Supplemental material

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Supplemental Methods

Phenotyping study:

We studied a total of 450 participants with Obesity (defined as BMI >30 kg/m^2). The study was approved by the Mayo Clinic Institutional Review Board, and all participants gave written informed consent following thorough explanation of the study details. Women of childbearing potential had a negative pregnancy test within 48 hours prior to testing. All the studies were performed at the Mayo Clinic Clinical Research Trials Unit after an 8 hour fasting period. Participants completed a screen visit and a phenotype visit with the following tests performed in the order described and completed in one day: resting energy expenditure by indirect calorimetry, gastric emptying of solids and liquids by scintigraphy, VAS for appetite score after gastric emptying breakfast, and satiation by means of an ad-libitum buffet meal ingested 4 hours after the meal used to assess gastric emptying and VAS for appetite score after buffet meal test. We included men and women, age 18-65 and have a stable weight for the previous 3 months. We excluded patients with recent use of weight loss medications (<6 months), history of abdominal GI surgery other than appendectomy, pregnancy, uncontrolled systemic disease, or medications that might interfere with motility, appetite or absorption.

Phenotype tests methods

- Resting energy expenditure was assessed by indirect calorimetry with a ventilated hood (Parvo Medics, Sandy, UT)(1, 2).
- Gastric emptying (GE) of solids was assessed by scintigraphy using a 320kcal ^{99m}Tc-radiolabeled egg, solid-liquid meal. The primary endpoint was gastric half-emptying time (GE $T_{1/2}$)(3, 4).
- Satiation was measured by ad-libitum buffet meal test measured total caloric intake and macronutrient distribution in the chosen foods from standard foods of known nutrient composition: lasagna (Stouffers, Nestle USA, Inc., Solon, OH, USA]; vanilla pudding (Hunts, Kraft Foods North America, Tarrytown, NY, USA); and skim milk. The total kilocalories of food consumed and macronutrients ingested at the ad libitum meal were analyzed by validated software (ProNutra 3.0; Viocare Technologies Inc., Princeton, NJ, USA)(3);
- Appetite Score (hunger, fullness, satisfaction and desire to eat) was assessed by 100-mm visual analog score visual analog score fasting and postprandial after the standard meal for GE and after the Ad-libitum meal test; scores were obtained every 30 minutes for 2 hours(5)
- DEXA scan (dual energy x-ray absorptiometry) was used to measure body composition(6).
- Self-administered questionnaires: assessing affect, physical activity levels, attitudes, body image, and eating behavior; details of each questionnaire are provided below:

    - AUDIT-C Alcoholism Screening Test(7) - 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.
    - Eating Disorders Questionnaire - The Questionnaire on Eating and Weight Patterns-Revised(8), is a valid measure of screening for eating disorders in obese populations(9).
    - Body Image Satisfaction - The Multidimensional Body-Self Relations Questionnaire(10, 11)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.

- Eating Self-Efficacy - The Weight Efficacy Life-Style Questionnaire (WEL)(12, 13) 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. Participants 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.
- Physical Activity Level - The four-item Physical Activity Stages of Change Questionnaire(14) was utilized to assess the physical activity level of participants.
- Exercise behavior- The Exercise Regulations Questionnaire (BREQ-3)(15) as a measure of the continuum of behavioral regulation in exercise psychology research, or as a unidimensional index of the *degree* of self-determination.
- Barriers to Increasing Physical Activity Participation - Barriers to Being Active Quiz, What keeps you from being more active?(16).
- Three Factor Eating Questionnaire - is a 21-item questionnaire, validated, to assess for emotional eating disorders and food cravings.(17)

Statistical Analysis: with the intention to translate a quantifiable and reproducible obesity-phenotype classification based on a specific *a priori* determined cutoff, we selected the 75th percentile of each measurement in the first 100 females and males and then applied this cut-off to characterize the function for each phenotype (table 1). We used this cutoff to identify the prevalence of the five distinct phenotypes among the patients with obesity (Figure 1b). No further statistical analysis was performed as the obesity phenotypes described in figures 2A-D as they were *a priori* determined cutoff, and therefore will be artificially statistically significant.

Phenotype-guided pharmacotherapy

Rationale: Our basic hypothesis is that the classification of obesity based on pathophysiological phenotypes, serves as a clinical tool to identify the best responders to obesity interventions directed at their unique pathophysiological abnormalities. We tested the 'actionable phenotype' classification by assessing weight loss in response to tailored obesity treatment. This approach could complement other approached in prevention of disease progression, consistent with the NIH -Precision Medicine Initiative(18).

We previously tested the obesity phenotype approach with three FDA-approved medications in, randomized, double-blinded, placebo-controlled, proof-of-concept clinical trials(3, 6, 19) and showed that a phenotype-tailored application may predict best responders for the following medications: Phentermine-topiramate ER for the hungry brain phenotype(3); and exenatide and liraglutide for the hungry gut phenotype(6, 19). Additionally, the best responders for FDA-approved intragastric balloons may be patients with a hungry gut phenotype(20).

Design and Eligibility Criteria:

A comprehensive, prospective, pragmatic trial of phenotype-guided anti-obesity medications in patients with obesity in a multidisciplinary weight loss clinic was approved by the Mayo Clinic Institutional Review Board (IRB 17-001068) and included patients enrolled between June 1, 2017 and June 30, 2019. Treatment decisions were determined *a priori* by a management approach; supported by our previously completed trials as well as the medications predominant mechanism of action (see below phenotype-guided anti-obesity medication guideline). We report the outcomes of 84 obesity phenotype-guided treated patients compared to 228 non-phenotype treated patients (standard of care), who were assigned to either group based on clinical schedule

availability. The phenotype-guided group had their phenotype measured by clinically available tests (satiation test: nutrient drink test; satiety test: gastric emptying by scintigraphy; emotional hunger: validated questionnaires; and slow burn: indirect calorimetry resting energy expenditure). Patients outcomes were tracked through integrated medical record query tools (ACE-Advanced Cohort Explorer- and i2b2-Informatics for Integrating Biology and the Bedside) and data was gathered from the electronic medical record. Participants included in the analysis met these inclusion criteria: 1) patients with a BMI ≥27 kg/m^2 with adiposity-related co-morbidities or patients with BMI ≥30 kg/m^2 with or without adiposity-related co-morbidities; 2) patients prescribed FDA-approved anti-obesity medications; 3) follow-up of at least 3 months; and, 4) two or more face-to-face visits with one of the physicians at the Mayo Clinic Weight Management Program. We excluded all patients who: 1) had prior major gastrointestinal surgery; 2) had prior endoscopic weight loss intervention; 3) did not fill the medication prescription due to health insurance coverage denial and/or high drug cost, and 4) were taking FDA-approved anti-obesity medications prior to the first visit to the Mayo Clinic Weight Management Program, since such a prior prescription may conceivably confound the weight loss outcomes. All the information was collected from physician's documentation including outcomes and adverse events. See CONSORT-extention guidelines checklist below.

Mayo Clinic Weight Management Program: The Mayo Clinic Weight Management program involves a multidisciplinary team that includes 8 physicians (with an average experience in obesity medicine practice of 15 years), registered dietitians, advanced practice providers (Nurse Practitioners, Physician Assistants) and behavioral psychologists. Upon initial evaluation, patients are encouraged to meet with a dietitian and with the behavioral psychology team. All patients are encouraged but not obligated to participate in a standardized 12 week behavioral program. The general recommendations are to 1) reduce dietary intake to 1200–1500 calories per day for women and 1500–1800 calories per day for men, 2) achieve a goal of 10,000 steps or more per day and 150 minutes or more of cardiovascular exercise per week, and 3) limit the consumption of liquid calories (sodas, juices, alcohol, etc.). Calorie restriction and counselling on activity might vary widely based on comorbidities and functional capacity.

