Individualized Pharmacological Approach to Obesity Management based on Obesity Phenotypes: A Randomized Clinical Trial (PHENO-MEDs trial)

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ABSTRACT

Introduction: Obesity prevalence continues to increase worldwide[7] and, in the United States, 69% of adults are overweight or obese[8]. Despite advances in understanding of aspects of obesity pathophysiology, <u>weight loss with current treatments including diet, exercise, medications, endoscopy and surgery is highly variable</u> [9]. However, there are usually great responders to each therapy, specifically "responders" to medications can lose as much weight and with less side effects than bariatric surgery. These individuals – the responders – can benefit from significant weight loss (>15% total body weight loss) which is known to reduce all-cause cardiovascular mortality and morbidity. With the current approach with pharmacotherapy, less than 35% of patients will lose more than 10% of body weight. Additionally, the high variability in weight loss response has resulted in a poor market penetration by new medications, devices and surgery. Clearly the one-treatment-fits all approach is not working and obesity management beyond diet and behavioral therapy continues to be a hit-or-miss intervention. One approach has been advocated where physicians select pharmacotherapy based on potential side effect and patient comorbidities [10], instead of choosing the right drug for the right patient based on its pathophysiology. To achieve the goal of individualizing treatment for obesity, it is essential to identify the best responders to each intervention, and hence maximize their weight loss. Recently, we acquired preliminary data to identify predictors of weight loss using gastrointestinal and behavioral traits (phenotypes) *[3]*.

Obesity can be sub-classified based on specific phenotypes in satiation (21%), gastric capacity (15%), behavioral (13%), gastric sensorimotor (11%) factors and others (40%)[3]. This obesity sub-classification may stratify patients for weight loss pharmacotherapy or bariatric endoscopy [3, 11-13]. Using this classification, the effect on weight loss of Phentermine-topiramate ER [3], and exenatide 5µg [11] can be enhanced, and the marked effect of Liraglutide 3mg on gastric emptying suggests it may be even more efficacious in patients with baseline acceleration of gastric emptying [11]. However, the results with Phentermine-topiramate ER [3], and exenatide 5µg [11] were determined in post-hoc analysis and each study was done independently. Thus, the identification of the obesity phenotype at baseline to guide obesity pharmacotherapy has not yet been tested and the outcome is unknown in the clinical setting. Thus, there is a **critical need to study the weight loss outcome using obesity phenotypes** to guide therapy for obesity.

We **hypothesize** that the identification of the obesity phenotype at baseline to guide obesity pharmacotherapy will enhance the weight loss response rate (i.e. percentage of patient with weight loss higher than 10% at 12 weeks).
Aim: To compare the weight loss response to phenotype-guided pharmacotherapy vs. control therapy (not guided by baseline phenotype) in patients with obesity and an abnormal obesity-related phenotype.
-Sub-aim 1: to study the effect of phenotype-guided pharmacotherapy in brain blood flow and gut mucosal changes.
Methods:
Design: In a 12 week, randomized, double-blinded, **active** controlled, with open-label extension trial of 250 participants with obesity; we will compare the weight loss response rate to obesity-phenotype-guided pharmacotherapy (intervention) vs. non-phenotype guided (randomly selected) pharmacotherapy (control) in patients with obesity and an abnormal obesity-related phenotype.

Baseline Phenotype: All 250 participants will be phenotype:
a) The DEXA scan (dual energy x-ray absorptiometry) will measure body composition.
b) <u>Resting energy expenditure:</u>
c) <u>Gastric emptying (GE) of solids</u> by scintigraphy
d) <u>Appetite</u> (hunger level) by visual analog score fasting and after standard meal for GE and prior to Satiation test
e) <u>Satiation</u> by *ad-libitum* buffet meal to measure total caloric intake and macronutrient distribution in the chosen food
f) Satiety will be measured in length of time of fullness at Ad-libitum meal
g) <u>Self-administered questionnaires</u> assessing affect, physical activity, attitudes, body image, and eating behavior;
h) <u>Plasma gastrointestinal hormones (Total and active Ghrelin, GLP-1, CCK, PYY and bile acids)</u> by RIA
i) <u>Targeted Metabolomics</u>
j) Blood DNA stored
k) Stool sample stored
l) Saliva samples stored
m) Flexible sigmoidoscopy and biopsies of left colon for mRNA, DNA, protein studies
n) Pseudo-continuous arterial spin labeling (pCASL) functional brain MRI study paradigm

<u>Medication selection</u> by a non-study pharmacist will be randomized and double blinded (physician, study team and participant) according to the FDA-approved medicine suggested by the phenotype or to another FDA-approved medicine not suggested by the phenotype.

<u>Follow-up:</u> All participants will be seen at 4 and 12 weeks (+/- 4 days) (current standard of practice). All participants will receive standard intense lifestyle interventions, which consists of one visit with CRTU registered dietitian. At 10 weeks (+/- 2 weeks) of treatment, participants will have a repeat flexible sigmoidoscopy and a pCASL functional brain MRI study paradigm. At the 12-week visit, participants will be unblinded to their "obesity-related phenotype" and they could contact their physician to discuss continuation of a FDA-approved medication as clinically indicated and recommended by their physician. Study team will prospectively follow the patients' weight and waist circumference, as well as any pharmacotherapy followed for obesity every 3 months for 1 year.

Primary endpoint: Total Body Weight Loss, kg (defined as weight changed from baseline to 12 weeks) in the obesity phenotype-guided pharmacotherapy (intervention) vs. the randomly assigned pharmacotherapy (control) group.

The secondary end points will be percentage of responders (defined as number of participants who loss 5% or more of total body weight) compared to baseline in the obesity phenotype guided pharmacotherapy (intervention) group vs. standard of care at 4 and 12 weeks; percentage of responders with at least 10 and 15% at 12 weeks, and 10% at 6 months and 12 months; percentage of responders at 5%, 10% and 15%; percentage of responders within each obesity-phenotype group at 4 and 12 weeks; side effects of medications; changes in blood flow in the brain areas, and transcriptomic changes in colonic mucosa. In the open-label extension, we will assess the total body weight loss at 24 and 52 weeks in both groups.

Sample size assessment: In order to account for dropouts and to detect an effect size in weight loss between groups of interest (intervention vs. control) we propose a randomized, double-blinded, active controlled trial of 250 participants with obesity to compare effects of Intervention compared to Control in weight loss. In our recent pilot study [with Liraglutide 3.0 mg vs. placebo], the SD for the overall weight change (pre-post) observed was 2.8kg. Using this SD, we have estimated the differences between groups that could be detected with approximately 80% power (2-sided α level of 0.05) for main effects. The analysis will involve an ANCOVA models, with the response being actual weight change; the covariates to be considered include gender, and weight at baseline. We noticed a 20% withdrawal rate during the study, thus, we extended the enrolled number to aim to have 200 participants complete the study.

Significance: Our study individualizes obesity treatment to maximize pharmacotherapy outcome based on phenotyping obesity at baseline.

BACKGROUND

Obesity prevalence continues to increase worldwide[7] and, in the United States, 69% of adults are overweight or obese[8]. Estimated costs to the healthcare system are more than $550 billion annually. Increased severity of obesity correlates with a higher prevalence of the associated co-morbidities. Likewise, obesity increases the risk of premature mortality [14]. Obesity affects almost every organ system in the body and increases the risk of numerous diseases including type 2 diabetes mellitus, hypertension, dyslipidemia, cardiovascular disease, and cancer. It is estimated that a man in his twenties with a BMI over 45 will have a 22% reduction (13 years) in life expectancy.

Despite advances in understanding of aspects of obesity pathophysiology, weight loss with current treatments including diet, exercise, medications, endoscopy and surgery is highly variable [9]. However, there are usually great responders to each therapy, specifically "responders" to medications can lose as much weight and with less side effects than bariatric surgery. These individuals – the responders – can benefit from significant weight loss (>15% total body weight loss) which is known to reduce all-cause cardiovascular mortality and morbidity. For example, the high dose of extended release (ER) phentermine-topiramate was associated with an average weight loss of 9.8%; only 48% of patients lost more than 10% of body weight, whereas 30% of patients lost less than 5% body weight [4]. Additionally, the high variability in weight loss response has resulted in a poor market penetration by new medications, devices and surgery. Clearly the one-treatment-fits all is not working and obesity management continue to be a hit-or-miss intervention. Leaving physicians to select pharmacotherapy based in potential side effect and patient comorbidities [10], instead of choosing the right drug for the right patient based on its pathophysiology. Thus, it is essential to identify the responders to each intervention, to maximize their weight loss. Recently, we made significant progress to identify predictors of weight loss using gastrointestinal and behavioral traits *[3]*.

Treatment for obesity:

The 2013 Obesity Guidelines suggest that to achieve weight loss, an energy deficit is essential. Reducing dietary energy intake below that required for energy balance can be achieved through a reduction of daily calories to 1200-1500 for women, and 1,500-1800 for men (kilocalorie levels are usually adjusted for the individual's body weight and physical activity levels); or estimation of individual daily energy requirements and prescription of an energy deficit of 500 kcal/d or 750 kcal/d. Recommendations for young children through adolescence vary in order to support normal growth and development occurring during these years. The Academy of Nutrition and Dietetics Evidence Analysis Library recommends no fewer than 900 kcal/day for 6-12 year olds who are medically monitored and no fewer than 1200 kcal/day for 13-18 year olds (Academy of Nutrition and Dietetics Weight Management Position Paper which provides an overview of a nutrition assessment: http://www.eatrightpro.org/resource/practice/position-and-practice-papers/position-papers/weight-management). Evidence supports greatest long-term success with an individualized, structured meal plan in place. A registered dietitian nutritionist can play an important role in designing the nutrition intervention tailored to address each patient's unique needs and circumstances, taking into consideration factors such as insulin resistance. Any diet program that meets this required energy deficit is appropriate to adopt, and comparative trials have shown no long-term superiority between different macronutrient composition or elimination diets. Furthermore, it is important to adhere to a balanced diet that provides a variety of items from all food groups and limits potentially harmful food ingredients like added sugars, sodium and alcohol. Additionally, guidelines recommend limiting or avoiding liquid calories (i.e. sodas, juices, alcohol, etc.). And, finally, the meal plan should be designed in such a way that the individual is likely to follow it.

Along with the prescription for a reduced calorie diet, a comprehensive lifestyle intervention program should prescribe increased aerobic physical activity (such as brisk walking) for ≥150 min/week (equal to ≥30 min/d most days of the week), and a goal of >10,000 steps per day. Higher levels of physical activity, approximately 200 to 300 min/wk., are recommended to maintain the weight lost or minimize weight regain in the long term (>1 year) [15]. The diet and physical activity can be in combination with a hospital/university or commercial behavior program; these are comprehensive lifestyle interventions that usually provide structured behavior strategies to facilitate adherence to diet and activity recommendations. These strategies include regular self-monitoring of food intake, body weight, physical activity, and food cravings. These same behaviors are recommended to maintain lost weight, with the addition of frequent (i.e., weekly or more frequent) monitoring of body weight[16].
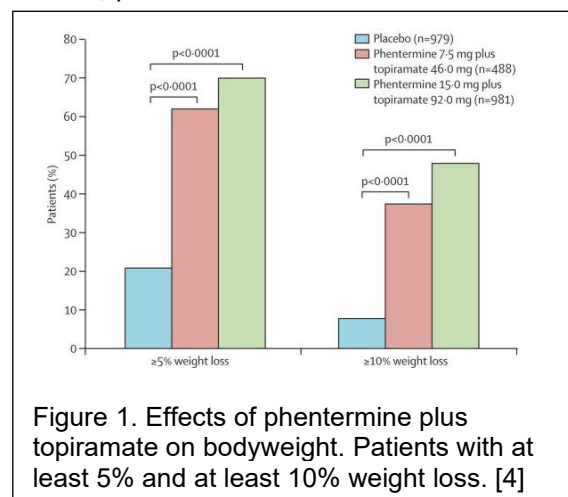
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Pharmacotherapy

In addition to diet, exercise and behavioral modification, pharmacotherapies should be considered as an adjunct to lifestyle changes in patients who have been unable to lose and maintain weight with diet and exercise alone. They should also be considered in people whose history or clinical circumstances require expedited weight loss. Medication should not be used alone, but in combination with an intensive lifestyle program.

