# SUPPLEMENTAL MATERIALS

## Supplement 1. Methods

### Phenotyping Visit:

Participants were asked to report with prior 8-hour fasting and abstain from caffeine the day prior. Participant's weight, height, and hip and waist circumference were measured using standard clinical techniques. Their resting energy expenditure (REE) was measured by indirect calorimetry. Afterwards, participants had a baseline fasting blood draw, consumed a standardized radiolabel breakfast of 320kcal, 30% fat for gastric emptying (GE) to evaluate half emptying time (GE T ½, min and percent emptied at 120 min), and 4 hours later, for lunch they had an *ad libitum* meal to evaluate calories to fullness (CTF, kcal). Appetite sensations were assessed with visual analog scales (VAS) before starting an *ad libitum* meal and every 30 minutes after lunch for 2 hours. Questionnaires [i.e., Hospital Anxiety and Depression Scale (HADS) and Three-Factor Eating Questionnaire (TFEQ-R21) were completed at home and returned to study staff at the in-person phenotype testing day.). Body composition was measured by dual-energy x-ray absorptiometry (QDA 4500A, Hologics, Bedford, Mass) at baseline and at week 12.

### Gastric emptying for solids

Participants in the trial reported with a minimum of an 8-hour fasting. 1.0 mCi $^{99m}$Tc-sulfur colloid was added to two raw eggs during the scrambling cooking process. The eggs were served on one slice of brown bread with 120 ml of skim milk (total calories: 320 kcal, 32% protein, 35% fat, 33% carbohydrate). Images were acquired by SPECT/CT (Siemens Diacam, GE XR/T), in a standing position with the anterior portion of the body in apposition with the detector. Anterior and posterior gamma camera images were obtained immediately after radiolabeled meal ingestion and every 15 minutes for the first 2 hours, then every 30 minutes for the next 2 hours (with a total of 4 hours after the radiolabeled meal).[1-3] Geometric mean of decay-corrected counts in anterior and posterior gastric regions of interest were used to estimate the proportion of 99mTc emptied at each time point (gastric emptying [GE]). GE is summarized by the half-emptying time (T1/2) in minutes.

### *Ad libitum* meal

The *ad libitum* meal included: vegetable lasagna [Stouffers®, Nestle USA, Inc, Solon, OH; nutritional analysis of each 326g box: 420kcal, 17g protein (16% of energy), 38g carbohydrate (37% of energy), and 22g fat (47% of energy)]; vanilla pudding [Hunts®, Kraft Foods North America, Tarrytown, NY; nutritional analysis of each 99g carton: 130kcal, 1g protein (3% of energy), 21g carbohydrate (65% of energy), and 4.5g fat (32% of energy)]; and skim milk [nutritional analysis of each 236mL carton: 90kcal, 8g protein (36% of energy), 13g carbohydrate (64% of energy), and 0g fat]. The total amount (g and kcal) of food consumed and the kcal of each macronutrient at the *ad libitum* meal were analyzed by a registered dietitian, using a validated software (ProNutra 3.0; Viocare Technologies Inc, Princeton, NJ).

Participants were served the prepared meal. While participants were consuming part of their meal, they were asked if they desired any additional lasagna, pudding, or milk. Participants were asked to eat any or all the food products until they reached maximum fullness. Once they were done consuming all components of the meal, the remaining of the plate, pudding container, and/or milk volume were weighed, accordingly. When subject reached maximum fullness, the total weight of food product consumed was summarized. The primary measurement was the total number of kcal consumed in one sitting.

**Resting energy expenditure**

REE was measured by indirect calorimetry (ParvoMedics, Sandy, UT). The gas analyzer and the flow meter were calibrated before each test. The REE was assessed in the morning, after reclining in a steady-state and supine position in a quiet room, for 20 minutes. The measurements involved recording the rate of oxygen consumption (VO2) and carbon dioxide production (VCO2) every 30 seconds for 40 minutes with the participants lying in a supine position and being fully awake. The respiratory exchange ratio and oxygen uptake were analyzed within the middle 20 minutes of the test period. Expected REE was determined using the Harris-Benedict equation, which takes into account the weight, height, and age of the participant[4]. For every subject, the measured REE was divided by the expected REE and multiplied by 100.

