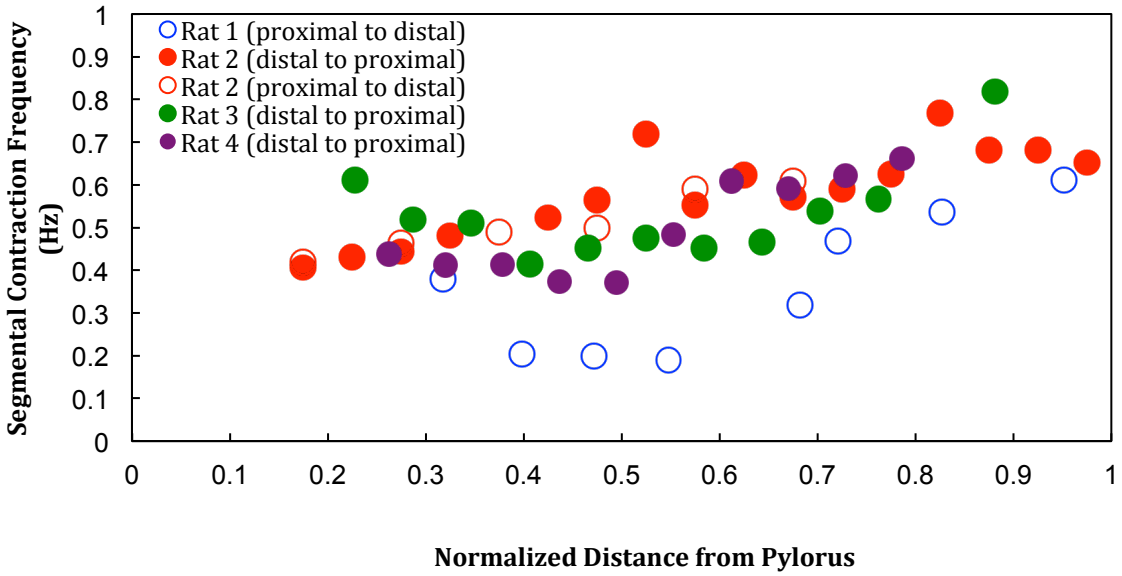

Figure 1. Segmental contraction frequency gradients in rat small intestine. Body weight of rats 1, 2, and 3 were 350 g, 310 g, and 320 g, respectively.

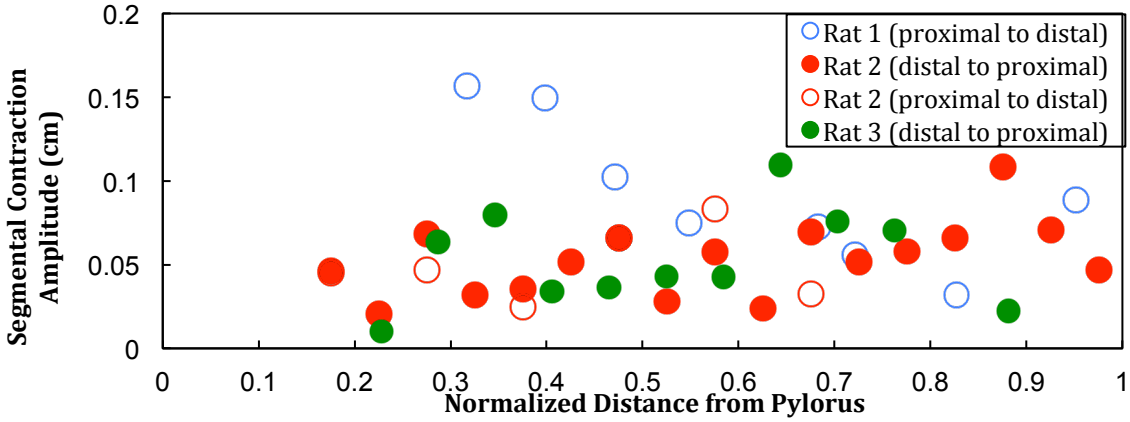

Figure 2. Segmental contraction amplitude gradients in rat small intestine. Body weight of rats 1, 2, and 3 were 350 g, 310 g, and 320 g, respectively.