Flow of Participants in Pragmatic trial: Participants were assigned to a clinical appointment with a provider (MD/NP) offering either standard of care or phenotype-guided pharmacotherapy or phenotype-guided pharmacotherapy. The random assignment was performed when patients requested an appointment, not by a randomization code and allocation sequence, in order to reflect as much as possible, the principles of a pragmatic trial (21, 22) (supplemental figure 1).

Interventions: Patients prescribed with anti-obesity pharmacotherapy were divided into two groups: a group that received pharmacotherapy based on their phenotype, and a group of patients that were prescribed an anti-obesity medication for other reasons (insurance coverage, previous success/non-success, avoidance of side effects, and patient preference). Patients were scheduled to return for follow-up visits at 3, 6, 9 and 12 months after anti-obesity pharmacotherapy is initiated. During each visit, information on weight loss, adherence to medications and recommendations, and side effects were collected.

Non-Phenotype-guided Pharmacotherapy for Obesity (Control group or Standard of care)

Standard of care pharmacotherapy for obesity recommends the following doses and regimen for weight loss:

- Phentermine: 15-37.5 mg oral daily
- Phentermine-Topiramate Extended Release (Qsymia®) at dose of 3.23/23 mg to 15/82 mg oral daily
- Locarserin (Belviq®) at 10 - 20 mg 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 selected the medication to use on their patients based on physician/patient preference, medication interactions, comorbidities, risk of potential adverse events or insurance coverage(23-25).

Phenotype-guided Pharmacotherapy for Obesity (Intervention group)

Participants in the phenotype-guided pharmacotherapy for obesity group received their medication based on their obesity phenotype:

- Hungry brain (abnormal satiation): Phentermine-Topiramate Extended Release (Qsymia®) at dose of 7.5/46 mg oral daily; or Locarserin (Belviq®) at 20 mg daily
- Hungry Gut (abnormal satiety): Liraglutide (Saxenda®) 3 mg SQ daily
- Emotional Hunger (abnormal hedonic eating): 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
- Slow Burn (low predicted energy expenditure): Phentermine 15 mg daily plus increase resistance training.

Study End-Points: In this comprehensive, prospective, pragmatic trial of phenotype-guided anti-obesity medications the primary end-point was the percentage weight loss during 1-year follow-up for patients prescribed a FDA-approved anti-obesity medication. Thirty-two patients were excluded because they did not meet the inclusion criteria due to "no" phenotype or were not able to start 'assigned' medication. There were no difference in demographics in the excluded patients (age [mean ± SEM)]: age 45.7 ± 2.3 years old, BMI 40.6 ± 1.6 kg/m^2, 75% females, 100% white, fasting glucose 107 ± 4.3 mg/dl).

The modified intention-to-treat cohort was defined, in accordance with protocol, as patients who were enrolled, received treatment and had at least 1 follow-up visit during the following 12 months. We used the last observation carried forward to appraise treatment efficacy. A per protocol analysis was based on participants who completed 3, 6, 9, or 12 months of treatment and follow-up. Secondary endpoints included the proportion of patients who had a reduction from baseline body weight of ≥5%, ≥10%, ≥15% and ≥20%.

Statistical analysis: All continuous data are summarized as means and SEM. Categorical data are presented as frequencies and percentages. We used Pearson χ^2 and unpaired Student, two-tailed t-test for between-group comparisons for baseline nominal and ordinal variables. All P-values <0.05 were considered statistically significant.

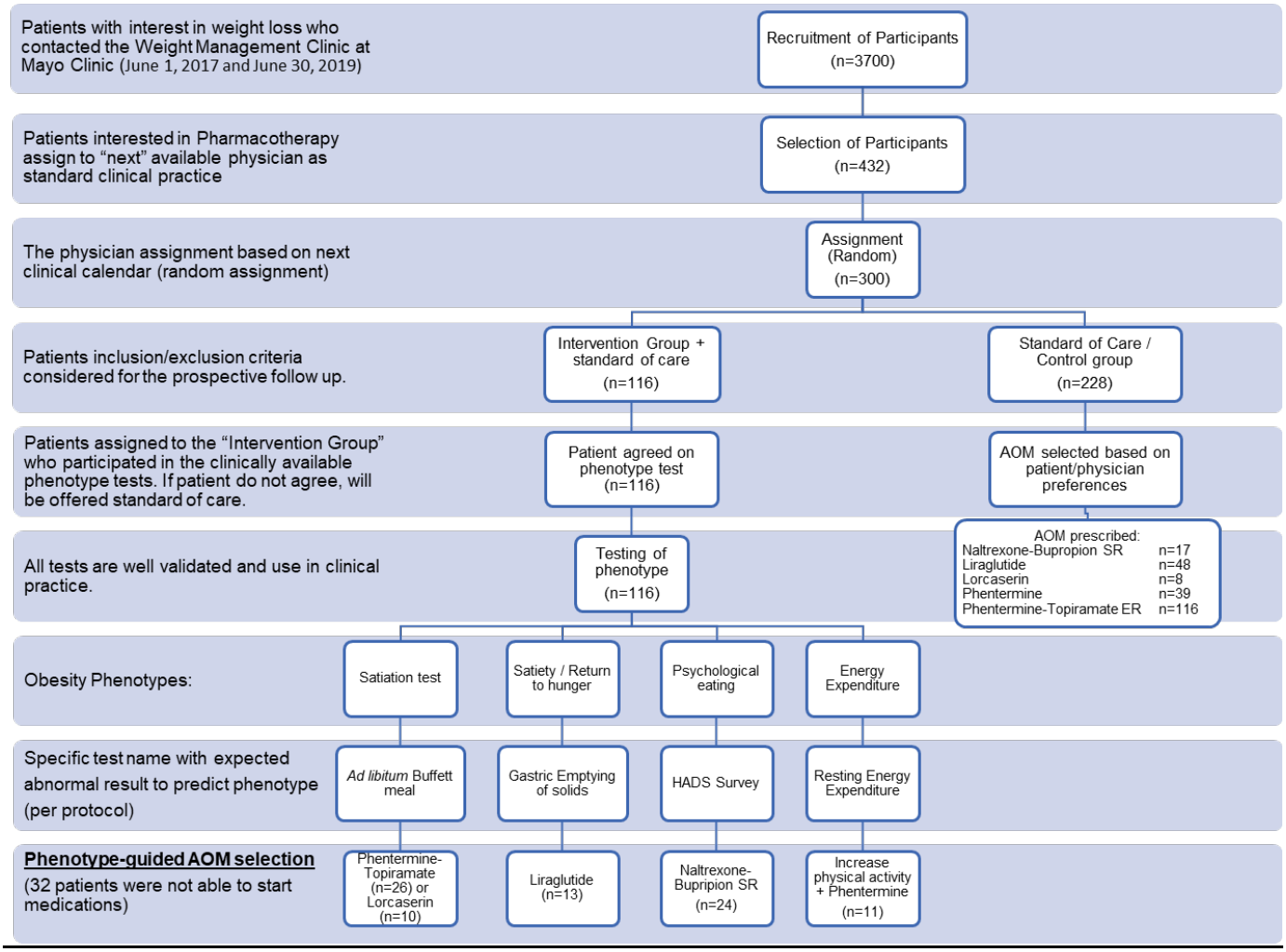
Supplemental Table

Supplemental table 1: Participants characteristics and outcomes of anti-obesity pharmacotherapy in a phenotype-guided intervention vs. non-phenotype guided intervention (standard of care) in sub-cohort of patients with obesity and type 2 diabetes. Data shown in Mean ± SEM.

