

Effects of Alpha-1 Antagonists,
Stress and Relaxation on Anal
Pressures

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Title of the study	Effects of Alpha-1 Antagonists, Stress and Relaxation on Anal Pressures
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Abstract

Modification on 6/20/16 shown in blue

The sympathetic nervous system provides tonic excitatory input mediated by adrenergic α_1 receptors to the internal anal sphincter. Defecatory disorders (DD) are attributed to inadequate rectal propulsive forces and/or impaired anal/pelvic floor relaxation during defecation. Anecdotally, many patients with DD have anxiety, but the effects of anxiety on anal sphincter pressures are unknown. DD are ideally managed by pelvic floor retraining (biofeedback therapy). However, a majority of patients with DD do not have access to biofeedback therapy and at best, 70% of patients respond to biofeedback therapy. There are no pharmacological options for DD.

This proposal will assess the contribution of adrenergic α_1 receptors and separately anxiety/relaxation to anal pressures in healthy and DD. The major objectives are to (1) study the effects of adrenergic α_{1A} receptor antagonists on anal resting pressure, with the intent of developing pharmacological approaches to manage DD in future, (2) evaluate the effects of anxiety and mental relaxation on anal pressures, and (3) compare rectal compliance in DD and healthy subjects. Our overall hypothesis is that anxiety increases while mental relaxation reduces anal resting pressure via α_1 adrenoreceptor-mediated increased and reduced sympathetic tone respectively. The specific aims of this study are to evaluate the following hypotheses in healthy subjects and patients with DD: (i) α_1 receptors contribute to anal resting pressure but not the squeeze pressure increment; (ii) Mental stress will increase anal resting but not squeeze pressure via α_1 adrenoreceptors; (iii) Mental relaxation will reduce anal resting pressure via α_1 adrenoreceptors; hence the α_1 adrenoreceptor antagonist will attenuate the reduction in anal resting pressure resulting from mental relaxation; (iv) Rectal compliance is higher and the contractile response to sinusoidal oscillation is lower in DD than in healthy people; and (v) Alfuzosin will improve bowel symptoms in chronic constipation.

Anal resting and squeeze pressures will be evaluated at baseline, after randomization to the highly selective α_1 adrenoreceptor antagonist alfuzosin (2.5 mg) or

placebo and finally after randomization to active stress or mental relaxation in 36 healthy women and 36 female patients with defecatory disorders. Thereafter, bowel habits will be recorded at baseline (2 weeks) then during treatment with placebo or alfuzosin ER (UroxatralTM 10 mg daily) for 2 weeks. The most significant anticipated findings are that alfuzosin and mental relaxation will reduce anal resting pressures, thereby providing additional options for patients with DD.

A. Specific Aims

The anorectum maintains fecal continence and is responsible for defecation¹. Defecatory disorders (DD) result from inadequate rectal propulsive forces and/or impaired anal/pelvic floor relaxation during defecation². Pelvic floor retraining by biofeedback therapy is the cornerstone for managing defecatory disorders. However, in the United States, pelvic floor retraining is only available at a handful of centers and not covered by many insurance programs. A substantial proportion of patients with defecatory disorders do not respond to pelvic floor retraining. Hence, additional options for managing DD are necessary. High anal pressures at rest and during defecation may impair rectal evacuation in DD. Conceptually, increased anal resting pressure may be attributed to the internal or external anal sphincters, which are responsible for approximately 70% and 30% of anal resting tone respectively. In humans, monkeys and dogs, the internal sphincter is functionally innervated by sympathetic motor nerves. Ongoing sympathetic activity may contribute to as much as 50% basal anal pressure^{3,4} predominantly via α_1 adrenoreceptors. Moreover, in vitro studies suggest that stimulation of sympathetic nerves to the monkey IAS can double spontaneous contraction⁵.

Similar to other functional GI disorders, many patients with defecatory disorders have anxiety. However, the effects of anxiety on anal pressures have not been evaluated. We postulate that anxiety may increase anal resting pressure via sympathetic stimulation of the internal sphincter and/or predispose to conscious or subconscious voluntary contraction of the external anal sphincter, which may also increase resting tone. Hence, our overall hypothesis is that anxiety increases anal resting pressure via α_1 adrenoreceptors while mental relaxation reduces anal resting pressure. α_1 -adrenergic stimulation also affects hypothalamic stress-mediated release of CRF⁶ and the startle response, possibly via CRF release from brainstem nuclei mediating startle⁷. Indeed, prazosin reverses the excessive startle response induced by alpha-1 agonists⁸. In this study, we propose to use the α_1 -adrenergic antagonist alfuzosin which is highly selective for urological tissues relative to the prostate, has a low risk of orthostatic hypotension and penetrates the brain poorly.

Finally, rectal contraction contributes to the desire to defecate. There is limited evidence, more in adolescents than in adults, that chronic constipation is associated with increased rectal compliance. Increased rectal compliance is associated with rectal hyposensitivity⁹. In children and adolescents, increased rectal compliance is associated with severe symptoms but does not predict treatment failure^{10,11}. However, it is unclear if increased rectal compliance causes or is consequent to chronic constipation or if increased rectal compliance reflects reduced rectal contractile response to distention. We now propose to explore this question by using approaches that we have extensively utilized to evaluate rectal pressure-volume relationships in fecal incontinence i.e., distention with a barostat and separately with sinusoidal oscillation¹²⁻¹⁴.

