Pharmacodynamics, Pharmacogenetics, Clinical Efficacy and Safety of Cannabidiol for Gastroparesis and Functional Dyspepsia

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PROTOCOL TITLE:
Pharmacodynamics, Pharmacogenetics, Clinical Efficacy and Safety of Cannabidiol for Gastroparesis and Functional Dyspepsia

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ABSTRACT

Gastroparesis is defined as a gastrointestinal motility disorder with objectively delayed gastric emptying in the absence of mechanical obstruction. Gastroparesis is associated with upper gastrointestinal symptoms including early satiety, postprandial fullness, nausea, vomiting, bloating, and upper abdominal pain. The diagnosis of gastroparesis is based on the combination of symptoms of gastroparesis, absence of gastric outlet obstruction or ulceration, and delay in gastric emptying (4 hour gastric emptying test). Similar symptoms may also accompany other mechanisms of gastric dysfunction including reduced gastric accommodation and gastric hypersensitivity. Together, these gastric motor and sensory abnormalities may cause functional dyspepsia. Functional dyspepsia is a very common cause of substantial morbidity; it is estimated to affect 10% of the population and manifests as abdominal pain/discomfort after eating for at least three days per week. It has been estimated that 40% of patients with this symptom complex consult their physicians, with impact on their workplace attendance and productivity and an economic impact in excess of $18 billion in 2009. Development of effective treatments of these disorders is desirable, given significant unmet medical need. The only approved drug for gastroparesis is metoclopramide, a dopamine D_2 antagonist and 5-HT_4 agonist; it can be prescribed for a minority of patients for up to 3 months because of endocrine, cardiac and neurological side effects. There is no currently approved treatment for functional dyspepsia. The non-selective cannabinoid receptor agonist, dronabinol, was previously shown to retard gastric emptying and enhance gastric accommodation. $^{\Delta 9}$THC and non-pharmaceutical grade cannabidiol are used for diverse pain-related disorders; the effects and benefit-risk ratio of these agents are unclear. With recent FDA approval of cannabidiol, our **general hypothesis** is that cannabidiol relieves symptoms in patients with gastroparesis and functional dyspepsia without deleterious effects on gastric emptying, accommodation, satiation or satiety. Our **aims** are:
1. To compare the *pharmacodynamics and clinical effects* of cannabidiol vs. placebo on satiation, fasting gastric volume, gastric accommodation, gastric emptying, and symptoms in patients with:
1A. gastroparesis (symptoms based on Gastroparesis Cardinal Symptom Index-Daily Diary (GCSI-DD); and
1B. functional dyspepsia (+ non-delayed gastric emptying) and symptoms based on Nepean Dyspepsia Index
2. To assess *pharmacogenetics effects* of variants in *FAAH* and *CNR1* genes on the pharmacodynamics effects of cannabidiol compared to placebo on fasting and accommodation gastric volumes, gastric emptying and satiation.
Anticipated Results and Significance: We expect these studies will lead to understanding the mechanisms of action of cannabidiol in improving gastrointestinal functions and patient reported outcomes, including pain, in patients with gastroparesis or functional dyspepsia, addressing unmet needs of millions of American citizens.

Specific Aims

Gastroparesis is defined as a gastrointestinal motility disorder with objectively delayed gastric emptying in the absence of mechanical obstruction that is associated with upper gastrointestinal symptoms including early satiety, postprandial fullness, nausea, vomiting, bloating, and upper abdominal pain. The most common etiologies of gastroparesis are diabetes mellitus, post-surgical, and idiopathic; less common are iatrogenic, extrinsic neuronal (such as Parkinsonism and paraneoplastic disease), and infiltrative disorders (such as scleroderma). The diagnosis of gastroparesis is based on the combination of symptoms of gastroparesis, absence of gastric outlet obstruction or ulceration, and delay in gastric emptying (4 hour gastric emptying test). The initial management of gastroparesis is based on dietary therapy for restoration of fluids and electrolytes, nutritional support, and treating the underlying etiology such as optimization of glycemic control in diabetics. The dietary therapy should be supported with prokinetic agents to accelerate gastric emptying and improve gastroparesis symptoms. Development of effective pharmacologic therapies for such disorders is desirable, as these conditions represent a significant unmet medical need; the only approved drug is metoclopramide, a dopamine D_2 antagonist and 5-HT_4 agonist, which is approved for a minority of patients for a period of only three months because of endocrine, cardiac and neurological adverse effects. Given the paucity of available efficacious treatments, devices (such as stents), per-oral endoscopic myotomy of the pylorus, and electric stimulation or surgery are being performed without proven benefit in sham controlled trials.

Functional dyspepsia is estimated to affect ~10% of the population and manifests as abdominal pain after eating for at least three days per week. It causes substantial morbidity, impacts workplace attendance and productivity, and had an economic impact in excess of 18 billion dollars in 2009. There is no currently approved treatment for functional dyspepsia. Current medical treatment includes eradication of H pylori, acid suppression, prokinetic drugs, antidepressants, and psychological and alternative therapy; yet, despite this, many patients remain refractory to treatment, experiencing continued disabling symptoms including postprandial pain. Although subgroups of functional dyspepsia patients also have delayed or accelerated gastric emptying, other mechanisms include reduced gastric accommodation and gastric hypersensitivity. Given the extensive use of $^{\Delta 9}$THC and cannabidiol for diverse pain-related conditions, the effects and benefit-risk ratio of these agents is unclear. With recent FDA approval of pharmaceutical grade cannabidiol, there is potential to provide patients with relief.

In our prior studies [NIH R01-DK079866 (09-11)], the non-selective cannabinoid receptor agonist dronabinol (DRO) was previously shown to retard gastric emptying especially in female patients, increase fasting gastric volume in males, and inhibit colonic tone and phasic pressure activity. However, DRO did not significantly alter thresholds for first, gas or pain sensation during stepwise distension in the descending colon; paradoxically, DRO increased sensory rating for pain during random-order phasic distensions. The endo-cannabinoid system consists of CB1 and CB2 receptors; the ligands of these receptors are anandamide and 2-arachidonyl glycerol (2-AG), and their respective ligand-inactivating enzymes are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGLL). It is conceivable that variants in pivotal genes involved in the inactivation of the endocannabinoids or the CBR1 receptor could impact the response to administered cannabinoid agonist. Indeed, we previously showed two significant treatment-by-genotype interactions: DRO preferentially delays colonic transit in those with the *CNR1* rs806378 (CT/TT vs. CC) genotypes; DRO influences fasting colonic motility index in IBS (with diarrhea or alternating bowel function) who carry *FAAH* rs324420 (CA/AA vs. CC).

Our **general hypothesis** is that cannabidiol relieves symptoms in patients with gastroparesis and functional dyspepsia without deleterious effects on gastric emptying, satiation or satiety. We shall assess effects on symptoms using validated patient reported outcomes.

Overall Aim 1: To compare the *pharmacodynamics and clinical effects* of cannabidiol vs. placebo on satiation, fasting gastric volume, gastric accommodation, gastric emptying, and symptoms in patients with:

Aim 1 a. gastroparesis with symptoms based on American Neurogastroenterology Motility Society's Gastroparesis Cardinal Symptom Index (GCSI)-Daily Diary; or **Aim 1 b**. functional dyspepsia (with normal gastric emptying of solids) with symptoms based on Nepean Dyspepsia Index.

Aim 2. To assess *pharmacogenetics effects* of variants in *FAAH* and *CNR1* genes on the pharmacodynamics effects of cannabidiol vs. placebo on fasting and accommodation gastric volumes, gastric emptying and satiation in the same patients.

Anticipated Results and Significance: We expect these studies will lead to understanding the mechanisms of action of cannabidiol in improving gastrointestinal functions and patient reported outcomes including pain in the same patients with gastroparesis or functional dyspepsia; thus, our studies will address unmet needs of millions of American citizens.

Background on Gastroparesis and the Related Disorder, Functional Dyspepsia

Gastroparesis is defined as a gastrointestinal motility disorder with objectively delayed gastric emptying in the absence of mechanical obstruction that is associated with upper gastrointestinal symptoms including early satiety, postprandial fullness, nausea, vomiting, bloating, and upper abdominal pain (1,2). The most common etiologies of gastroparesis are diabetes mellitus, post-surgical, and idiopathic; less common are iatrogenic, extrinsic neuronal (such as Parkinson's disease and paraneoplastic disease), and infiltrative disorders (such as scleroderma) (3,4). The diagnosis of gastroparesis is based on the combination of symptoms of gastroparesis, absence of gastric outlet obstruction or ulceration, and delay in gastric emptying [4 hour gastric emptying test (2)]. The management of gastroparesis is based on dietary therapy for restoration of fluids and electrolytes, nutritional support, and treating the underlying etiology such as optimization of glycemic control in patients with diabetes. Dietary therapy should be supported with prokinetic agents to accelerate gastric emptying and improve gastroparesis symptoms. Devices such as stents, and endoscopic approaches such as per-oral endoscopic myotomy of the pylorus, electric stimulation, or surgery are being proposed (5).

Functional dyspepsia (FD) is a very common cause of substantial morbidity, estimated to affect 10% of the population, and manifest as abdominal pain/discomfort after eating that is present at least three days per week. It has been estimated that 40% of patients with this symptom complex consult their physicians, with impact on their workplace attendance and productivity and an economic impact in excess of 18 billion dollars in 2009 (6). The pathophysiology of FD is most likely heterogeneous, with different underlying mechanisms contributing to somewhat more specific diverse symptom patterns (7). Impaired gastric accommodation to a meal and *delayed gastric emptying and hypersensitivity* to gastric distention are the mechanisms classically implicated in postprandial distress syndrome (a subtype of FD that is typically associated with normal gastric emptying). *Impaired gastric accommodation* to a meal is present in up to 50% of patients with FD, is associated with early satiation and weight loss, and may result in redistribution of the meal to the distal stomach and more *rapid* gastric emptying (8). Visceral hypersensitivity is present in approximately one-third of patients with FD and is associated with higher intensity ratings of all epigastric symptoms including pain (9). Visceral hypersensitivity is supported by functional brain imaging studies or lack of anti-nociceptive response to gastric signals and by co-morbid psychosocial disorders such as anxiety, depression and somatization. Delayed gastric emptying occurs in up to one-third of patients with FD and, in some series, has been associated with postprandial fullness, nausea and vomiting. More recently, impaired duodenal mucosal integrity, with low-grade mucosal inflammation characterized by eosinophils and mast cells, has been reported as a putative pathophysiological mechanism in FD (10,11).