Characteristic	Variable	Phenotype Guided Therapy	Non-Phenotype Guided Therapy	Difference (95% CI)	P-value
Patient Demographics	N	21	67		
	Age, y	48 ± 3.3	54 ± 1.9	-6.5 (-14 to 1.3)	0.10
	Gender (F), %	63	55		0.53
	Race (White), %	84	97		**0.04**
	Weight (kg)	123 ± 4.7	128 ± 2.7	-5.0 (-16 to 6.1)	0.36
	Height (cM)	170 ± 1.8	170 ± 1.2	0.3 (-3.9 to 4.6)	0.88
	BMI, kg/m^2	42.4 ± 1.4	44.4 ± 0.9	-1.9 (-5.4 to 1.4)	0.25
	Blood Presure (SBP),mmHg	128 ± 5.5	132 ± 2.1	-4.7 (-17 to 7.5)	0.43
	Blood Presure (DBP),mmHg	79 ± 3.9	76 ± 1.4	2.5 (-6.2 to 11)	0.55
	Fasting Glucose, mg/dl	158 ± 28	157 ± 13.4	1.1 (-184 to 186)	0.97
	Hemoglobin A1C, %	6.5 ± 0.3	7.7 ± 0.3	-1.2 (-1.9 to -0.3)	**0.008**
	DM medications, #	0.9 ± 0.3	1.5 ± 0.1	-0.6 (-1.3 to 0.1)	0.09
Medications use	Naltrexone-Bupropion SR	6 (32%)	6 (9%)		**0.004**
	Liraglutide	7 (37%)	40 (60%)		
	Lorcaserin	2 (11%)	0 (0%)		
	Phentermine	0 (0%)	6 (9%)		
	Phentermine-Topiramate ER	4 (20%)	15 (22%)		
Intervention	Follow up, Months	9.6 ± 1.0	8.4 ± 0.5	1.3 (-1 to 3.5)	0.27
	# Follow up Visits	3.3 ± 0.2	3.1 ± 0.1	0.3 (-0.2 to 0.7)	0.24
	Pts with ≥ 1 follow up visit 0-6 months with physician	17 (81%)	60 (91%)		0.50
	Pts with ≥ 1 follow up visit 6-12 months with physician	11 (52%)	33 (50%)		0.40
	Pts with ≥ 1 dietitian visit	4 (21%)	33 (52%)		**0.02**
	# Dietitian visits	0.9 ± 0.4	0.82 ± 0.1	0.07 (-0.8 to 1.0)	0.86
	Patients with > 1 psych visit	1 (11%)	25 (39%)		0.10
	# Behavioral Psych Visits	0.1 ± 0.1	0.7 ± 0.2	-0.6 (-0.9 to -0.2)	**0.006**
Intervention Outcomes	Weight loss at 3 months, %	-5.3 ± 0.9	-4.1 ± 0.6	-1.2 (-3.4 to 1.1)	0.29
	Weight loss at 6 months, %	-9.7 ± 1.4	-5.3 ± 0.7	-4.4 (-7.7 to -1.1)	**0.01**
	Weight loss at 12 months, %	-12 ± 1.7	-6.3 ± 1.0	-5.7 (-9.8 to -1.5)	**0.009**
	Weight loss at LOCF, %	-10.5 ± 1.3	-6.2 ± 0.7	-4.3 (-7.3 to -1.3)	**0.007**
Adverse Events	Documented adverse events	3 (16%)	17 (25%)		0.37

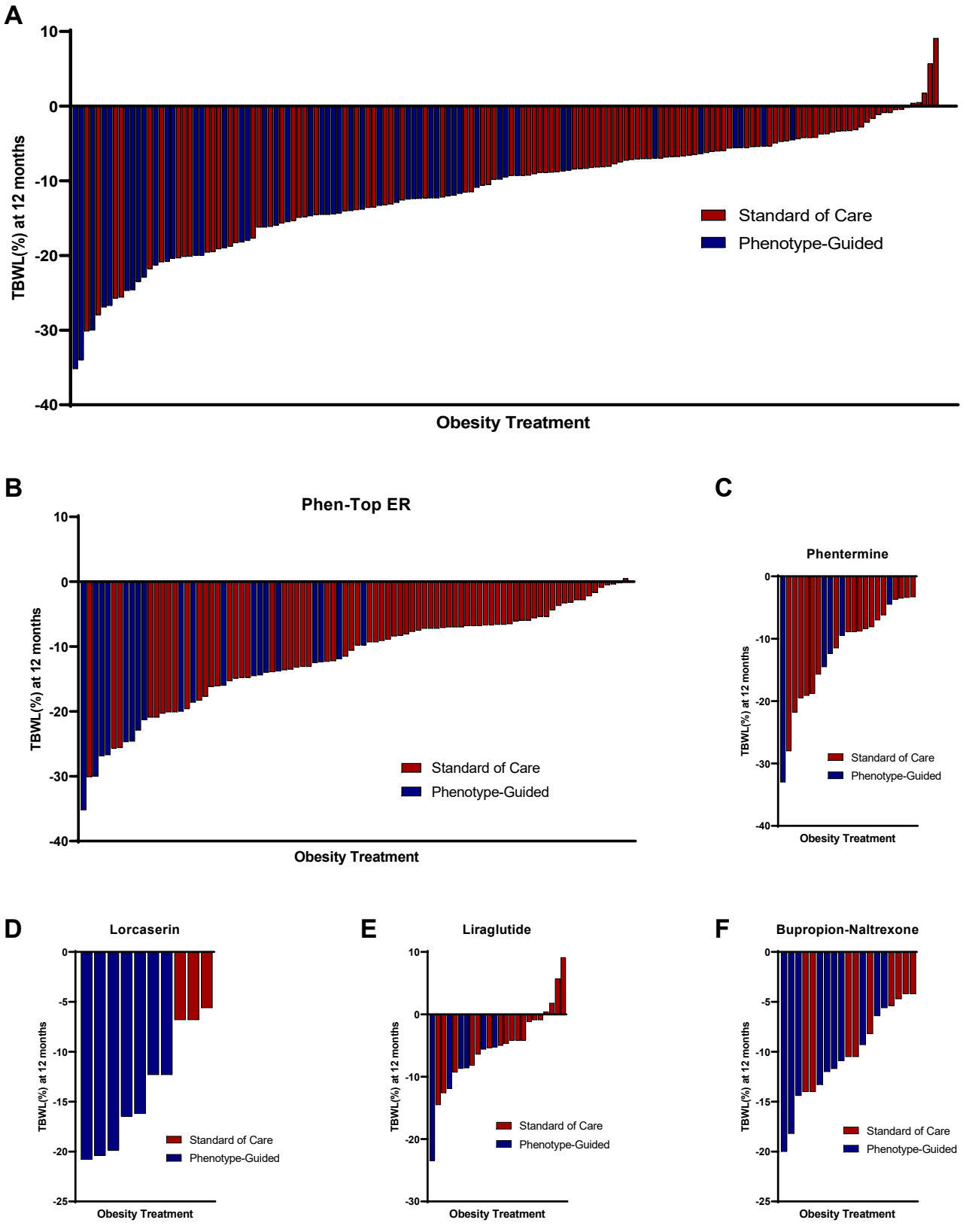
Supplemental Figures

Supplemental Figure 1. CONSORT-extension flow chart.

