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Orally-Dosed Citalopram Stimulates Small Intestinal Mucosal Growth

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Contents

Abstract

ORALLY-DOSED CITALOPRAM STIMULATES SMALL INTESTINAL MUCOSAL GROWTH

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Parenterally-administered selective serotonin reuptake inhibitors (SSRI), such as citalopram, increase intestinal mucosal absorptive surface by day 7 of treatment. We hypothesized that enteral citalopram would also induce intestinal mucosal growth, thus allowing for therapy with an oral agent. In the study's first phase, C57BL/6 mice received peanut butter (PB) pellets containing 10, 50, or 100 mg/kg/day citalopram for 7 days; or 25 mg/kg/day citalopram for 14 or 21 days; or plain PB pellets for 7, 14, or 21 days. In the second phase, C57BL/6 mice received 0, 10, 25, or 50 mg/kg/day citalopram in drinking water for 2, 3, 6, or 8 weeks, or for 6 weeks followed by 2 weeks of drug withdrawal. Two-centimeter ileal segments were harvested and prepared for microscopic assessment of villus height (VH), crypt depth (CD), villus width (VW), and crypt width (CW). Mucosal surface area (MSA) was calculated and data were compared using Student's t-test. Enteral citalopram given for 14 days in PB pellets resulted in an increased VH ($p < 0.0001$), VW ($p = 0.0058$), and ileal MSA per mm^2 ($p = 0.0007$). The increase in MSA was sustained at 21 days ($p < 0.0001$). When citalopram was given in drinking water, VH was greater than controls by week 3 ($p < 0.0001$ across all three doses); however, MSA per mm^2 was not significantly increased, regardless of treatment dose or duration. Further work is needed, but SSRIs show some promise in the short-term period as oral therapy for serious intestinal disorders such as short bowel syndrome.

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So find something new to try, something to change. Count how often you succeed and how often you fail. Write about it. Ask people what they think. See if you can keep the conversation going.
—Atul Gawande, *Better: A Surgeon's Notes on Performance* (1)

Introduction

1.1. Short Bowel Syndrome and Intestinal Failure

In 1968, Wilmore and Dudrick revolutionized the care of children with short bowel syndrome (SBS) when they described the first such infant to grow and gain weight while receiving only intravenous parenteral nutrition (PN) over the course of over 6 weeks (2). She had undergone major bowel resection for intestinal atresia, and shortly after the operation, developed a functional intestinal obstruction. Despite receiving intravenous fluids containing plasma, glucose, and protein, she continued to lose significant weight and deteriorate in condition. At that point, the decision was made to initiate PN. Within 24 hours, she was noticeably resuscitated, and by day 45, her remaining bowel had adapted sufficiently to allow for tolerance of full enteral nutrition (2).

Fifty years later, PN remains a mainstay of SBS treatment, yet the outcomes are poor. Any investigation into better treatments for SBS, however, first requires an understanding of SBS.

1.1.1. Definition and Epidemiology

SBS is characterized by malabsorption that stems from major loss of small intestinal surface area, typically due to surgical resection or congenital disease, and can affect both children and adults (3). The most common etiologies in the neonatal and young pediatric populations are necrotizing enterocolitis and intestinal anomalies, such as midgut volvulus, gastroschisis, Hirschsprung disease, and as Wilmore and Dudrick encountered, intestinal atresia (3-5). Among older children and adults, SBS is more often associated with Crohn disease, radiation, ischemia, trauma, and malignancy (3). In turn,

SBS is the most common cause of intestinal failure (IF), which is a blanket term used to describe a condition of grossly insufficient nutrient absorption resulting in not only an inability to support homeostasis or growth, but also a dependence on PN. Causes of IF that are not associated with shortened bowel length include disorders of intestinal motility as well as congenital enteropathies (3, 6).

Given the lack of a consensus definition for SBS/IF, however, considerable variability exists in the incidence of SBS/IF that is reported in the literature. One retrospective cohort study found that, at their institution, the incidence of newborns with SBS was 353.7 cases per 100,000 live births in preterm infants and 3.5 per 100,000 in term infants, for an overall rate of 24.5/100,000 (3, 7). Other studies have found SBS incidence rates of 700 and 1200 per 100,000 live births, among neonates weighing less than 1500 grams (4, 6, 8, 9).

Despite many advances in treatment options over the past 50 years since PN was introduced for SBS/IF, the outcomes are still devastating. Several factors, including length of remaining small bowel (less than 50 cm), the absence of intact colon and/or ileocecal valve, older patient age, and dependence on PN, portend a poorer prognosis. Mortality rates range consistently from approximately 10% to 30% (4-6, 10-15).

1.1.2. Economics

The economic impact of SBS/IF is almost as staggering. One study, adjusted for inflation to the year 2005, found that the total cost of care for patients with SBS in the first five years after diagnosis was on average more than 1.6 million US dollars, with the first year typically costing a little over $500,000 and years 2-5 costing roughly $250,000 to $300,000 each year (5). Inpatient hospitalizations and readmissions accounted for the large

majority of this cost, especially in the first few years after diagnosis. As the years went by, patients spent progressively less and less time in the hospital, and this was reflected in the costs of care: in-hospital expenses shrank from over $400,000 in the first year to less than $50,000 in the fifth year, while home care costs more than doubled from less than $90,000 in the first year to almost $185,000 by year 5 (5). PN was the predominant home care expense, even as most patients were eventually able to wean off of it. The categories of "antimicrobials," "injectables," "intravenous hydration," and "enteral nutrition" additionally contributed to home costs (5). These data clearly suggest that SBS/IF is a costly and morbid disease that warrants aggressive study.

1.1.3. Intestinal Adaptation and Complications

Much of the morbidity of SBS/IF derives from the disease process itself as well as sequelae of the treatment, which begs the need for investigation into better treatment options. In response to major resection, the intestine gradually undergoes changes to compensate for the reduced absorptive surface and capacity. This process, termed "intestinal adaptation," includes gastric hypersecretion, small bowel dilatation, and, to some extent, lengthening, as well as increases in villus height and crypt depth at the microscopic level (6, 11, 12). These changes are thought to occur in an attempt to restore mucosal surface area that was lost due to resection.

Fifty percent of patients who ultimately wean from PN to enteral autonomy do so within the first 6 months, and 95% are able to be free from PN within 2 years (11, 13). However, those most severely affected patients who require long-term PN, or those who never successfully wean from PN, are at significant ongoing risk for several complications.