**Appetite sensations**

The visual analog scales (VAS) were measured using a validated, standard, 100mm appetite VAS for hunger, fullness, desire to eat, and satisfaction score.[5,6] Appetite VAS was assessed 15 minutes before the *ad libitum* meal, then every 30 minutes for the first 120 minutes following meal consumption.

**Body composition**

Body composition was measured using dual-energy x-ray absorptiometry (DXA) (QDA 4500A; Hologics, Bedford, MA). For the analysis of total mass, bone mass, and lean body mass (LBM) enCORE Software v16 was used. FFM and fat mass (FM) were calculated from weight and percent body fat. Appendicular LBM was calculated as the sum of arms and legs LBM.

**Symptoms of Anxiety**

The Hospital Anxiety and Depression Scale (HADS) is a questionnaire widely used for detecting symptoms of anxiety and depression.[7] HADS comprises 14 items, seven of which relate to anxiety symptoms and seven others to depressive symptoms. Each item is coded with a scale ranging from 0 to 3. The scores for each of the anxiety and depression components can therefore vary from 0 to 21, depending on the presence and severity of the symptoms. The interpretation of the obtained score is as follows: $< 7$: no symptoms of anxiety and $\geq 7$: symptoms of anxiety.[8]

### Eating behaviors

The Three-Factor Eating Questionnaire (TFEQ-R21) is a measure that has been designed to assess eating and weight control behaviors [9]. Participants completed the TFEQ-R21 at their baseline assessment. The TFEQ-R21 is a 21-item instrument that measures three domains of eating behavior: cognitive restraint (CR), uncontrolled eating (UE), and emotional eating (EE) [10]. The first twenty items are rated on a 4-point likert scale, and item 21 is answered through an 8-point likert scale. Before calculating domain scores, items 1–16 were reverse coded, and item 21 was recorded as follows: 1–2 scores as 1; 3–4 as 2; 5–6 as 3; 7–8 as 4. For each of the three domains, scores were then calculated as a mean of all items within the respective domain; hence, domain scores also ranged from 1 to 4 (CR [six items], UE [nine items], and EE [six items]), with higher scores being indicative of a greater likelihood of CR, UE, and EE.

## Interventions

The Mayo Clinic Healthy Living Program (HLP)[11] is a health and wellness program that is individualised to each patient. The program's interventions are based on three essential pillars: diet and nutrition, physical activity and exercise, and resilience-related topics. The comprehensive program includes a physical activity assessment, an individualised health and wellness coaching, didactic teaching for health education, and an active participation in classes discussing various topics, as healthy living practices in nutrition, physical exercise significance, and concept of resiliency. The program has the potential to reduce stress levels, minimize burnout symptoms, and increase health habits and state of well-being among participants. Participants are given didactic educational sessions such as 'Burnout and Healthy Living Program Philosophy on Resilience' and 'Nutrition Controversies' during the two-day event. Other classes, such as 'Non-Exercise Activity Thermogenesis (NEAT): Sit Less and Move More' (taken while walking on a treadmill) and 'Lunch: Cooking Well,' are immersive and require active involvement from participants (the latter being a cooking class where participants gain the opportunity to learn basic culinary skills and prepare their own lunch meals). Participants are encouraged to utilize this acquired knowledge, along with the help of a health and wellness coach, to construct a rigid personalised health plan.