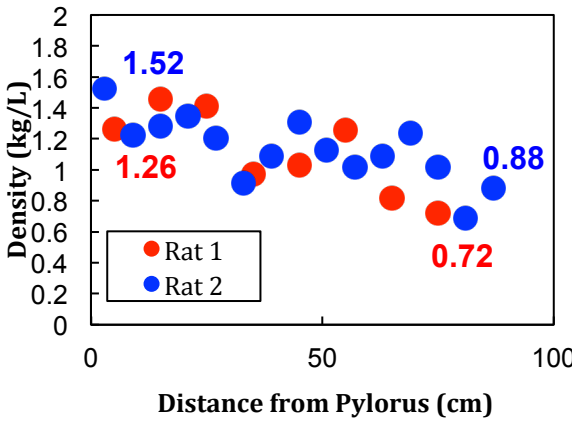

Figure 3. Density gradients in rat small intestine. Density was determined at 37˚C.

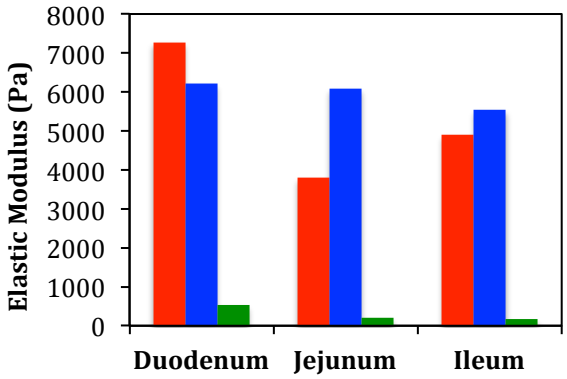

Figure 4. The circumferential wall stress-strain distributions from the ileum, jejunum, and duodenum. (Red values)[8], (Blue Values)[9], (Green values)[10]

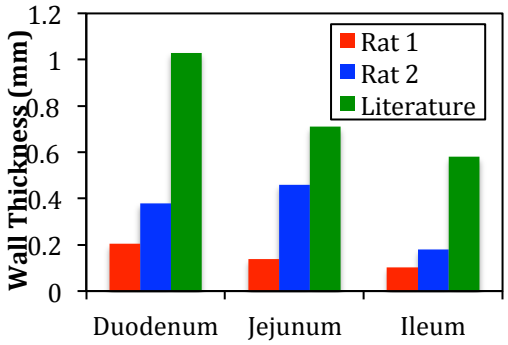

Figure 5. Average wall thickness (mm) of the three small intestinal segments in rat 1 (432 g), rat 2 (340 g), and from literature [10].

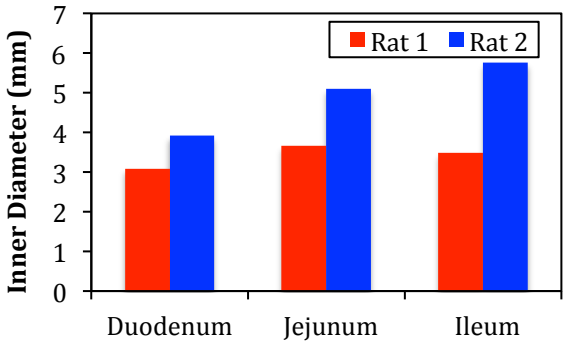

Figure 6. Average inner diameter (mm) of the three small intestinal segments in rat 1 (432 g), and rat 2 (340 g)

Table 1. Summarized calculations from figures 1-6.