Malabsorptive diarrhea is one such symptom that plagues patients with SBS/IF, and can precipitate fluid and electrolyte imbalances. Furthermore, the major resection of small bowel prevents the absorption of vitamins and minerals, particularly vitamin B_{12}, zinc, magnesium, calcium, and the fat-soluble vitamins A, D, E, K, and has been shown to remove an inhibitory mechanism and trigger hypergastrinemia, hyperacidity, and peptic ulcer occurrence (4, 11). Ileal resection disrupts the enterohepatic circulation of bile acids, which not only contributes to fat malabsorption and both secretory diarrhea and steatorrhea in patients with SBS, but also cholelithiasis and calcium oxalate nephrolithiasis (11).

Small intestinal bacterial overgrowth (SIBO) is another common consequence of the changes in intestinal anatomy, motility, and secretion associated with SBS (6, 16). Of note, the bacteria *Lactobacilli* can cause D-lactic acidosis in patients with an intact colon, by fermenting carbohydrates into D-lactic acid, which is then absorbed in the colon. The condition is characterized by mental status changes and an anion gap metabolic acidosis that is unexplained because laboratory tests typically measure for the L-lactic acid stereoisomer (11).

While myriad issues arise from the malabsorption and intestinal resection of SBS/IF, another considerable set of complications stems from the use of PN. Central venous access carries its own substantial risks: catheter breakage, thrombosis, infection, and sepsis (4). Ethanol lock therapy, in which 70% ethanol is infused into the catheter during off-cycles of PN, seems to be effective in reducing the number of central catheter-associated bloodstream infections; however, there is evidence to suggest that ethanol may adversely affect the catheter material (silicone) and integrity (6, 17-19). Moreover,

reduced bone mineral density and metabolic bone disease, in the form of osteomalacia or osteoporosis, has been found in up to 84% of patients receiving PN (11, 20).

Arguably the most notorious and potentially fatal complication, however, is intestinal failure-associated liver disease (IFALD). IFALD can lead to steatosis, cholestasis, fibrosis, and even cirrhosis, and affects up to 40-60% of infants on PN (4, 11, 21-23). Furthermore, a strong correlation has been found between SBS mortality and IFALD. One study found that Kaplan-Meier survival was less than 20% among children with cholestasis; in those without cholestasis, survival was closer to 80% (4, 24). Thus, minimizing the need for PN and maximizing functional intestinal surface area and intestinal motility are important goals in the treatment of patients with SBS/IF.

1.1.4. Treatment

Both SBS and IF are complex conditions that require long-term care from multiple disciplines, whether nutritional, surgical, medical, or some combination of the three. Notably, intestinal rehabilitation programs have been developed worldwide to more easily coordinate care for these complex patients, and these efforts have been shown to prolong survival (10, 25, 26). The various treatment options and approaches for patients with SBS/IF are described below.

Nutritional Therapy

Much of the long-term management of fluid, electrolyte, and nutritional balance in patients with SBS/IF is achieved through a combination of oral nutrition, enteral nutrition, and PN. Nutritional needs are heavily dictated by which segments and what lengths of bowel remain intact (4, 16, 26). For instance, in patients without a colon, small,

frequent meals high in fat, soluble fiber, and complex carbohydrates are recommended. Isotonic oral rehydration solutions (ORS) make use of the sodium-glucose cotransporter in the small bowel and are therefore especially important in these patients for fluid uptake and hydration (16, 26). In contrast, those individuals with intact colon are better suited to diets of limited fat content and greater proportions of complex carbohydrates; ORS are not necessary. Similarly, maintenance of vitamin B_{12} and methylmalonic acid levels is especially important in cases of resection of the distal ileum (16, 26). It is suggested that all patients with SBS take calcium, magnesium, and zinc supplements in addition to a daily multivitamin. In those not receiving PN, supplementation with the fat-soluble vitamins A, D, E, and K is encouraged (4, 16, 26).

Safe PN is life-saving for patients with SBS/IF. Recent advances in our understanding of the relationship between PN and IFALD have shed some light on how to best provide PN safely. PN preparations containing soy-derived omega-6 fatty acids may play a role in IFALD. There have been preliminary reports to suggest that, in fact, fish oil-based omega-3 fatty acids may be beneficial in pediatric IFALD (6, 11). For example, Smoflipid (Fresenius Kabi), an intravenous fat emulsion containing fish, soy, olive, and medium-chain oils, was approved in 2016 for adults. Thus far, one pediatric randomized controlled trial has shown promising results: lower levels of conjugated bilirubin were found in infants treated with Smoflipid as compared to those treated with the soy-derived Intralipid (Fresenius Kabi) (6, 27). Further studies are needed, however, as laboratory markers of cholestasis do not always correlate with liver histology (6).

Early enteral nutrition, whether via nasogastric/gastrostromy tube or by mouth, has been shown to promote intestinal absorption and adaptation; the ability to wean from PN and transition to enteral feeding is associated with higher rates of survival (4, 5,

16). Ultimately, the ideal goal in patients with SBS/IF is enteral autonomy: growth and maintenance of nutrition status and liver function, preferably without PN (4).

Surgical Therapy

Surgical procedures are a cornerstone of the management of patients with SBS. The spectrum of available options includes the placement of central venous catheters to deliver fluids and PN, as well as the insertion of gastrostomy, gastrojejunostomy, or jejunostomy devices to facilitate continuous feedings and enteral nutrition (4, 6). Other patients may be appropriate candidates for longitudinal intestinal lengthening and tailoring (LILT), the serial transverse enteroplasty (STEP) procedure, or even intestinal transplantation.

LILT was first introduced by Bianchi in 1980 (28). In the operation, the mesenteric vasculature is carefully preserved before the intestinal lumen is divided longitudinally in half with a gastrointestinal anastomosis (GIA) stapler. The two "hemiloops" of bowel are then anastomosed end-to-end, to essentially double the intestinal length (28).

In the STEP procedure, the GIA stapler is serially used on alternating sides of the bowel, to create a zig-zag pattern. This method, which effectively narrows the bowel lumen while increasing intestinal length, preserves the vasculature, avoids enterotomies and anastomoses, and has been very successful (4, 29).

Lastly, intestinal transplantation is an option for patients with "permanent" IF and critically serious PN complications: portal hypertension, impaired liver synthetic function, numerous central line infections, multiple instances of central venous thrombosis, and recurrent severe dehydration (4). Though five-year survival rates are

approximately 50%, the one-year rates range from 80% to over 90%, far surpassing the 60% of patients who survive five years on PN. Taking into consideration the high cost of PN, the value of intestinal transplantation in patients with permanent IF has become a matter of small debate (4).