This health and wellness coaching strategy revolves around creating a base of strength within participants, whether be it physically or mentally. Our team has reported that participation in our 12 week health and wellness coaching program can improve quality of life, reduce stress and improve the health behaviors of adults that engage in health and wellness coaching.[12,13] Counseling psychology, positive psychology, preventative research, social work, solution-focused therapy, and motivational interviewing have all made contributions to build strength-based methods. Through a strength-based approach, health and wellness coaches discuss with each individual the incorporation of the Mayo Clinic 5E Coach Training Model: (1) engage, build a trusting relationship with the individual; (2) explore, assist individuals in identifying their values and desires; (3) envision, facilitate a personal vision for wellness; (4) experiment, enhance self-confidence for wellness and translate values and goals into action; and (5) evolve, facilitate, and promote long-term positive lifestyle. The wellness coaching program starts with a 60-minute introductory session with a goal of creating a vision, discussing the participant's strengths, motivation for change, challenges, and personal goals; and finally determining strategies to

achieve the participant's wellness goals. The initial session is followed by 11 weekly 30- to 60-minute in-person follow-up sessions to discuss and complete self-identified wellness goals. The follow-up sessions allow for the health and wellness coach and participants to discuss the steps that have been taken to achieve the end goal, and, in contrast, the lessons learnt along the way when exploring behavior change methods to motivate the individual in pursuing successful continued efforts.

## Supplement 2. Rationale for Phenotype-tailored lifestyle intervention

Our four obesity phenotypes consider the main components of energy balance regulation, homeostatic and hedonic eating, and energy expenditure. We used the following rational for a Phenotype-Tailored approach (Figure 1a):

**Abnormal Satiation Phenotype** is characterised by a requirement of a higher number of calories at each meal to reach fullness. These patients might benefit from: i) reducing the allowed period of caloric intake during a day with prolonged fasting periods (time-restricted eating);[14] ii) increasing dietary non-soluble fiber (volumetric)[15-17]; and iii) healthy second servings, if needed. The purpose of this abnormal satiation diet is to: i) keep the brain hunger centre "off" for longer periods of time (as these patients have normal hunger, but abnormal satiation)[14]; ii) produce maximal gastric distention and accommodation to induce the sensation of fullness using a volumetric diet[18]; and iii) recognize the need for second servings and suggest healthy second servings which may also help in reaching satiation.[19] Recommendation: Low-calorie volumetric diet, increased fiber, fruits, and vegetables for second servings, with time-restricted eating typically eating during an 8-hour daytime window.

**Abnormal Postprandial Satiety Phenotype** is characterised by having an accelerated gastric emptying and increased post-prandial hunger. These patients might benefit from a high-protein diet with protein preloads to increase the early release of GI satiety hormones, like ghrelin.[20] High protein diets resulted in weight loss and improved the sensation of satiety on a long-term.[21] Protein preloads slowed gastric emptying in healthy participants and in patients with type 2 diabetes.[22,23] Recommendation: Low-calorie, high-protein diet with a pre-meal shake or healthy protein snack. Three to five meals a day to help with the increase sensation of hunger between meals.

**Emotional Eating Phenotype** is characterised by negative mood and reward-seeking behaviors in relation to negative and positive emotions.[24] These individuals might benefit from a behavioral intervention structured around emotion regulation, resiliency strategies, cognitive reframing, goal-setting, self-monitoring, and stimulus control[25]. Furthermore, mindfulness-based approaches were shown to decrease emotional eating and could help increase self-awareness[26]. Recommendation: Low-calorie diet, balanced diet with 3 meals a day. No snacks to avoid using food as coping mechanisms. Emphasis on high-intensity, cognitive behavioral program focused on identifying and changing emotional eating patterns, while building simultaneously a support network and leaning positive coping skills.