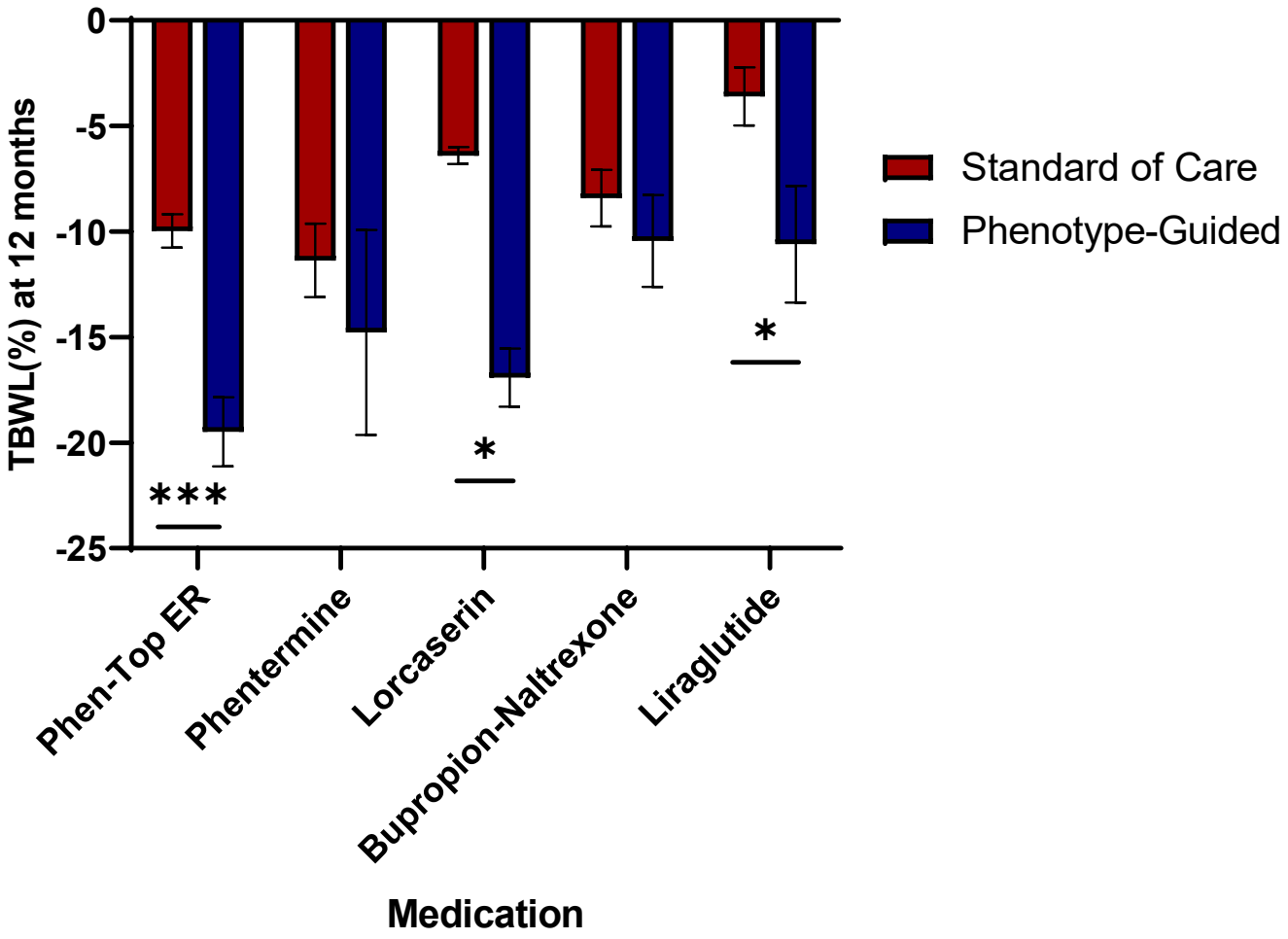


AOM: anti-obesity medication
HADS: Hospital anxiety and depression survey

Supplemental Figure 2. Waterfall plots for each individual weight loss in the whole cohort and per medication. Blue bars = phenotype-guided AOM; red bars = standard of care AOM. Data shown in percentage total body weight loss (TBWL %).



Supplemental figure 3. Phenotype-Guided Specific-Anti-obesity Medication Effect on Weight Loss Outcomes. The average percentage of total body weight loss non-phenotype guided (red columns), and phenotype-guided (blue columns) treatment at 12 months for each anti-obesity medication. * p<0.05. ***p<0.001. Data shown in mean +/- SEM



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STROBE Statement—Checklist of items that should be included in reports of ***case-control studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	1,2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	2-4, sup1-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2, sup 1-3
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	3, sup 1, 3
		(*b*) For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	2-4, sup 1-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	2-4, Sup1-5
Bias	9	Describe any efforts to address potential sources of bias	Sup 4-5
Study size	10	Explain how the study size was arrived at	Protocol 16
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	2-4, sup1-5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	4, Sup 3-5
		(*b*) Describe any methods used to examine subgroups and interactions	4, Sup4-5
		(*c*) Explain how missing data were addressed	4, Sup5
		(*d*) If applicable, explain how matching of cases and controls was addressed	Sup4-5
		(*e*) Describe any sensitivity analyses	NA
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	4-8, table 1-2
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	Supp 6
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	4-8, table 1-3,
		(b) Indicate number of participants with missing data for each variable of interest	table 1-3

Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	4-8, table 1-2
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	4-8, table 3
		(*b*) Report category boundaries when continuous variables were categorized	Table 1 - 3
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7-8, supp table 1
Discussion			
Key results	18	Summarise key results with reference to study objectives	8, 9
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8,9
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls.

CONSORT – Extension checklist of items for reporting pragmatic trials

Section	Item	Standard CONSORT description	Extension for pragmatic trials	Page No
Title and abstract	1	How participants were allocated to interventions (eg, “random allocation,” “randomised,” or “randomly assigned”)		1
Introduction				
Background	2	Scientific background and explanation of rationale	Describe the health or health service problem that the intervention is intended to address and other interventions that may commonly be aimed at this problem	1,2
Methods				
Participants	3	Eligibility criteria for participants; settings and locations where the data were collected	Eligibility criteria should be explicitly framed to show the degree to which they include typical participants and/or, where applicable, typical providers (eg, nurses), institutions (eg, hospitals), communities (or localities eg, towns) and settings of care (eg, different healthcare financing systems)	3,4, suppl 3-5
Interventions	4	Precise details of the interventions intended for each group and how and when they were actually administered	Describe extra resources added to (or resources removed from) usual settings in order to implement intervention. Indicate if efforts were made to standardise the intervention or if the intervention and its delivery were allowed to vary between participants, practitioners, or study sites	3,4, suppl 2-5
			Describe the comparator in similar detail to the intervention	4, suppl 4
Objectives	5	Specific objectives and hypotheses		2,4, suppl 2,3
Outcomes	6	Clearly defined primary and secondary outcome measures and, when applicable, any methods used to enhance the quality	Explain why the chosen outcomes and, when relevant, the length of follow-up	4