Current medical treatments include eradication of *H. pylori*, acid suppression, prokinetics, antidepressants, and psychological and alternative therapies; yet, despite this, many patients remain refractory to treatment, experiencing continued disabling symptoms (12).

Development of effective pharmacologic therapies for such disorders is desirable, as these conditions represent a significant unmet medical need. In clinical practice, more and more patients are resorting to the use of cannabis or opioids, or registering for medical marijuana or opioid treatment through their state-approved process for relief of abdominal symptoms. The symptoms include nausea, dyspepsia and abdominal pain based on functional gastrointestinal (GI) disorders (FGID) (13), as well as relief of symptoms unresponsive to biologics in patients with inflammatory bowel disease, in whom there are increasing trends of opioid use disorder (14), especially in those with sustained poor quality of life (15).

Potential of Cannabinoid Agents in Treatment of Non-malignant Gastrointestinal Diseases

The endocannabinoid system consists of CB1 and CB2 receptors; the ligands of these receptors are anandamide and 2-arachidonyl glycerol (2-AG), and their respective ligand-inactivating enzymes (16) are fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MGLL). Endocannabinoids are synthesized in post-synaptic neurons and act as retrograde messengers binding to the presynaptic CB1 receptor through various effectors, reducing activity of protein kinases, modulating ion channels and neurotransmitter release, e.g., acetylcholine. Endocannabinoids may also activate the transient receptor potential (TRP) vanilloid type 1 (TRPV1), which is mainly expressed by primary afferent neurons and the orphan G protein-coupled receptors such as GPR555. Exogenous or administered cannabinoids can activate the same presynaptic receptors to modulate neurotransmitter release or sensory receptors.

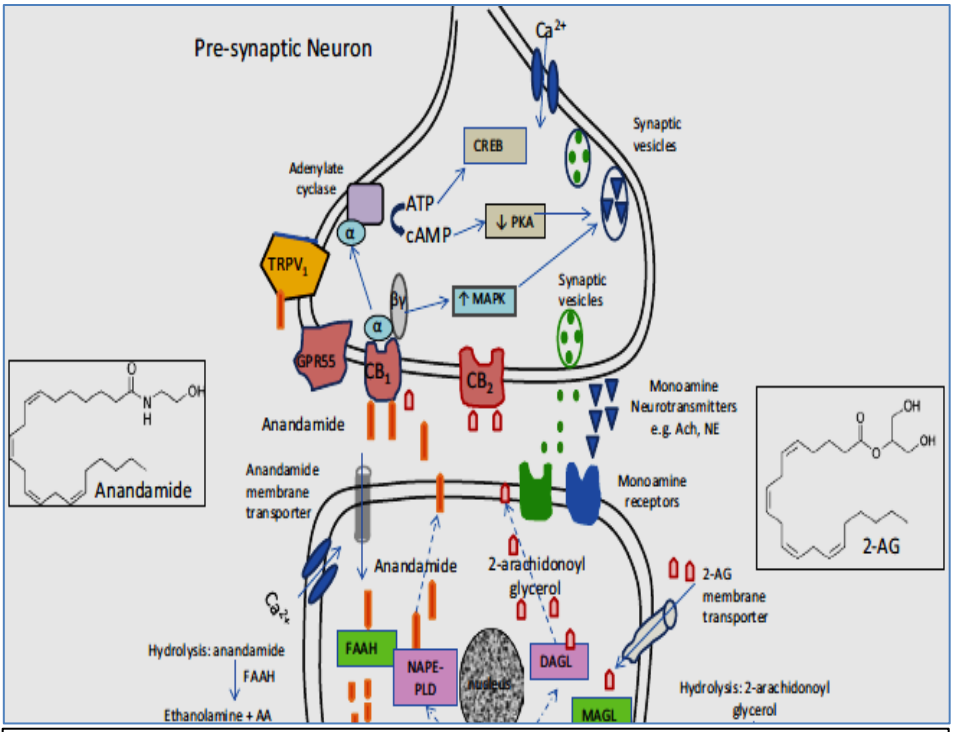


Figure 1. Synthesis, action, hydrolysis of endocannabinoids (from Camilleri 2018 (16)

Cannabinoids are involved in the regulation of food intake, nausea and emesis, gastric secretion and gastro-protection, gut motility, ion transport, visceral sensation, intestinal inflammation and cell proliferation in the gut (17). In prior studies, we assessed the potential role of genetic variations in cannabinoid genes for their associations with GI phenotypes as indirect evidence of the role of cannabinoid mechanisms in GI diseases and functions. The associations with **phenotypes such as the symptoms based on Rome III/IV criteria for FGID, including bowel dysfunction and pain, colonic transit, colonic sensation, gastric volume and satiation, support the notion that the endocannabinoid system modulates gastric and colonic functions**. These are documented by our published observations based on in 62 overweight/obese subjects for gastric phenotype, 75 irritable bowel syndrome (IBS) patients (35 IBS-D, 35 IBS-C, 5 IBS-A) for colonic sensation and motility index measurements, 102 IBS patients for rectal sensation (18,19). We also previously reported association of genetic variants in *FAAH* and *CNR1* and IBS symptoms, motor and sensory phenotypes based on 455 FGID patients and 228 healthy controls (18-20).

a. *FAAH* (C385A; rs324420) CA/AA genotype increases the odds (relative to HV) for IBS-D (P=0.008), IBS-alternating (IBS-A) (P=0.012) and chronic abdominal pain (P=0.055).
b. *FAAH* rs324420 CA/AA genotype was associated with accelerated colonic transit in IBS-D (P=0.037).
c. *CNR1* rs806378 (P=0.014; CC vs. CT/TT) was associated with colonic transit in IBS-D; the TT group had the fastest colonic transit at 24 and 48 hours.
d. *CNR1* rs806378 was associated with colonic higher sensation rating (CT/TT vs. CC) of gas (P=0.025) in the IBS-D or IBS-A groups.
e. *FAAH* rs324420 (CA/AA vs.CC) influences fasting colon motility index in patients with IBS-D and IBS-A.
f. *CNR1* rs806378 genotype associated with reduced fasting gastric volume (CC vs. CT/TT) (P=0.031).

g. *FAAH* rs324420 CC genotype was associated with a lower maximum tolerated volume (MTV) of Ensure® in a lab-based satiation test compared to CA/AA group (P=0.046).

These observations also provide further rationale for proposing pharmacological modulation of the cannabinoid mechanisms to normalize gastric functions in patients with functional gastrointestinal disorders.

We choose to focus on the upper gastrointestinal diseases because of the greater unmet clinical need.

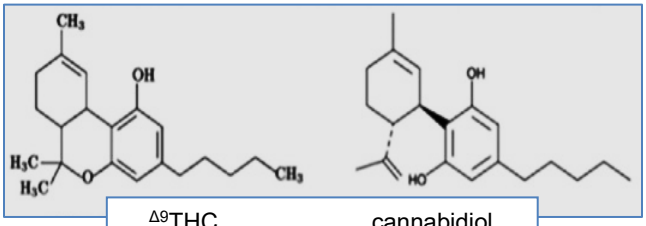
While there are several potential selective agonists and antagonists used in experimental animal studies (Table 1), to date the only one available for human studies has been dronabinol (DRO), a non-selective cannabinoid receptor agonist.

Agonists	
Plant derived	
Δ^9-THC	Main psychoactive cannabinoid in the marijuana plant
Δ^8-THC	Slightly less potent than Δ^9-THC
11-OH-Δ^9-THC	Bioactive compound formed when the body breaks down Δ^9-THC
Animal derived	
Anandamide	2-AG
THC analogs	
Dronabinol	Nabilone, CP-55,940, HU-210, levonantradol
Different chemical structure	
WIN-55,212	Binds to both cannabinoid receptors
Antagonists	
SR-241716A	Synthetic CB1 antagonist
SR-144528	Synthetic CB2 antagonist
AM841	CB1 mega-agonist with negligible central effects at doses that potently reduce GI motor function
WIN-55,212-2	A weaker CB1R/CB2R non-selective cannabinoid agonist

Table 1. Potential selective agonists and antagonists used in experimental animal studies

Pharmacology and Actions of Cannabidiol

A novel phytocannabinoid agent has been recently approved [Epidiolex® (cannabidiol)] by the FDA for use in seizure disorders. A selective CB2 receptor agonist (APD371) is being studied in IBD and pain (Clinical Trials .gov Identifier: NCT03155945) but is not available for research. The rationale for focusing on CB2 receptor agonist is provided below**.** From a recent review in Nature Reviews in Drug Discovery (21), several synthetic CB1 or CB2 modulators are being tested for diverse indications (GW842166, S-777469, JBT-101 [all CB2 selective agonists], SAB378, NEO1940 [both peripherally-restricted CB1 and CB2 receptor agonists], and PF-04457845, URB597, V158866, JNJ-42165279, BIA 10-2474 [FAAH inhibitors] and PF-06818883 and ABX-1431 [both MAGL inhibitors]), but they are not available for our studies.



$^{\Delta 9}$THC cannabidiol

Figure 2. 2-dimensional molecular structures of $^{\Delta 9}$THC and cannabidiol

We propose to focus our studies on cannabidiol (CBD) because of its approval status by FDA, its new availability as a pharmaceutical grade preparation, and increased evidence of its potential in the relief of pain and discomfort cannabidiol while being generally regarded as being devoid of "euphoric" cannabinoid-like side effects in contrast to $^{\Delta 9}$THC; 2-D structures of the 2 compounds are shown in Figure 2.