The theoretic foundation for the 12-session Emotional Eating group was based on standard behavioral weight management strategies: goal setting during each session, enhancing social support, encouraging mindful eating, promoting a physically active lifestyle, and building relapse preventing strategies.[27] Our intervention was targeted for emotional eaters, so the intervention also included emotion regulation strategies, cognitive restructuring, and harm reduction approaches when faced with high-risk eating situations.[28,29] Additionally, we included health and wellness coaching which was shown to improve health behaviors[30], build confidence towards these behaviors[13] and reduce perceived stress [12]. To further provide skills aimed at managing emotional eating, we referred back to our previous experiences with the work site wellness programs and supported the incorporation of stress management strategies[31], strategies meant to build resilience,

grow self-compassion, and improve overall happiness.[32] Mindful moment activities were also practiced within participants and included expression of gratitude, reflective questioning, and identification of personal strengths; these activities focused on linking participants with positive emotions that lead to increased social connection, curiosity and desire to further learn about the healthy lifestyle.[33] These groups were colead by a clinical health psychologist (KG or MMC) and a health and wellness coach (JF).

**Mayo Clinic Emotional Eating Group Program**

**Content for Weekly Group Sessions**

1. SMART Goal Setting
2. Self-monitoring
3. Triggers for Overeating
4. Thoughts, Feelings and Behaviors
5. Resiliency and Positive Coping Strategies
6. Emotional Regulation
7. Mindfulness and Mindful Eating
8. Body Positivity and Self-Compassion
9. Support Network
10. High Risk Eating Situations
11. Behavior Chain
12. Maintaining Motivation for Change

**Abnormal Resting Energy Expenditure Phenotype** is characterised by a reduced resting energy expenditure and decreased muscle mass. These patients might benefit from i) a structured exercise plan to increase muscle mass, which accounts for most of the overall energy expenditure ratio[34]; ii) increasing protein intake. There is an excellent dose-response relationship between resistance training and muscle hypertrophy[35]. Moreover, low-carbohydrate, high-protein diets enhanced changes in muscle strength and size and subsequently contributed to an increase in total energy expenditure[36,37]. Recommendation: Low-calorie high-protein diet, with 3 meals a day and post-workout shakes or healthy protein snack. Emphasis on resistance training and high-intensity interval training (HIIT). Exercise program with online interactive videos incorporating various types of resistance training, such as free weights, resistance bands, and the patient's own body weight, as well as interval training workout sessions (HIIT) in training zones 1 to 5. Once a week, a specialised physical therapist supervises the training program to keep track of the activities and participant performance.

**Supplement 3. Statistical Analysis Plan**

**Primary endpoint:** Total Body Weight Loss in kg (defined as weight changed from baseline to the 12-week time point) in the whole cohort in the Modified Healthy Living Program – Mayo Clinic Diet Experience compared with the standard Mayo Clinic diet program.

**Secondary endpoints:**
1. Total Body Weight Loss in percentage (defined as weight changed from baseline to 12 weeks) in each obesity-related phenotype in the Modified Healthy Living Program – Mayo Clinic Diet Experience compared with the standard Mayo Clinic diet program.
2. Responder rate (>5%, >10% TBWL)
3. Change in waist circumference (cm),
4. Change in lean mass percentage (DEXA, %),
5. Change in phenotypes:
    a) total calories consumed in 24 hours for abnormal satiation
    b) calories to fullness in ad libitum meal for abnormal satiation
    c) gastric emptying t ½ in minutes for abnormal postprandial satiety
    d) gastric emptying % after 120 minutes abnormal postprandial satiety
    e) HADS-A score for abnormal emotional eating
    d) emotional eating score on the TFEQ for abnormal emotional eating
    e) body composition Fat free mass (%) for abnormal energy expenditure
    f) kcal/day measured by indirect calorimetry abnormal energy expenditure,
6. Adherence and compliance with program defined by the number of contacts with the team,
7. Changes in plasma low-density lipoprotein (LDL) and high-density lipoprotein (LDL) cholesterol, and triglycerides,
8. Changes in C-reactive protein,
9. Changes in glucoregulatory factors including fasting glucose, HbA1C, fasting insulin.
10. Changes in blood pressure (systolic blood pressure and diastolic blood pressure), and hear rate

**General Approach:** The primary analyses was conducted on primary analysis cohorts (PA), defined as subjects who have at least one post-baseline assessment. Subjects who did not formally withdraw from the study but did not have a final assessment at 12 weeks were defined as dropouts. The primary approach for missing values due to drop-out or withdrawal in the primary analysis cohort was to use last-value-carried-forward (LVCF). The completers cohort consisting only of subjects who had an assessment at 12 weeks was used for sensitivity analyses. All hypothesis tests were 2-sided with a 0.05 significance level. All effect estimates were reported with 95% confidence intervals.