Section	Item	Standard CONSORT description	Extension for pragmatic trials	Page No
		of measurements (eg, multiple observations, training of assessors)	are considered important to those who will use the results of the trial	
Sample size	7	How sample size was determined; explanation of any interim analyses and stopping rules when applicable	If calculated using the smallest difference considered important by the target decision maker audience (the minimally important difference) then report where this difference was obtained	4, suppl 4
Randomisation—sequence generation	8	Method used to generate the random allocation sequence, including details of any restriction (eg, blocking, stratification)		NA
Randomisation—allocation concealment	9	Method used to implement the random allocation sequence (eg, numbered containers or central telephone), clarifying whether the sequence was concealed until interventions were assigned		3,4, suppl 3-4
Randomisation—implementation	10	Who generated the allocation sequence, who enrolled participants, and who assigned participants to their groups		NA
Blinding (masking)	11	Whether participants, those administering the interventions, and those assessing the outcomes were blinded to group assignment	If blinding was not done, or was not possible, explain why	NA
Statistical methods	12	Statistical methods used to compare groups for primary outcomes; methods for additional analyses, such as subgroup analyses and adjusted analyses		4, suppl 4
Results				
Participant flow	13	Flow of participants through each stage (a diagram is strongly recommended)—specifically, for each group, report the numbers of participants randomly assigned, receiving intended treatment, completing the study protocol, and	The number of participants or units approached to take part in the trial, the number which were eligible, and reasons for non-participation should be reported	5-8, sup figure 1, table 3

Section	Item	Standard CONSORT description	Extension for pragmatic trials	Page No
		analysed for the primary outcome; describe deviations from planned study protocol, together with reasons		
Recruitment	14	Dates defining the periods of recruitment and follow-up		3
Baseline data	15	Baseline demographic and clinical characteristics of each group		5-6, table 3
Numbers analysed	16	Number of participants (denominator) in each group included in each analysis and whether analysis was by "intention-to-treat"; state the results in absolute numbers when feasible (eg, 10/20, not 50%)		5-8, table 3
Outcomes and estimation	17	For each primary and secondary outcome, a summary of results for each group and the estimated effect size and its precision (eg, 95% CI)		5,8, table 3, sup table 1
Ancillary analyses	18	Address multiplicity by reporting any other analyses performed, including subgroup analyses and adjusted analyses, indicating which are prespecified and which are exploratory		7,8 sup table 1
Adverse events	19	All important adverse events or side effects in each intervention group		8, table 3
Discussion				
Interpretation	20	Interpretation of the results, taking into account study hypotheses, sources of potential bias or imprecision, and the dangers associated with multiplicity of analyses and outcomes		8, 9
Generalisability	21	Generalisability (external validity) of the trial findings	Describe key aspects of the setting which determined the trial results. Discuss possible differences in other settings where clinical traditions, health service organisation, staffing, or	8, 9

Section	Item	Standard CONSORT description	Extension for pragmatic trials	Page No
			resources may vary from those of the trial	
Overall evidence	22	General interpretation of the results in the context of current evidence		8, 9

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Protocol:

Individualized Pharmacological Approach to Obesity Management: A Pragmatic Clinical Trial

Andres Acosta MD, PhD
Mayo Clinic

PROTOCOL

May 18, 2017

Individualized Pharmacological Approach to Obesity Management: A Pragmatic Clinical Trial

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Conflict of Interest: none

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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, 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. With the current approach with pharmacotherapy, less than 20% of patients will lose more than 10% of body weight. Additionally, the high variability in weight loss response has resulted in a poor market penetrance 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. 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 (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 predict weight loss response to pharmacotherapy and bariatric endoscopy [3, 10-12]. Using this classification, the effect on weight loss of Phentermine-topiramate ER [3], exenatide 5ug and Liraglutide 3mg is maximized [10]. However, these results were determined retrospectively (post-hoc analysis) and each study was done independently. Thus, the identification of the obesity phenotype at baseline 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 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 rate to obesity-phenotype-guided pharmacotherapy vs. controls.

Methods: In a 12 week, pragmatic, 1:1 paralleled controlled trial of 200 participants with obesity; we will compare the weight loss response rate to obesity-phenotype-guided pharmacotherapy vs. standard of care in the Mayo Clinic Weight Management and Nutrition Clinic (See flow diagram below, Figure 1). Patients that are interest in Weight Management with medications will be randomized (at the clinical triage scheduling system) to be offered a diagnostic test to use an obesity-phenotype-guided pharmacotherapy (Intervention). A similar cohort who will not be offered the test will be followed in parallel in a prospective manner (standard of care group). When patients are assigned to the physician in the Intervention clinic, they will be offered the diagnostic tests as part of their care, but at no cost for the patient and the tests will be done under informed consent. The results of the tests will guide obesity pharmacotherapy (figure 1). All participants will be contacted at 4 and seen at 12 weeks (current standard in practice). All participants will receive a standard intense lifestyle intervention, which consists of 2-4 visits with registered dietitian and one visit with a behavioral psychologist. The phenotypic studies include (all performed in same day in the following order): Fasting blood collection, resting energy expenditure, gastric emptying with meal for breakfast, behavioral questionnaires, and nutrient drink test for lunch. Blood will be collected assessment of metabolomic biomarkers, gastrointestinal hormones (GLP-1, PYY, CCK, bile acids), DNA (blood and buccal swab) and pharmacogenomics. Stool samples for microbiome.

Primary endpoint: Percentage of responders (defined as number of participants who loss 10% or more of total body weight) compared to baseline in the obesity phenotype guided pharmacotherapy (intervention) group vs. standard of care at 12 weeks. The secondary end points will be percentage of responders with at least 5% at 4 weeks, 15% at 12 weeks, and 10% at 6 months and 12 months; side effects of medications and patient satisfaction.

Sample size assessment: We propose a 1:1 design to compare effects of Intervention compared to Standard of Care in weight loss. The analysis will involve use of 2-way analysis of covariance (ANCOVA) models with treatment; and the covariates to be considered include gender, BMI (at baseline). The current standard of care suggests that approximate 35% of patients will lose more than 10% of body weight [1, 6, 13-15]. We estimate a detectable effect size difference (Δ) in percentage of responders for the intervention group compare to standard of care of more than 20% (55% intervention vs. 35% control). Using this proportion of responders [2, 5, 16-18], the sample size will be 100 participants in intervention group and 100 participants in standard of care group to demonstrate a treatment-related difference in weight loss (using $\alpha=0.05$, 80% power, 2-sided t test).