Pharmacotherapy for the treatment of obesity can be considered if a patient has a body mass index (BMI) ≥ 30 kg/m^2 or a BMI ≥ 27 kg/m^2 with weight-related co-morbidities such as hypertension, type 2 diabetes, dyslipidemia and obstructive sleep apnea[16]. Medical therapy should be initiated with dose escalation based on efficacy and tolerability to the recommended dose. An assessment of efficacy and safety at least monthly for the first three months and then at least every three months. In patients who have cardiovascular disease, guidelines recommend against prescribing sympathomimetic agents such as phentermine and phentermine/topiramate extended release (ER). Lorcaserin and orlistat are safer alternatives. In patients with T2DM, the guidelines suggest antidiabetic agents that promote weight loss such as glucagon-like peptide (GLP-1) analogs which reduce hyperglycemia in addition to the first-line agent for T2DM, metformin[17].

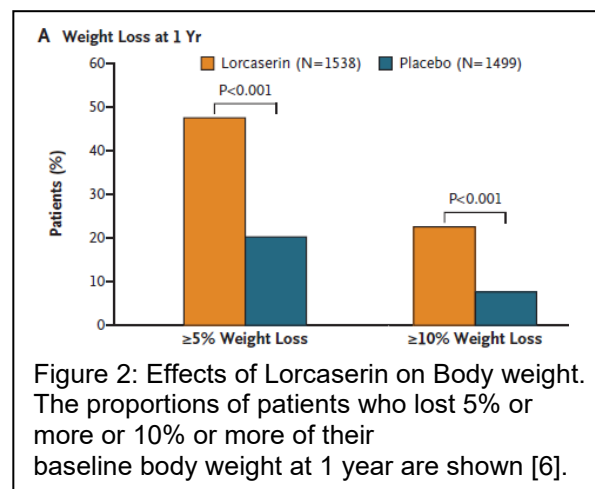| Medication / dose	Clinical data	Mean weight change from baseline after 1 year	Weight loss after 1 year (Proportion of participants)			References
			>5%	>10%	>15%	
Orlistat 120 mg TID	Clinical data from three trials	−6.0 to 10.3 Kg vs −2.6 to 6.1 Kg with placebo	**36–67%** (vs.16–43.6%)	**17 - 38.9** (vs. 8.8 – 24.8)	**NA**	[18-20]
Phentermine/ topiramate ER 15 mg/92 mg QD	1-year trial, people with obesity (BMI ≥35 kg/m^2)	−10.9% vs −1.6% with placebo	**70%** (vs.21%)	**48%** (vs. 7%)	NA	[21]
Lorcaserin 10 mg BID	2-year trial, people with obesity or overweight and ≥1 comorbidity	−5.8% vs −2.5% with placebo	**47%** (vs. 23%)	**22.6** (vs. 7.7)	NA	[6]
Naltrexone/ bupropion SR 32 mg/360 mg	Four 56-week trials, people with obesity and ≥1 comorbidity	−5.4% vs −1.3% with placebo (COR-I)	**42%** (vs. 17%)	**28.3** (vs. 5.7)	**13.5** (vs. 2.4)	[1]
Liraglutide 3.0 mg QD	56-week trial, people with obesity or overweight and ≥1 comorbidity	−7.4% vs −3.0% with placebo	**62%** (vs. 34%)	**33.1%** (vs. 10.6%)	**14.4%** (vs. 3.5%)	[22]

Phentermine-Topiramate Extended Release: When low-dose, controlled-release, phentermine was combined with the glutamatergic and GABA-ergic antiepileptic topiramate in a large phase III study (more than 1400 participants on treatment arms with different doses), subjects lost 10.2 kg on 15/92 mg combination therapy vs. 1.4 kg on placebo over 56 weeks [21]. The most common adverse events were dry mouth, paresthesias, constipation, insomnia, dizziness, and dysgeusia. Depression- and anxiety-related adverse events were also observed. The medication had favorable effects on glycemia, including prevent progression to diabetes, improvements in lipids, blood pressure, sleep apnea, and quality of life measures. There was also, as previously noted, a small but consistent increase in pulse rate [23]. The overall rate of adverse effects decreased in weeks 56–108 compared to weeks 0–56; among which dry mouth, constipation and paresthesias were the most prevalent There were 19 pregnancies carried to term

Figure 1. Effects of phentermine plus topiramate on bodyweight. Patients with at least 5% and at least 10% weight loss. [4]

during these studies none of which resulted in congenital abnormalities [23-25].
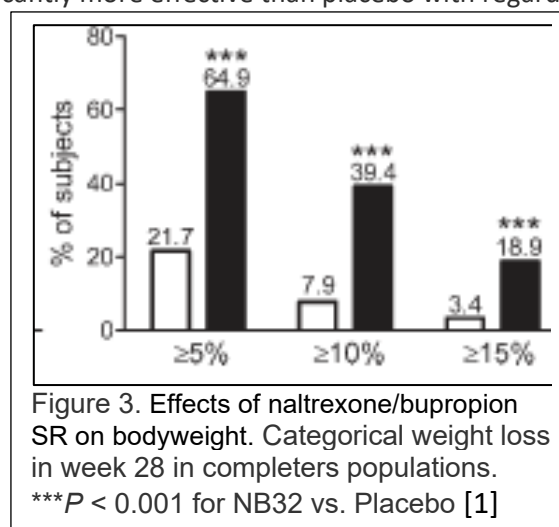
In July 2012, the FDA voted for approval of phentermine (3.75–15mg/d) plus extended release topiramate (23–92mg/d) as an adjunct to diet and physical activity for treatment of obesity among adult individuals with BMI≥30kg/m^2 or BMI≥27kg/m^2 with at least one obesity-related comorbid condition. The drug will carry a warning of potential increased risk for orofacial clefts in neonates exposed to topiramate during the first trimester of gestation and will be subject to a Risk Evaluation and Mitigation Strategy (REMS) that will restrict prescribing to trained clinicians, will require effective contraception and monthly pregnancy tests for reproductive age women, and will restrict dispensing to specific mail-order pharmacies. The company is also required to carry a long-term cardiovascular outcomes trial. No randomized pediatric studies have as yet been reported. Noteworthy, the high dose of PhenTop was associated with a mean weight loss of 9.8%; however, only 48% of patients lost >10% of their body weight, and 30% of patients lost <5% of their body weight (Figure 1). The high variability of weight loss response to treatment with PhenTop is unclear.

Lorcaserin (Belviq®): The second medication approved by the FDA in 2012 for chronic weight management is lorcaserin[26]. It is a serotonin receptor agonist thought to reduce food intake and increase satiety by selectively activating receptors on anorexigenic POMC neurons in the hypothalamus. At the recommended dose, lorcaserin selectively binds to 5-HT2C receptors instead of 5-HT2A and 5-HT2B receptors, which are associated with hallucinations and cardiac valve insufficiency respectively [27]. The recommended dose of lorcaserin is 10 mg twice daily. The medication should be discontinued if ≥ 5% weight loss is not achieved after 12 weeks (Figure 2)[6].

Figure 2: Effects of Lorcaserin on Body weight. The proportions of patients who lost 5% or more or 10% or more of their baseline body weight at 1 year are shown [6].

Oral naltrexone extended-release/bupropion extended-release (NBSR; Contrave®, Mysimba™) is available as an adjunct to a reduced-calorie diet and increased physical activity in adults with an initial body mass index (BMI) of ≥30 kg/m2 (i.e. obese) or a BMI of ≥27 kg/m2 (i.e. overweight) in the presence of at least one bodyweight-related comorbidity, such as type 2 diabetes mellitus, hypertension or dyslipidemia. In 56-week phase III trials in these patient populations, oral naltrexone ER/bupropion ER 32/360 mg/day was significantly more effective than placebo with regard to percentage bodyweight reductions from baseline and the proportion of patients who achieved bodyweight reductions of ≥5 and ≥10 % (table 1)(figure 3)[1, 28, 29]. Significantly greater improvements in several cardiometabolic risk factors were also observed with naltrexone ER/bupropion ER versus placebo, as well as greater improvements in glycated hemoglobin levels in obese or overweight adults with type 2 diabetes. Naltrexone ER/bupropion ER was generally well tolerated in phase III trials, with nausea being the most common adverse event (table 2) [28, 29]. Thus, naltrexone ER/bupropion ER 32/360 mg/day as an adjunct to a reduced-calorie diet and increased physical activity is an effective and well-tolerated option for chronic bodyweight management in obese adults or overweight adults with at least one bodyweight-related comorbidity.

Figure 3. Effects of naltrexone/bupropion SR on bodyweight. Categorical weight loss in week 28 in completers populations. ***P < 0.001 for NB32 vs. Placebo [1]

Noteworthy, the high dose of NBSR was associated with a mean weight loss of 8.1%; however, only 34% of patients lost >10% of their body weight, and 62% of patients lost <5% of their body weight. The high variability of weight loss response to treatment with NBSR is unclear.

Liraglutide (Saxenda®) is a glucagon-like peptide-1 (GLP-1) analogue with 97% homology to human GLP-1, a gut derived incretin hormone[26]. Liraglutide was approved in 2010 for the treatment of type 2 diabetes at doses up to 1.8 mg daily. In phase III studies many patients on liraglutide for diabetes lost weight in a dose-dependent manner [30] and the

efficacy was similar in patients with obesity without diabetes [22]. The FDA approved liraglutide in 2014 as Saxenda at 3.0 mg dose for chronic weight management in patients with obesity. Weight loss is mediated by reduced energy intake by reducing appetite, increasing satiety and delaying gastric emptying [11, 31]. Liraglutide is administered as a subcutaneous injection once daily. It is initiated at 0.6 mg daily for one week with instructions to increase by 0.6 mg weekly until 3.0 mg is reached. Slower dose titration is effective in managing gastrointestinal side effects. The medication should be discontinued if a patient has achieved ≤ 4% weight loss at 16 weeks.

The average weight loss in a large NEJM-published trial [2] of liraglutide was ~8% of body weight; 33% of participants lost >10% and 14.4% lost >15% of body weight. However, 36.8% of patients did not respond to treatment with liraglutide (figure 4). The reason for the high variability of weight loss response to treatment with liraglutide is unclear.

Figure 4 shows the proportions of patients who lost at least 5%, more than 10%, and more than 15% of their baseline body weight. Data shown are the observed means for the full-analysis set (with LOCF). [2].