**Primary Analysis:** The primary endpoint was total body weight loss (TBWL), defined as the change in body weight from baseline to the 12-week time point. Weight loss was assessed using ANCOVA models with sex, age, and weight at baseline as covariates in all participants. Mean TBWL and 95% confidence intervals were reported overall and for each phenotype.

**Secondary Analyses:** Additional analyses included analysis of secondary endpoints (e.g. responder rate, change in waist circumference, body composition via DEXA scan, change in

phenotype, changes in phenotype defining measurements). Interactions were used to test subgroup effects of modified diet within phenotype groups. Additionally, we investigated whether the magnitude of change in phenotyping defining measures had a greater association with weight loss within the phenotype subgroup relative to other phenotypes. Linear regression models for the final weight at 12-week time point were modeled with treatment, age, sex, baseline weight, and changes in phenotype measurements plus an interaction between phenotype subgroups and changes in phenotype measurements. These analyses explored the effectiveness of the modified diet on the phenotype defining measures within each phenotype after weight loss. The measures of specific interest for this analysis were dependent on each phenotype: total calories consumed in 24 hours and ad libitum meal caloric intake for the abnormal satiation, HADS-A score, and emotional eating score on the TFEQ for the abnormal emotional eating, gastric emptying time and gastric emptying % after 120 minutes for the abnormal satiety, measured versus predicted REE and lean mass percentage for the abnormal resting energy expenditure**.**

**Sample size assessment:** In our recent pilot study (with Liraglutide 3.0 mg vs. placebo), the SD for the overall weight change (pre-post) observed in placebo (lifestyle intervention alone) was 3kg at 3 months. Recently the DIETFITS trial observed a standard deviation of 10kg for weight loss at 12 months. Enrollment of 223 subjects total with a 15% drop out rate results in 95 subjects per group, which provides 90% power (2-sided test of 0.05 Type I error rate) to detect a standardised difference of 0.47. Given our standard deviation estimates, we were well powered to detect a mean difference of 1.4kg to 4.7kg between the groups.