The **potential modes of benefit of cannabidiol** include:

a. targeting (as an inverse agonist) of G protein-coupled receptors 3, 6, and 12 [GPR3, GPR6 (22), and GPR12 (23)], a family of closely-related orphan receptors that are phylogenetically most closely related to the cannabinoid receptors, and are reported to play important roles in many normal physiological functions and to be involved in a variety of pathological conditions. For example, GPR3 (24) orphan receptor is involved in *neuropathic pain* after peripheral nerve injury and regulates morphine-induced anti-nociception.

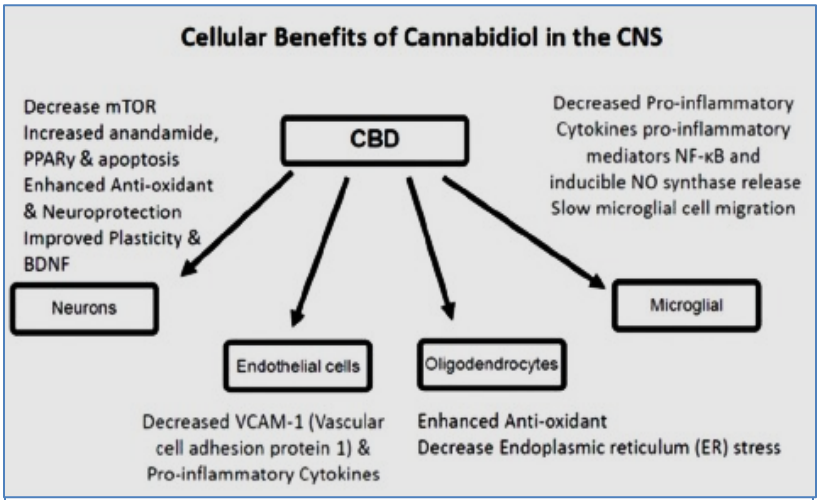


Figure 3. Cellular targets of cannabidiol (and endocannabinoids) include neurons, endothelial cells, oligodendrocytes and microglial cells (from reference 26)

b. non-psychotropic plant cannabinoids, including cannabidiol, dose-dependently activate and rapidly desensitize TRPV1, as well as TRP channels of subfamily V type 2 (TRPV2) and subfamily A type 1 (TRPA1), based on patch clamp analysis in transfected HEK293 cells (25).

c. anti-inflammatory effects, which are thought to be mediated by effects of cannabidiol on CB2 receptors (26), impacting diverse cellular functions, as summarized in Figure 3, that may lead to neuroprotection. Cannabidiol appears to stimulate synaptic plasticity and facilitates neurogenesis that may explain its positive effects on attenuating psychotic, anxiety, and depressive behaviors.

d. targeting 5-HT_{1A} receptors (25) for relief of experimental pain; note that 5-HT_{1A} receptors modulate gastric accommodation (27).

Conversely, the literature suggests the effects of *other* cannabinoid agents on pain are controversial:

A. A Cochrane review (28) concluded that potential benefits of cannabis-based medicine (herbal cannabis, plant-derived or synthetic THC, THC/CBD oromucosal spray) in chronic neuropathic pain might be outweighed by their potential harms.

B. A separate systematic review that included 24 RCTs and 1334 patients concluded that, though some RCTs showed a clinically significant improvement with a decrease of pain scores, the majority of the studies did not show an effect on pain (29), possibly indicating an effect on well-being or mood, rather than pain sensation.

C. An experimental brain imaging study showed that different cannabinoid-based drugs had heterogeneous effects on brain imaging (30), anxiety and the perception of experimentally-induced pain.

D. Thus, $^{\Delta 9}$THC *increased anxiety*, and levels of intoxication, *sedation, and psychotic symptoms*, whereas there was a trend for a reduction in anxiety following administration of cannabidiol in addition to a reduction in only the affective, but not the sensory perception of pain, which was associated with reduced blood oxygenation level-dependent signal in "affective regions" such as amygdala and the anterior and posterior cingulate cortex rather than the frontal and somatic cortex.

E. A randomized, controlled trial of $^{\Delta 9}$THC and placebo in 3 forms of chronic abdominal pain showed no significant effects of treatment with $^{\Delta 9}$THC (31).

Published Human Data on Pharmacodynamics and Pharmacogenetics with Cannabinoid Agonist

In our prior studies (16,32,33) [*NIH R01-DK079866 Cannabinoid mechanisms in human GI motor & sensory functions*], we have shown that DRO retarded gastric emptying especially in female patients (Figure 4) and increased fasting gastric volume in males, and inhibited colonic tone and phasic pressure activity (Figure 5).

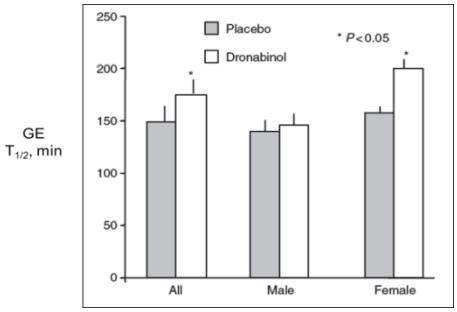


Figure 4. DRO retards gastric emptying especially in females

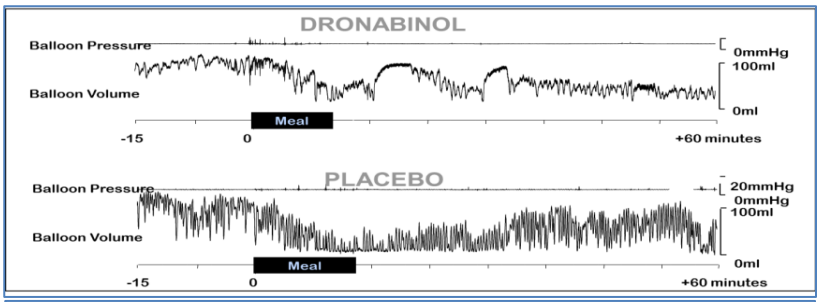


Figure 5. Colonic tone and phasic pressure activity measured with intracolonic DRO inhibits postprandial phasic contractility and tone (reduced volume under barostat conditions

However, DRO did not significantly alter thresholds for first gas or pain sensation during stepwise distension in the descending colon, but increased sensory rating for pain during phasic distensions. DRO may worsen pain.

It is conceivable that biological variations in inactivation of the endocannabinoids or in the CBR1 receptor could impact the response to exogenously administered cannabinoids. Our prior studies also assessed the potential role of genetic variations in cannabinoid genes for their associations with pharmacological responses to DRO (pharmacogenomics) (34). Thus, we demonstrated, in patients with IBS-D, a treatment-by-genotype effect whereby DRO preferentially delayed colonic transit in those with the *CNR1* rs806378 CT/TT genotypes. There was no significant interaction of treatment with *FAAH* rs324420 detected.

Gaps in Current Knowledge and Unmet Clinical Needs

There have been **no new medications approved for patients with gastroparesis and functional dyspepsia since 1979.** The only currently approved medication, metoclopramide, is poorly efficacious, is associated with an FDA black box warning, and often results in adverse neurological effects, some of which, like tardive dyskinesia, can be permanent. There is a need for new medications. While there are multiple potential positive effects of cannabidiol on pain mechanisms, it is still unclear whether they benefit **visceral pain and other symptoms** in humans. However, the potential combination of effects on pain mechanisms, as well as neuroprotective anti-inflammatory effects suggest cannabidiol differs significantly in its potential efficacy and safety (without inducing anxiety or euphoric effects) relative to $^{\Delta 9}$THC.

Our lab is uniquely poised to conduct the proof of concept and clinical studies necessary to start new approaches to treat patients with these conditions which are associated with high morbidity.

APPROACH: GENERAL METHODS

Study Design, Randomization and Allocation

We shall conduct **randomized, double blind, placebo-controlled, parallel-group trials** of the effects of cannabidiol on gastric motor functions, satiation, and symptoms during 4 weeks' treatment in single-center studies in patients with gastroparesis (aim 1A) or functional dyspepsia with non-delayed gastric emptying of solids (aim 1B). In addition to 4 weeks of treatment there is another week required for de-escalating the study medication, so the patients will be on treatment for a total of 5 weeks. A randomization schedule, computer-generated by the study statistician's office, will be submitted to the Mayo Clinic CCaTS Research Pharmacy. Allocations will be concealed; studies will be blinded until locked data are transmitted to the statistician.

Participants Eligibility Criteria

Patients with gastroparesis or functional dyspepsia (Appendix 1), age 18-70 years, will be recruited and enrolled after providing written informed consent. Patients will be identified from among Mayo Clinic patients or non-Mayo patients through Clinicaltrials.gov .Patients will have symptoms consistent with gastroparesis based on a national guideline (2) for gastroparesis (symptoms PLUS delayed gastric emptying of solids). Patients with Rome IV criteria for postprandial distress syndrome (a subset of functional dyspepsia) (35) will be selected based on *gastric emptying of solids which is NOT delayed*, in addition to standard FD criteria:

1. Symptoms fulfilled for the last 3 months with onset ≥6 months before diagnosis:
2. ≥ One symptom being bothersome: postprandial fullness, early satiation, epigastric pain or burning
3. Must include one or both of the following at least 3 days per week:
   a. Bothersome postprandial fullness (i.e., severe enough to impact on usual activities)
   b. Bothersome early satiation (i.e., severe enough to prevent finishing a regular-size meal)
4. No evidence of organic, systemic, or metabolic disease to explain the symptoms on routine investigations.