Pharmacotherapy

The pharmacotherapy for SBS is largely limited to antisecretory and antimotility agents, as well as agents thought to promote intestinal growth and adaptation (12, 16, 26). Therapies targeting the gastric hypersecretion and secretory diarrhea that commonly ail patients with SBS include H_2 blockers, proton-pump inhibitors, octreotide, cholestyramine, and the α_2 agonist clonidine (4, 16, 26, 30-32). Similarly, antimotility medications such as loperamide, diphenoxylate/atropine, and certain opioids (codeine, opium tincture) have been shown to provide relief and help slow intestinal transit time (4, 16, 26). Unfortunately, these therapies have been primarily studied in adult SBS/IF patients; few have been explored in the pediatric population (6).

Various other treatments have found utility in SBS. Broad-spectrum antibiotics, namely metronidazole, ciprofloxacin, and rifaximin, and probiotics have been used to combat small intestinal bacterial overgrowth (6, 16, 26). Ursodeoxycholic acid has also been effective in managing PN-associated cholestasis (4).

One of the most notable pharmacotherapies for SBS available to date, however, is teduglutide, a glucagon-like peptide 2 analog. Administered via subcutaneous injection, it is currently approved for adult PN-dependent patients, but its propensity to stimulate the development of neoplasms has limited its use, especially in children (33). Thus, there are no approved therapies for children that specifically aim to increase intestinal surface

area and absorptive function. For these reasons, new therapies that specifically and actively increase intestinal mucosal surface area, especially those administered orally, are desperately needed for children with severe SBS and IF.

1.2. Serotonin and the Enteric Nervous System

The neural crest gives rise to the enteric nervous system (ENS), which is a complex network of nerves found in the bowel wall extending from the esophagus to the anus. It is comprised of the myenteric and submucosal plexuses. The ENS notably functions and carries out reflexes independently of the central nervous system (CNS), but nevertheless, there is much interaction between the two nervous systems (34, 35).

Indeed, one of the most well-known neurotransmitters in the brain, serotonin or 5-hydroxytryptamine (5-HT), is also found in great abundance in the ENS. Its receptors and functions are found in every major organ system in the body, but in fact, an overwhelming 95% of the body's 5-HT stores are found in the gastrointestinal (GI) system (35, 36). Serotonin's synthesis occurs in enterochromaffin cells of the intestinal mucosa, where it then acts on extrinsic nerves involved in sensory neurotransmission to the CNS, 5-HT_3 receptors of intrinsic primary afferent neurons (IPANs) in the myenteric plexus, and 5-HT_{1P} receptors of IPANs in the submucosal plexus. Aided by 5-HT_4, submucosal nerves release acetylcholine and calcitonin gene-related peptide (CGRP), which ultimately influence intestinal secretion and peristalsis. Myenteric IPANs release only acetylcholine and have been implicated in giant migrating contractions (35). There has also been evidence to show that serotonin plays a role in bone formation and liver regeneration, as well as in enteric neurogenesis (35, 37-39).

The ubiquity of 5-HT in the bowel and the ability of the ENS to control multiple aspects of GI function drove us to explore serotonin's potential as an intestinal mucosal growth factor, with the ultimate goal of targeting the 5-HT signaling system as a therapy for SBS/IF.

1.2.1. Previous Work

Due to the distribution of serotonin and the availability of pharmacologic agents that have been designed to alter its physiology, we have focused on its action in the bowel. Our laboratory has shown previously, via both genetic knock-out models and pharmacologic inhibition, that neuronal serotonin induces intestinal mucosal growth (40). In these experiments, wild-type (WT) mice receiving selective serotonin reuptake inhibitors (SSRIs) parenterally via intraperitoneally implanted osmotic micropumps were found to have increased villus height (VH), crypt depth (CD), and enterocyte proliferation, by day 7 of treatment (40). We therefore aimed to extend these findings to allow for a more translational approach and hypothesized that enteral dosing of the SSRI citalopram would also induce intestinal mucosal growth, thus allowing for therapy with a commonly available oral agent.

Statement of Purpose

Specific Aims:

1. To develop an enteral route of administering SSRI to mice in a reliable, safe, and palatable manner.
2. To examine the effect of enteral citalopram on intestinal mucosal growth in WT mice.

Hypothesis:

1. The enteral administration of citalopram is strongly associated with increases in VH and CD in WT mice in a dose- and time-dependent fashion, when compared to WT mice that do not receive SSRI.

Methods

This protocol (#2016-11567) was approved by the Yale University Institutional Animal Care & Use Committee.

3.1. Author Contributions

All data acquisition (animal experiments, intestinal resection, morphometric measurements), statistical analysis, and data interpretation were completed by LZ. All authors (LZ, CJG, RAC) participated in the conception and design of the study as well as drafting and critical revision of the associated manuscript.

3.2. Vehicle of SSRI Administration

Our experiments were conducted in two phases. In the first, we opted to use peanut butter (PB) as the vehicle of drug delivery, in lieu of more stressful approaches such as oral gavage, and adapted our methods from previously described studies (41, 42). For our second phase of longer-term studies, we explored the administration of SSRI in drinking water. Both methods of SSRI administration are described below in greater detail.

3.2.1. Peanut Butter Pellets

Peanut Delight creamy PB (Aldi Inc., Batavia, IL, USA) was heated in an 850-watt microwave oven for 40-80 seconds until a thick liquid consistency was achieved. PB pellets with SSRI were prepared by thoroughly mixing citalopram hydrobromide (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan; Acros Organics, New Jersey, USA) at

10, 25, 50, or 100 mg/kg per 0.2 mL pellet into the PB. All pellets were aliquoted onto a Parafilm membrane using a small-volume syringe, then stored for use at -20°C. Fresh pellets were made every 4-5 days (Figure 1). Mice underwent a 7-day habituation period and then either a 7-, 14-, or 21-day experimental period. Throughout both phases, they were transferred temporarily to individual cages for the duration of the administration of the PB pellet.

Figure 1. Peanut Butter (PB) Pellets.

Habituation Period

Every day for seven consecutive days, mice were presented with a 0.2 mL plain PB pellet that was placed on the nozzle of a water bottle. Mice did not immediately take to the PB pellet—perhaps due to its novelty—but as described in the literature, by day 7, they consumed it within minutes of presentation (41).