## Supplement Appendix Table 1 Baseline Demographics and Clinical Characteristics per Phenotype and treatment group

| | Abnormal Satiation | | | Abnormal Satiety | | | Reduced REE | | | Emotional Eating | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **SLI** | **PLI** | **p-value** | **SLI** | **PLI** | **p-value** | **SLI** | **PLI** | **p-value** | **SLI** | **PLI** | **p-value** |
| | 30 | 28 | | 7 | 13 | | 31 | 37 | | 19 | 26 | |
| Age, years | 42.3 (12.78) | 39.93 (11.39) | 0.46 | 39.29 (7.36) | 46.08 (13.19) | 0.16 | 40.23 (11.92) | 44.84 (12.77) | 0.13 | 39.63 (13.95) | 40.15 (13.03) | 0.90 |
| Weight, kg | 114.11 (22.45) | 114.09 (24.36) | 1 | 117.16 (15.54) | 125.55 (28.67) | 0.41 | 116,37 (21.17) | 116.45 (27.37) | 0.99 | 115.62 (24.5) | 114.93 (27.72) | 0.93 |
| BMI, $kg/m^2$ | 38.37 (6.63) | 38.85 (8.4) | 0.81 | 38.06 (6.38) | 41.72 (7.56) | 0.27 | 39.23 (6.18) | 39.48 (8.44) | 0.89 | 39.76 (5.85) | 40.19 (8.7) | 0.85 |
| Waist Circumference, cm | 119.07 (14.09) | 118.57 (18.05) | 0.91 | 113.21 (9.26) | 121.78 (17.67) | 0.18 | 120.79 (12.08) | 119.92 (17.52) | 0.81 | 119.52 (14.25) | 117.18 (15.91) | 0.61 |
| Heart Rate, bpm | 77.17 (9.41) | 78.75 (7.95) | 0.49 | 85.57 (9.27) | 82.31 (13.61) | 0.53 | 77.03 (11.41) | 79.65 (10.32) | 0.33 | 81.26 (8.78) | 80.5 (11.19) | 0.80 |
| Blood Pressure Systolic, mmHg | 118.87 (12.51) | 118.14 (8.67) | 0.8 | 119.71 (8.77) | 127.08 (10.23) | 0.11 | 119.71 (13.47) | 120.43 (11.61) | 0.82 | 117.74 (10.59) | 119.85 (10.65) | 0.51 |
| Blood Pressure Diastolic, mmHg | 79.4 (7.6) | 79.29 (5.82) | 0.95 | 82 (7.23) | 83.38 (8.25) | 0.7 | 79.48 (10.51) | 80.24 (7.03) | 0.73 | 79.11 (9.3) | 79.62 (8.57) | 0.85 |
| Phenotype Characteristics | | | | | | | | | | | | |
| Daily Calory Intake, kcal | 1632.12 (626.25) | 1945.14 (804.79) | 0.18 | 1620.22 (545.83) | 2067.04 (1047.41) | 0.31 | 1686.06 (543.91) | 1889.83 (718.38) | 0.28 | 1628.45 (538.68) | 1878.03 (742.63) | 0.30 |
| Calories to fullness, kcal | 1272.9 (305.68) | 1186.81 (270.25) | 0.26 | 743.59 (398.92) | 901.96 (438.95) | 0.43 | 1075.72 (427.45) | 939.74 (374.93) | 0.18 | 1093.73 (427.97) | 787.19 (276.47) | 0.01 |
| Gastric Emptying $T_{1/2}$, minutes | 131.89 (32.45) | 124.5 (28.29) | 0.36 | 79.39 (16.32) | 89.14 (11.05) | 0.19 | 138.54 (35.09) | 130.27 (32.29) | 0.32 | 136.21 (33.63) | 128.79 (45.29) | 0.53 |
| Resting Energy Expenditure, kcal/day | 1876.6 (371.56) | 1856.68 (289.28) | 0.82 | 2223.99 (277.13) | 2085.86 (320.36) | 0.34 | 1761.95 (310.28) | 1701.69 (305.33) | 0.42 | 1975.07 (352.66) | 1908.89 (392.92) | 0.56 |
| REE predicted/measured, % | 96.3 (15.79) | 95.73 (14.68) | 0.89 | 110.83 (16.37) | 105.72 (18.87) | 0.55 | 87.18 (9.42) | 86.02 (6.45) | 0.56 | 99.45 (13.69) | 100.26 (14.47) | 0.85 |
| Lean Mass, % | 200.78 (23.49) | 202.14 (27.81) | 0.84 | 202.66 (32.97) | 214.85 (22.34) | 0.4 | 206.6 (22.9) | 205.46 (26.92) | 0.85 | 202.56 (16.83) | 212.25 (28.41) | 0.18 |
| HADS-Anxiety, score | 4.57 (3) | 4.04 (3.26) | 0.52 | 2.86 (2.19) | 5.77 (3.17) | 0.03 | 4.45 (3.63) | 4.92 (3.54) | 0.6 | 8.58 (2.32) | 8.92 (1.72) | 0.59 |
| Emotional eating (6 items), score | 21.9 (4.54) | 22 (3.96) | 0.93 | 20.43 (7.3) | 21.18 (5.21) | 0.82 | 22.5 (6.38) | 21.64 (3.91) | 0.52 | 23.32 (5.06) | 23.27 (3.77) | 0.98 |