Participants will have previously undergone test of gastric emptying of solids using the standardized Mayo Clinic scintigraphic method (4h 320kcal egg, 30% fat meal). Subjects may be involved in study activity for up to 10 weeks from screening to completion. In the event that a potential participant with reasonable clinical evidence of gastroparesis has not previously undergone testing of gastric emptying of solids using the standardized Mayo Clinic scintigraphic method, gastric emptying testing done at baseline may be used to determine eligibility.

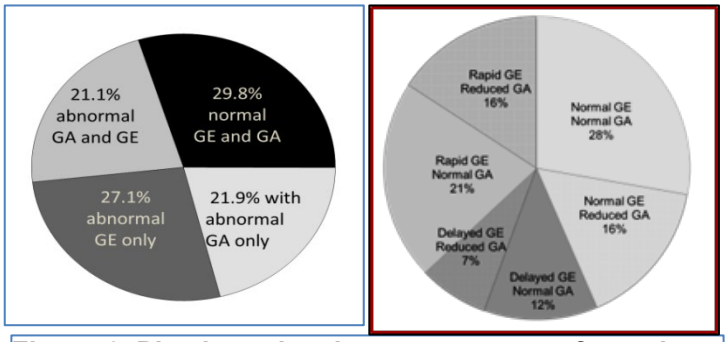


Figure 6. Pie chart showing percentages of gastric motor functions in 1276 patients (37) with functional upper GI symptoms and 108 patients with diabetes

In recent studies from our clinical motility group (37,38), we identified 1276 patients with upper gastrointestinal symptoms who were evaluated at Mayo Clinic over a 15-year period, and whose records were accessed through the electronic medical record: 953 females (74.0%); mean age was 43.1±15.4 years (SD), and the mean BMI was 24.7±6.2 kg/m^2. There were 175 (13.6%) who had treated hypothyroidism, 109 (8.54%) with diabetes, and 20.8% with psychological or psychiatric disorders. These data (37) suggest that results from studies on patients included in our study will provide generalizable data.

Standard inclusion criteria will include: ability to provide informed consent, absence of other diseases (structural or metabolic) which could interfere with interpretation of the study results, body mass index of 18-40 kg/m^2 and, for females, must not be pregnant or lactating due to administration of study medications and radiation exposure, 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 for secretin PAM) are permissible. Also subject will be included if they are on stable (no change in past month) doses of the following medications listed in table 1b.

Specific eligibility criteria regarding gastric emptying (320kcal, 30% fat meal) in different specific aims will be:

a. For gastroparesis (aims 1a): <25% emptied at 2 hours for females and <28.4% emptied at 2 hours for males, and/or >23.8% retained for females (<76.2 emptied) at 4 hours and >23% retained for males (<77% emptied) at 4 hours

Table 2 (A) Values of gastric emptying at different times and inter-individual variation (B) Values of gastric emptying $T_{1/2}$ on two repeat studies, and intra-individual variation

(A)

	GE $T_{1/2}$ min	GE 1 h, %	GE 2 h, %	GE 3 h, %	GE 4 h, %
All participants					
Mean ± SD	121.7 ± 29.8	18.1 ± 9.5	51.4 ± 15.7	78.1 ± 14.5	93.2 ± 8.9
Median (5th, 95th %ile)	120 (78.4, 174.0)	17 (4.4, 35.0)	50 (25.0, 78.5)	80 (52.0, 98.0)	96 (76.2, 100.0)
N	319	319	319	314	315
COV_{inter}, %	24.5	52.7	30.6	18.6	9.6
Females					
Mean ± SD	127.7 ± 28.7	16.5 ± 8.3	47.8 ± 14.3	75.3 ± 14.2	92.1 ± 9.4
Median (5th, 95th %ile)	125 (89.0, 180.0)	16 (4.3, 31.4)	47.2 (25.0, 71.0)	76 (50.0, 95.9)	94.8 (76.2, 100.0)
N	214	214	214	211	211
COV_{inter}, %	22.5	50.5	29.9	18.8	10.2
Males					
Mean ± SD	109.9 ± 28.6	21.3 ± 10.9	58.6 ± 15.1	83.8 ± 13.6	92.1 ± 9.4
Median (5th, 95th %ile)	105 (73.2, 165.0)	19 (4.7, 40.0)	60.0 (28.4, 82.0)	88 (55.0, 100.0)	98.3 (77.0, 100.0)
N	105	105	105	103	104
COV_{inter}, %	26.0	51.3	27.5	16.2	7.7

b. For functional dyspepsia (aim 1b): <25% retained at 4 hours (i.e., normal gastric emptying of solids)

Participants will have baseline screen for anxiety and depression by questionnaire **(Appendix 2).**

Exclusion Criteria:

1. Patients with current *H. pylori* infection will be excluded.
2. Pregnancy or lactation
3. Ultra-rapid metabolizers for CYP2C19 [estimated prevalence of 17% and 18% respectively based on literature review (36)] will be excluded since this could impact assessment of effects of cannabidiol
4. Patients with abnormal baseline liver transaminases (greater than two times the upper limit of normal), since up to 3-fold, dose-related elevations of liver transaminases (ALT and/or AST) occur in 13% of treated patients (vs. 1% placebo);
5. Hypersensitivity to cannabidiol or any of the ingredients in EPIDIOLEX
6. Concomitant use of valproate, other hepatotoxic drugs

7. The subject has HbA1c > 12%
8. The subject is unable to withdraw any of the following medications listed in table 1a 48 hours prior to the study
9. The subject has participated in another interventional clinical study within the past two weeks.
10. History of recent surgery (within 60 days of screening)
11. The subject has a history diagnosis of post-surgical gastroparesis
12. A subject who in the determination of the investigator, possesses any condition that the investigator believes would put the subject at risk or would preclude the subject from successfully completing all aspects of the study.
13. Concomitant use of CNS depressants and/or alcohol within 48 hours of GI testing, unless able to maintain a consistent dosage throughout the study

Table 1a. Excluded Medications

- Drugs known to affect gastrointestinal transit or motor functions
- GLP-1 receptor or amylin agonists in patients with diabetes mellitus.
- Drugs known to alter NI transit including laxatives, magnesium or aluminum-containing antacids, prokinetics, erythromycin. Laxatives may be allowed per physician discretion if participants are still experiencing symptoms and if able to maintain a stable dose throughout the study.
- Opiates, including: dextropropoxyphene, tramadol, ketobemidone, methadone
- Antidiabetic treatment with pramlintide or glucagonlike peptide-1 receptor agonists.
- Phencyclidine
- Participants using Zofran for symptom management may be included per physician discretion if the dosage is stable and remains consistent throughout study participation.

Table 1b. Included Medications:

- Amitriptyline up to 50mg per 24 hours
- Nortriptyline up to 50mg per 24 hours
- Venlafaxine up to 150mg per 24 hours
- Duloxetine up to 120mg per 24 hours
- Paroxetine up to 50mg per 24 hours
- Escitalopram up to 20 mg per 24 hours
- Bupropion up to 300mg per 24 hours
- Buspirone up to 30mg per 24 hours
- Quetiapine up to 50mg per 24 hours
- Citalopram up to 40 mg per 24 hours
- Fluoxetine up to 60 mg per 24 hours
- Sertraline up to 100 mg per 24 hours
- Fluvoxamine up to 100 mg daily
- Gabapentin up to 1200mg daily
- Pregabalin up to 150mg daily

Baseline safety blood tests in all patients:

a. Serum creatinine, bilirubin, ALT, AST
b. CYP3A4 or CYP2C19 (pharmacogenetics)
c. Urine pregnancy test

Medication, Doses, and Mode of Administration

Cannabidiol or placebo will be administered as in trials for childhood syndromic seizure disorders (39-41), orally twice daily in equally divided doses starting at 2.5mg/kg per day and increasing by 2.5 to 5.0mg/kg weekly until the target dose of 20mg/kg is reached. The active treatment is a plant-derived pharmaceutical formulation of purified cannabidiol oral solution (100mg per milliliter). Cannabidiol and the matching placebo

solution (excipients alone) will be provided in identical 100ml amber glass bottles. At the end of the treatment period, the treatment solutions will be tapered (10% volume each day) over 7 days.
In accordance with FDA guidance, prior to starting treatment and at end of 1 month treatment, we shall obtain serum transaminases (ALT and AST) and total bilirubin levels. These tests will also be performed if patient develops clinical signs or symptoms suggestive of hepatic dysfunction (e.g., unexplained nausea, vomiting, right upper quadrant abdominal pain, fatigue, anorexia, or jaundice and/or dark urine); if such features develop the treatment will be interrupted or discontinued.

Common adverse events (>10% frequency) in the cannabidiol trials for childhood seizure disorders were somnolence, vomiting, fatigue, pyrexia, upper respiratory tract infection, decreased appetite, convulsion, lethargy, somnolence, and diarrhea. Elevated aminotransferase levels have been observed. In the trials to date, cannabidiol lacked the psycho-activity of the archetypal cannabinoid, $^{\Delta 9}$THC, consistent with lack of appreciable affinity or activity at the cannabinoid receptors. Cannabidiol did not provoke suicidality. As a precaution, we shall use the approved assessment instrument, the Columbia Suicide Severity Rating Scale (**Appendix 3**) and the study team will assess the patients by phone at weeks 1 and 2, and in person at week 4. If suicidal thoughts and behavior emerge during treatment, the treatment will be discontinued.

Randomization: A randomization schedule, computer-generated by the study statistician's office, will be submitted to the Mayo Clinic CCaTS Research Pharmacy. Allocations will be concealed. Medications will be *stored* in a monitored, climate-controlled environment according to manufacturer's directions. Monitoring records will be available for review to ensure quality control.