Unit	Symbol	Linear Fit Line	Correlation (R^2)	Min Point	Max Point
Frequency (Hz)	*f*	y = 0.306x + 0.3457	0.25762	0.189	0.819
Amplitude (cm)	A	y = 0.0145x + 0.052	0.01065	0.010	0.157
Density (kg/L)	ρ	y = -0.5764x + 1.4132	0.55779	0.688	1.524
Elastic Modulus (Pa)	E	y = -556.74x + 4992.2	0.63584	3363.489	4673.107
Wall Thickness (mm)	δ	y = -0.125x + 0.6706	0.98804	0.288	0.538
Inner Diameter (mm)	δ	y = 0.5507x + 3.0587	0.89452	3.500	4.602

RESULTS

Upon analyzing the data in figure 1, we found that the contraction frequency tended to increase distally in the small intestines of all three rats. The contraction frequencies for rats 1 and 2 fall between a range of ~0.4—0.8 Hz. Rat 1 notably has a slightly lower contraction frequencies range of ~0.2—0.6 Hz. Though there is a general increasing trend, the contraction frequency in rats 1 and 3 appear to drop in contraction frequency in the proximal small intestine sections by ~0.2 Hz, and then steadily increase ~0.4 Hz down the rest of the small intestine. Rat 3 does not show this initial decrease in contraction frequency, but rather shows a more linear increase along the entirety of the small intestine (Fig. 1). Additionally, analysis of the contraction frequency data collected from rat 2 showed that the slope of the data from the videos taken in the direction of the distal small intestine to the proximal small intestine, m = 0.0029, was relatively similar to the slope of the data from the videos taken in the direction of the proximal small intestine to the distal small intestine, m = 0.0032 (Fig. 1).

Contraction amplitude data in rat 1 showed a fairly steady decrease; however, contraction amplitude data in rats 2 and 3 were very scattered and showed no statistical trend. In addition, the amplitudes in rats 2 and 3 were notably lower than the amplitudes found in rat 1 (Fig. 2).

Both trials in figure 3 show that density decreases distally in the small intestine at the physiological body temperature of the rat (~37˚C).

The cross-sectional areas (CSA) for the jejunum were higher than those in the duodenum and smaller than those in the ileum ($P < 0.001$) (Fig. 4) [10].

Figure 4 illustrates the circumferential wall stress-strain distributions in the duodenum, jejunum, and ileum. The duodenum was significantly the stiffest segment, followed by the jejunum. While the jejunum and ileum have similar elastic moduli, the jejunum appears to be slightly stiffer [8-10]. The elastic modulus values for appear drastically smaller in the green values due to the use of a different strain constant known as the circumferential Green's strain (dimensionless) [10].

The wall thickness data show that wall thickness generally decreases distally in the small intestine (Fig. 5). For rat 1, the duodenum, jejunum, and ileum had a wall thickness of 0.204 mm, 0.139 mm, and 0.102 mm, respectively. For rat 2, the duodenum, jejunum, and ileum had a wall thickness of 0.379 mm, 0.460 mm, and 0.180 mm, respectively. For the literature values, the duodenum, jejunum, and ileum had a wall thickness of 1.03 mm, 0.71 mm, and 0.58 mm, respectively [10].

The inner diameter data indicated that the inner diameter slightly increases distally in the small intestine (Fig. 6). For rat 1, the duodenum, jejunum, and ileum had an inner diameter of 3.09 mm, 3.66 mm, and 3.478 mm, respectively. For rat 2, the duodenum, jejunum, and ileum had an inner diameter of 3.91 mm, 5.10 mm, and 5.76 mm, respectively.

DISCUSSION

The general increasing trend in the contraction frequency of all three rats is important in that it shows a repeatable pattern in several individuals (Fig. 1). The variations in frequency ranges may be due to the differing times of each rat's last meal or failure to maintain a consistent temperature of the APSS across all three procedures. The variation could of course be based on the individual characteristics of each rat. Further trials would need to be conducted to determine if this variation is abnormal or expected. Furthermore, to determine if time was a factor that affected the observed contraction frequency trend, data was collected starting at the distal end of the small intestine of rat 2 and then was compared to the data that was collected starting at the proximal end of the small intestine of rat 2. The similar slopes suggest that time does not significantly affect the trends (Fig. 1).