Pharmacogenomics

Pharmacogenomics (PGx) is a new field in individualized medicine generally concerned with genetic polymorphisms in drug-metabolizing enzymes, transporters, receptors, and drug targets that explain inter-individual variation in drug efficacy and toxicity [41]. PGx has the potential to improve clinical outcomes by using an individual's genotype to inform personalization and optimization of drug therapy. A large number of PGx variants with demonstrated clinical utility are known and have been incorporated into drug labeling by the US Food and Drug Administration (FDA) [2]. As the availability of high throughput genomics technology becomes more widespread and the associated cost of genetic testing more economical, opportunities for patients to have precision genomic information to guide healthcare decisions is expected to increase. Integration of genetic data into the clinical decision making process has the potential to significantly advance the practice of precision medicine and in the case of PGx, ultimately affect every patient. Mayo Clinic's Individualized Medicine Clinic (within the Center for Individualized Medicine) has established a Pharmacogenomics Testing Service. Largely an interest for "otherwise healthy" patients, or those self-reporting medication struggles, these patients can be referred for testing and/or consultation/evaluation by a PGx expert pharmacist who will help facilitate a PGx laboratory test in partnership with the referring physician. The PGx pharmacists can also assist with interpretation of the results—given the newness of the field and level of exposure to PGx testing across physicians. Though single gene PGx testing has long been used at Mayo Clinic in certain, focused departments for diagnostic or therapeutic reasons, the clinical evidence is expanding to implicate a greater number of genes and medications, laboratories are creating panel tests that cover more genes at a lesser cost than previous single gene tests. The clinical value of these tests is now becoming more broadly understood. Prescribers who believe their patients have medication metabolism issues can currently tap into the PGx Testing Service by ordering PGx gene tests for diagnostic purposes and requesting a PGx e-consult or patient face to face consult with the PGx pharmacist for results interpretation assistance. Additionally, a limited number of pilots offering clinical PGx testing primarily for predictive reasons are offered with the Center for Individualized Medicine. The value of the service includes utilization of PGx testing as a tool for assisting health care providers improve the medication experience of their patients. Patients may also benefit from understand their own PGx variations and the relevance of their results and other family members. The service is also assisting providers in this new and growing field, by providing expertise and support to help prescribers tailor medications for their patients--adjusting current mediations according to the patient's genetic variations and/or providing valuable information for future prescribing events.
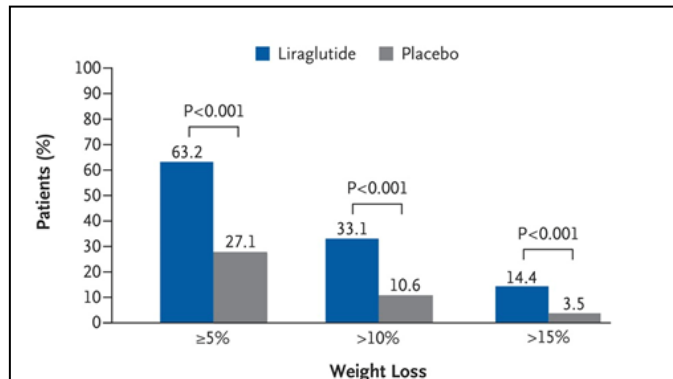
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PRELIMINARY DATA

Gastrointestinal traits (phenotypes) associated with obesity: Recently we published the characterized gastrointestinal functions, satiation and satiety, in 509 participants across the normal weight to obesity spectrum. We found that obesity is associated with decreased satiation (higher caloric intake before feeling full, measure by volume to fullness [VTF] p=0.038), large fasting gastric volume (GV, p=0.03), accelerated gastric emptying (GE) $T_{1/2}$ (solids: p<0.001; liquids: p=0.011), and lower postprandial peak plasma levels of PYY (p=0.003). In addition, principal components (PC) analysis identified latent dimensions (LDs) accounting for ~81% of OW-OB variation and sub-classifies obesity (figure 5) in satiation (21%), gastric capacity (15%), behavioral (13%), gastric sensorimotor (11%) factors and others (40%)[3]. This obesity sub-classification may predict weight loss response to pharmacotherapy and bariatric endoscopy [3].

Obesity phenotypes to predict weight loss response:
Thus far, we validated the applicability of obesity-related gastrointestinal quantitative traits in two randomized clinical trials [3, 11]. In a single-center, randomized, parallel-group, double-blind, placebo-controlled, 14-day study, we evaluated the effects of Phentermine-topiramate-ER (PhenTop) (7.5/46mg, orally, daily) on GE, GV, satiation, satiety, and fasting and postprandial gut hormones in 24 obese adults using validated assays. PhenTop is approved for the treatment of obesity. However, its effects on gastric functions, satiation, satiety and relevant gut hormones are unknown. PhenTop was associated with reduced food intake at buffet meal (mean Δ 260kcal, p=0.032) and delayed GE solids (mean Δ GE4h 6%, p=0.03; and Δ GE T½ 19min, p=0.057). There were no significant differences in GV, satiation, GE of liquids and GI hormones. Patients on PhenTop had greater mean weight loss of 1.4kg than placebo (p=0.03). Weight loss on PhenTop was significantly associated with kcal intake at a prior satiety test. We concluded that PhenTop reduces food intake and delays GE of solids, suggesting central as well as peripheral mechanisms of action in inducing weight loss and that **a prior satiety test predicts weight loss with PhenTop** (Figure 6) [3].

In another placebo-controlled trial, we studied the effect of exenatide, 5µg, SQ, twice daily for 30 days, on GE, satiety, satiation and weight loss in 20 obese participants with accelerated GE. Exenatide had a very significant effect on GE of solids (p<0.001) and reduced calorie intake at a buffet meal by an average 130kcal compared to placebo. The average weight loss was 1.3kg for exenatide and 0.5kg for the placebo group. We concluded from this relatively short duration study that exenatide reduces food intake and delays GE of solids; and that **a prior accelerated gastric emptying test predicts weight loss with exenatide [11]**.

In a recent retrospective analysis, we have identified that the best responders to the intragastric balloon therapy are those individuals with an accelerated gastric emptying (p<0.001) and the greater delay in gastric emptying after intragastric balloon placement (p<0.001)[13].

We recently completed a prospective, randomized clinical trial with liraglutide, a long-acting GLP-1 receptor agonist, is approved for treatment of obesity. The objective was to compare effects of liraglutide and placebo over 16 weeks on gastric motor functions,
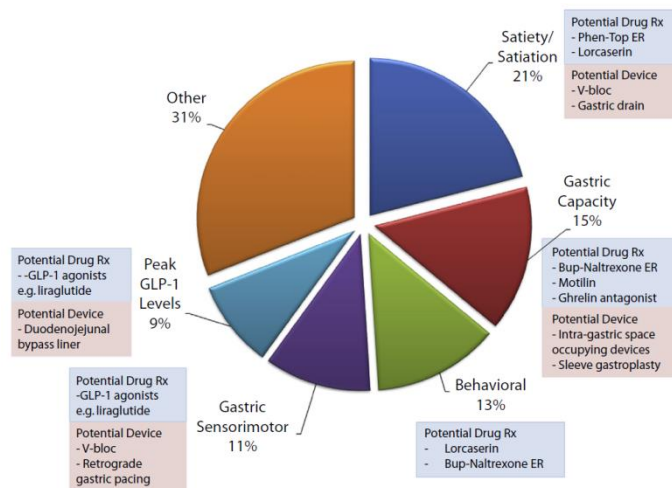
Figure 5: Potential application of medications and devices directed at phenotypes associated with obesity. Rx, therapy; ER, extended release; GLP-1, glucagon-like peptide 1. Adapted from Camilleri and Acosta, GIE, 2016 [5]
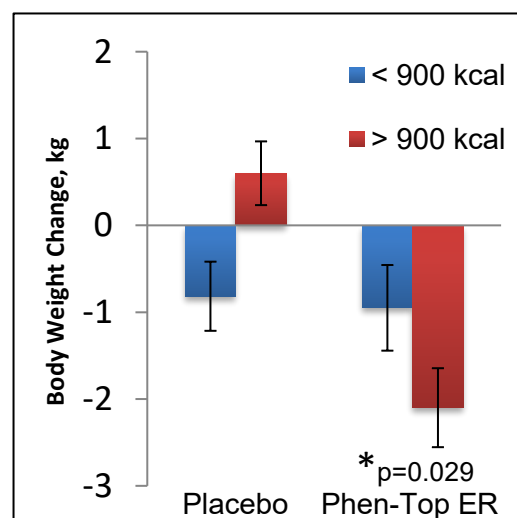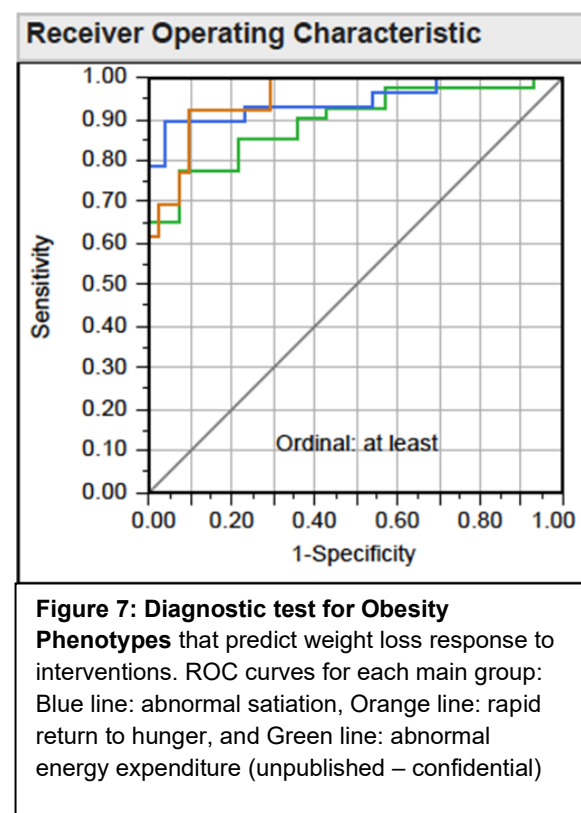
Figure 6: *Body weight change by prior satiety test. Association of change in body weight (in response to randomized treatment with placebo or PhenTop) and kcal intake at prior ad-libitum meal. This is shown (p=0.029) for the drug* treatment interaction.[3]*

satiation, satiety and weight in obese patients. This study was a randomized, double-blind, placebo-controlled trial of subcutaneous liraglutide, 3mg, with standardized nutritional and behavioral counseling at Mayo Clinic, Rochester, MN. Forty adult, otherwise healthy local residents with BMI ≥30kg/m^2 were randomized between December 2015 and September 2016. Liraglutide or placebo was escalated by 0.6mg/day each week for 5 weeks and continued until week 16. At baseline and after 16 weeks' treatment, we measured weight, gastric emptying of solids (GES, primary endpoint), gastric volumes, satiation, and satiety. GES was also measured at 5 weeks. Statistical analysis compared treatment effects using ANCOVA (with baseline measurement as covariate). Effect of liraglutide on GES T$_{1/2}$ at 5 and 16 weeks in the liraglutide group was analyzed by paired t-test. Seventeen participants were analyzed in the liraglutide group (n=19 randomized) and 18 in the placebo group (n=21 randomized). Compared to placebo, liraglutide retarded GES at 5 (p<0.0001) and 16 (p=0.025) weeks, caused significant weight loss and increased satiation. In 16 weeks, the total body weight loss for the liraglutide group was 6.1±2.8 kg (SD) compared to 2.2±5 kg control group (p=0.0096). There was tachyphylaxis to GES effects of liraglutide from 5 to 16 weeks' treatment. At 5 and 16 weeks, GES T$_{1/2}$ correlated with Δ weight loss on liraglutide (all p<0.02). Nausea was the most common adverse event in the liraglutide group (63.2%) compared to placebo (9.5%). Our results suggests that Liraglutide, 3.0mg, significantly delays GES after 5 and 16 weeks' treatment; effects on weight loss are associated with absolute value of GES T$_{1/2}$ on liraglutide [32].