Psychological factors such as stress or depression are known to influence the natural history of chronic gastrointestinal illnesses such as inflammatory bowel disease (IBD) [15] and irritable bowel syndrome (IBS) [16] via the gut-brain axis. These conditions, and also constipation are also associated with dysbiosis. [17] Furthermore, the gut microbiota-brain axis appears to be bidirectional.[18] It is thus important to also evaluate the gut microbial profile in patients and controls to identify potential interactions between gut microbiota and

the response to stress, in health and in constipation. We propose to assess this using 16s rRNA analysis of stool samples.

This study will evaluate the following specific aims in healthy subjects and in patients with defecatory disorders:

- 1) To assess the effects of a highly-selective α_{1A} receptor antagonist alfuzosin on anal resting pressure, squeeze increment, and rectoanal gradient during simulated evacuation. Our hypothesis is that α_1 receptors contribute to anal resting pressure but not the squeeze pressure increment in healthy people and defecatory disorders.
- 2) To study the effects of mental stress on anal resting and squeeze pressures via α_1 adrenoreceptors. Our hypothesis is that mental stress will increase anal resting but not squeeze pressure via α_1 adrenoreceptors in healthy people and defecatory disorders.
- 3) To evaluate the effects of active mental relaxation on anal resting and squeeze pressures. Our hypotheses is that mental relaxation will reduce anal resting pressure via α_1 adrenoreceptors; hence the α_1 adrenoreceptor antagonist will attenuate the reduction in anal resting pressure resulting from mental relaxation in healthy people and defecatory disorders.
- 4) To compare rectal compliance and the response to sinusoidal oscillation. Our hypotheses are that rectal compliance is higher and the contractile response to sinusoidal oscillation is lower in defecatory disorders than in healthy people.
- 5) To explore the effects alfuzosin on bowel symptoms in patients with chronic constipation. Our hypotheses are that alfuzosin will improve bowel symptoms in chronic constipation.

6) To evaluate the role of microbiota in the relationship between stress/relaxation and defecatory mechanisms. Our hypothesis is that the susceptibility to stress is associated with differences in fecal microbiota. **B. Research Design and Methods**

This study comprises 2 parts. Part A will compare the effects of oral alfuzosin (IR 2.5 mg) and placebo on anorectal functions on 1 day in all subjects (controls and patients). Part B will compare the effects of oral alfuzosin (10 mg ER) and placebo on bowel habits in constipated patients (but not in healthy subjects). 36 healthy women and 36 women with defecatory disorders will be enrolled in this study. This protocol is limited to women only because the vast majority of patients with constipation and defecatory disorders in our practice are women.

1. Human subjects.

a) Inclusion Criteria for Controls (Part A only)

- i. Healthy women volunteers aged 18-80 years
- ii. Able to provide written informed consent before participating in the study

- iii. Able to communicate adequately with the investigator and to comply with the requirements for the entire study.

b) Inclusion Criteria for Patients (women aged 18-80 years) (Parts A and B)

- i. Constipation. Women with chronic constipation for 1 year with any 2 or more of the following symptoms for 3 months or longer, i.e. <3 bowel motions/week, straining $\geq 25\%$ of time, hard or lumpy stools $\geq 25\%$ of time, incomplete evacuation $\geq \square 25\%$ of time, feeling of anorectal blockage $\geq 25\%$ of time.
- ii. Able to provide written informed consent before participating in the study
- iii. Able to communicate adequately with the investigator and to comply with the requirements for the entire study.

c) Exclusion Criteria for Controls; Items indicated with an asterisk (*) are also exclusion criteria for patients

- i. Clinical evidence of significant cardiovascular, respiratory, renal, hepatic, gastrointestinal, hematological, neurological, psychiatric or other disease that may interfere with the objectives of the study and/or pose safety concerns.*
- ii. Current symptomatic orthostatic hypotension or history of hypotensive response as defined by a reduction of ≥ 30 mmHg in systolic or ≥ 20 mmHg in diastolic BP.*
- iii. Current symptoms of a functional gastrointestinal disorder assessed by questionnaire^{15, 16}
- iv. Putative risk factors for pelvic floor trauma, i.e. six or more vaginal deliveries, birthweight >4500gms (macrosomia), or known 4th degree perineal tear
- v. Inability to withdraw medications prior to the baseline period and throughout the study (except as protocol defined rescue medications):
 - Medications that substantially alter GI transit * including laxatives, magnesium and aluminum containing antacids, prokinetics, erythromycin, narcotics, anti-cholinergics, tricyclic antidepressants, SNRI and newer antidepressants
 - Selective serotonin reuptake inhibitor (SSRI) antidepressants are permissible at low, stable doses. All medications shall be reviewed and dis/approved by the principal investigator on a case by case basis. *
 - Potent Cyp 3A4 inhibitors such as ketoconazole, itraconazole and ritonavir, nitrates and phosphodiesterase inhibitors *