Experimental Period

One cohort of mice was fed PB pellets containing either 10 mg/kg, 50 mg/kg, or 100 mg/kg citalopram, or plain PB pellets, once daily for 7 days. Significant difficulty was noted in ensuring that mice consumed the PB pellets with the higher doses of citalopram, and therefore a more modest dose of drug was chosen for additional

experiments. The second cohort was provided with pellets containing 25 mg/kg citalopram or plain PB pellets, once daily for 14 or 21 days. Pellets were again presented via water bottle nozzle and mice consumed these without difficulty.

3.2.2. Drinking Water

In order to more feasibly conduct longer term studies, we used drinking water as a vehicle of delivery of enteral SSRI. Given that mice take in anywhere between 2.12 mL and 6.7 mL of water per day, we assumed a daily average of 4 mL water (43, 44). Solutions of citalopram hydrobromide were prepared at concentrations of 10, 25, or 50 mg/kg/day; dissolved into tap water by vortexing; and administered for 2, 3, 6, or 8 weeks, or for 6 weeks immediately followed by 2 weeks of plain tap water. Opaque water bottles were used throughout all drinking water experiments in order to protect both drug and water from light, and fresh solutions were made weekly.

3.3. Animals

The average mouse lives for 1 to 3 years. Weaning occurs at age 3-4 weeks, and puberty at 4-7 weeks (43). We chose to utilize 6- to 10-week old mice as our model of the pediatric age group.

Wild-type C57BL/6 mice were housed with access to standard chow and water ad libitum under standard 12 hour light/dark conditions in the Yale Animal Resource Center. For the entire duration of our experiments, mice were weighed daily (PB pellet studies) or weekly (drinking water studies). One mouse, of all animals studied in both the PB pellet and drinking water studies, was euthanized due to illness prior to the end of the experimental period. In order to reduce bias and facilitate investigator blinding,

all animals and subsequent tissue specimens and histologic slides were each identified by a nondescript cage number and ear clipping; the treatment condition assigned to each mouse was referred to only when absolutely necessary during treatment (PB pellet or water bottle) administration.

3.4. Histology

At the conclusion of the experimental period, mice were euthanized by CO_2 asphyxiation. A midline laparotomy was performed, and the small intestine was isolated, harvested, and gently flushed free of stool with 10% neutral buffered formalin (NBF). Two-centimeter segments of the distal ileum were fixed overnight at room temperature in 10% NBF. We enlisted the help of Yale Pathology Tissue Services to paraffin-embed and section the tissue specimens into thin slices for staining with hematoxylin and eosin. Lastly, the slides were visualized under standard brightfield microscopy at 20x magnification (Zeiss Axio Imager.M1, Oberkochen, Germany).

3.5. Measurements and Calculations

Using ImageJ (NIH, Bethesda, MD, USA), we measured VH, CD, villus width (VW), and crypt width (CW), as defined by the crypt-villus junction (Figure 2).

As detailed above, investigators carrying out the measurements were blind to the experimental treatment condition corresponding to each histologic slide. Intestinal villi and crypts selected for measurement were unique and fully intact with the entire length of the central lacteal or crypt lumen clearly visible. We did not measure villi or crypts that had evidence of damage, artifact from histological preparation, or oblique sectioning (45).

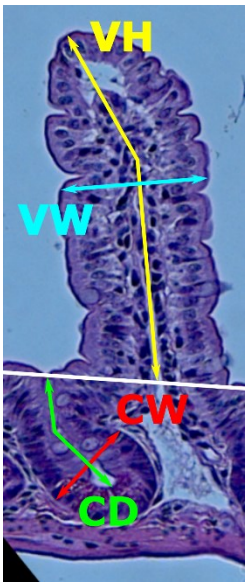

Figure 2. Example Villus Unit with Corresponding Villus and Crypt. VH = villus height, VW = villus width, CD = crypt depth, CW = crypt width.

Figure 2 demonstrates a representative villus unit. For each villus unit with corresponding values for VH, VW, CD, and CW, we used a previously described mathematical approximation of intestinal mucosal surface area (MSA) to calculate MSA per mm^2 (46):

$$\text{MSA per mm}^2 = \frac{(\text{VW}\times\text{VH}) + \left(\frac{\text{VW}}{2} + \frac{\text{CW}}{2}\right)^2 - \left(\frac{\text{VW}}{2}\right)^2}{\left(\frac{\text{VW}}{2} + \frac{\text{CW}}{2}\right)^2} \qquad (46)$$

All measured and calculated data were compared using Student's t-test. Statistical significance was defined when $p < 0.05$.

Results

Treatment with oral citalopram caused no obvious adverse effects. SSRI-treated animals experienced similar patterns of weight gain—and namely, did not experience weight loss—when compared to control animals.

4.1. Peanut Butter Pellets

Seven days of oral citalopram given at 10, 50, or 100 mg/kg/day resulted in virtually no differences compared to controls treated with PB pellets alone in three of the measured parameters, VH, VW, and CD, regardless of citalopram dose; the one exception was an increase in VW ($p = 0.0093$) in mice receiving 10 mg/kg/day. At all three doses, however, CW decreased significantly (p-values ranging from 0.0002 to <0.0001), likely contributing to the increases in MSA (p-values from 0.0578 to 0.0006) (Table 1).

Table 1. Measured Mucosal Parameters after the Enteral Administration of Citalopram in Peanut Butter (PB) Pellets for 7 Days.

	Vehicle (n)	10 mg/kg/day (n)	p-value	50 mg/kg/day (n)	p-value	100 mg/kg/day (n)	p-value
VH (μm)	209.7 ± 5.8 (40)	210.6 ± 2.6 (103)	0.8703	217.9 ± 4.4 (34)	0.2774	198.7 ± 3.4 (63)	0.0829
VW (μm)	58.8 ± 1.7 (40)	63.8 ± 0.9 (103)	**0.0093**	57.0 ± 2.2 (34)	0.5119	62.2 ± 1.2 (63)	0.1124
CD (μm)	69.8 ± 8.0 (6)	82.0 ± 2.4 (52)	0.1138	74.5 ± 2.6 (22)	0.4718	70.9 ± 3.0 (26)	0.8855
CW (μm)	46.4 ± 2.2 (6)	33.0 ± 0.7 (52)	**<0.0001**	36.3 ± 1.0 (22)	**0.0002**	35.2 ± 0.8 (26)	**<0.0001**
MSA (per mm^2)	5.2 ± 0.1 (6)	6.4 ± 0.1 (52)	**0.0045**	6.3 ± 0.1 (22)	**0.0006**	5.8 ± 0.2 (26)	0.0578

VH = villus height, VW = villus width, CD = crypt depth, CW = crypt width, MSA = mucosal surface area

After 14 days of treatment, enteral citalopram at 25 mg/kg/day significantly increased VH and VW compared to controls, by approximately 12.3% ($p < 0.0001$) and 12.2% ($p = 0.0058$), respectively. CD remained statistically similar ($p = 0.2960$), and CW

decreased by 12.7% (p = 0.0020). Overall, calculated ileal MSA per mm² increased by 22% from 5.0 ± 0.2 to 6.1 ± 0.1 (p = 0.0007) (Figure 3).