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

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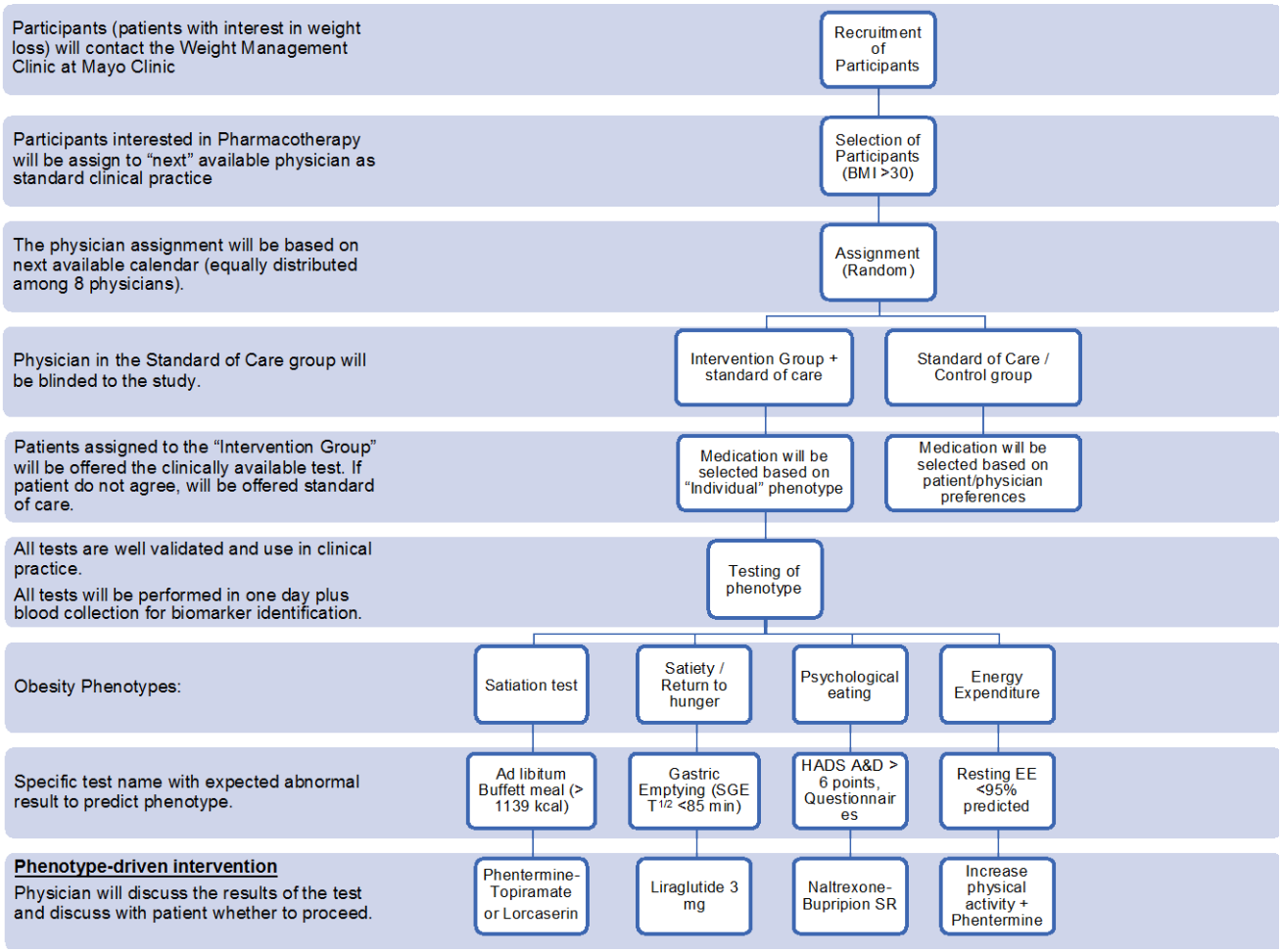
STUDY FLOW DESIGN:

Pragmatic clinical trial to identify weight loss responders to obesity pharmacotherapy



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 [19]. 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 [5]. Additionally, the high variability in weight loss response has resulted in a poor market penetrance 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. 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) [20]. 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[21].

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[21]. 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[22].

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**	[13, 23, 24]
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	[14]
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%)	[15]

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 on15/92 mg combination therapy vs. 1.4 kg on placebo over 56 weeks [14]. 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 [25]. 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 during these studies none of which resulted in congenital abnormalities [17, 25, 26].

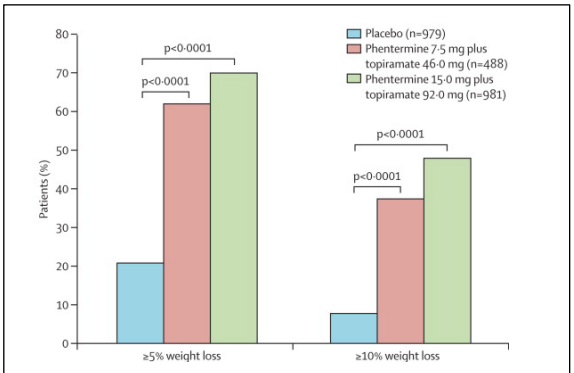


Figure 2. Effects of phentermine plus topiramate on bodyweight. Patients with at least 5% and at least 10% weight loss. [5]

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. The high variability of weight loss response to treatment with PhenTop is unclear.

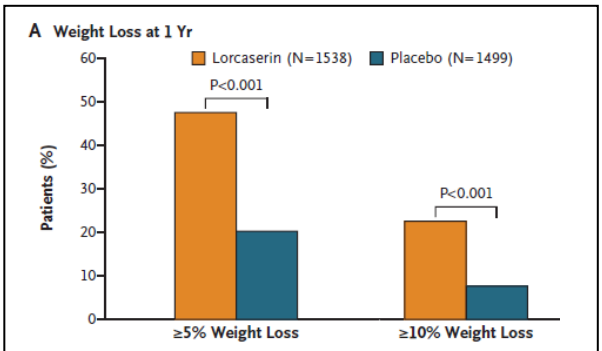


Figure 3: Effects of Lorcaserin on Body weight. The proportions of patients who lost 5% or more or 10% or more of their baseline body weight at 1 year are shown [6].

Lorcaserin (Belviq®): The second medication approved by the FDA in 2012 for chronic weight management is lorcaserin[27]. 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 [28]. 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 3)[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)[1, 16, 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) [16, 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.

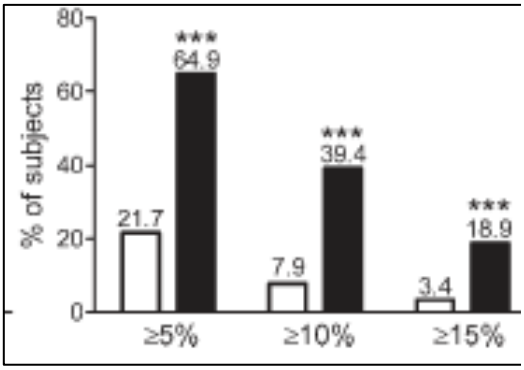


Figure 4. Effects of naltrexone/bupropion SR on bodyweight. Categorical weight loss in week 28 in completers populations. ***P < 0.001 for NB32 vs. Placebo [1]

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[27]. 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 [15]. 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 [10, 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.

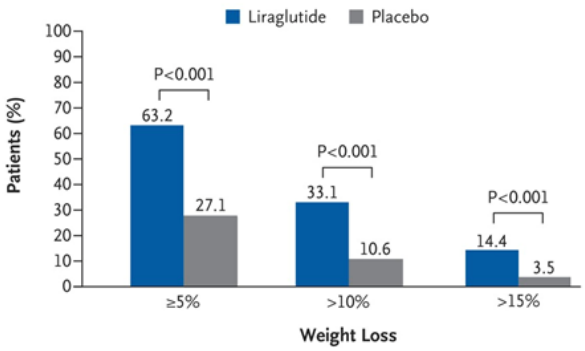


Figure 5 shows the proportions of patients who lost at least 5%, more than 10%, and more than 15% of their baseline body weight. Data shown are the observed means for the full-analysis set (with LOCF). [2].

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.

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 2) 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, 10]. 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 3) [3].

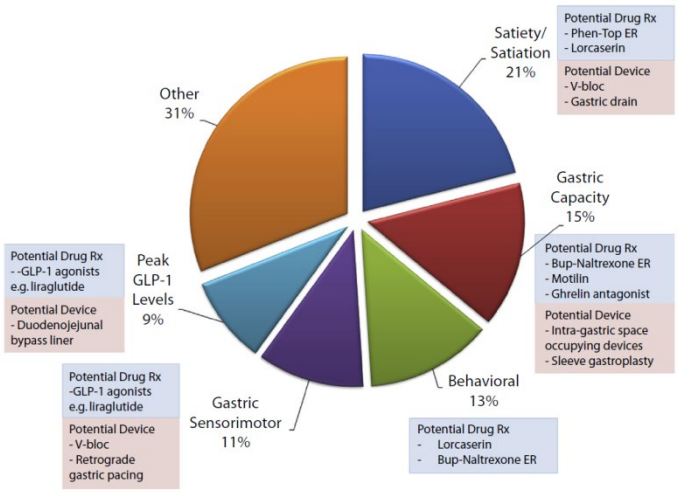


Figure 6: Potential application of medications and devices directed at phenotypes associated with obesity. Rx, therapy; ER, extended release; GLP-1, glucagon-like peptide 1. Adapted from Camilleri and Acosta, GIE, 2016 [4]

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 [10]**.