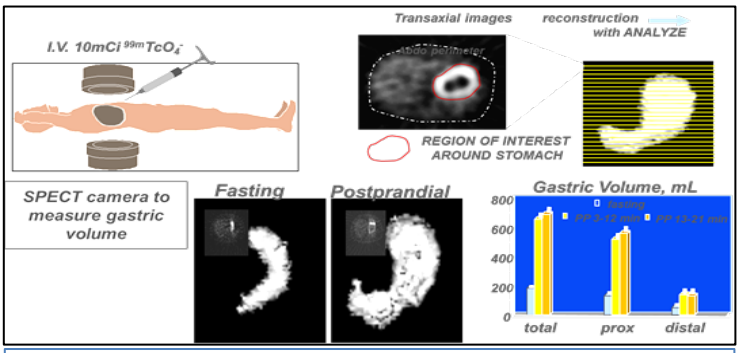


Figure 7. SPECT measures gastric accommodation

Quantitative Traits: On different days, participants will attend Mayo Clinic Clinical Research Trials Unit (CRTU) after an 8-hour fasting period, and validated quantitative traits will be measured.
Gastric Accommodation: Fasting, postprandial and accommodation gastric volumes will be measured by single photon emission computed tomography (SPECT) (42) (Figure 7), which was developed and validated in our lab.
Gastric Emptying: Gastric emptying (GE) will be measured by radioscintigraphy after overnight fast using a ^{111}In radiolabeled meal consisting of 2 scrambled eggs, one slice of whole wheat bread and one glass of skim milk (320kcal, 30% fat). Performance characteristics and normal values (214 female and 105 male volunteers) are published (43). A variable region of interest program will be used to quantitate counts in the stomach. Data will be summarized as GE $T_{1/2}$ and GE % emptied at 2 and 4 hours. Note that in healthy controls, gender, but not age or BMI, was associated with GE of solids (GE $T_{1/2}$, proportion emptied at 1 hour and 2 hours, all $p<0.001$) (43).

Table 2. Mean ± SD for gastric emptying (healthy adults) using the same method (43)

	All controls, n=319	Male controls, n=104	Female controls, n=215
Age , y	36.2 ± 13.1	34.6 ± 13.3	37.2 ± 12.8
BMI, kg/m^2	26.9 ± 5.1	27.8 ± 4.5	26.5 ± 5.3
Solid GE $T_{1/2}$	121.7 ± 29.8	109.9 ± 28.6	127.7 ± 28.7

***Glucose monitoring in relation to measurement of GE*:** If the fasting glucose is >250mg/dl on either test day, diabetic subjects will be managed following standard of care in clinical setting. The recommended insulin doses will follow standard care: "Insulin sliding scale with Insulin regular: glucose 250-259 mg/dl = 3 units; 260-299 mg/dl = 4 units; 300-339 mg/dl = 5 units; 340-379 mg/dl = 6 units; 380-399 mg/dl = 7 units; ≥400 mg/dl = call physician to assess subject and to determine continued progression of study activities."
Symptoms during GE study: We shall measure aggregate and individual symptom scores (nausea, bloating, fullness, pain) area under the curve (AUC) during the 4 hours after the standard meal during the GE test.

Satiation

Ensure® nutrient drink test (1kcal/mL, 11% fat, 73% carbohydrate, and 16% protein), which was developed and validated in our lab (44), with ingestion at a constant rate of 30ml/min will measure volume to

fullness (VTF) and maximum tolerated volume (MTV). Briefly, participants record their sensations every 5min using a numerical scale from 0 to 5, with level 0 being no symptoms, level 3 corresponding to fullness sensation after a typical meal, and level 5 corresponding to the MTV (maximum or unbearable fullness/satiation). Nutrient intake is stopped when subjects reach the score of 5. Postprandial symptoms of fullness, nausea, bloating, and pain are measured 30min after the meal using 100mm horizontal visual analog scales, with the words "none" and "worst ever" anchored at each end (**Appendix 4**).

Patient Reported Outcomes (PROs): Symptoms during 4 Weeks of Randomized Treatment

1. Assessment of upper gastrointestinal symptoms in patients with gastroparesis: ANMS GCSI-DD

Every day, participants will fill in the GCSI-DD questionnaire (**Appendix 5**) (44) which covers the relevant symptoms from the patient's perspective. It appraises 9 symptom severity items, which cover the following domains: nausea/vomiting (three items); fullness/early satiety (four items); and bloating (two items). The diary also contains items on the frequency of vomiting and retching, symptom severity items for upper abdominal pain and upper abdominal discomfort, and an overall rating of gastroparesis severity. Symptoms are rated by the patients from none (0) to very severe (5). The total GCSI-DD is calculated representing the mean of the three subscale scores of nausea/vomiting, postprandial fullness/early satiety, and bloating. Responsiveness of this instrument has been demonstrated by prospective assessment of 69 patients (46 idiopathic, 19 diabetic, and 4 post-fundoplication gastroparesis). Excellent test-retest reliability was seen for GCSI-DD scores, and there were significant correlations between GCSI-DD scores and clinician ratings of symptom severity. Responders to treatment reported improvements in nausea [effect size (ES) =0.42, P<0.001], postprandial fullness (ES=0.83, P<0.001), bloating (ES=0.34, P<0.001), and early satiety (ES=0.53, P<0.001), but there were lower responses for upper abdominal pain (ES=0.29) and vomiting (ES=0.22; P=0.119). Minimal important differences were estimated based on baseline to 4 weeks changes in symptom scores for small improvements. Minimal important differences were 0.55 for nausea, 0.97 for excessive fullness, 0.63 for bloating, 0.77 for postprandial fullness, and 0.30 for abdominal pain (45).

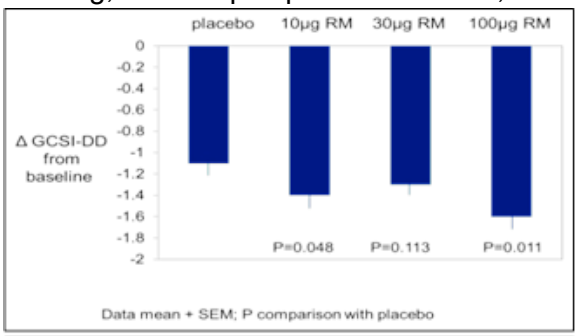


Figure 8. Effects of 12 weeks' treatment with relamorelin on symptoms using GCSI-DD

Recent data also demonstrated GCSI-DD responsiveness in diabetic gastroparesis with treatment of the pentapeptide ghrelin agonist, relamorelin (46). Therefore, the GCSI-DD is a valid patient reported outcomes instrument for use in patients with gastroparesis, and demonstrates responsiveness to a prokinetic.

2. Assessment of upper gastrointestinal symptoms in patients with functional dyspepsia

As in a previous large, multicenter study of pharmacotherapy in functional dyspepsia (47) anchored at Mayo Clinic, we shall assess patient reported outcomes (PROs) using validated instruments:

a. Weekly global symptom assessment will be measured through *adequate relief* of dyspepsia symptoms during the prior week (48,49). This self-report measure is considered clinically relevant and has been tested for responsiveness in functional dyspepsia (50,51).

b. Disease-specific validated Nepean Dyspepsia Index (NDI) (**Appendix 6**) will be used to assess functional dyspepsia quality of life (52) at baseline and post-treatment. NDI scores are summarized as overall quality of life and 5 subscales: interference, knowledge/control, eating/drinking, sleep disturbance, work/study (range: 0 to 100) (52). NDI also evaluates mean symptom scores for abdominal pain and postprandial distress.

c. Validated daily symptom diaries assessing upper abdominal pain, nausea, bloating, fullness, and early satiety on a scale of 0 to 3 (0 nil; 1 mild; 2 moderate; 3 severe) using a validated instrument (53) and simply tabulating the information.

d. Leuven Postprandial Distress Scale (LPDS), the most validated symptom scale available with psychometric evidence supporting the LPDS's individual items, as well as evidence of responsiveness to treatment (54-56), comprises eight symptoms (early satiation, postprandial fullness, upper abdominal bloating, epigastric pain, epigastric burning, nausea, belching and heartburn) with verbal descriptors rated for severity (0-4). The symptoms early satiation, postprandial fullness, and upper abdominal bloating constitute the most valid symptom group for postprandial distress in FD with an established minimal clinically important difference.

e. Functional Dyspepsia Symptom Diary (FDSD), a content-valid measure (57), with psychometric evidence supporting the FDSD's individual items (stomach pain, stomach burning, nausea, bloating, stomach fullness,

early satiety, burping/belching rating, burping/belching bothersome) measured on 11-point (0-10) scale and total score (stomach pain, stomach burning, bloating, stomach fullness, early satiety: maximum score of 50).

Pharmacogenomics: Collection and storage of venous blood for DNA studies: All participants in the trials in aim 1 will have 10mL venous blood sample for DNA extraction and storage. Genotyping of FAAH (rs324420), and CNR1 (rs806378) will be performed using Taqman™ SNP Genotyping assays (Applied Biosystems Inc., Foster City, CA, USA) in accordance with the manufacturer's instructions.

Rationale for the selection of candidate cannabinoid genetic polymorphisms

CNR1 (the gene for CB1 receptor) has 2 synonymous single nucleotide polymorphisms (SNPs), rs35057475 and rs1049353; neither results in a change in amino acid sequence. There is an (AAT)n repeat at the 3' end of *CNR1*, 18,000 bases downstream from the start site of exon 4 of CNR1. The number of repeats is highly variable (from 2 to 6 repeats) and, although associations with psychiatric disease, i.v. drug use, and response to antipsychotics have been described (58-62), the functional significance of this genetic variation is unclear. The T allele of ***CNR1* polymorphism rs806378** is associated with altered nuclear protein binding in an electrophoretic mobility shift assay, suggesting that rs806378 is a **functional** polymorphism (63). We previously demonstrated **association of this genotype** with gastric and colonic functions (see Background). ***FAAH* 385C to A allelic variant (rs324420)** leads to a Pro129Thr amino acid sequence change, which decreases expression of the FAAH protein due to reduced protein stability (64). The prevalence of the A allele is 16%–25% in studies of Caucasians in the NCBI database. Our laboratory has previously confirmed the A allele frequency to be 25% in our sample population of healthy controls and IBS patients in southeastern Minnesota (18). Reduction in FAAH protein level and activity compromises inactivation of the endocannabinoid anandamide, which leads to higher synaptic concentrations of anandamide and resulted in a greater effect of the exogenously administered cannabinoid agent, dronabinol, via activation of CB1 and CB2 receptors.