Quantitative traits - phenotypes are associated with higher BMI, distinguish obesity phenotypes, and may predict response to obesity pharmacotherapy and endoscopic devices [3]. However, the tests of quantitative GI traits are currently limited to a few research/academic centers. Thus, we have **developed a novel and simple diagnostic-blood-test that predicts weight loss in obesity**. The diagnostic test is based on an algorithm that combines candidate gene variants (SNPs), metabolites and metabolic peptides. We recently completed the analysis of 102 patients with obesity, matched for gender, age and BMI. These individuals were non-diabetic and were in not medications for weight loss. Based on the profile of each patient we were able to validate the main groups in obesity in 1) abnormal satiation, 2) rapid return to hunger, 3) behavioral eating (identified by questionnaire) and 4) abnormal energy expenditure; plus a "mixed" group. Once these variables were tested, we first created a combined logit regression model using stepwise variable selection to identify variables that are significantly associated with each of the phenotypic classes. The result included a combination 14 metabolites (amino-compounds, neurotransmitters and fatty acids), no candidate gene or metabolic peptide were included/make the cut (The 14 metabolites are knowingly not disclosed per MCV/legal request - Mayo IP disclosure No. 2017-040 and DR16-520 – unpublished/confidential). Figure 7 shows the sub-classification prediction accuracy of this combined model and an ROC analysis showed that this model has >0.90 AUC for all four classes. Next, we set out to derive binary classification models that can predict whether a patient belongs to one group over the others. As

Figure 7: Diagnostic test for Obesity Phenotypes that predict weight loss response to interventions. ROC curves for each main group: Blue line: abnormal satiation, Orange line: rapid return to hunger, and Green line: abnormal energy expenditure (unpublished – confidential)

preliminary data, we derived Bayesian covariate predictors for abnormal satiation, behavioral eating, and abnormal energy expenditure (detailed models not shown here for lack of space). These models yielded an ROC AUC of 0.9414, 0.9668, and 0.8775. These data suggested that the serum metabolite levels hold all the information needed to predict obesity subclasses. We propose to develop a novel targeted panel-based blood assay using the metabolites in these models (both integrated model and independent binary models) and validate them against an independent cohort. We will also extend our statistical analysis to develop a binary model for predicting whether a patient has rapid return to hunger phenotype.

Quantitative traits are associated with higher BMI, distinguish obesity phenotypes, and may predict response to obesity pharmacotherapy and endoscopic devices (Figure 5) [3]. However, these results were determined retrospectively and each of them was done independently. Thus, the identification of the obesity phenotype at baseline

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to guide obesity pharmacotherapy has not been tested yet and the outcome is unknown in the clinical setting. Thus, there is a **critical need to do a prospective randomized study to evaluate the weight loss outcome using obesity phenotypes** to guide therapy for obesity.

PRELIMINARYA DATA FOR SUB-AIM 1:

The heterogeneity of human obesity has confounded our ability to translate research findings to understand the mechanisms underlying obesity pathophysiology. Thus, we hypothesized that our pathophysiological classification will assist in the elucidation the uniqueness among obesity phenotype and to address this hypothesis, we decided to conduct a series of studies aiming to further characterize each phenotype in depth, and identify unique biological perturbations underpinning each phenotype. In all the measurements described below, there were no differences in weight, BMI, age or gender among the different groups, unless otherwise noted.

Hungry Brain Phenotype – Abnormal Satiation: The sensation of satiation, which leads to the termination of a meal[33], requires close coordination between the brain and the gut[34, 35]. These "stop eating signals" are mainly driven by the distention of the stomach, and vagal nerve afferents, transmitting the sensation to the hypothalamus to induce satiation and stop the meal[34, 36]. Previously, we have shown that individuals with obesity consumed more calories prior to reaching 'usual' fullness – for every 5 units of BMI increase, participants consumed 50 calories more[37]. Here, we showed that patients with a hungry brain phenotype consumed significantly more calories (62% more) to reach satiation, thus determining their unique phenotype. Interestingly, individuals without the hungry brain obesity phenotype consumed similar calories to historical normal weight controls[37]. The prevalence of hungry brain obesity was 28% in the new cohort (n=46/165) and 42% in the previously published[37] cohort of patients completing the phenotype test (n=68/163).

These participants completed a well-validated "nutrient-drink test", another feeding paradigm, that identifies different degrees of the sensation of fullness. During a nutrient drink test, participants with hungry brain obesity consumed 107 calories more calories to reach volume to "usual" fullness (VTF) ($p < 0.05$) and 241 calories more calories to reach 'maximal' fullness – maximal tolerated volume (MTV)($p < 0.001$) compared to individuals with the non-hungry brain obesity (Supplemental table 1). Thus, the hungry brain obesity phenotype may have a deficiency in the mechanisms that mediate the "stop eating" signals.

To further investigate and to understand this association, we performed brain MRI in patients, using pseudo-Continuous Arterial Spin Labeling (pCASL) MRI sequence to study the hypothalamic function of individuals with and without the hungry brain obesity phenotype, and lean controls (patient demographics Supplemental table 3). The patients were scanned at 4 time points - i.e., at baseline, after drinking 240ml of Ensure (VTF), after reaching maximal tolerable volume (MTV) of Ensure representing peak fullness and 30 minutes after reaching MTV (MTV+30). There was no difference in hypothalamic blood flow at any time point in patients with obesity (n=22) compared to lean (n=7) controls (Figure 2H) or in a control area, which was frontal lobe white matter that is considered unrelated to food regulation and therefore represents an area where, theoretically, blood flow would be stable at all four time points (Figure 2 G, I). In patients with hungry brain obesity (n=8), the average blood flow within the right side of the hypothalamus was consistently lower at all four time points and significantly lower at MTV ($p = 0.02$), compared to patients with non-hungry brain obesity (n=14)(Figure 2 A-F, J). These findings are consistent with previous observations of obesity in animal models and humans associated with hypothalamic injury[38], neuronal plasticity[39] and/or gliosis[40].
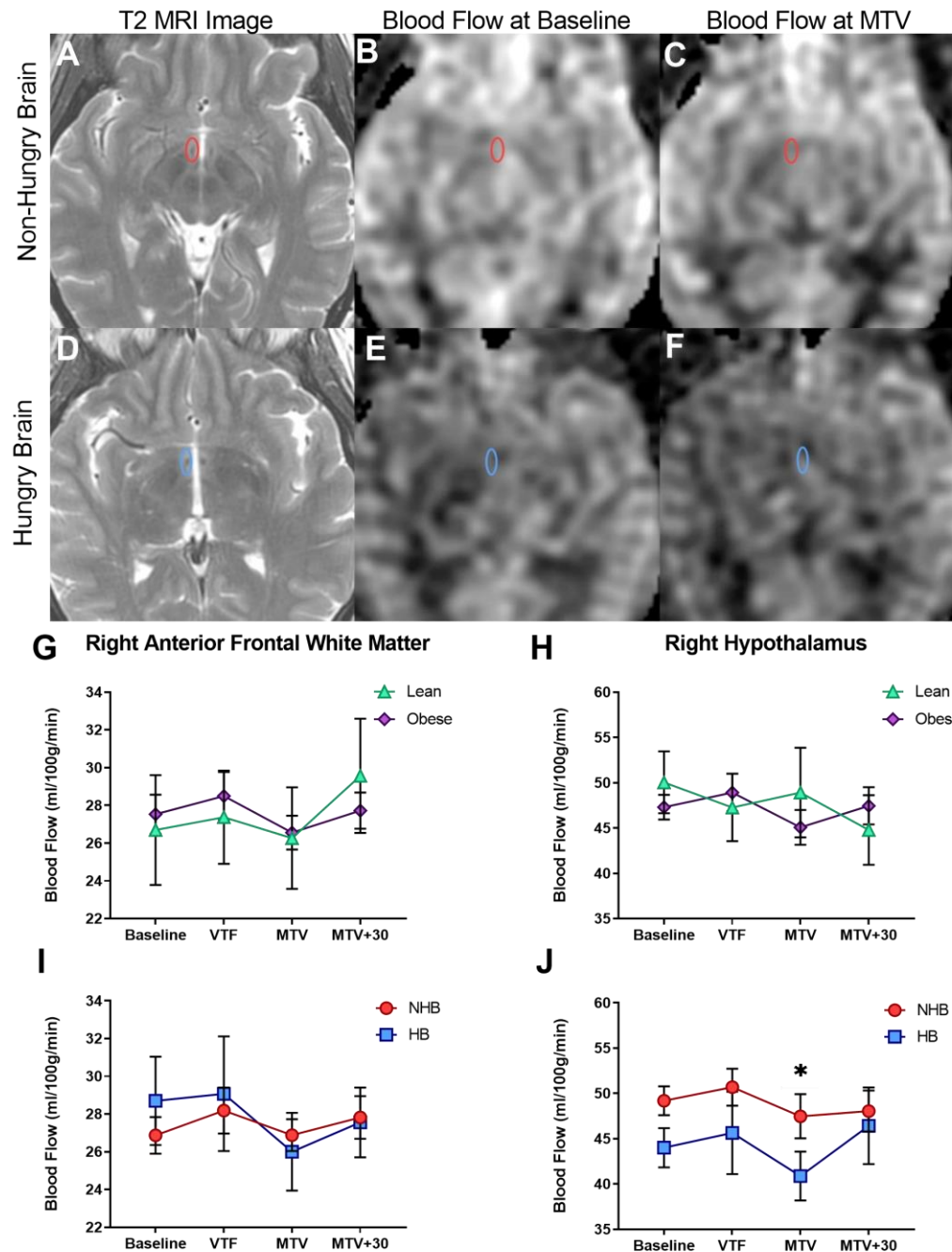
Figure 2. Hungry brain phenotype assessed by Pseudo-Continuous Arterial Spin Labeling (pCASL) MRI sequence. The blood flow to the right hypothalmus, region of interest, shown in red and blue circles for non-hungry brain and hungry brain, respectively, visible by T2 MRI imaging (A, D), pCASL imaging at baseline (B, E) and at maximal fullness (MTV) (C,F). Quantitated blood flow at baseline, volume to usual fullnes (VTF), MTV, and 30 minutes after reaching MTV (MTV+30) was compared between groups for the right anterior frontal white matter, a control area (G, I), and the right hypothalamus (H,J). Green triangles= lean; purple diamonds= "all" obesity; red circles=NHB; blue-squares=HB. *p<0.05.