NOTE: stable doses of thyroid replacement, estrogen replacement, low dose aspirin for cardioprotection, and birth control (but with adequate backup contraception as drug-interactions with birth control have not been conducted) are permissible. *
- vi. Stable dose of thyroxine will be permitted *
- vii. Prolonged Q-Tc interval > 500 msec on ECG within the last three months*
- viii. Estimated glomerular filtration rate (eGFR) < 60 mL/minute. * Based on guidelines and recommendations from the National Kidney Disease Education Program (NKDEP) of the National Institutes of Health (NIH) and the Kidney Disease Outcomes Quality Initiative (KDOQI) of the National Kidney Foundation, the an eGFR using the Modification of Diet in Renal Disease (MDRD) Study

equation is more accurate than a creatinine clearance calculated from serum and urine measurements. The formula is $eGFR (mL/min/1.73 m^2) = 175 \times (Scr)^{-1.154} \times (Age)^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$. Based on our extensive experience in clinical practice and research studies, it is anticipated that all potentially eligible participants will have normal serum creatinine.

- ix. History of allergies to $\alpha 1$ adrenoreceptor antagonist *
 - x. Active rectal inflammation, cancer; perianal sepsis; history of pelvic radiation, rectosigmoid surgery or inflammatory bowel disease*
 - xi. Pregnant women, prisoners and institutionalized individuals*
 - xii. Persons with a latex allergy.
2. Overall experimental design. Study Assessments are shown in Table 1. During the screening visit, a clinical assessment, a medical history and physical exam, including height, weight and "vital signs" (blood pressure, temperature, heart and breathing rates), Draw a blood sample, and test urine for pregnancy if you are a female able to become pregnant, along with, questionnaires (bowel questionnaire, Hospital Anxiety and Depression Scale ¹⁷, a health-status questionnaire ¹⁸), screening laboratory tests to exclude renal insufficiency, an ECG and a urine pregnancy test if necessary, will be completed.

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Table 1. Schedule for Study Assessments and Monitoring

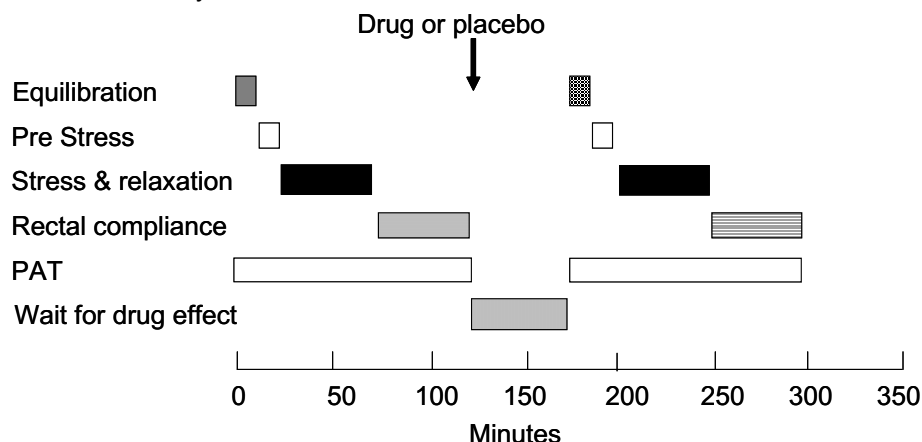
	Screening	Acute Study	Bowel Symptoms (Alfuzosin – ER 10 mg)			
	Days –30 to 1	Day 1	Baseline Period (Days 3-16)	Treatment Period (Days 17-30)		
Procedure			Daily	Other	Daily	Other
Informed consent	X					
Review eligibility	X					
Physical examination	X					
Concomitant medication review	X	X				
Vital signs, weight	X	X ¹				
Serum creatinine, urine pregnancy test, ECG (12-lead)	X ²					
Stool sample	X ³			X ³		
Anorectal study		X				
Randomization to treatment		X				
Study treatment administration		X				X ³
Adverse event assessment		X		X ⁴		X ⁴
Stool diary (Appendix 1)			X		X	
Abdominal pain (Appendix 2)			X		X	
Global assessments (relief, severity) (Appendix 3)				Weekly		Weekly
Global assessment – satisfaction with treatment, (Appendix 4)	X			X (day 17)		X (day 31)
Other abdominal symptoms (Appendix 5)				Weekly		Weekly
Telephone contact				X ⁴ (q3-5 days)		X ⁴ (q3-5 days)

1. During the acute study, heart rate and blood pressure will be recorded at hourly intervals. Height (cm) and weight (kg) will be recorded at the screening visit but not on Day 1.
2. Urine pregnancy test to be performed for women of childbearing potential within 2 weeks of Day 1. Serum creatinine and ECG (12-lead) to have been done within three months of Day 1.
3. A single stool sample can be collected either before the acute study visit, or 3 days after the acute study visit, but before the treatment period.

4. Patients will be treated with placebo or alfuzosin ER (10 mg) once daily during this phase. While the protocol is planned for the treatment period to follow directly the baseline period, if needed for scheduling, there can be up to a 5-day interval between the baseline and treatment period.
5. Patients will be contacted by telephone every ~3-5 days between clinic visits to address questions, ascertain side effects and remind subjects to complete diaries. The final phone call will be within 5 days after completing the treatment period. Subjects will be asked to return unused medication by mail.