It is important to note that we attempted to obtain frequency data from rat 3 by filming from the proximal to distal segments of the small intestine, however, there was very little to no segmentation observed. Instead, the small intestine as a whole seemed to shake. Data was not included in figure 1 as it was not segmentation contractions. We attribute this behavior to how long the rat was under anesthesia for. By this point, the rat was in surgery for over two hours. In addition, small tears in the blood vessels caused from manipulating the segments in the first set of data might also have caused the irregular shakes in the intestines. We will try to minimize surgery time to avoid this outcome in the future.

Though analysis of the contraction amplitudes in rat 1 showed that amplitude decreases distally, the amplitude data collected from rats 2 and 3 proved inconclusive. The small intestines of rats 2 and 3 showed notably less movement than the small intestine of rat 1. It is my suspicion that the timing of the rats' last meal is what causes this apparent variation in overall motion. Rat 1, for instance, may have eaten more recently prior to the procedure than rats 2 and 3 had. This possibility can be further

supported by the observations that rats 2 and 3 had less ingesta in their small intestines than rat 1 had (Fig. 2).

I can reasonably conclude that the density of the chyme in small intestine appears to decrease distally (Fig. 3). It has been observed that the chyme in the proximal and distal portions of the small intestine differ greatly in composition, with the later being much more gaseous. Knowing that the main function of segmentation is to mix digestive enzymes in with the chyme, the proximal portions of the small intestine would contain less digested and denser chyme than the distal portions because the digestive enzymes have not yet been thoroughly mixed in with the chyme. Having been in the small intestine for a longer duration, the chyme in the distal portion is much more digested and has a larger fraction of fermentative gases. This would explain the decrease in density along the small intestine.

The passive elastic properties of the small intestinal wall are very important for both the function of the small intestine and our understanding of small intestinal motility. Knowing that the major tensile stress during distension of the small intestine is in the circumferential direction, we compiled literature data to determine the passive elastic behavior in the circumferential direction in the duodenum, jejunum, and ileum in response to luminal pressure loadings. Variations in the biomechanical parameters were found among the three segments of the small intestine. Literature values indicate differences in the luminal CSA, elastic properties, and wall thickness between the duodenum, jejunum, and ileum

All of the segments showed stress-strain distributions that were exponential by nature (Fig. 4) [10]. Figure 4 indicates that the duodenum is the stiffest of all three segments, and that the stiffness decreases distally. Additionally, it was found that the wall thickness in the small intestine decreased in the distal direction [9]. The stiffness and wall thickness appear to be directly correlated. The differences in stiffness and wall thickness may be associated with the specialized functions of the proximal and distal segments. For instance, perhaps the duodenum has thicker and stiffer walls because it has a large influence on gastric emptying, whereas the distal ileum acts as a larger space to hold slower moving chyme [11, 12]. Though the density of the intestinal contents may partly explain why the transit time is slower distally, the elastic properties of each segment in the small intestine may also contribute to the differences in intestinal flow patterns: the chyme is propelled faster in the proximal section where the wall stiffness and wall thickness are high, while the more flexible walls of the ileum bulge, leading to pooling and decreased flow of chyme. A faster transit time in the proximal end of the small intestine would allow for faster gastric emptying, while a slower transit time in the distal end of the small intestine would aid in digestion and absorption of nutrients and water. On the other hand, the differences in the stiffness and wall thickness may be associated with the density of the intestinal contents. Because the intestinal contents are most dense at the proximal duodenum, perhaps thicker, stiffer walls are necessary to produce contractions that have the power to adequately propel and mix the intestinal contents.

I propose that, contrary to previous publications, the segmental contraction frequency increases distally along the small intestine, and varies in response to the decreasing density and slower propulsion of chyme. As the chyme moves down the small intestine, it is further mixed with digestive enzymes that break it up and alter its composition. As the composition of chyme changes and decreases in density, the small intestine may respond to sensory signals detected by the gut wall by adjusting the rate of segmental contraction.

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