Hungry Gut Phenotype – Abnormal Satiety: From a physiological perspective, the sensation of satiety or persistent fullness[33] is mainly driven by communication of the gut with the brainstem and the hypothalamus[34, 36, 41]. Factors regulating satiety, which is usually recorded via subjective 100 mm visual analog scales, include gastric emptying and gastrointestinal satiety peptides, and these variables are reproducible and objective measurements of satiety[37, 42, 43]. Therefore, rapid gastric emptying was selected as a surrogate for abnormal satiety in assessment of the hungry gut phenotype, as it is an objective, reproducible test. The prevalence of hungry gut obesity was 28% in the new cohort (n=46/165) and 34% in the previously published[37] cohort (n=56/164). In female participants with hungry gut obesity, gastric emptying $T^{1/2}$ (GE) was accelerated by 30% for solids (p<0.001) and by 22% for liquids (p=0.01) compared to non-hungry gut obesity. In male participants with hungry gut obesity, GE $T^{1/2}$ was accelerated by 38% for solids (p<0.001) and 33% for liquids (p=0.05) compared to non-hungry gut obesity. The observation is of clinical relevance as gastric emptying is correlated with the caloric consumption in the next meal[43] and with the sensation of persistent fullness[34, 44]. Interestingly, this observation both elucidates and builds upon our previous findings that "all" obesity experiences rapid gastric emptying compared to lean controls; demonstrating only a subgroup of obesity experience this pathophysiological abnormality that alters satiety[37, 42, 43].

The pathophysiology underlying the hungry gut phenotype might be secondary to an abnormal negative feedback of gastrointestinal satiety hormones, Glucagon-like peptide 1 (GLP-1), and Peptide YY (PYY), secreted from L-type enteroendocrine cells of the ileum and colon. Enteroendocrine (EE) cells are real-time nutrient, bile and microbiota sensors that regulate food intake, brain-gut communication, gastrointestinal motility, and glucose metabolism. EE cell function can be studied indirectly by measuring plasma levels of hormones such as GLP-1 or PYY, and less frequently EE cells are studied as part of whole intestinal tissue. We have examined the characteristics of the EE cells in participants with or without the hungry-gut obesity phenotype in mucosal biopsies obtained during unsedated flexible sigmoidoscopy. Both mucosal biopsies and blood were collected from previously phenotyped participants (demographics in Supplemental table 4 and 5). There was no difference in plasma fasting or 15, 45 and 90 minutes postprandial gastrointestinal peptides PYY3-36 or GLP-1 concentration (Figure 3A, E) nor in colonic mucosal GCG mRNA expression (Figure 3C) in obesity compared to healthy weight controls; though PYY mRNA expression was significantly increased in 'all' obesity compared to lean (Figure 3G; p=0.04). However, participants with the hungry gut obesity phenotype, defined by accelerated GE, had significantly lower plasma concentration of GLP-1 (AUC p=0.007) and PYY (AUC p=0.03) when compared with non-hungry gut obesity (Figure 3B, F). Furthermore, participants with hungry gut obesity had decreased mRNA expression of GLP-1 (p=0.01) and PYY (p=0.04) in colonic mucosa when compared to non-hungry gut obesity (Figure 3D, H). The observed reductions in mucosal expression and circulating levels of GLP-1 and PYY are consistent with reduced negative feedback that normally results in slow gastric emptying and reduced signaling to the hypothalamus, thereby reducing the hypothalamic-mediated sensation of fullness in this phenotype. These observations also suggest the importance of the EE cells, of the distal gut in food intake regulation, in addition to their role in direct communication with luminal nutrients, toxins and microbiota.
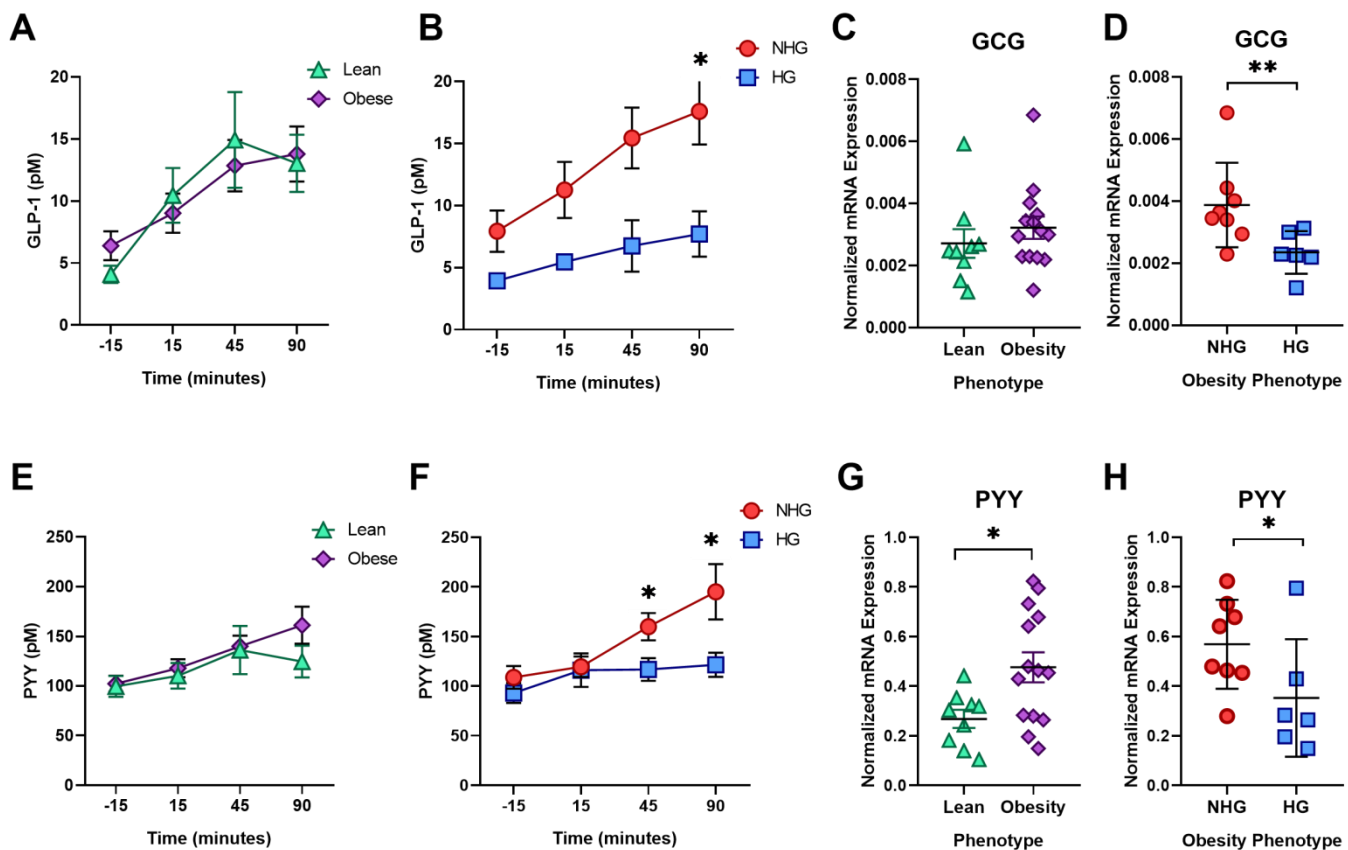
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Figure 3. Hungry gut phenotype characterized by comparing plasma concentration and colonic mucosal gene expression of gastrointestinal satiety hormones, Glucagon-like peptide-1 (GLP-1, GCG), and Peptide YY3-36 (PYY). Comparisons are made between lean versus "all" obesity, as well as between the Hungry gut-obesity phenotype (HG), and the non-hungry gut obesity phenotype (NHG). Plasma concentrations during fasting (-15 minutes) and at 15, 45 and 90 minutes postprandially for GLP-1 (A, B) and PYY (E, F) were investigated. Normalized mRNA expression of GCG (GLP-1) (C,D), and PYY (G, H) was measured at baseline from colonic mucosal tissue.

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Emotional Hunger Phenotype - Hedonic Behavior: Obesity is often regarded as a behavioral disease, in which patient's emotions drive obesogenic behavior in search of reward through compensatory mechanisms[45]. Here we report that there is a sub-group within obesity possessing a strong psychological component, potentially predisposing them to obesity, thus, we have labeled this sub-group as the emotional hunger phenotype. The prevalence of emotional hunger obesity was 34% in the new cohort (n=56/165) and 27% in the previously published[37] cohort (n=44/164). Additionally, individuals with the emotional hunger obesity phenotype are characterized as having higher levels of symptoms of anxiety (p<0.001), symptoms of depression (p<0.001), emotional restraint –TEFQ21 (30% higher, p=0.04), emotional eating (Disinhibition on the Eating Inventory, p=0.007) and lower levels of self-esteem (p=0.002) and body image (P<0.001) when compared to non-emotional eating obesity phenotypes. This emotional hunger group is likely acquiring most of their calories from emotional eating, cravings and reward-seeking behaviors, despite having appropriate sensations of satiation and satiety – normal homeostatic eating behavior. Identifying this phenotype validates decades of eating behavior research, which describes a hedonic eating behavior, mechanistically different that the "homeostatic" eating behavior[46].