3. Experimental procedures. Items a-d below exclusively pertain to Part A,
- a) Anorectal sensorimotor functions. After rectal cleansing with 1-2 sodium phosphate (Fleets®, C.B. Fleet, Lynchburg, VA) enemas, anorectal resting and squeeze pressures and rectal compliance and sensation will be evaluated before and after randomization to alfuzosin (2.5 mg oral) or placebo (Figure 1) in the Charlton 7 CRU. During each epoch (i.e., baseline or drug), pressures will be evaluated before and after patients are randomized to one of 6 sequences (orders) of 3 interventions (i.e., 2 stressors and active relaxation). The same stressor/relaxation sequence will be applied during pre-drug and post drug epochs. Anorectal assessments, which will be performed with standard techniques, include:
- Anal resting and squeeze pressures using high resolution manometry, similar to the procedure employed in the clinical lab and in recent studies¹⁹;
 - Rectal compliance and sensation using a barostat/ polyethylene balloon. A conditioning distention from 0-40 mmHg in 4 mm steps at 15 second intervals will be performed. Ten minutes thereafter, the balloon will be distended from 0-44 mmHg or maximum tolerated pressure in 4 mmHg steps; sensory thresholds for first sensation, desire to defecate, and urgency will be recorded^{13, 20}
 - Rectal ramp distention. Because the contractile response to distention is influenced by the rate of distention²⁰, a ramp distention to 200 mL over 1 minute will be performed; this will be sustained for 2 minutes. While (ii) is useful for characterizing quasi-static pressure-volume relationships representing colonic tone, ramp distentions can uncover the active contractile response to distention.
 - Rectal sinusoidal oscillation will be performed at 5 cpm for 20 minutes after distention to 125 mL. This paradigm is more sensitive than barostat-driven distentions for uncovering increased rectal stiffness in fecal incontinence²¹ but has not been evaluated in constipation

Figure 1. Experimental Design for Anorectal Study. During the stress/relaxation epoch, subjects will receive 2 stress and 1 relaxation intervention in randomized order. The duration of this epoch includes two 10 minute rest periods between the 3 interventions. The manometry catheter will be removed and limited ambulation will be permitted while we are waiting for drug concentrations to increase. Combined, this wait period and the second equilibration period are 70 minutes long, which is within the range of t_{max} for alfuzosin (1.1 – 1.5 hours). PAT = peripheral arterial tonometry



- v. Rectal balloon expulsion will also be assessed in the seated position to confirm presence (or absence) of an evacuation disorder.
- b) Mental stressors and relaxation method. The mental stress testing protocol will be administered under the supervision of a licensed clinical psychologist who is familiar with these tests and routinely administers the same. Two stress interventions and a relaxation protocol will be provided in randomized order with 10-minute equilibration periods. Two different mental stress tasks of 6-minute duration will be performed. Each test is designed to address a different mental skill set. This was designed specifically to prevent mental fatigue and keep subjects motivated. All tasks will have intra-test levels of varying difficulty and will be externally paced, with the goal of stressing subjects as much as tolerated as judged by the psychologist administering the tests. It is known that external task pacing accentuates the induced mental stress.²² The aim is to keep subjects engaged and focused. The tasks, which will be performed in randomized order are as follows:
 - i. A computerized version of the Stroop word-color conflict.^{23, 24} Stroop word-color conflict test consists of three colored words (red, blue, and green) displayed in random order on a computer screen. Each word could appear in either its own color (visually concordant), or in one of the other 2 colors (visually discordant). The subject's task will be to enunciate the color of the word, not the actual word itself. A rectangular box will surround the stimulus color-word, and this task will also be externally paced, with the subject having to keep pace with the advancing box.
 - ii. Number subtraction of increasing difficulty (1 digit from 2 digits up to 3 digits from 3 digits) testing math skills.
 - iii. Relaxation will be induced by asking patients to view a CD obtained from Mayo Patient education. In essence, this CD encourages patients to close their eyes, be comfortable, monitor their breathing, and relax the entire body.

Before and at the end of each task, subjects will be asked to rate their stress and arousal on four 10 mm long visual analog scales labeled tired-energetic, peaceful-tense, worried-relaxed, and active-drowsy²⁵.
- c) Blood pressure and heart rate measurements will be taken 30 seconds before, 2 minutes into, and at the end of each 6-minute mental stress task. Subjects will have 5-minute baseline rest periods between each mental stress task, during which they will be instructed to relax. At the end of the mental stress tasks, the reactive hyperemia protocol will be repeated. Double product will be calculated as systolic blood pressure multiplied by heart rate.
- d) Peripheral arterial tonometry (PAT) will provide an independent biological marker of mental stress, which causes vasoconstriction, and the α_1 antagonist, which causes vasodilatation. There is a characteristic PAT signal response to mental stress, with diminution of the signal amplitude during stress. A ratio of the stress PAT amplitude to baseline PAT amplitude (PAT score) of 0.8 or less is considered a positive response to mental stress and considered a marker for future stress-related cardiovascular events.²⁶

e) Drugs. After carefully reviewing the pharmacokinetic properties and side effects of all α_1 adrenoreceptor antagonists, we have opted to use alfuzosin, because it (i) is highly selective for urological tissues relative to the vascular system, (ii) has a low risk of orthostatic hypotension and (iii) penetrates the brain poorly^{27, 28}. For example, in isolated human tissues, alfuzosin displays the highest selectivity ratio for the prostate over the vascular tissue (ratio, 144) compared with tamsulosin (90), doxazosin (51), and terazosin (19). In experimental animal models, the primary effect of alfuzosin is a urethral relaxant activity and not an antihypertensive effect. Administered intravenously to conscious normotensive rats at doses 3 to 10 times higher than those necessary to induce significant urethral relaxation, alfuzosin displays the lowest hypotensive activity compared with doxazosin, terazosin, and tamsulosin.