Significant differences in VH, VW (data not shown), and CD were no longer seen by day 21 of enteral citalopram at 25 mg/kg/day. Crypt width decreased by 37.7% (p <0.0001), with a concurrent increase in MSA of 36.8% (p <0.0001) (Figure 3).

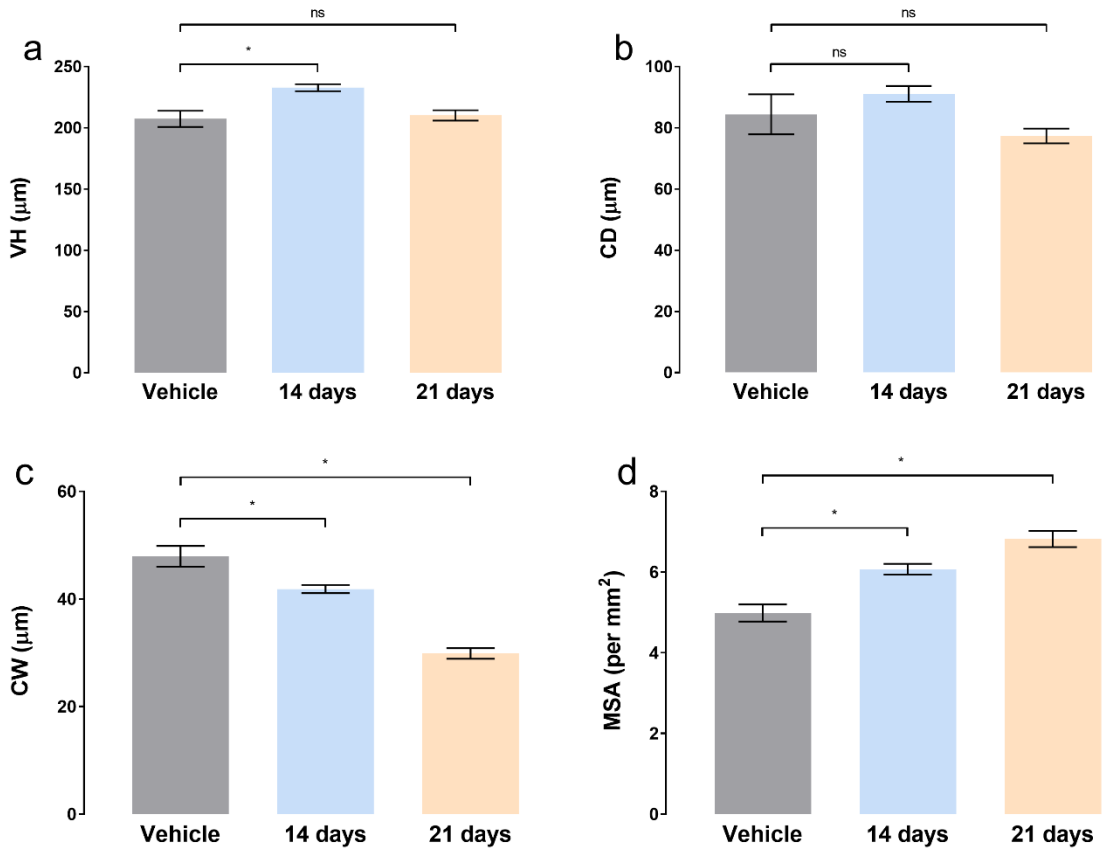

Figure 3. Measured Mucosal Parameters after the Enteral Administration of Citalopram in Peanut Butter (PB) Pellets for 14 or 21 Days.
Citalopram was dosed at 25 mg/kg/day. Panels A-D show VH, CD, CW, and MSA, respectively. VH = villus height, CD = crypt depth, CW = crypt width, MSA = mucosal surface area. * denotes p <0.05.

4.2. *Drinking Water*

Citalopram was administered in drinking water at 10, 25, or 50 mg/kg per day, for 2, 3, 6, or 8 weeks; Figure 4 depicts MSA per square millimeter under these experimental conditions. Notably, with one exception at week 2 (Table 2), no differences in MSA were seen until week 6 when comparing controls and the three treatment doses. At the 6-week and 8-week time points, mucosal surface was consistently greater in controls than in any animals receiving enteral citalopram ($p < 0.0001$ across all doses at both time points).

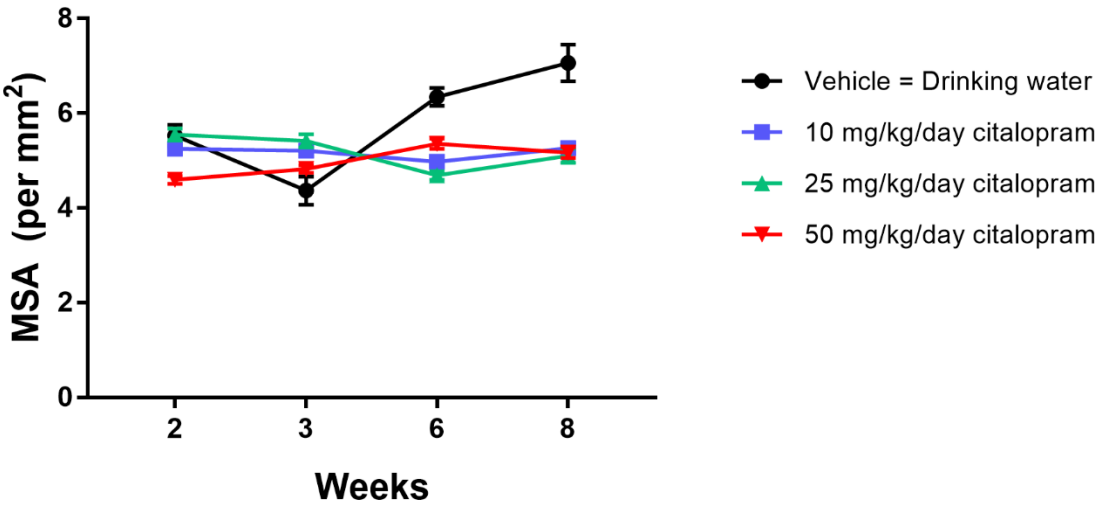

Figure 4. Mucosal Surface Area (MSA) after Long-Term Enteral Administration of Citalopram.