In addition, we shall screen patients' ***CYP3A4*** and ***CYP2C19*** genes, which impact the main CYP450 enzymes responsible for cannabidiol metabolism (65). Ultra-rapid metabolizers for CYP2C19 [estimated 17% and 18% based on literature review (36)] will be excluded since this could impact assessment of effects of cannabidiol.

General Approach for Statistical Analyses

The analyses will be based on a 2-treatment design to compare the effects of each medication tested and placebo on *post-treatment gastric functions, satiation, incretins and symptoms*. The analyses will use a 2-way analysis of covariance (ANCOVA) model with treatment as the quantitative trait, and a treatment by quantitative trait interaction term. It is anticipated that all primary and secondary endpoints will be normally distributed. If the data are not normally distributed, we shall use an empirical normal quantile transformation also known as the van der Waerden normal scores (66,67), or the generalized linear mixed models (GLMM) that provides a rich class of statistical models to model correlated data with responses from the exponential family of distributions including Gaussian, Binomial, Poisson among others (68). Covariate analysis may be conducted using gender and BMI as covariates, since these may significantly affect gastric functions such as satiation maximum tolerated volume, and using smoking and gender as covariates, since these may significantly affect drug levels of secretin. Symptom relief in aim 2b will be evaluated for treatment effects using a logistic regression model, with adequate relief as the binary dependent variable (responder definition based on at least 50% of weeks of symptom relief).

Analysis: Primary endpoints for this study will be:

- Fasting gastric and accommodation volumes measured by SPECT
- Volume to fullness (VTF, mL) on satiation test
- Gastric emptying $T_{1/2}$ of solids on scintigraphy
- Average weekly GCSI-DD score on treatment in patients with gastroparesis
- Postprandial distress score on Nepean Dyspepsia Index in patients with functional dyspepsia

Secondary endpoints will be**:**

- Absolute postprandial gastric volume by SPECT
- Gastric emptying of solids at 2h and 4h on scintigraphy
- Aggregate symptoms and individual symptom scores (nausea, bloating, fullness, pain) area under the curve (AUC) during the 4 hours after the standard meal during the gastric emptying test
- Maximum tolerated volume (MTV) and aggregate symptoms score 30min after MTV on satiation test
- Individual symptom scores (nausea, bloating, fullness, pain) on satiation test
- Individual subscales of GCSI-DD (3 subscales: nausea/vomiting, postprandial fullness/early satiety, and bloating) in patients with gastroparesis (aim 1a)

- Overall Nepean Dyspepsia Index (NDI) score in functional dyspepsia (aim 1b)
- Abdominal pain score on NDI in functional dyspepsia (aim 1b)
- Proportion of responders based on adequate relief in functional dyspepsia (aim 1b)
- Average daily aggregate symptom score for upper abdominal pain, nausea, bloating, fullness, and early satiety (each on a scale of 0 to 3) in functional dyspepsia (aim 1b)

LPDS score and FDSD scores will be exploratory endpoints since they have only recently been validated.

Sample Size Assessment

This is based on results of the primary endpoints in our Mayo Clinic lab. Expected (80% power, α=0.05, assuming parallel-group study and unpaired analysis with n=22 in aim 1a and **n=24 aim 1b**) demonstrable differences compared to placebo show that the study is powered to detect clinically relevant effects of cannabidiol based on the variation (SD) observed from our Mayo prior studies (42-44,46,47).

TABLE 3. Sample Size Assessment: Response	Mean	SD	Effect size detectable (absolute [% of mean], n=24 per group
Fasting gastric volume, mL	273	57	47mL (17%)
Postprandial gastric volume, mL	848	111	91.7mL (10.8%)
Gastric emptying of solids $T_{1/2}$, min	122	29.8	24.62 (20.2%)
Volume to fullness, mL	755	330	273mL (36.2%)
Maximum tolerated volume, mL	1283	400	330.5mL (25.8%)
Δ from baseline in average (of 3 subscales in) GCSI-DD	-1.4	0.5	0.413 (29.5%)
Average Aggregate Symptoms Score for dyspepsia	1.42	0.64	0.50 (35.2%)
NDI overall quality of life score	63.6	22.5	18.6 (29.2%)
NDI abdominal pain score	29.4	10.4	8.6 (29.3%)
NDI postprandial distress score	12.8	7.5	6.2 (48.4%)

Therefore, the study has sufficient power to detect clinically relevant effects on quantitative traits. The study will provide important information on the mean response in clinically important endpoints in the two trials and, therefore, provide important preliminary information, such as coefficient of variation of the response in the current hypothesis-generating trials in order to plan future hypothesis-testing trials.

Interim Analysis: As part of an on-going method to compare the effects of each medication tested and placebo, an interim analysis will be conducted when 50% of the first specific aim has been reached.

Rationale for Interim Analysis:

a. To assess power of the study based on the actual coefficient of variation as measured in the actual study with >50% completed; this is because the coefficient of variation could be different from the COV used in the a priori power calculation used in development of the protocol.

b. As part of the analysis, the statisticians will also provide a comparison of the results on the primary and secondary endpoints; this assessment will be communicated to the investigators as average data (e.g. median and IQR) for the treatment GROUPS rather than individual patient data. These group data could be used for preparation of preliminary reports in the form of abstract for national meetings specifically, Digestive Disease Week to be held in May 2021 for which the deadline for abstract submission is December 1, 2020.

c. As a result of the power analysis, it is possible that the sample size may be either reduced or increased or the study could be stopped because of “futility” if there is no evidence of a trend to suggest efficacy of treatment.

Outcome of Interim Analysis:

In accordance with the approval from IRB to conduct an interim analysis after 50% of planned participants with gastroparesis had completed the study activities according to protocol, the data of 24 patients (that is 50% of the number originally proposed for the study) were analyzed by the study statistician, W Scott Harmsen MS.

The **objective of the interim analysis** is to determine what is the SAMPLE SIZE (currently planned at 24 per treatment arm, that is cannabidiol vs placebo, 1:1 ratio) required, based on the current data (group difference and coefficient of variation) to demonstrate EFFICACY based on the STATED PRIMARY endpoints in the NIH grant application:

Primary endpoints: Physiological traits and patient reported outcomes for cannabidiol vs. placebo groups:
(a). Gastric emptying T1/2 solids

(b). Fasting and accommodation gastric volumes
(c). Satiation by the maximum (max) tolerated volume
(d). Average daily (total) GCSI-DD score

Secondary endpoints:
1. Satiation assessed by the volume to fullness
2. Aggregate symptom score 30 min. after max. tolerated volume
3. GCSI-DD subscores of: nausea, vomiting, postprandial fullness, satiety, bloating, pain

Data Included in Interim Analysis: Among these 24 patients with documented diagnosis of gastroparesis, one randomized patient did qualify as the baseline gastric emptying was within the normal range at the time of this study. Therefore, data from 23 patients were included in the analysis. Physiological measurements at end of treatment were unavailable in 2 patients (one developed COVID infection, one had a family emergency). The summary data were provided by the study statistician, but the study blind was maintained and randomization code was only provided to the statistician from the research pharmacy. Gastric emptying $T_{1/2}$ values were censored to a maximum of 240 minutes since this was the last time point of data collection. The 2 groups were well-matched at baseline.

Data show median (IQR)	**Cannabidiol**	**Placebo**	**P value**
N (F and M)	10 (9 and 1)	13 (12 and 1)	1.0
Age (y)	45.3 (32, 57)	44.5 (36, 55)	0.93
BMI (kg/m^2)	26.1 (20.7, 29.1)	27.3 (25.7, 32,2)	0.42
Baseline GE $T_{1/2}$, min	208.2 (140.8, 240)	198.4 (172.5, 230.3)	0.88
Baseline GE lag time, min	84.5 (65.0, 111.8)	73.6 (64.0, 101.3)	0.64
Baseline feeling excessively full score	2.0 (2.0, 3.0)	2.0 (1.0, 2.0)	0.58
Baseline upper abdominal pain score	1.2 (0.8, 2.0)	1.0 (0.8, 1.8)	0.95
Baseline # vomiting episodes (maximum 5)	0.0 (0.0, 1.0)	0.0 (0.0, 1.0)	0.60
Baseline average GCSI-daily diary score	1.8 (1.4, 2.4)	1.2 (0.8, 1.8	0.29

Sample Size re-estimation based on the observed SD
To observe statistically significant differences in effects of cannabidiol and placebo with 80% power, the interim analysis shows the following changes relative to baseline (baseline MINUS end of treatment values shown) in the 2 treatment arms, conditional power and estimated number of patients required in each treatment arm:

	Placebo		**Cannabidiol**				
Measurement	Mean	SD	Mean	SD	T-stat	Conditional Power	Sample Size Re-est. (n_1=n_2)
Analyzed using ANCOVA for Change in measure Baseline to Post-Tx							
Δ TrGEt50LinearMax240	16.4	26.8	-7.7	48.9	-1.50	0.61	38
Δ Total GCSI Sx scores *(Q's 1 to 5)*	0.1	0.4	0.6	0.8	1.84	0.85	**22**
Δ Daily Q3 *Feel excess full*	0.2	0.6	0.5	0.6	0.93	0.80	64
Δ Daily Q4 *Upper Abdominal Pain*	0.0	0.8	0.6	1.1	1.50	0.62	37
Δ Daily Q5 *# Vomit (max=5)*	-0.2	0.6	0.8	1.5	2.16	0.93	**15**
Measurements without a Baseline measure							
Nutrient Drink *max tolerated Kcal*	639.5	286.7	856.3	343.7	1.65	0.71	30
Nutrient Drink *Kcal to fullness*	384.6	200.7	506.6	236.3	1.34	0.49	47

Based on Δ values, a positive number indicates an improvement in the parameter on treatment relative to baseline. A priori, conditional power of <0.20 was identified as indicative of futility to continue the trial. Given that several parameters of interest were associated with conditional power >0.20, and the sample size required to identify clinically important differences with n<24 per treatment group (which was originally planned in study protocol), the conclusions reached following the interim analysis were:

1. To continue the clinical trial with the same treatments (cannabidiol and placebo)
2. Reduce total sample size from 48 to 44
3. Retain strategies of concealed allocation, study blind of all investigators on the study

With larger sample size in the two treatment groups, it is conceivable that the SD and coefficient of variation will be smaller in the final study database, thereby providing potential to appraise other endpoints shown in the table, such as upper abdominal pain, maximum tolerated kcal, and GE $T_{1/2}$.