Alfuzosin (2.5 mg) has a mean bioavailability of 64%, t_{max} of 1.0 – 1.5 hours and a terminal t_{half} of 3-5 hours^{27, 28}. The drug is extensively metabolized by the liver with only 11% of the administered dose excreted unchanged in the urine. This immediate release formulation, which is also manufactured by Sanofi-Aventis, not approved in the US but widely available in other countries, will be imported from UK (Xatral™, 2.5 mg) under an investigator-initiated IND (117098) from the FDA because the long t_{max} for alfuzosin ER limits its use in this acute study.

f) Stool collection. Using stool kits and standardized instructions, patients will collect stool samples according to the procedure in the Appendix. Stool samples will be frozen and stored in a -20°C freezer. The first stool specimen will be collected (a) without a laxative in controls and (b) if necessary, after their usual laxative regimen in patients.

In Part B, the effects of alfuzosin extended-release (10 mg, Uroxatral) on bowel symptoms will be evaluated. Since such use satisfies the requirements of 21 CFR 312.2 (b) (1), an IND is not required. We confirm that all the following apply to Part B of this study:

a) The investigation is not intended to be reported to FDA as a well-controlled study in support of a new indication for use nor intended to be used to support any other significant change in the labeling for the drug; and

b) The investigation is not intended to support a significant change in the advertising for the product; and

c) The investigation does not involve a route of administration or dosage level or use in a patient population or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product; and

d) The investigation will be conducted in compliance with the requirements for institutional review set forth in 21 CFR 56 Institutional Review Boards and with the requirements for informed consent set forth in part 21 CFR 50 Protection of Human Subjects; and

e) The investigation will be conducted in compliance with the requirements of 21 CFR 312.7 - Promotion of Investigational Drugs.

f) Genome wide DNA analysis. Towards our long-term objective of uncovering associations between SNPs and DD, we propose to extract DNA from 10 ml of blood, to be drawn from study participants. DNA extraction will be conducted at the BAP Lab. Genome-wide analysis will be conducted using Illumina 610 QUAD microarray or

comparable approaches. Genotype-phenotype correlations will be examined using these patients and other patients in ongoing studies.

g) **Sequencing and analytical methods.** Using resources available in the UIUC-Mayo consortium, two complementary approaches will be utilized to analyze stool specimens from each subject. First, microbial DNA will be extracted from stool and tissue samples using standardized methods [33]. Phylotype profiles of the microbiome from control and constipated populations will be generated using deep rDNA hypervariable tag sequencing of the hypervariable V3-V5 region of the SSU rRNA gene, which has been validated for use with human microbiomes and is one of the methods of choice for the HMP. With the longer reads from the MiSeq (250x250 paired end reads), our sequencing will include both the V3-V5 RGTs making for a more optimal phylogenetic analysis [34]. Phylotype profiles of the mucosa-associated microbiome will be generated using rDNA sequencing of the hypervariable V3-V5 region, which has been validated for standard use by the human microbiome project. The 300 base pair reads will ensure a better optimal phylogenetic identification. Barcoding samples prior to sequencing will yield approximately 20,000 reads/sample, ensuring detection of both dominant (core microbiome) and poorly represented taxa (variable microbiome). Identifying the existent taxa, diversity, and ecological relationships within each sample requires that we process large volumes of 16S DNA sequence data. In order to accomplish this task we will follow protocols that we have quantitatively shown to be optimal. Paired end reads will be stitched, aligned, and classified using a custom pipeline (TORNADO v2.0).[35] Briefly, low base quality reads will be either trimmed or discarded,[36] and these reads will not be classified as a bacteria kingdom[37] or matched to the bacteria 16S rRNA secondary structure.[38] To evaluate the microbial diversity and abundance, UPARSE will be used for Operational Taxonomical Units (OTU) clustering,[39] and FastTree for phylogeny.[40] The 16S data will be clustered into OTUs at 97% sequence similarity, and the taxonomy will be assigned using the Ribosomal Database Project classifier. h) Effects of alfuzosin in bowel habits (Part B). During the first 2 weeks, bowel habits will be recorded at baseline. During the next 2 weeks, patients will be treated (double-blind) with placebo or alfuzosin (10 mg) ER once daily, which is FDA-approved dose for urinary symptoms. Symptom assessments are shown in Table 1 and in Appendices 1-5. Per eligibility criteria, subjects will discontinue medications which can affect GI transit during the baseline and treatment periods. Patients who need rescue medication, after three full days without a bowel movement, can receive 1-2 bisacodyl 5 mg tabs or a bisacodyl suppository (10 mg) or up to Fleet's enemas. In addition, patients who need relief of mild pain can use analgesic drugs such as NSAIDs and COX-2 inhibitors for up to two doses per week. During the procedure, intake of acetaminophen up to 1.5g per day in divided doses will be permissible.