While an interesting, and somewhat expected finding, the questionnaires remain a subjective measurement of emotional eating. The Nucleus accumbens is an important brain structure in the reward response system[47] and previously reported to be highly associated with obesity[48, 49].   Indeed, deep brain stimulation of the nucleus accumbens is attempted as a treatment for obesity[50]. For this reason we utilized pCASL MRI, to study the relationship between perfusion of nucleus accumbens and the emotional hunger status (See cohort demographics in Supplemental table 6). There was no difference in blood flow in the nucleus accumbens or in the anterior frontal white matter control area, in obesity compared to lean controls (Figure 4 A-G, I). In contrast, in the emotional hunger obesity phenotype (n=6) there was increased blood flow in the left nucleus accumbens at all 4 time points: baseline (p=0.06), VTF (p=0.21), MTV (p=0.07), and significantly higher at MTV+30 (p=0.015) compared to the non-emotional eating phenotypes (n=14) (Figure 4 A-F, H, J). Although the right side nucleus accumbens showed similar trends (i.e., higher flow in all time points), none of the differences were statistically significant. Our MRI findings support the unique nature of the phenotype, and the importance of our classification in identifying patients with abnormal emotional eating behavior.
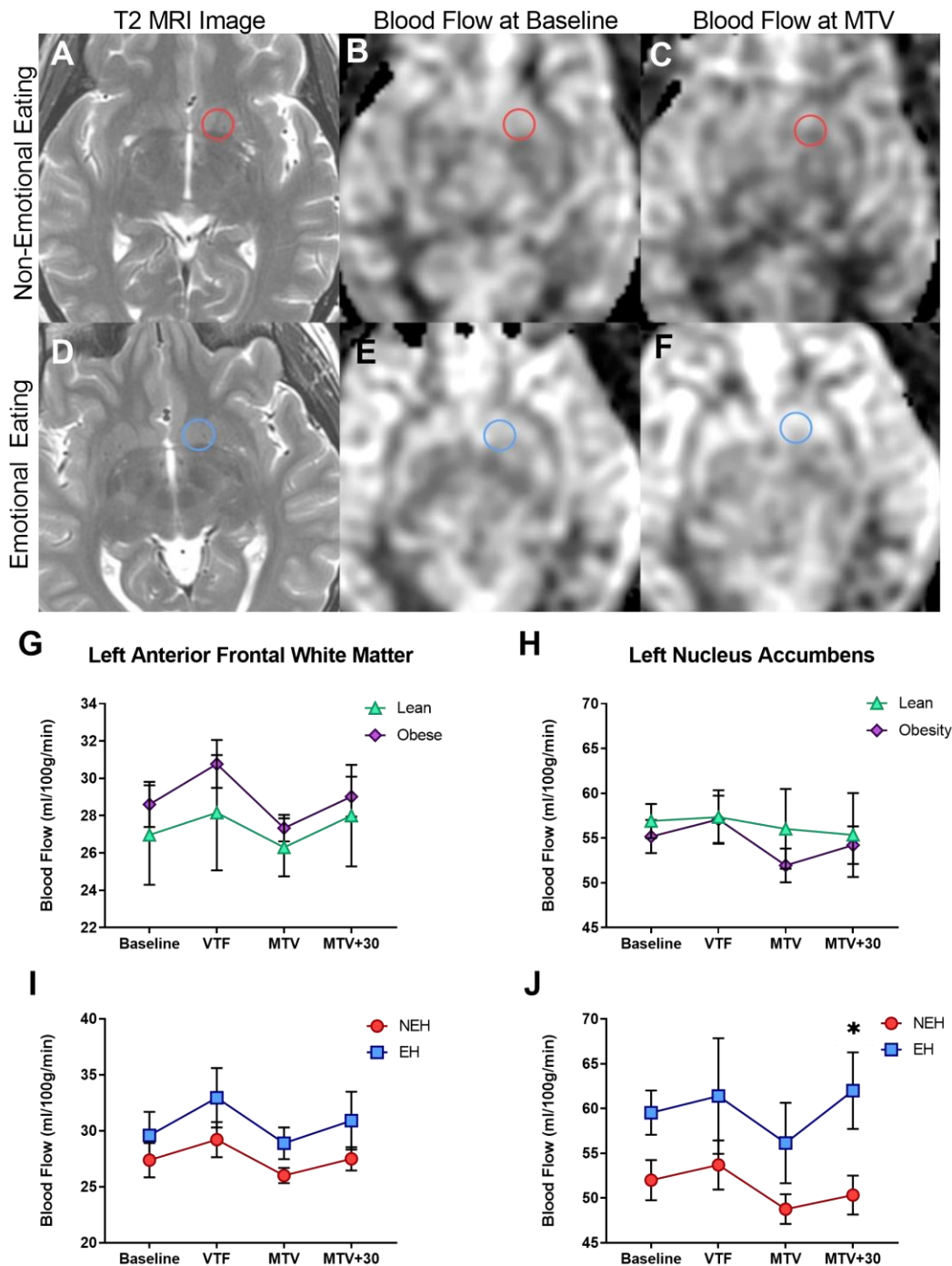
Figure 4. Emotional Hunger phenotype assessed by Pseudo-Continuous Arterial Spin Labeling (pCASL) MRI sequence. The blood flow to the left nucleus accumbens, region of interest, shown in red and blue circles for non-emotional hunger and emotional hunger, respectively, visible by T2 MRI imaging (A, D), pCASL imaging at baseline (B, E) and at maximal fullness (MTV) (C,F). Quantitated blood flow at baseline, volume to usual fullness (VTF), MTV, and 30 minutes after reaching MTV (MTV+30) was compared between groups for the left anterior frontal white matter, a control area (G, I), and the left nucleus accumbens (H,J). Green triangles= lean; purple diamonds= "all" obesity; red circles=NEH; blue-squares=EH. *$p<0.05$.

HYPOTHESIS AND AIMS

The unique quantitative data garnered in our preliminary studies led to the **overall hypothesis** that weight loss with pharmacological agents may be individualized, based on the baseline abnormality in obesity phenotype of each patient. Thus, each baseline trait could be targeted by pharmacological actions of specific obesity medications.

Hypothesis: We **hypothesize** that the identification of the obesity phenotype at baseline to guide obesity pharmacotherapy will enhance the weight loss response rate (i.e. percentage of patient with weight loss higher than 10% at 12 weeks).

Aim: In a 12 week, randomized, double-blinded, **active** controlled trial, with open-label 9-month extension of 250 participants with obesity; we will compare the weight loss response rate to obesity-phenotype-guided pharmacotherapy (intervention) vs. non-phenotype guided (randomly selected) pharmacotherapy (control) in patients with obesity and an abnormal obesity-related phenotype.
-Sub-aim 1: to study the effect of phenotype-guided pharmacotherapy in brain blood flow and gut mucosal changes.

SIGNIFICANCE

This proposal addresses a significant unmet public health need: the development of effective management approaches to treat obesity based in individual phenotypes. Currently, there are several safe and effective FDA-approved medications and devices for the treatment of obesity. Unfortunately, the response to obesity treatment (medicines, devices or surgery) is highly variable. Obesity phenotypes can be used to predict weight loss response to pharmacotherapy and devices. Thus, it essential that we understand the predictors of response to each intervention for obesity to be able to select the right tool for the right patient with minimal or no side effects – Individualized approach for obesity.
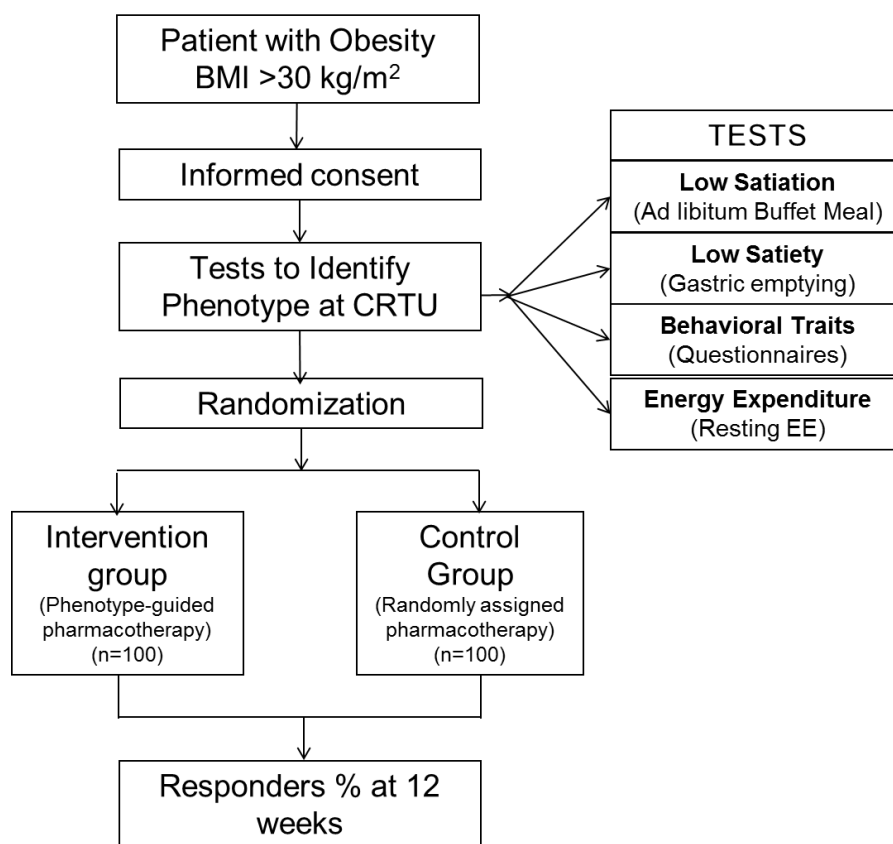
RESEARCH PLAN

Study Design:

In a 12 week, randomized, double-blinded, **active** controlled trial, with 9 month open-label extension of 250 participants with obesity; we will compare the weight loss response rate to obesity-phenotype-guided pharmacotherapy (intervention) vs. non-phenotype guided (randomly selected) pharmacotherapy (control) in patients with obesity. All 250 participants will be phenotyped and the medication selection will be randomly and double blinded (to physician, study team and participant) to the FDA-approved medicine suggested by the phenotype or to another FDA-approved medicine not suggested by the phenotype. A computer generated randomization schedule generated by the study statistician's office will be submitted to the Mayo Clinic CCaTS Research Pharmacy. Allocations will be concealed. This study will be blinded until data are transmitted to the statistician for data lock. All participants will receive a standard intense lifestyle intervention, which consists of 1 visit with registered dietitian. The phenotypic studies include (all performed in same day in the following order): Fasting blood collection, resting energy expenditure, gastric emptying

FIGURE 8: STUDY DESIGN - Randomized clinical trial to identify weight loss responders to obesity pharmacotherapy

with meal for breakfast, behavioral questionnaires, and buffet meal test for lunch. Blood will be collected assessment of metabolomic biomarkers, gastrointestinal hormones, and DNA (blood and buccal swab). Stool samples for microbiome and bile acid. Saliva samples for assessment of metabolomic biomarkers. Participants will return to the CRTU to pick up medication based on the randomization and a personal fitness tracker. All participants will be seen at 4 and 12 weeks (+/- 4 days) (current standard in practice). Participants will keep a medication diary, which will have to be returned on the 12-week visit. A saliva, stool and fasting blood sample, and DEXA will be done at the 12-week visit. At the 12-week visit, participants will be unblinded to their "obesity-related phenotype" and they could contact their physician to continue a FDA-approved medication as part of clinical care. Study team will prospectively follow the patients' weight, waist circumference and use of obesity medications every 3 months for 1 year. In a sub-study, 40 participants will complete at baseline and at 10 weeks (+/- 2 weeks), a flexible sigmoidoscopy and a pCASL functional MRI; with the intention to study the effects of pharmacotherapy in gut mucosa and brain blood flow changes at baseline and after treatment.

Randomization and Allocation

A computer generated randomization schedule generated by the study statistician's office will be submitted to the Mayo Clinic CCaTS Research Pharmacy. Randomization will be based on guiding pharmacotherapy based on the phenotype or randomly as current standard of care. Allocations will be concealed. This study will be blinded until data are transmitted to the statistician for data lock. All subjects will be given a verbal explanation of the study, provided time to read and study the written consent form and its information, given opportunities to ask questions and a copy of the consent form. Participants will be informed of their right to withdraw from the study at any time without prejudice to their clinical management now or in the future. Consent will be sought by one of the medical doctor investigators or the study coordinator, and consent will be documented by the participant's signature on the consent form. Mayo's Institutional

Review Board will approve the process and protocol. All the members of multidisciplinary team for weight management (i.e. physicians, coordinators, clinical assistants, registered dietitians will remain blinded).

Selection Participants

We plan to study a cohort of 200 patients with obesity (BMI>30 kg/m^2) and an abnormal obesity-related phenotype. Participants will be recruited from the Mayo Clinic Weight Management and Nutrition Clinic when they are offered a medication for weight loss as standard of care for obesity; from our gastroenterology clinics; from our previous existing database of more than 1000 participants with obesity and from advertising and Mayo Clinic classifieds. Two hundred and fifty participants that agree to pharmacotherapy treatment will be invited to participate in the phenotypic assessment of their obesity, that will guide (or not) the pharmacotherapy.

Inclusion criteria
- Adults with obesity (BMI >30Kg/m^2); these will be otherwise healthy individuals with no unstable psychiatric disease and controlled comorbidities or other diseases.
- Age: 18-75 years.
- Gender: Men or women. Women of childbearing potential will have negative pregnancy tests before each radiation exposure.
- Participant must have an abnormal phenotype based on testing done in visit 2.