In mice that received citalopram-treated drinking water of 10 or 50 mg/kg/day for 2 weeks, VH decreased from controls by 7.3% ($p = 0.0016$) and 13.7% ($p < 0.0001$), respectively, while in those that received 25 mg/kg/day citalopram, VH remained statistically similar ($p = 0.6394$). CD was significantly reduced among all animals given citalopram in water for 2 weeks ($p < 0.0001$ across all three doses). The only change in

MSA per mm^2 for this treatment duration, a reduction from 5.5 ± 0.2 in controls to 4.6 ± 0.1 ($p = 0.0006$), occurred at a drug concentration of 50 mg/kg/day.

By 3 weeks, citalopram-treated villi were taller than controls by roughly 32.9% (10 mg/kg/day, $p < 0.0001$), 28.3% (25 mg/kg/day, $p < 0.0001$), and 13.8% (50 mg/kg/day, $p < 0.0001$). No differences, however, were seen in CW ($p = 0.8309$, $p = 0.1231$, and $p = 0.1394$, corresponding respectively to 10, 25, and 50 mg/kg/day citalopram) or MSA ($p = 0.2053$, $p = 0.1628$, and $p = 0.3609$).

VH was increased from controls by 3.1% after treatment with 10 mg/kg/day citalopram for 6 weeks ($p = 0.0308$); dosing at 50 mg/kg/day resulted in an 11.5% increase ($p < 0.0001$). A 4.4% reduction in height was seen with the 25 mg/kg/day dose ($p = 0.0057$) (Figure 5). Furthermore, after 6 weeks, all villi and crypts of citalopram-treated mice were significantly wider than controls, while crypt depth was decreased. As noted above, calculated mucosal surface by week 6 in animals of the three treatment doses was 20.6%, 25.4%, and 14.3% less than that of controls (corresponding to 10, 25, and 50 mg/kg/day citalopram, respectively; $p < 0.0001$ across all three doses).

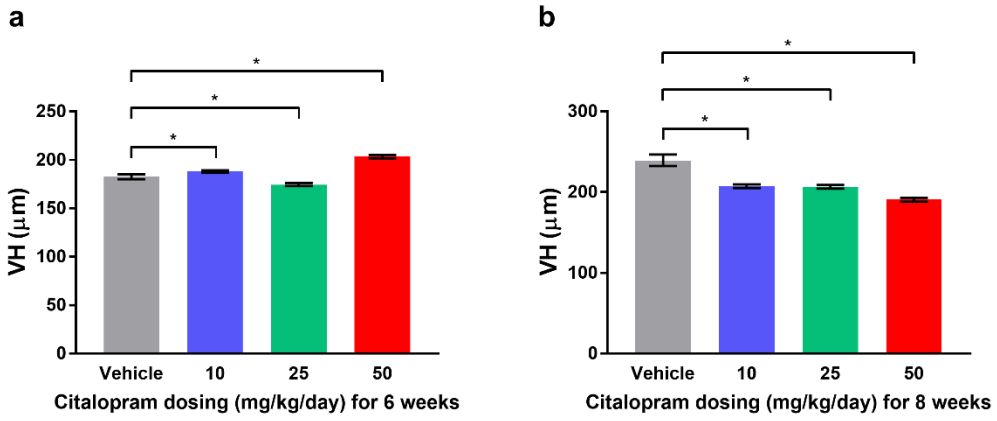

Figure 5. Villus Height (VH) after Administration of Citalopram in Drinking Water.
Panels A and B correspond to 6- and 8-week treatments, respectively.
* denotes $p < 0.05$

The time point at 8 weeks was notable for shorter and wider villi in all citalopram-treated animals when compared to controls ($p < 0.0001$ across all three doses, for both VH and VW) (Figure 5). Again, MSA per mm^2 in animals receiving 10, 25, or 50 mg/kg/day citalopram in water for 8 weeks was decreased from controls by 25.4%, 28.2%, and 26.8%, respectively ($p < 0.0001$ across all three doses).

Drug withdrawal for 2 weeks following 6 weeks of citalopram treatment also showed villi that were significantly shorter and wider than controls. While CW was statistically similar between control and all treatment groups in this cohort, MSA per mm^2 in treated animals was reduced from controls by 21.1%, 33.8%, and 31.0% (respectively 10, 25, and 50 mg/kg/day citalopram; $p < 0.0001$ across all three doses).

Mean values for VH, VW, CD, CW, and MSA under all experimental doses and durations are detailed in Table 2.

Table 2. Measured Mucosal Parameters after the Enteral Administration of Citalopram in Drinking Water.

Treatment for 2 Weeks

	Vehicle (n)	10 mg/kg/day (n)	p-value	25 mg/kg/day (n)	p-value	50 mg/kg/day (n)	p-value
VH (µm)	206.3 ± 6.3 (30)	191.2 ± 1.3 (351)	**0.0016**	209.3 ± 2.4 (174)	0.6394	178.0 ± 1.6 (295)	**<0.0001**
VW (µm)	68.8 ± 1.6 (30)	65.3 ± 0.5 (351)	0.0616	63.5 ± 0.6 (174)	**0.0012**	65.5 ± 0.6 (295)	0.0707
CD (µm)	97.0 ± 2.2 (18)	75.2 ± 2.1 (116)	**<0.0001**	67.8 ± 3.6 (49)	**<0.0001**	63.1 ± 1.1 (103)	**<0.0001**
CW (µm)	36.4 ± 1.1 (18)	39.3 ± 0.8 (116)	0.1362	41.3 ± 0.7 (49)	**0.0004**	43.0 ± 0.5 (103)	**<0.0001**
MSA (per mm^2)	5.5 ± 0.2 (8)	5.3 ± 0.1 (97)	0.5155	5.6 ± 0.1 (52)	0.9475	4.6 ± 0.1 (71)	**0.0006**