Subsequent Interim Analysis: As part of an on-going method to compare the effects of each medication tested and placebo, a subsequent interim analysis will be conducted when >85% of the gastroparesis group participants have completed study procedures.

Rationale for Subsequent Interim Analysis:
The objective of the interim analysis is not to change the current goal of evaluating 44 patients randomized to either cannabidiol or placebo as is currently detailed in the statistical analysis plan and was confirmed following an interim analysis performed for the purposes of evaluating statistical power and appropriate sample size.
This subsequent interim analysis is intended to provide an evaluation of data collected from more than 85% of the gastroparesis group participants. In this analysis, statisticians will provide a comparison of the results on the primary and secondary endpoints; this assessment will be communicated to the investigators as average data (e.g. median and IQR) for the treatment GROUPS rather than individual patient data in order for the investigators to remain blinded to the individual participant data and to the treatment group to which each individual had been assign by randomization. These group data would be used for preparation of preliminary reports in the form of abstract for national meetings, specifically Digestive Disease Week to be held in May 2023, for which the deadline for abstract submission is December 1, 2022.

Approval of this request for the subsequent interim analysis is necessary for the research pharmacy to send the randomization code exclusively to the statistician.

APPROACH

AIM 1a: Effects of treatment with cannabidiol on gastric functions, satiation and symptoms in patients with gastroparesis

Hypothesis 1a: Cannabidiol enhances gastric accommodation and reduces post-nutrient challenge symptoms without altering gastric emptying in patients with gastroparesis.

Specific Aim 1a: To compare effects of cannabidiol vs. saline on satiation, fasting gastric volume, gastric accommodation and emptying, and GCSI-DD in patients with gastroparesis

Rationale: Cannabidiol has multiple effects on GPCRs, 5-HT_{1a} receptors and neuroprotection to potentially impact gastric functions and pain in patients with gastroparesis. Pain is a significant unmet need in patients with gastroparesis. In the NIH Gastroparesis Consortium Patient Cohort, the predominant symptoms in 393 patients were pain/discomfort in 21% and nausea/vomiting in 44%. Pain was rated moderate or severe in 66% of those with pain. Idiopathic gastroparesis (256 patients) was correlated with opioid and antiemetic use, depression, anxiety, and poor QOL. Pain presentation was also not associated with the results of gastric emptying test, or with diabetic neuropathy or control of diabetes (69).

Experimental design: We will perform a randomized, double-blinded, placebo-controlled study of cannabidiol, 20mg/kg/day (achieved after dose escalation), and placebo, each in two divided doses with n=22 per treatment arm. All participants will have gastroparesis symptoms, prior documentation of delayed gastric emptying (GE) at Mayo Clinic, and will undergo *baseline measurements of GE to be used as a covariate* in assessing the response to treatment with cannabidiol. Randomization will be stratified on gender and BMI.

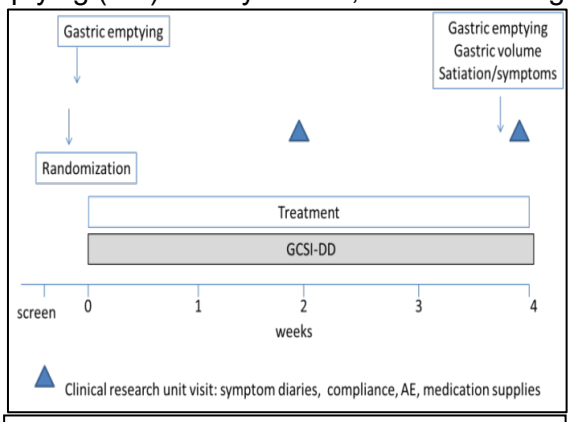


Figure 9. Experimental protocol to compare the effects of cannabidiol and placebo in gastroparesis

Experimental procedures and measurements: All participants will undergo measurements of GE of solids, gastric volume (fasting and postprandial), Ensure® nutrient drink satiation test (which also serves as a dyspeptogenic meal) and symptom assessment.
Details of each are provided in the Common Methods section.
Statistical analysis: ANCOVA will be used to compare the 2 treatment groups, including baseline GE $T_{1/2}$ as covariate.
Primary endpoints: Physiological traits and patient reported outcomes for cannabidiol vs. placebo groups:
(a). Gastric emptying $T_{1/2}$ solids
(b). Fasting and accommodation gastric volumes
(c). Satiation by the maximum (max) tolerated volume
(d). Average GCSI-DD score

Secondary endpoints:
1. Satiation assessed by the volume to fullness
2. Aggregate symptom score 30 min. after max. tolerated volume
3. GCSI-DD subscales: nausea/vomiting, postprandial fullness/satiety, bloating

Statistical power: Addressed in the Common Methods/Statistics section

Anticipated results compared to placebo:
a. Cannabidiol will increase fasting and accommodation gastric volumes, and calorie intake at satiation test without significantly retarding gastric emptying.
b. Cannabidiol will reduce average weekly symptoms score based on GCSI-DD.
c. We anticipate <5% dropouts due to medication intolerance or adverse events in this single-center study, consistent with experience from multicenter studies conducted in patients with syndromic seizures.

Potential pitfalls, precautions taken, and alternative strategies:
a. Feasibility: Given our 100-person database of patients with gastroparesis residing within 150 miles of Rochester, MN and with prior documentation of upper gastrointestinal symptoms consistent with gastroparesis, as well as documented delay in gastric emptying, we are confident that we can recruit the required number of participants for the studies that involve only noninvasive tests.
b. Consideration of sex as a biological variable: In our past cohort of 100 patients, 69% were female. Therefore we anticipate recruitment of 44patients will include sufficient numbers of male and female patients.
c. Scientific premise for inclusion of both genders: Although gastroparesis and functional dyspepsia are more prevalent in females, there is no a priori evidence that the effect of cannabidiol differs between genders and the pathophysiological mechanisms do not appear to be different between genders. Therefore, our study will assess treatment efficacy in **both genders;** we shall recruit numbers of men and women in ratio of 1:1.5.
d. Drop-outs: The hypothesis-testing study will utilize intention-to-treat principles. The analysis population will include all randomized subjects. We plan to include in the informed consent forms clear information to differentiate treatment discontinuation from study withdrawal, as well as a statement educating patients about the continued scientific importance of their data, even if they discontinue study treatment early. Therefore, patients will be followed after treatment discontinuation in order to preserve the ability to analyze endpoints for all participants who underwent randomization and, thus, to make possible intention-to-treat inferences (70).
e. Missing data: We shall explore three methods for data imputation:
(i) Imputation of any missing data for the primary endpoint (e.g. gastric emptying $T_{1/2}$) will use the overall mean $T_{1/2}$ for all subjects randomized, adjusting the error degrees of freedom (df) in the ANCOVA model by subtracting one df for each value imputed to provide a more accurate estimate of residual error variance.
(ii) Using a mixed-model repeated measures method, we shall assume that continuous repeated measures have a normal distribution with a specified form of mean and covariance matrix. With multiple imputations, multiple sets of plausible values for missing data will be created from their model-based predictive distribution, and estimates and standard errors will be obtained using multiple-imputation combining rules (50).

(iii) We shall use the last rank carried forward (LRCF) approach as described in O'Brien et al. (71).

In order to develop pre-specified monitoring and analysis plans, we shall establish a DATA SAFETY MONITORING PLAN which will involve review by experts independent of the study, using a team comprised of researchers with track records in biostatistics and clinical trials and expertise in the quantitative traits. We have established definite stopping rules for the trial.

Significance: This study will evaluate efficacy, safety, and mechanisms of action of cannabidiol in the largest single-center study to date, and it will provide information on the coefficient of variation in patient reported outcomes to plan future hypothesis-testing, multicenter studies of the effects of cannabidiol in gastroparesis.

AIM 1b: Acute effects of cannabidiol on gastrointestinal functions and symptoms in patients with functional dyspepsia and non-delayed gastric emptying

Hypothesis 1b: Cannabidiol enhances gastric accommodation and reduces post-challenge symptoms without altering gastric emptying in patients with functional dyspepsia with non-delayed baseline gastric emptying.

Specific Aim 1b: To compare the effects of cannabidiol vs. placebo on satiation, fasting gastric volume, gastric accommodation and emptying, and symptoms in patients with functional dyspepsia with normal baseline gastric emptying,

Rationale: A prominent pathophysiologic feature of functional dyspepsia is defective gastric accommodation, with inadequate relaxation of the fundus of the stomach in response to eating, which is responsible for the post-cibal pain. This was observed in 47% of 151 patients with functional dyspepsia in a Mayo Clinic study performed in the clinical practice of the PI (72). There are presently no generally effective medications to restore normal gastric accommodation, and there is only limited support, based on single-center studies, for use of such diverse medications as clonidine, sumatriptan, buspirone (many of which have central or vascular side effects), or for acotiamide, a cholinesterase inhibitor, approved for use in Japan, but not in the USA.