Exclusion criteria
a) Abdominal bariatric surgery
b) Positive history of chronic gastrointestinal diseases, or systemic disease that could affect gastrointestinal motility, or use of medications that may alter gastrointestinal motility, appetite or absorption, e.g., orlistat, within the last 6 months.
c) Significant untreated psychiatric dysfunction based upon screening with the Hospital Anxiety and Depression Inventory (HAD), and the Questionnaire on Eating and Weight Patterns (binge eating disorders and bulimia). If such a dysfunction is identified by an anxiety or depression score >11 or difficulties with substance or eating disorders, the participant will be excluded and given a referral letter to his/her primary care doctor for further appraisal and follow-up.
d) Hypersensitivity to any of the study medications.
e) No contraindications to the FDA-approved medications: Phentermine-Topiramate Extended Release; Phentermine; Oral naltrexone extended-release/bupropion extended-release (NBSR; Contrave®, Mysimba™); and Liraglutide (Saxenda®).
f) Participants who meet the following criteria:
    a. No match to any phenotype based upon 90th and 75th percentile criteria; or
    b. Matches 2 or more phenotype based upon 90th percentile criteria; or
    c. No match to phenotype based on 90th percentile criteria and 2 or more match to phenotype based upon 75th percentile criteria
g) Sub Study specific exclusion criteria
    a. Any contraindications to MRI
    b. Claustrophobia

Anthropometrics and phenotype studies

Anthropometrics Measurements: will be taken of hip-waist ratio, height, weight, blood pressure, pulse at baseline, randomization day and week 12.
Phenotype studies at baseline:
Participants will attend the Mayo Clinic Clinical Research and Trials Unit after an 8-hour fasting period, and the following validated quantitative traits (phenotypes) will be measured at baseline:
o) The DEXA scan (dual energy x-ray absorptiometry) will measure body composition.
p) Resting energy expenditure: was assessed by indirect calorimetry with a ventilated hood (Parvo Medics, Sandy, UT).

q) Gastric emptying (GE) of solids by scintigraphy: The primary endpoint is gastric half-emptying time (GE $t_{1/2}$) [3, 51, 52].

r) Appetite (hunger level) by visual analog score fasting and after standard meal for GE and prior to the Satiation test [3].

s) Satiation will be measure by *ad-libitum* buffet meal to measure total caloric intake and macronutrient distribution in the chosen food. Satiation will be reported in calories consumed at fullness (satiation) [3].

t) Satiety by visual analog score postprandial after standard meal for GE and after to the Ad-libitum meal test for every 30 minutes for 2 hours [3]. Satiety will be measured in length of time of fullness.

u) Self-administered questionnaires assessing affect, physical activity levels, attitudes, , body image, and eating behavior; details of each questionnaire are provided below.

v) Samples collection, handling and storage: Samples were collected after an overnight fast (of at least 8 hours) in the morning. Plasma was preserved following standard guidelines and protein degradation inhibitors, kalikrein and DPP-IV inhibitors were added to preserve the samples. Samples are stored at -80ºC in the PI's laboratory.

   a. Plasma gastrointestinal hormones (Total and active Ghrelin, GLP-1, CCK, PYY and bile acids) by radioimmunoassay, measured fasting, and 15, 45, and 90 minutes postprandial, with the primary endpoint being the peak postprandial level (test should be done simultaneously to GE).

   b. Targeted Metabolomics: We will perform quantitative, targeted metabolomics of salient classes of compounds in plasma and saliva samples using mass spectrometry. These assays are well-established, validated, and routinely performed in the Mayo Clinic Metabolomics Core Laboratory. Amino acids plus amino metabolites will be quantified in plasma by derivatizing with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate according to Waters MassTrak kit. A 10-point calibration standard curve will be used for quantification of unknowns using a triple-stage quadrupole mass spectrometer (Thermo Scientific TSQ Quantum Ultra) coupled with an ultra-performance liquid chromatography (UPLC) system (Waters Acquity UPLC). Data acquisition will be performed using multiple-reaction monitoring (MRM). Concentrations of 42 analytes in each sample are calculated against their respective calibration curves with a measurement precision of < 5%. Essential nonesterified fatty acid (NEFA) concentrations, such as myristic, palmitic, palmetoleic palmitoelaidic, stearic, oleic, elaidic, linoleic, linolenic and arachidonic, will be measured against a six-point standard curve by LC/MS/MS, underivatized after extraction from plasma via negative electrospray ionization (ESI) and multiple reaction monitoring conditions. This technique was developed to replace the GC/MS method where NEFAs required methylation before analysis. This technique reduces the uncertainty as to whether the methylation step increases FFA concentrations by inadvertently hydrolyzing other lipid classes. Intra CV is < 3% for all analytes.

   c. Blood DNA.

w) Stool will be collected and stored to study microbiome, short chain fatty acids and bile acids.

x) Saliva will be collected by passive drooling using a Saliva Collection aid from SalivaBio (State college, PA). Saliva sample will be stored at -80C for metabolomics and GI peptides testing.

Sub-study at baseline and 10 weeks

- Flexible Sigmoidoscopy visit:
  1. After an overnight fasting, participants will come to the CRTU in Charlton 7.
  2. Flexible sigmoidoscopy: It will be performed in the CRTU with a prior tap water enema by the CRTU nurse. Using standard biopsy forceps, 24 mucosal biopsies will be obtained each from the left colon (descending, sigmoid and rectum; 8 mucosal biopsies for each).
     - Standard EE-cell immunohistochemistry: Tissues and sorted cells will be processed by standard clinical methods as published previously [53, 54].
     - Flow cytometry - Fluorescence-activated cell sorting (FACS)[55]:
     - Purified EE-cells from each group (healthy, obesity without hungry gut and obesity with hungry gut) will be sorted to validate the cell counts, cell morphology, DNA, total RNA content, mRNA expression and protein concentration of GLP-1 and PYY. mRNA will be extracted from the EE-cells as described previously[56]. Protein will be studied as described previously by Dr. LaRusso [57]. Supernatants and lysates will be assayed for active GLP-1 and/or total PYY by ELISA (Millipore, USA). RNA and protein will be stored for further studies. Collected tissue will be used for Standard EE-cell

immunohistochemistry, Flow cytometry - Fluorescence-activated cell sorting (FACS). EE cells sorted will be used for RNA, IHC and protein.

-

- Pseudo-continuous arterial spin labeling (pCASL) MRI visit
    1. After overnight fasting, participants will be provided a 300 kcal breakfast (breakfast granola bar and protein shake).
    2. Partipants will report to the Charlton LN or any other Mayo Clinic MRI facility four hours later and complete the MRI patient safety screening form
    3. pCASL MRI: (3 Tesla MRI scanner): will be performed three times during the nutrient drink test (baseline, after reaching maximal fullness and 30 minutes after maximal fullness). A single experienced radiologist (JDP) will be blinded to the timing of the scans performed all the ROI analyses and the groups.
    4. Nutrient Drink test: using Ensure, Abbott Labs, Abbott Park, IL will be done as described previously [3]. The main outcomes will be volume to usual fullness (VTF), maximal tolerated volume (MTV) and 30 minutes post-MTV.

Participants, who are not compensated for participating in the study, will be compensated for completing each visit of the sub-study.

Studies at 12-week visit:
- Saliva, stool and fasting blood sample will be collected and stored. Stool will be used to measure microbiome, short chain fatty acids and bile acids (as above). Fasting saliva and blood will be used to GI hormones and metabolomics (as above).
- The DEXA scan (dual energy x-ray absorptiometry) (as above).
- Behavioral questionnaires

Questionnaires to Assess GI Symptoms and Behavioral Disorders
Participants will complete a series of questionnaires (all included in the APPENDIX): Weight management Questionnaire (Mayo Clinic®) and the Hospital Anxiety and Depression Inventory [HAD [58]] to appraise the contribution of affective disorder.

Behavioural Questionnaires
a. AUDIT-C Alcoholism Screening Test [59] - The AUDIT-C is a 3-item alcohol screening questionnaire that reliably identifies participants who are hazardous alcohol drinkers or have active alcohol use disorders. This score will be used in screening by the study physician/nurse coordinator. The AUDIT-C is scored on a scale of 0-12. Each AUDIT-C question has 5 answer choices. Points allotted are: a=0 points; b=1 point; c=2 points; d=3 points; e=4 points. In men, a score of 4 or more is considered positive, optimal for identifying hazardous drinking or active alcohol use disorders. In women, a score of 3 or more is considered positive (same as above).
b. Eating Disorders Questionnaire - The Questionnaire on Eating and Weight Patterns-Revised [60], is a valid measure of screening for eating disorders which has been used in several national multi-site field trials. Respondents are classified as binge eating disorder, purging bulimia nervosa, non-purging bulimia nervosa, or anorexia nervosa. We have used this instrument to screen for eating disorders in obese populations.
c. Body Image Satisfaction - The Multidimensional Body-Self Relations Questionnaire [61, 62]provides a standardized attitudinal assessment of body image, normed from a national body-image survey. Items are rated on a 5-point scale, ranging from 1=Definitely Disagree to 5=Definitely Agree. In this study, we will use one of the sub-scales, *the Body Areas Satisfaction Scale*, which measures feelings of satisfaction with discrete aspects of physical appearance (e.g., face, weight, hair). Cronbach's α values range from .70 to .89 [62].
d. Eating Behaviors - The Weight Efficacy Life-Style Questionnaire [WEL [63]] is a 20-item eating self-efficacy scale consisting of a total score and five situational factors: negative emotions, availability, social pressure, physical discomfort, and positive activities. Subjects are asked to rate their confidence about being able to successfully resist the urge to eat using a 10-point scale ranging from 0=not confident to 9=very confident.

e.   Physical Activity Level - The four-item Physical Activity Stages of Change Questionnaire [64]will be utilized to assess the physical activity level of participants. Mayo Clinic investigators, led by co-investigator Dr. Clark, have used these items to explore the relationship between quality of life and physical activity in an NCI-funded study on long-term lung cancer survivors [64].

f. Exercise behavior- The Exercise Regulations Questionnaire (BREQ-3)*[65]* and its subsequent modifications have become the most widely used measures of the continuum of behavioural regulation in exercise psychology research. It has been used either as a multidimensional instrument giving separate scores for each subscale, or as a unidimensional index of the *degree* of self-determination.

g. Barriers to Increasing Physical Activity Participation - Barriers to Being Active Quiz, *What keeps you from being more active?[66].*

h. Three Factor eating questionnaire is 51-item questionnaire, validated, to assess for emotional eating disorders and food cravings.

i. Bowel Disease Questionnaire

Standard of Care:

All participants will received standard of care which consists of 1) Intense lifestyle intervention, behavioral evaluation and treatment, and a medication as part of the regular clinic management for obesity.