Treatment for 3 Weeks

	Vehicle (n)	10 mg/kg/day (n)	p-value	25 mg/kg/day (n)	p-value	50 mg/kg/day (n)	p-value
VH (µm)	153.9 ± 6.7 (14)	204.5 ± 1.6 (234)	**<0.0001**	197.5 ± 2.0 (203)	**<0.0001**	175.2 ± 1.0 (342)	**<0.0001**
VW (µm)	73.5 ± 3.2 (14)	70.2 ± 0.8 (234)	0.3306	65.4 ± 0.7 (203)	**0.0064**	64.4 ± 0.5 (342)	**0.0002**
CD (µm)	89.0 ± 4.1 (6)	76.6 ± 1.8 (100)	0.1027	69.8 ± 2.5 (68)	**0.0278**	53.3 ± 0.9 (89)	**<0.0001**
CW (µm)	42.1 ± 2.8 (6)	42.8 ± 0.8 (100)	0.8309	38.0 ± 0.7 (68)	0.1231	39.0 ± 0.5 (89)	0.1394
MSA (per mm^2)	4.4 ± 0.3 (2)	5.2 ± 0.1 (60)	0.2053	5.4 ± 0.1 (53)	0.1628	4.8 ± 0.1 (79)	0.3609

Treatment for 6 Weeks

	Vehicle (n)	10 mg/kg/day (n)	p-value	25 mg/kg/day (n)	p-value	50 mg/kg/day (n)	p-value
VH (µm)	182.5 ± 2.5 (125)	188.1 ± 1.3 (285)	**0.0308**	174.5 ± 1.6 (286)	**0.0057**	203.4 ± 1.7 (193)	**<0.0001**
VW (µm)	52.8 ± 0.7 (125)	63.7 ± 0.5 (285)	**<0.0001**	67.8 ± 0.5 (286)	**<0.0001**	62.9 ± 0.7 (193)	**<0.0001**
CD (µm)	68.8 ± 1.4 (68)	61.2 ± 1.3 (82)	**0.0001**	62.3 ± 1.2 (96)	**0.0006**	62.1 ± 1.7 (77)	**0.0032**
CW (µm)	29.4 ± 0.6 (68)	40.9 ± 0.7 (82)	**<0.0001**	41.6 ± 0.5 (96)	**<0.0001**	41.5 ± 0.6 (77)	**<0.0001**
MSA (per mm^2)	6.3 ± 0.2 (49)	5.0 ± 0.1 (52)	**<0.0001**	4.7 ± 0.1 (70)	**<0.0001**	5.4 ± 0.1 (44)	**<0.0001**

Treatment for 8 Weeks

	Vehicle (n)	10 mg/kg/day (n)	p-value	25 mg/kg/day (n)	p-value	50 mg/kg/day (n)	p-value
VH (µm)	239.5 ± 7.1 (39)	207.4 ± 2.3 (221)	**<0.0001**	206.6 ± 2.3 (224)	**<0.0001**	190.5 ± 2.4 (163)	**<0.0001**
VW (µm)	56.2 ± 1.8 (39)	66.5 ± 0.7 (221)	**<0.0001**	67.2 ± 0.8 (224)	**<0.0001**	63.4 ± 0.6 (163)	**<0.0001**
CD (µm)	69.3 ± 4.0 (9)	71.5 ± 1.2 (102)	0.5897	68.9 ± 1.6 (80)	0.9353	54.9 ± 0.9 (68)	**<0.0001**
CW (µm)	39.7 ± 2.1 (9)	42.7 ± 0.5 (102)	0.0774	44.4 ± 0.7 (80)	**0.0451**	40.0 ± 0.7 (68)	0.8856
MSA (per mm^2)	7.1 ± 0.4 (12)	5.3 ± 0.1 (60)	**<0.0001**	5.1 ± 0.1 (49)	**<0.0001**	5.2 ± 0.1 (46)	**<0.0001**

Treatment for 6 Weeks + Withdrawal for 2 Weeks

	Vehicle (n)	10 mg/kg/day (n)	p-value	25 mg/kg/day (n)	p-value	50 mg/kg/day (n)	p-value
VH (µm)	239.5 ± 7.1 (39)	220.3 ± 2.5 (142)	**0.0017**	176.4 ± 2.5 (293)	**<0.0001**	181.2 ± 2.1 (321)	**<0.0001**
VW (µm)	56.2 ± 1.8 (39)	67.8 ± 0.9 (142)	**<0.0001**	70.2 ± 0.6 (293)	**<0.0001**	66.0 ± 0.7 (321)	**<0.0001**
CD (µm)	69.3 ± 4.0 (9)	72.3 ± 1.2 (72)	0.4099	67.1 ± 2.1 (98)	0.7645	60.7 ± 1.3 (84)	**0.0461**
CW (µm)	39.7 ± 2.1 (9)	43.6 ± 0.7 (72)	0.0777	42.3 ± 0.6 (98)	0.1986	42.6 ± 0.9 (84)	0.2915
MSA (per mm^2)	7.1 ± 0.4 (12)	5.6 ± 0.1 (50)	**<0.0001**	4.7 ± 0.1 (81)	**<0.0001**	4.9 ± 0.1 (64)	**<0.0001**

Discussion

In previous work, we studied VH and CD, among other parameters, after parenteral administration of citalopram at 25 mg/kg/day. By 7 and 14 days of treatment, VH was increased from controls by roughly 15% and 12%, respectively, and CD by 7% and 18% (40). That same investigation examined genetic mouse models where the serotonin reuptake transporter is absent (SERTKO), and found an increase in VH by approximately 40% compared to WT mice (40).

Building on this past work, in the first phase of the current study, we delivered citalopram via PB pellets to WT mice for 7, 14, or 21 days, at varying doses. No changes in either VH or CD were seen when citalopram was given for 7 days, at 3 doses ranging from 10 to 100 mg/kg/day. On the other hand, fourteen days of citalopram in PB pellets at 25 mg/kg/day caused a statistically significant increase in VH of approximately 12%, though CD remained unchanged compared to controls. The slower changes seen with this enteral route of administration of SSRI are most likely pharmacokinetic in nature and secondary to first-pass metabolism, which is not involved when the drug is given parenterally (47, 48). Our findings also suggest that changes in CD may be slowest to occur, if at all, of all measured parameters.

In the second phase of our study, citalopram was delivered in drinking water for 2, 3, 6, or 8 weeks, as well as for 6 weeks immediately followed by a 2-week washout period. In contrast to the findings seen with citalopram in PB pellets, dosing of the drug in drinking water resulted in taller villi by three, not two, weeks. As we expected both PB pellets and drinking water to be comparable enteral routes of administration, this result was somewhat surprising. One explanation may lie in the difference between the

bolus-style drug delivery of PB pellets and relatively continuous drug supply of drinking water. Moreover, although mice had unlimited access to their water treatment, we had assumed an average daily water intake of 4 mL, and they were otherwise relatively unmonitored in the animal housing facility. It is very possible that the citalopram water was poorly palatable, that mice drank less water than assumed, and that steady-state drug levels were low. Overall, we did not monitor daily water intake or citalopram levels in the body at any point during the study.