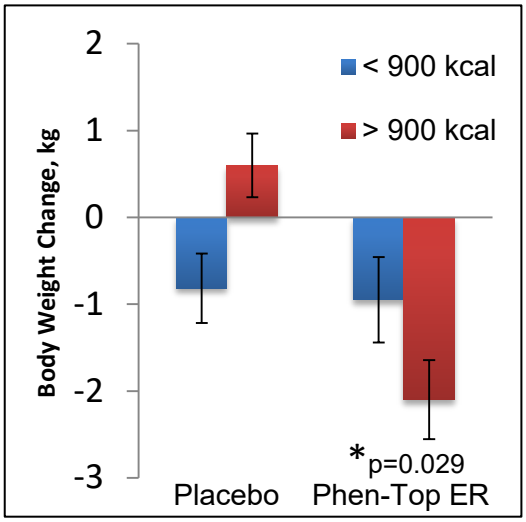


Figure 7: *Body weight change by prior satiety test.* *Association of change in body weight (in response to randomized treatment with placebo or PhenTop) and kcal intake at prior ad-libitum meal. This is shown (p=0.029) for the drug* treatment interaction.[3]*

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)[12].

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 2 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 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.

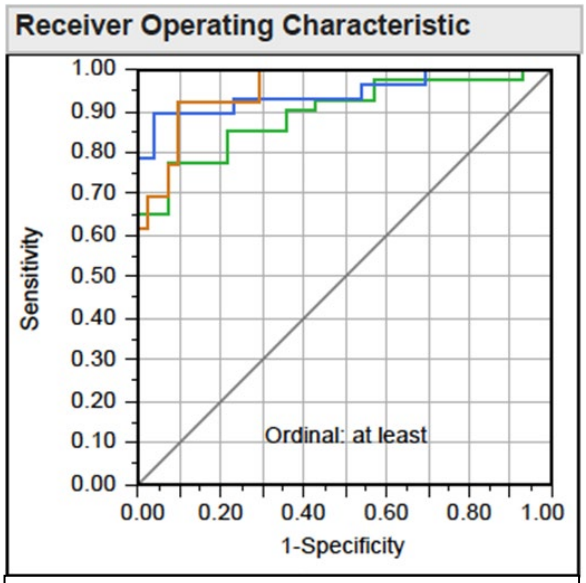


Figure 8: Diagnostic test for Obesity Phenotypes that predict weight loss response to interventions. ROC curves for each main group: Blue line: abnormal satiation, Orange line: rapid return to hunger, and Green line: abnormal energy expenditure (unpublished – confidential)

Quantitative traits are associated with higher BMI, distinguish obesity phenotypes, and may predict response to obesity pharmacotherapy and endoscopic devices (Figure 1) [3]. However, these results were determined retrospectively and each of them was done independently. Thus, the identification of the obesity phenotype at baseline 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 study the weight loss outcome using obesity phenotypes** to guide therapy for obesity.

Additionally, these tests of quantitative GI traits are currently limited to a few research/academic centers. Thus, we recently developed a Blood Test that may predict response to obesity therapy (Mayo IP disclosure No. 2017-040 and DR16-520 – unpublished/confidential). This blood assay has been validated against standard obesity phenotypes that predict weight response to obesity pharmacotherapy (Sensitivity/specificity >90%). The blood assay was developed after testing candidate gene variants (SNPs), metabolic peptides and metabolomics panel (amino-compounds, neurotransmitters, short chain fatty acids, bile acids). The next logical step in the development of this test is to validate the assay in clinical trials of pharmacotherapy for obesity.

Our *overall hypothesis* is that this obesity-phenotypes guided therapy will predict weight loss response to obesity therapy and will be able to identify the "responders" to each specific intervention compared to standard of care (hit-or-miss medication selection). Thus, we propose to validate the assay in two aims:

HYPOTHESIS AND AIMS

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

Hypothesis: The identification of the obesity-phenotype-guided 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 rate to obesity-phenotype-guided pharmacotherapy vs. control group.

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, pragmatic, 1:1 paralleled controlled trial of 200 participants with obesity; we will compare the weight loss response rate to obesity-phenotype-guided pharmacotherapy vs. standard of care in the Mayo Clinic Weight Management and Nutrition Clinic (See flow diagram below, Figure 1). Patients that are interest in Weight Management with medications will be randomized (at the clinical triage scheduling system) to be offered a diagnostic test to use an obesity-phenotype-guided pharmacotherapy (Intervention). A similar cohort who was not offered the test will be followed in parallel in a prospective manner (standard of care group). When patients are assigned to the physician in the Intervention clinic, they will be offered the diagnostic tests as part of their care, but at no cost for the patient and the tests will be done under informed consent. The results of the tests will guide obesity pharmacotherapy (figure 1). All participants will be contacted clinically at 4 and seen at 12 weeks (current standard in practice). All participants will receive, as standard of care, a standard intense lifestyle intervention, which consists of 2-4 visits with registered dietitian and one visit with a behavioral psychologist. The phenotypic studies include (all performed in same day): Fasting blood collection, resting energy expenditure, DEXA, gastric emptying with meal for breakfast, behavioral questionnaires, and nutrient drink test for lunch. Blood will be collected for assessment of metabolomic biomarkers, gastrointestinal hormones (GLP-1, PYY, CCK, bile acids), DNA (blood and buccal swab) and pharmacogenomics. A stool kit will be given at the screen and will be returned at some point during the testing day or day after. Stool sample will be analyzed for microbiome.

Randomization and Allocation

The randomization will be done at the level of the clinical appointment coordinators. Patients will call to request an appointment or will referred for Weight Management Consultation. As standard in a multiple-physician practice, patients will be “randomly” assign to each physician calendar (unless patient or referral requests a specific physician). Patients will be assigned to a physician offering standard of care or a physician offering obesity-phenotype-guided pharmacotherapy. Patients in the standard of care group will not be aware of the other group (intervention); those patients will be followed in a prospective by chart review in a paralleled control group. The patients in the intervention group will be offered the testing to identify their phenotype; they may select only standard of care and decline the testing. Patients who are randomly assign, are willing to start a medication for weight management, and are willing to be tested for phenotype will be enroll in the study. Once they agree to the testing, participants will be offered an informed consent. 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. The rest of the member of multidisciplinary team for weight management (i.e. clinical assistants, registered dietitians, psychologist, pharmacy will remain blinded). Physicians in the standard of care group will be blinded too. The allocation will be concealed at the level of randomization.

Selection Participants

We plan to study a cohort of 200 patients with obesity (BMI>30 kg/m^2). 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. Our Weight Management and Nutrition Clinic see more than 40 patients per week for obesity management (>1200 patients per year). One hundred participants (controls) will be followed prospectively (chart review) for their weight loss after standard of care is provided. These participants will be blinded to the other arm of the study, and to their participation. One hundred participants (Intervention group) will be invited to participate in the phenotypic assessment of their obesity, will agree to guide their pharmacological intervention based on their phenotype results and agree to follow up their weight loss. We will attempt to enroll approximately 25 patients in each group of the obesity-phenotypes (total=100).