4. Data and statistical analysis

a) Part A. Anal manometry will be analyzed by established techniques and summarized as anal resting and squeeze pressures, rectal and anal pressure and rectoanal gradient during evacuation^{19, 29}. Rectal pressure-volume relationships will be summarized by the Pr_{half} during rectal compliance, rectal capacity, pressure and volume sensory thresholds for first sensation, desire to defecate, and urgency¹⁴. During

sinusoidal oscillation, average rectal pressures and the variability in pressures over 20 min will be summarized¹⁴.

The analysis will be based on analysis of covariance (ANCOVA) models to assess the pre-drug period for intervention effects with subject status and intervention type as the primary factors (a check for order effects of the intervention sequences will also be made, and the corresponding pre-stress response will be included as a covariate). Models with a subject status by intervention type interaction will also be examined. During period 2, subjects will have been randomized to drug or placebo (balanced on subject status) and use the same intervention sequence as the pre-drug period). The analysis of the post drug period will also be based on ANCOVA models incorporating subject status, intervention type, treatment group, and corresponding interactions (a check for order effects will again be made). The corresponding responses from the pre-stress interval from period 1 and VAS scores for stress and arousal will be used as covariates,

b) Part B.

- i. Endpoints. Symptoms and Signs of Chronic Constipation. The effects of alfuzosin compared to placebo will be assessed by evaluating
 - Bowel habits using a daily diary (Appendix 1) to record stool consistency (Bristol Stool Form scale), frequency, ease-of-passage and sensation of complete bowel emptying (yes/no)
 - Daily Abdominal Pain Scores (Appendix 2) collected as (a) most severe daily score and (b) overall average daily score).
 - Global Patient Treatment Assessments reporting outcomes on a weekly basis (Appendix 3) including overall relief and severity of constipation
 - Global Patient reported outcomes on a 2-week basis (Appendix 5)
 - Patient reported outcomes of other abdominal symptoms (Appendix 6)

Two outcomes will be derived from primary data.

- Weekly rate of SBMs: a bowel movement (BM) is considered an SBM if no laxative, enema or suppository was taken in the preceding 24 hours.
 - Weekly rate of CSBMs: if the patient indicated that the SBM was associated with a sensation of complete bowel emptying, the SBM will be counted as a CSBM.
- ii. Analysis. Daily bowel diary responses will first be averaged over study periods (baseline and treatment) and in the case of stool frequency, per week. These responses will also be assessed using ANCOVA models, with the corresponding baseline value as the primary covariate. For stool frequency, the two weekly values will be assessed using a repeated measures ANCOVA model with the overall average baseline stool frequency value as a covariate. The global assessment scores (adequate relief and severity) will first be summed over the two treatment weeks, and then assessed using the Wilcoxon rank sum test. Individual adverse events (AEs) will be summarized by treatment group and the overall total frequency (per subject) of AEs assessed using the Wilcoxon rank sum test. The analyses will follow the intent to treat (ITT) paradigm with all

- patients randomized included in the analyses. Patients with missing values for a particular endpoint will have their missing values imputed using the corresponding overall mean value from all patients with non-missing data for that endpoint. An adjustment in the ANCOVA error degrees of freedom (subtracting one for each imputed value) will be made to account for the imputation. A similar adjustment for the Wilcoxon rank sum test (adjusting the degrees of freedom for the t-approximation) will also be examined.
5. Sample size assessment. The sample size assessment is based on the variation in anal resting pressures in healthy subjects and patients with defecatory disorders and the effect of the α_1 receptor antagonist (indoramin) on anal resting pressure in a previous study³⁰. The CV (%) in anal resting pressure evaluated by the same technique in healthy women (<50 years old) and patients with defecatory disorders (<50 years old and resting pressures >90) was 20% (mean=93.7{SD=18.5}) and 11% (mean=102.9{SD=11.7}), respectively. In period 2 the sample size of 36 controls and 36 patients will yield 18 subjects per treatment arm (drug vs. placebo) in each subject group (control vs. patients). This should provide 80% power to detect a treatment effect size of roughly 15-20%, based on a two sample t-test with a two-sided alpha level of 0.05 separately in each subject group. The effect size is defined as the difference in group means as a percentage of the overall (both groups) mean value. The previous study suggested an effect size of 47% for drug effects³⁰. For part B, data pooled from constipation subjects imply differences between treatment groups of ~4 stools per week, differences in stool consistency scores of ~1.1 and differences in ease of passage scores of 0.8 could be detected with 80% power (2-sided alpha level of 0.05)³¹. It is anticipated that the ANCOVA models will provide similar power for somewhat smaller differences by incorporating the baseline values as covariates.
 6. Anticipated results and interpretation. We anticipate that: (i) alfuzosin will reduce anal resting pressure and increase the rectoanal gradient during simulated evacuation but not affect the squeeze increment; (ii) alfuzosin will attenuate the augmentation of anal pressure by mental stress; (iii) mental relaxation will reduce anal resting pressure to a greater extent after placebo than alfuzosin; and (iv) rectal compliance and the contractile response to sinusoidal oscillation will be lower in chronic constipation than in healthy subjects.
 7. Potential pitfalls and precautions taken. The anorectal procedures to be used in this study are established in our laboratory. While the effects of alfuzosin on anal pressures are unknown, the dose used in this study is safe and relaxes the urethral sphincter to increase urinary flow. Peripheral arterial tonometry will provide an independent biological marker for effects of stress and alfuzosin. We recognize the given the phenotypic variability in defecatory disorders³², it is conceivable that only a subset of patients will respond to an α_1 antagonist. To maximize the likelihood of a response, patients with high anal resting pressure by digital rectal examination or manometry will be recruited for initial studies. Established perturbations administered in a standardized format will be used to modulate arousal and anxiety and the effects of these