Another unexpected finding was that all doses and durations of the citalopram-treated water showed either no difference in MSA from controls, or in some cases, a significant decrease. Most notably, mucosal surface in the control group progressively increased from week 3 to week 6 to week 8, as the animal grew in age and size. This trend was not seen in any of the experimental groups, raising the question of an inhibitory effect with chronic treatment. By comparison, 25 mg/kg/day citalopram in PB pellets increased mucosal surface per mm^2 by 21.8% by week 2 and by 36.8% by week 3. It is perhaps worth noting, however, that VH, VW, and CD, were statistically similar between the PB experimental and control groups at the 3-week mark; narrower crypts, and not taller, thinner villi or deeper crypts, were the most likely driver of the increases in MSA in that cohort.

One group of mice received citalopram in drinking water for six weeks, and then was immediately transitioned to plain tap water for two weeks. Comparison with the 8-week control group showed shorter, wider villi, corresponding to a decreased MSA per mm^2. These trends were identical to those observed in the 8-week experimental group, suggesting that citalopram's effects on intestinal mucosal morphology may begin to

plateau at the 6- to 8-week mark, or that it may simply take longer than two weeks for the mucosa to return to its starting baseline.

With respect to currently available medical therapies for SBS, teduglutide remains the only one approved for use in adult disease. In the study demonstrating efficacy of this agent, mice injected with glucagon-like peptide 2 were found to have increases in VH, from controls, of approximately 25% by 6 days of treatment and 50% by 10 days, with no significant differences in CD (49). By contrast, we found that 14 days of enteral citalopram resulted in a more modest increase in VH of 12.3%. Whether this is a mechanistic and/or pharmacokinetic phenomenon remains to be seen; nevertheless, the oral route of citalopram administration was associated with a concurrent increase in VW and decrease in CW, likely contributing to the 22% increase in calculated MSA.

Many questions remain regarding the precise role that serotonin plays in the ENS in the maintenance of intestinal mucosa, as well as the implications of intestinal mucosal growth on intestinal function. While further work is needed to refine our current understanding of this complex signaling environment in the ENS, we have presented some data to demonstrate the potential efficacy of an orally administered SSRI to promote intestinal mucosal growth, especially in the short-term period.

5.1. Future Directions

Long-term parenteral studies would provide invaluable data to compare with the results presented here. Further avenues of investigation into the enteral route should include analysis of other segments of the small bowel, such as the jejunum, as well as other measures of intestinal mucosal growth, such as intestinal weight and the crypt proliferation index (a marker of cell division). Given that the palatability of citalopram

may be a limiting factor, future work should also explore other methods of enteral delivery, such as oral gavage, to ensure that the drug is ingested fully and in the expected amounts. While we have preliminary data showing increased absorption of solutes and nutrients in genetic mouse models, functional assays in the setting of enteral SSRI treatment would be of great interest. The ultimate goal would be to examine enteral SSRI administration in a large animal model of intestinal injury or after massive resection.

The population of patients who have short bowel syndrome and are also prescribed SSRIs for a mood disorder may prove to be enlightening. In 2014, Faye et al. examined the pharmacokinetics of oral and intravenous citalopram or escitalopram in eight such patients (50). Though the authors' focus was primarily on steady-state concentrations and concentration-dose ratios (CDRs), they also included in their analysis such variables as frequency of PN, small bowel length, and percentage of intact colon. Patients required PN anywhere from 0 to 7 times per week, and their remaining lengths of small intestine ranged from 5 cm to 200 cm. A small intestinal length of less than 20 cm was associated with poor absorption (low steady-state concentration and CDR), while patients with greater than 180 cm of small bowel remaining, or more than 80 cm small bowel and at least half their colon, had normal serum levels of drug (50). While this was a small study, it introduces a special group of patients who are highly relevant to the research question posed here.

5.2. Limitations

Though both plain and SSRI PB pellets were indistinguishable in appearance, investigators were not blinded to the type of pellet they administered. Higher doses of

citalopram were not as readily accepted by mice when presented as part of a PB pellet, and the final dose absorbed by each animal was difficult to control entirely when compared to a parenteral dosing regimen. Similarly, investigators were not blinded to the drug concentration when preparing, refilling, and administering citalopram solutions.

Moreover, the sample sizes of the drinking water control groups (that is, no drug) were very small. Only one animal was studied per experimental condition, which therefore limited the number of intact villi and crypts that were candidates for measurement. Additionally, both male and female mice were used indiscriminately throughout the study, as well as mice anywhere from 6 to 10 weeks of age. Our study did not control or balance for sex, as recommended in 2014 by the National Institutes of Health's "Policy on Sex as a Biological Variable" (51, 52). These potentially confounding factors, in addition to random variation itself, could have been mitigated with a more robust sample size.

To truly isolate the effect of citalopram, it would have been ideal to measure villi and crypts before and after drug treatment from the same animal. Lastly, the equation we favored to estimate mucosal surface area is merely one of several mathematical models that have been proposed (46, 53, 54).

5.3. Conclusions

This year, 2018, marks 50 years since Wilmore and Dudrick first used parenteral nutrition to treat pediatric short bowel syndrome. Though we now have a great number more and a wider variety of treatment options for SBS and IF, PN remains fundamental to management of these patients, and the outcomes are still devastating.

Building on previous work on parenteral citalopram, we sought to investigate the effect of the enteral citalopram on intestinal mucosal morphometrics and surface area in WT mice, at a range of treatment doses and durations. In this study, we utilized both PB pellets and drinking water as vehicles to deliver our drug. Fourteen days of citalopram in PB pellets resulted in increased VH, VW, and ileal MSA, and the increased mucosal surface was sustained at day 21. Drinking water seemed to be less effective. VH in citalopram-treated animals was greater than controls by week 3, but this finding dissipated by weeks 6 and 8. MSA per mm^2 in the treatment group, across all doses and durations, was statistically similar to or decreased from the control group.

While the drinking water results were unexpected, much of the PB pellet data showed promise. Further studies would be invaluable for shedding light on the serotonin signaling system in the bowel, as well as on the potential for an orally and commonly available agent to treat serious intestinal disorders such as short bowel syndrome.

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If you can't control your peanut butter, you can't expect to control your life.
—Bill Watterson, *The Complete Calvin and Hobbes* (55)