Intervention group inclusion and exclusion criteria:

Inclusion criteria

a) Adults with obesity (BMI >30Kg/m^2); these will be otherwise healthy individuals with no unstable psychiatric disease and controlled comorbidities or other diseases.
b) Age: 18-75 years.
c) Gender: Men or women. Women of childbearing potential will have negative pregnancy tests within 48 hours of enrolment and before each radiation exposure.

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.

Control group inclusion and exclusion criteria:

Inclusion criteria

a) Adults with obesity (BMI >30Kg/m2); these will be otherwise healthy individuals with no unstable psychiatric disease and controlled comorbidities or other diseases.
b) Prescribed a medication for weight loss by their physician, as standard of care
c) Age: 18-75 years.
d) Gender: Men or women.

Exclusion criteria

a) Abdominal bariatric surgery
b) Use of medications that may alter gastrointestinal motility, appetite or absorption, e.g., orlistat, within the last 6 months
c) Hypersensitivity to any of the study medications.

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 a registered dietitian nutritionist, and behavioural psychologist as standard of care in our clinical practice. All participants will guided to 1) Nutrition: Reduce dietary intake below that required for energy balance by consuming 1200-1500 calories per day for women and 1500-1800 calories 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.). Participants will meet with RDN at baseline at week ~2 for guidance and monitoring.

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[21]. 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.

Control group: Pharmacotherapy for obesity

Standard of care pharmacotherapy for obesity recommends the following doses and regimen for weight loss:

- Phentermine: 15-37.5 mg oral daily
- Phentermine-Topiramate Extended Release (Qsymia®) at dose of 7.5/46 mg oral daily
- Lorcaserin (Belviq®) at dose of 10 mg oral twice 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
- Orlistat (Xenical®) at 120 mg oral three times daily

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 1 (above), pharmacotherapy will by guide based on the “abnormal” phenotype. In case of a mixed pattern or multiple abnormal phenotypes, the most prominent phenotype will be tackled.

Algorithm diagnostic:

1. satiation: Phentermine-Topiramate Extended Release (Qsymia®) at dose of 7.5/46 mg oral daily or Lorcaserin (Belviq®) at dose of 10 mg oral twice 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.

Anthropometrics and phenotype studies

Anthropometrics Measurements: will be taken of hip-waist ratio, height, weight, blood pressure, pulse at baseline and week 12.

Phenotype studies:

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:

a) The DEXA scan (dual energy x-ray absorptiometry) will measure body composition.
b) Resting energy expenditure: was assessed by indirect calorimetry with a ventilated hood (Parvo Medics, Sandy, UT).

c) Gastric emptying (GE) of solids by scintigraphy: The primary endpoint is gastric half-emptying time (GE $t_{1/2}$) [3, 18, 32].
d) Appetite (hunger level) by visual analog score fasting and after standard meal for GE and prior to the Satiation test [3].
e) 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].
f) 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.
g) Self-administered questionnaires assessing affect, physical activity levels, attitudes, , body image, and eating behavior; details of each questionnaire are provided below.
h) Samples collection, handling and storage: Samples were collected after an overnight fast (of at least 8 hours) in the morning within 7 and 9 am. 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 in the Guggenheim Bldg.
    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 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.
    d. Bucal Swab DNA for OneOme pharmacogenomics testing.
        i. Pharmacogenomics: Patients who have met the inclusion and exclusion criteria and have signed the informed consent will be asked to provide a one-time buccal scraping. This sample will be sent to OneOme™ Laboratory for use for the CLIA/CAP approved RightMed™ test (PGx gene panel). The OneOme RightMed test is an end-to-end pharmacogenomics solution that includes pharmacogenomic testing services, data analysis, clinical interpretation, and interactive reporting. The RightMed panel assesses 72 variants in 22 pharmacogenes, with seven cytochrome P450 enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5) covering approximately 90 percent of human drug oxidation and nearly 50 percent of commonly used medications, and 15 genes related to drug action or metabolism (COMT, DPYD, DRD2, F2, F5, GRIK4, HTR2A, HTR2C, IL28B, NUDT15, OPRM1, SLCO1B1, TPMT, UGT1A1, and VKORC1). These genes were selected because their relationships with various medications are supported by substantial clinical evidence (Appendix 1). Results for the patient will be returned to Mayo Clinic and placed into the patient EHR to be utilized for clinical treatment decisions. Through chart review, including the patient's current medication list as stated in the EHR, we will document previously reported medication inefficacy and intolerance. This data will be entered into a database. To assist in interpretation of the PGx test results, a PGx-trained pharmacist will review the test report, and document recommendations for the ordering provider via the e-Consult mechanism. A patient letter based on their results may be recommended by the

pharmacist on a case-by-case basis. During this process, we will document PGx variants, identify "actionable" variants potentially impacting current medications, all prescription(s) changes considered, and identify variants that might explain past medication issues. If indicated due to PGx variants or current medication list, the patient may have a face-to-face or virtual visit with a pharmacist to discuss their PGx results. If the patient participates in a face-to-face visit with a pharmacist as part of standard patient care, medication reconciliation would be performed and the data will be collected via interview. As this is a clinical test, patients will be given information and education materials and references about PGx testing as appropriate, and will also have access to the lab results report and consult notes via the Mayo Clinic Patient Online Services portal. Patients may also request a copy of their results through May Clinic Medical Records. A follow-up survey will be sent via U.S. mail to collect information regarding patients' perceptions, satisfaction and outcomes of PGx testing, as well as behaviors that may result from having this PGx test. Information collected includes, but is not limited to: prescription adjustments, satisfaction, perceived understanding, and sharing PGx results or information.

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

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®), the and the Hospital Anxiety and Depression Inventory [HAD [33]] to appraise the contribution of affective disorder.

Behavioural Questionnaires

a. AUDIT-C Alcoholism Screening Test [34] - 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 [35], 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 [36, 37]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 [37].

d. Eating Behaviors - The Weight Efficacy Life-Style Questionnaire [WEL [38]] 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 [39]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 [39].

General Principles of Statistical Analyses

Primary endpoint: Percentage of responders (defined as number of participants who loss 10% or more of total body weight) compared to baseline in the obesity phenotype guided pharmacotherapy (intervention) group vs. standard of care. The secondary end points will be percentage of responders with at least 5% at 4 weeks, 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 and patient satisfaction.

Sample size assessment: We propose a 1:1 design to compare effects of Intervention compared to Standard of Care in weight loss. The analysis will involve use of 2-way analysis of covariance (ANCOVA) models with treatment; and the covariates to be considered include gender, BMI (at baseline). The current standard of care suggests that approximate 35% of patients will lose more than 10% of body weight [1, 6, 13-15]. We estimate a detectable effect size difference (Δ) in percentage of responders for the intervention group compare to standard of care of more than 20% (55% intervention vs. 35% control). Using this proportion of responders [2, 5, 16-18], the sample size will be 100 participants in intervention group and 100 participants in standard of care group to demonstrate a treatment-related difference in weight loss (using α=0.05, 80% power, 2-sided t test, and 95% Confidence level).

Percentage difference (Δ) of participants with a weight loss 10% or more [% of responders compare to expected responders with standard of care (approx. 35% in standard of care)]	Intervention (# of participants)	Control (# of participants)
Percentage difference of 10% [45 vs. 35%)	376	376
Percentage difference of **2**0% [55 vs. 35%)	96	96
Percentage difference of 30% [65 vs. 35%)	43	43

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. Feasibility - Given high volume weight management clinic, we are confident we will recruit sufficient participants for these studies that involve only noninvasive tests and standard of care treatment.
b. Statistical power 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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