perturbations will be evaluated by standardized and validated VAS instruments. While the sample size estimate is primarily designed to detect acute effects on anorectal functions, it is also sufficient to identify clinically-significant effects on bowel symptoms (eg, bowel symptoms)³¹.

8. Innovative aspects, significance, and future directions. The concepts that mental stress can increase anal resting pressure via α -1 adrenergic receptors and the application of α -1 antagonists to manage these disorders are novel. Alternative approaches to pelvic floor retraining, which is the cornerstone for managing defecatory disorders, are necessary because (i) many insurance programs do not cover pelvic floor retraining; and (ii) our experience suggests that a considerable proportion of patients do not respond to pelvic floor retraining in clinical practice. If alfuzosin reduces anal resting pressures and increases the rectoanal gradient during defecation, further studies with the long-acting formulation, either alone or as an adjunct to biofeedback therapy, will be considered. Likewise, further studies to evaluate the effects of mental relaxation on symptoms and to understand the extent to which the effects of biofeedback therapy are mediated by mental relaxation in patients with DD may be considered. Conceivably, the response to these approaches may vary among patients and the acute response to these perturbations may predict response to chronic therapy.

C. Human Subjects

1. Only subjects who satisfy stringent inclusion and exclusion criteria and provide written informed consent will be included in the protocol. The consent form details issues related to privacy, withdrawal from studies, and potential risks of studies among other items. The anorectal assessments are extremely safe. Subjects may experience the urge to defecate during rectal distention. Consistent with all our studies, distention will be terminated if patients experience severe discomfort. The listed side effects of alfuzosin include faintness/dizziness, vertigo, malaise, headache, hypotension (postural), nausea, abdominal pain, diarrhea, dry mouth, and asthenia. Hypotension is a listed side effect. However, even with chronic administration, the risk of hypotension is low. For example, in a study comparing alfuzosin 2.5 mg immediate release tid with 5 mg sustained-release twice daily in 2442 patients, the incidence of hypotension at 1 year was 1.6%; there were no clinically significant changes in systolic and diastolic blood pressure or heart rate between baseline and 24 months, i.e., -3.1mmHg, -1.4mmHg, and -0.05 bpm, respectively³³. In the largest experience of 13,389 patients (average age 67 years old) treated with alfuzosin 2.5 mg tid for benign prostatic hypertrophy for 3 months, two-thirds of the adverse events leading to discontinuation were vasodilatory and occurred in 2.7% of the patients: vertigo/dizziness (1.4%); malaise (0.6%); hypotension (0.4%), and headache (0.4%)³⁴. While alfuzosin ER is approved by the FDA, the 2.5 mg immediate release formulation is not. Hence, this formulation will be imported from Europe after obtaining an IND from the FDA.
2. Data and Safety Monitoring Plan (DSMP)

- a) Subject Safety. Only subjects who are eligible based on pre-defined criteria, an interview and a physical examination, and screening laboratory tests will be enrolled. Study procedures are conducted by trained and experienced personnel. Interventions (alfuzosin and anorectal tests) pose a low risk to participants. Monitoring will be performed during and after the study by CRU RNs and the Study Coordinator. The PI will also review side effects. UPIRTSOs and non-UPIRTSOs will be reported per protocol to IRB and FDA (IND required for alfuzosin). Each study is reviewed and analyzed; study discussions and communications are generally by e-mail and stored. Study will be stopped if any rectal perforation occurs.
- b) Data Integrity. Only subjects who are eligible based on pre-defined criteria, an interview and a physical examination will be enrolled. Transcription of data is accurate and complete. Calculations will be standardized and accurate. The study technician will monitor data after every study. The PI will also review data after every 6 studies.
- c) Subject Privacy. Subjects will be consented in a private room. Adequate time for questions will be provided.
- d) Data Confidentiality. All records are maintained in password protected electronic files. Hard copies are filed in private offices.
- e) Product Accountability. Research Pharmacy will obtain and dispense medication. Unused medication will be collected from subjects at the end of the study and destroyed.
- f) Study Documentation. Documentation will be per established guidelines during the study (hard copy and electronic data capture). These files will be sampled annually.
- g) Study Coordination. Annual debriefing will be conducted to ensure expectations are clear and if educational needs exist.