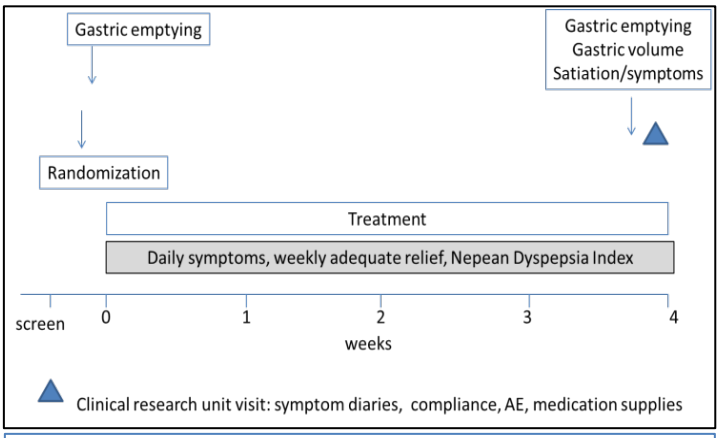


Figure 10. Experimental protocol

Experimental design: We shall perform a randomized, double-blinded, placebo-controlled study of cannabidiol. b.i.d., and placebo, b.i.d., with n=24 per treatment arm. All participants will have a history of functional dyspepsia with prior documentation of normal or accelerated gastric emptying at Mayo Clinic and will undergo *baseline measurements of gastric emptying to be used as a covariate* in assessing the response to treatment with cannabidiol. Randomization will be stratified on gender, and BMI.

Experimental procedures and measurements: All participants will undergo gastric emptying of solids, gastric volume (fasting and postprandial), satiation test (which also serves as a dyspeptogenic meal), symptoms assessment. Details of each are provided in the Common Methods section.

Statistical analysis: ANCOVA will compare the 2 treatment groups, including baseline gastric emptying $T_{1/2}$ as covariate. The endpoints of the study will be the same as in specific aim 1a, with the exception that symptoms/patient response outcomes will be based on proportion of average weekly adequate relief responders, NDI and daily symptom scores over the 28-day treatment trial, as in Common Methods section.

Statistical power: See Methods/Statistics section

Anticipated results compared to placebo: Cannabidiol will increase fasting and accommodation gastric volumes and calorie intake at satiation test without significantly retarding gastric emptying. Cannabidiol will increase the proportion of responders with at least 50% weeks with reported adequate relief, QOL scores and abdominal pain scores based on NDI at end of 4 weeks of treatment compared to baseline.

Potential pitfalls, precautions taken, and alternative strategies:

a. Feasibility: Given the community prevalence of functional dyspepsia and the review of medical records which identified >250 patients with functional dyspepsia symptoms without delayed gastric emptying seen in the past 5 years at Mayo Clinic (37), we are confident that we can recruit the required number of participants for the studies that involve only noninvasive tests.

b. Consideration of sex as a biological variable: In the ~1270 person database, 60% were female (37). We anticipate a female to male ratio of 1.5:1 to ensure we can assess sex as a biological variable.

c. Scientific premise for inclusion of both genders includes: Although functional dyspepsia is more prevalent in females, there is no a priori evidence that the effect of cannabidiol differs between genders, and the pathophysiological mechanisms do not appear to be different between genders. Therefore, our study will assess treatment efficacy in **both genders, and we shall recruit ~ equal numbers of men and women**.
d. Drop-outs and missing data will be managed as for specific aim 1a.
Significance: This study will evaluate efficacy, safety, and mechanisms of action of cannabidiol in the largest single-center study in functional dyspepsia to date, and it will provide information on the coefficient of variation in patient reported outcomes to plan *future hypothesis-testing, multicenter studies* of the effects of cannabidiol in patients with functional dyspepsia.

Aim 2: Treatment by genotype interaction of cannabidiol and *FAAH* and *CNR1* genes and gastric functions in patients with functional dyspepsia or gastroparesis
Hypothesis 2: *FAAH* rs324420 and *CNR1* rs806378 genotype significantly affect the response of gastric accommodation, gastric emptying and satiation to treatment with cannabidiol in patients with gastroparesis or functional dyspepsia with normal baseline gastric emptying.
Specific Aim 2: To assess pharmacogenetics effects of variants in *FAAH* and *CNR1* genes on the pharmacodynamics effects of cannabidiol vs. placebo on gastric accommodation, gastric emptying and satiation in the same patients with gastroparesis or functional dyspepsia.
Rationale: In prior studies, *FAAH* rs324420 and *CNR1* rs806378 gene variants were associated with gene by treatment interactions on diverse gastrointestinal motor functions in response to the non-selective cannabinoid agonist DRO (see background). Genotype distributions (18,19) in participants in prior Mayo Clinic studies are shown in Figures 11 and 12. We have previously demonstrated (18,19) the MAFs of the *FAAH* and *CNR1* variants in our catchment population are consistent with NCBI and other literature reports with *FAAH* MAF of 0.25 and *CNR1* MAF of 0.29.

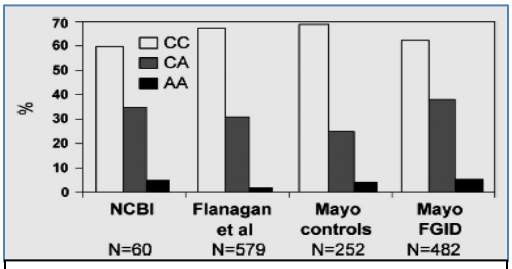


Figure 11: *FAAH* rs324420 genotype distribution in FGID and healthy controls studied at Mayo Clinic and reported distribution in NCBI and in a study mostly in Caucasians.

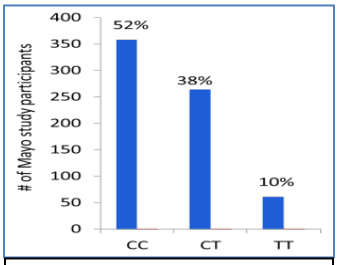


Figure 12: *CNR1* rs806378 genotype distribution in FGID and healthy controls at Mayo Clinic (mostly Caucasians)

Experimental design: From specific aim 1, there will be 46 patients (22 with gastroparesis and 24 with functional dyspepsia) treated with cannabidiol; we shall compare in these patients the association of gene variants on the effects of cannabidiol on gastric emptying using a dominant genetic model. The model will include gender, treatment (yes/no cannabidiol), gene variants, plus the interaction gene x treatment.
Experimental procedures and measurements: All participants will have undergone gastric emptying of solids, gastric volume (fasting and postprandial) and satiation test in specific aim 1. Genotyping for *FAAH* rs324420 and *CNR1* rs806378 and *CYP 3A4* and *2C19* will be performed. See common methods section.
Endpoints: (a) Gastric emptying $T_{1/2}$ solids; (b) Accommodation gastric volume; (c) Satiation by the MTV
Statistical considerations and analysis:
Applying the dominant genetic model, we anticipate there will be a 60:40 split of *FAAH* rs 324420 CC vs. CA/AA, and a 50:50 split for *CNR1*rs806378 CC vs. CT/TT.
Given there will be 46 participants receiving cannabidiol and assuming an approximate 50:50 split of the participants based on both genotypes (that is, 23 patients CC vs. 23 CA/AA *FAAH* rs 324420 or 23 CC vs. 23 CT/TT for *CNR1* rs806378), we anticipate the following effect sizes attributable to the gene variants (Table 4):

Table 4. Response	Mean	SD	Effect size detectable ([% of mean], n=24/ genotype group
Fasting gastric volume, mL	273	57	47mL (17%)
Postprandial gastric volume, mL	848	111	91.7mL (10.8%)
Gastric emptying solids $T_{1/2}$, min	122	29.8	24.62 (20.2%)
Volume to fullness, mL	755	330	273mL (36.2%)
Maximum tolerated volume, mL	1283	400	330.5mL (25.8%)

ANCOVA will compare the genotypes for each gene variant, including baseline GE $T_{1/2}$ as covariate.

Anticipated results: Effect of cannabidiol will be significantly associated with *FAAH* and *CNR1* genotypes consistent with the hypothesis that the gene variants impact the function of the receptor target or the inactivation of endocannabinoids that modulate gastric motor functions, or calorie intake at satiation test.

Potential pitfalls, precautions taken, and alternative strategies:

Feasibility: The study participants will be enrolled in specific aim 1; the power calculation based on our patient community gene minor allele frequency shows that the study has sufficient power to detect clinically relevant gene * treatment interactions.

Genetic variation in metabolism (inactivation) of cannabidiol: Ultra-rapid metabolizers for *CYP2C19* [estimated 17% and 18% based on literature review (36)] will be excluded since this could impact assessment of effects of cannabidiol.

Consideration of sex as a biological variable: In the ~1270 person database, 60% were female (37). We anticipate a female to male ratio of 1.5:1 to ensure we can assess sex as a biological variable.

Scientific premise for inclusion of both genders includes: Although functional dyspepsia is more prevalent in females, there is no a priori evidence that the effect of cannabidiol differs between genders, and the pathophysiological mechanisms do not appear to be different between genders. Therefore, our study will assess treatment efficacy in **both genders, and we shall recruit men and women in the ratio 1:1.5**.

Drop-outs and missing data will be managed as for specific aim 1.

Significance: This study will evaluate impact of common functional genetic variants in CB1 receptor and cannabinoid inactivation on the pharmacodynamics responses to cannabidiol, potentially important in individualizing therapy with cannabidiol in the treatment of gastroparesis and functional dyspepsia that may impact plan and design of *future hypothesis-testing, multicenter studies* of the effects of cannabidiol in patients.

Overall Significance of Application: The proposed studies provide the ability to validate a recently approved pharmaceutical grade cannabidiol for the treatment and addressing the unmet need of millions of American citizens with gastroparesis or functional dyspepsia, including personalization of treatment based on genotype by treatment interactions. This research team is uniquely poised to deliver on this vision.

Time Table: **Years 1-4:** specific aims 1, 2; **Year 5:** data analysis and publications

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