Intense Lifestyle Intervention and Behavioral Treatment

All the participants will meet the multidisciplinary team which consists of an Obesity Expert physician and a CRTU registered dietitian nutritionist as standard of care in our clinical practice. These appointments will be scheduled in the CRTU. All participants will guided to 1) Nutrition: Reduce dietary intake below that required for energy balance by consuming 1200calories per day for women and 1400calories per day for men; 2) Physical Activity: reach the goal of 10,000 steps or more per day; 3) Exercise: reach the goal of 150 minutes or more of cardiovascular exercise/week; 4) Limit consumption of liquid calories (i.e. sodas, juices, alcohol, etc.). All participants will receive a personal fitness tracker, where their activity and calories will be tracked. This information will be given in a booklet format.
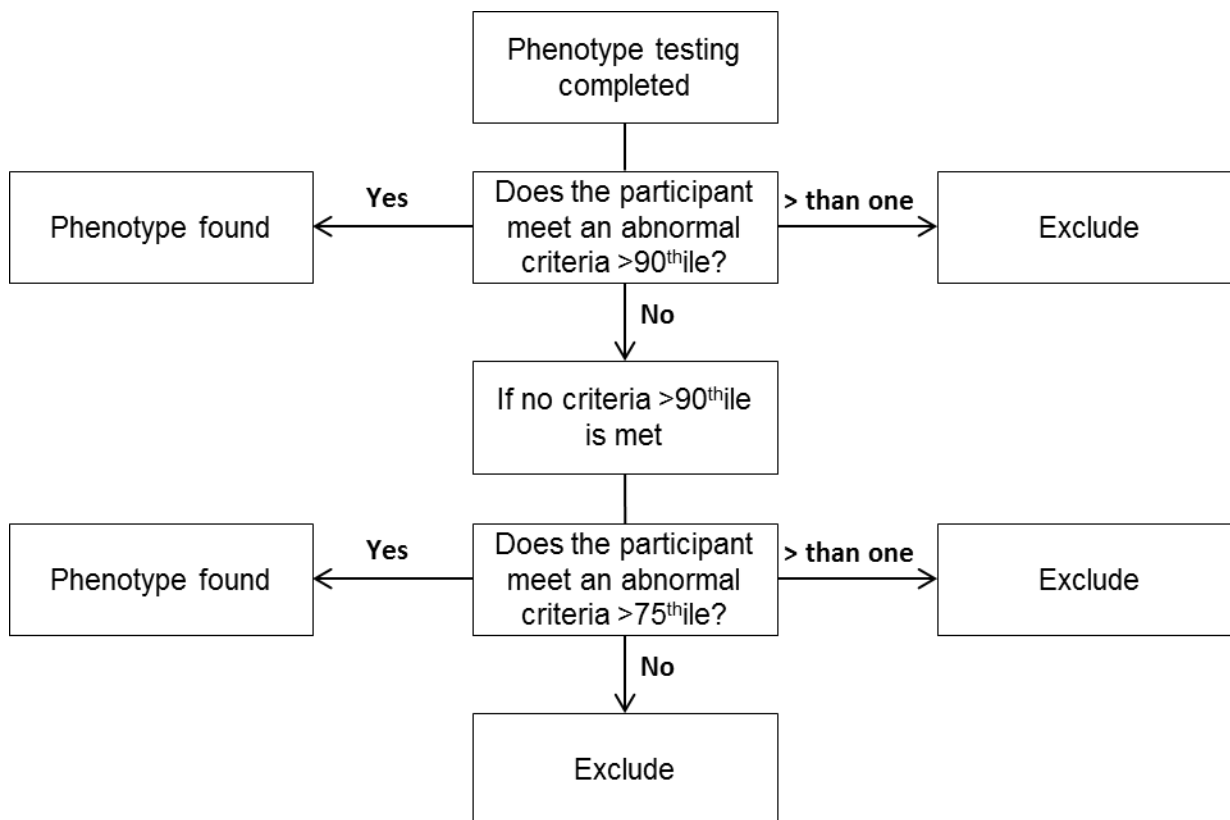
Pharmacotherapy for obesity

Pharmacotherapy for the treatment of obesity can be considered if a patient has a body mass index (BMI) ≥ 30 kg/m^2 or BMI >27 kg/m^2 with a comorbidity such as hypertension, type 2 diabetes, dyslipidemia and obstructive sleep apnea[16]. Medical therapy should be initiated with dose escalation based on efficacy and tolerability to the recommended dose. We do an assessment of efficacy and safety at 4 weeks. In both groups, medications will be assessed for drug interactions and potential side effects as standard of care.

Medication selection: Once the phenotype tests are completed the results will be filled in an algorithm to assist on the decision of the medication selection based on our previous data [3, 5, 11, 13]. The algorithm for phenotype selection will be based on the following decision tree (as figure below) 1) Participant will complete the ALL phenotype testing; 2) abnormal phenotype will be selected if participant meets criteria for one variable greater or lower than 90% of median (values described in table below); 3) if match to two or more abnormal 90% percentile) values, participant will be excluded; 4) if no abnormal (< or > 90%) value, the abnormal phenotype will be selected based on the value with < or > 75%; 5) if there are two or more abnormal values (75% percentile), patient will be excluded; 6) if participants do not have any abnormal value (based upon 90$^{%}$ of 75%) will be excluded.

TEST	Abnormal result				Example 1	Example 2	Example 3	Example 4
	Females		Males					
	(90%)	(75%)	(90%)	(75%)	Female	Male	Male	Female
Satiation (Ad libitum	> 1065 kcal	> 894 kcal	> 1962 kcal	>1376 kcal	1400 kcal	1000 kcal	1100 kcal	850 kcal

Buffet Meal)								
Satiety (Gastric emptying SGE $T^{1/2}$)	< 97 min	< 101 min	< 76 min	< 86 min	102 min	80 min	105 min	110 min
Behavioral Traits (Questionnaires)	HADS > 9 points	HADS > 7 points	HADS > 10 points	HADS A > 7 points	5	4	9	3
Energy Expenditure (Resting EE)	< 93% predicted	< 96% predicted	< 88% predicted	<94% predicted	102%	98%	95%	92%
Phenotype				-	Ab Satiation	Ab Satiety	Ab Psych	Ab E.E.

Once the decision is made on the "phenotype-guided" medication, pharmacy will assess whether patient is randomized to "intervention" or "control". Based on the randomization, patient will pick up the prescription for 3 months. Phentermine prescribed subjects will be recommended to involve into resistance training routines. During the 3 month visit, participants will be offer a prescription to continue the medication (if randomized to the intervention group) or switch to the phenotype guided medication (if randomized to the control group). Patients who continue obesity pharmacotherapy will be contact every three months for one year to monitor their weight and comorbidities.

Control group: Pharmacotherapy for obesity

Standard of care pharmacotherapy for obesity recommends the following doses and regimen for weight loss:
- Phentermine: 15 mg oral daily
- Phentermine-Topiramate Extended Release (Qsymia®) at dose of 7.5/46 mg oral daily
- Oral naltrexone extended-release/bupropion extended-release (NBSR; Contrave®) at dose of 32/360 mg oral daily (divided in 2 tables in morning and 2 tablets in evening)
- Liraglutide (Saxenda®) at dose of 3 mg subcutaneous daily

There is currently no gold-standard or first choice for obesity pharmacotherapy and physicians select the medication to use on their patients based on comorbidities, previous experience, safety or adverse events. Thus, in this protocol, the FDA-approved medications will be randomly selected. The physician will review the "selection" to assess for potential contraindications, adverse events and/or drug-drug interactions per inclusion and exclusion criteria.

Intervention group: by obesity phenotype guided pharmacotherapy

Participants in the intervention group will have 4 tests to assess 1) satiation, 2) Satiety/return to hunger, 3) behavioral, or 4) energy expenditure. As described on Figure 8 pharmacotherapy will by guide based on the "abnormal" phenotype (following algorithm described above). In case of a mixed pattern or multiple abnormal phenotypes, patient will be excluded from study and referred to weight management clinic.
Algorithm diagnostic:
1. satiation: Phentermine-Topiramate Extended Release (Qsymia®) at dose of 7.5/46 mg oral daily
2. Satiety/return to hunger: Liraglutide 3 mg SQ daily
3. Behavioral/Psychological: Oral naltrexone extended-release/bupropion extended-release (NBSR; Contrave®) at dose of 32/360 mg oral daily (divided in 2 tables in morning and 2 tablets in evening); or
4. Energy expenditure: Phentermine 15 mg daily plus increase physical activity and resistance training.

General Principles of Statistical Analyses

Primary endpoint: Total Body Weight Loss, kg (defined as weight changed from baseline to 12 weeks) in the obesity phenotype-guided pharmacotherapy (intervention) vs. the randomly assigned pharmacotherapy (control) group in patients with obesity and an abnormal obesity-related phenotype.

The secondary end points will be percentage of responders (defined as number of participants who loss 5% or more of total body weight) compared to baseline in the obesity phenotype guided pharmacotherapy (intervention) group vs. standard of care at 4 and 12 weeks; percentage of responders with at least 10 and 15% at 12 weeks, and 10% at 6 months and 12 months; percentage of responders at 5%, 10% and 15%; percentage of responders within each obesity-phenotype group at 4 and 12 weeks; side effects of medications, pharmacogenomics role to weight loss,side effect and; changes in blood flow in the brain areas, and transcriptomic changes in colonic mucosa.; the performance of the PHENO-Test in predicting the obesity phenotype and the weight loss outcome based on the test when comparing phenotype-guided intervention vs. controls. In the open-label extension, we will assess the total body weight loss at 24 and 52 weeks in both groups.

Statistical Analyses: We propose a randomized, double-blinded, active controlled trial of 200 participants with obesity to compare effects of Intervention compared to controls in weight loss. The analysis will involve an ANCOVA models, with the response being actual weight change; the covariates to be considered include gender, and weight at baseline.
Sample size assessment and power calculation: The detectable effect size in weight loss between groups of interest (intervention vs. control) is given in the table below. In our recent pilot study [with Liraglutide 3.0 mg vs. placebo], the SD for the overall weight change (pre-post) observed was 2.8kg [32]. Using this SD, we have estimated the differences between groups that could be detected with approximately 80% power (2-sided α level of 0.05) for main effects. Thus, the sample size needed is 87 participants per group. In order to account for a maximum dropout rate of 13%, we will randomize 100 participants per group. An interim assessment will be done when 50% of the patients have completed the study for the purposes of assessing data quality and the power calculation assumptions. Specifically, that the weight change standard deviation is 2.8 and the dropout rate is 10-13%. No calculation of the treatment difference will be

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done at this assessment. Any suggested revision of the power and sample size calculation based on the standard deviation or dropout rate will be shared with the investigative team - possibly increasing the planned study enrollment. We have no intention of completing the study with less than the original sample size of 100 per group. In the subaim, we will use an ANCOVA to study 20 participants with phenotype-guided pharmacotherapy and compared to 20 participants randomly assigned pharmacotherapy adjusting for their baseline value as a covariate. Additionally, we will study the changes of each drug on treatment compared to baseline using a paired t-test. We noticed a 20% withdrawal rate during the study, we propose to extend to 250 participants enrolled and randomized to reach the 100 participants per group (total of 200 participants) who have completed the study.

Mean difference (Δ) of total body weight loss in controls group (mean average 6.1 kg) vs. intervention group.	Intervention (# of participants)	Control (# of participants)
Mean difference of 10% [6.7 vs. 6.1kg)	343	343
Mean difference of **2**0% [7.3 vs. 6.1kg)	87	87
Mean difference of 30% [7.9 vs. 6.1kg)	39	39

Anticipated results and significance:
Our study individualizes obesity treatment to maximized pharmacotherapy outcome based on phenotyping obesity at baseline.

Potential pitfalls, precautions taken, and alternative strategies:
a. <u>Feasibility</u> - Given high volume of patients interested in weight loss, we are confident we will recruit sufficient participants for these studies that involve only noninvasive tests and standard of care treatment.
b. <u>Statistical power</u> has been addressed with appropriate sample sizes to demonstrate a difference in weight change on NBSR with and with phenotype vs. placebo.

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