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Appendix 1. Sample Stool Diary Card

Daily Diary			
Date: 			
If you had no bowel movements today, please check this box: <input type="checkbox"/> No bowel movements today.			
Please record the time you took the study medication today: _____ : _____			
Please record the time of today's breakfast: _____ : _____			
	Describe the consistency of bowel movement	Describe the Ease of Passage of bowel movement	Did you feel like you completely emptied your bowels?
	1 hard lumps 2 lumpy sausage 3 cracked sausage 4 smooth sausage 5 soft lumps 6 Mushy 7 watery	1 Manual Disimpaction 2 Enema needed 3 Straining needed 4 Normal 5 Urgent w/o/pain 6 Urgent w/pain 7 Incontinent	1 no 2 yes
1 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
2 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
3 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
4 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
5 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
6 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
7 <input type="checkbox"/> am hr. min <input type="checkbox"/> pm	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>	<div style="border: 1px solid black; width: 40px; height: 40px;"></div>
Have you had any unusual negative events today? No <input type="checkbox"/> Yes <input type="checkbox"/> (complete below)			
Event _____ Mild / Moderate / Severe Resolved / Ongoing			
Have you taken any medications other than those you routinely use today? No <input type="checkbox"/> Yes <input type="checkbox"/> (complete below)			
Medication _____	Medication _____	Medication _____	
Dose _____	Dose _____	Dose _____	
Frequency _____	Frequency _____	Frequency _____	

Appendix 2. Daily Diary: Abdominal Pain Questionnaire

[illegible]

- [illegible]

[illegible]

Appendix 4. *Bi-Weekly Global Satisfaction with Constipation Treatment Questionnaire (adapted from PAC-QoL Question 28)*

- The following question is designed to measure the impact constipation has had on your daily life **during the past two weeks**. For this question, please tick one box.

This question asks about how satisfied you are.	Not at all	A little bit	Moderately	Quite a bit	Extremely
	0	1	2	3	4
1. To what extent, during the past 2 weeks, have you been satisfied with your treatment?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Appendix 5. Patient Reported Outcomes for Other Abdominal Symptoms

INSTRUCTIONS: The questions in this diary are designed to measure the severity of various abdominal symptoms. Please complete the diary each week as directed, thinking about the 24-hours prior to completion of the diary on a particular day

For each question, please choose the one response that best describes your experiences during the past 24 hours.

1. How would you rate your bloating at its worst in the past 24 hours?

0	1	2	3	4	5	6	7	8	9	10
No bloating										Worst possible bloating

2. How would you rate your abdominal pain at its worst in the past 24 hours?

0	1	2	3	4	5	6	7	8	9	10
No abdominal pain										Worst possible abdominal pain

3. How would you rate your nausea at its worst in the past 24 hours?

0	1	2	3	4	5	6	7	8	9	10
No nausea										Worst possible nausea

4a. How would you rate your vomiting at its worst in the past 24 hours?

0	1	2	3	4	5	6	7	8	9	10
No vomiting										Worst possible vomiting

4b. How many times have you vomited in the past 24 hours?

_____ times (*If no vomiting, enter 0*)

APPENDIX 6

Stool Specimen Collection Instructions

Attaching the collection container

1. Open the kit
2. Lay the collection container on a flat surface with the labeled side facing up. Fold up the two cardboard sides (Figure 1).

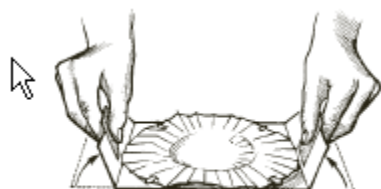


Figure 1

3. Remove the backing from the tape on each of the cardboard sides.
4. Insert the collection container into the toilet bowl and attach the tape to the top of the toilet seat toward the back half of the bowl (Figure 2). The cardboard sides should be up against the bottom of the toilet seat.

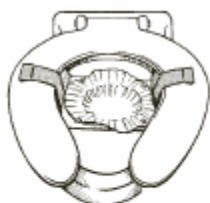


Figure 2

5. Shape the paper dish (middle part of the collection container) into a bowl by gently pushing down the center.

Collection a sample

1. Do not urinate into the collection container. (You may wish to urinate before attaching the collection container to the toilet seat.)
2. Have a bowel movement into the paper dish
3. Take out the collection tube and unscrew the cap. Use the spoon attached to the cap to scoop a marble-sized sample. Insert filled spoon

back into the tube and tightly screw the cap onto the collection tube (Figure 3).

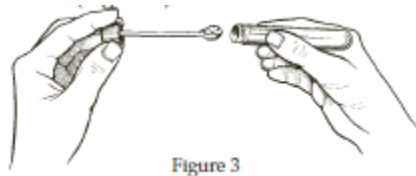


Figure 3

Disposal

1. Remove the paper dish holding the stool by gently lifting up the four attachment sites. Flush the paper dish and stool.
2. Remove the cardboard frame from the toilet seat and discard it in the wastebasket.
3. Wash your hands thoroughly with soap and water.

Returning the sample to Mayo Clinic

1. Be sure the collection tube cap is tightly fastened.
2. Place the collection tube containing your sample into the small white bag.



Collection tube

If you are at Mayo Clinic:

At your earliest convenience, return the white bag containing your specimen to: Station S/Specimen Collection Cart
Monday through Friday, 7 a.m. to 5 p.m.

If you are mailing your stool specimen to Mayo Clinic:

At your earliest convenience, mail your specimen to Mayo Clinic using the prepaid mailer provided.



Mailing tube