**Supplement Appendix Table 2. Changes in Body Composition and Cardiometabolic Risk Factors between Baseline and Week 12.**

| | Mean difference (95% confidence interval) | | | p-value† |
|---|---|---|---|---|
| | **Standard Lifestyle Intervention** | **Phenotype-tailored Lifestyle Intervention** | **Adjusted† group differences** | |
| **Body Composition** | | | | |
| Weight loss at 4-weeks, kg | -2·35 (-3·03, -1·67) | -4·18 (-5·52, -2·84) | -1·64 (-2·96, -0·31) | 0·018 |
| Weight loss at 12-weeks, kg | -4.28 (-5.81, -2.74) | -7.39 (-8.77, -6.01) | -3.04 (-4.99, -1.10) | 0.003 |
| Weight loss at 12-weeks, kg (completers only) | -3·72 (-4·77, -2·66) | -7·99 (-9·16, -6·81) | -4·06 (-5·61, -2·51) | <0·001 |
| Body mass index, kg/m$^2$ | -1.45 (-1.99, -0.91) | -2.61 (-3.17, -2.04) | -1.14 (-1.83, -0.46) | 0.002 |
| Waist circumference, cm | -4.11 (-9.50, 1.28) | -5.83 (-10.66, -1.00) | -1.87 (-7.61, 3.86) | 0.52 |
| Hip circumference, cm | -2.08 (-5.95, 1.78) | -2.18 (-7.26, 2.89) | 0.31 (-4.81, 5.43) | 0.91 |
| Fat mass, kg | -3.39 (-6.98, 0.19) | -6.64 (-10.72, -2.57) | -2.89 (-7.33, 1.55) | 0.21 |
| Lean mass, kg | 0.43 (-2.03, 2.88) | 0.35 (-2.88, 3.59) | -0.05 (-3.66, 3.56) | 0.98 |
| Lean mass, % | 2.38 (0.10, 4.67) | 3.83 (1.09, 6.56) | 1.39 (-1.51, 4.29) | 0.36 |
| **Glucoregulatory Factors** | | | | |
| Fasting glucose, mg/dl | -1.70 (-11.29, 7.88) | 0.70 (-6.93, 8.33) | -0.99 (-9.96, 7.99) | 0.83 |
| HbA1c (%) | -0.29 (-1.14, 0.55) | -0.24 (-0.69, 0.20) | -0.15 (-0.51, 0.22) | 0.44 |
| Fasting Insulin, mg/dl | -3.89 (-7.46, -0.32) | -2.67 (-6.28, 0.94) | -0.28 (-3.15, 2.58) | 0.85 |
| **Blood Pressure and Heart Rate** | | | | |
| Heart rate, bpm | -4.71 (-9.42, -0.01) | -7.54 (-14.16, -0.93) | -2.19 (-10.51, 6.13) | 0.61 |
| Systolic blood pressure, mmHg | -0.21 (-3.64, 3.22) | -0.02 (-4.58, 4.54) | -0.12 (-5.40, 5.15) | 0.96 |
| Diastolic blood pressure, mmHg | -2.61 (-6.56, 1.34) | -3.68 (-7.74, 0.37) | -1.45 (-5.33, 2.42) | 0.47 |
| **Plasma Lipids and Inflammation** | | | | |
| LDL cholesterol, mg/dl | -10.3 (-19.2, -1.37) | -9.34 (-18.1, -0.54) | 1.05 (-8.98, 11.1) | 0.84 |
| HDL cholesterol, mg/dl | 0.75 (-3.76, 5.27) | 0.27 (-3.82, 4.35) | -1.25 (-6.22, 3.72) | 0.62 |
| Triglycerides, mg/dl | 9.95 (-9.15, 29.1) | -12.9 (-28.1, 2.23) | -16.1 (-38.0, 5.78) | 0.16 |
| C-reactive protein HS, mg/dl | -0.12 (-2.96, 2.71) | 0.70 (-1.97, 3.37) | 0.76 (-2.26, 3.77) | 0.63 |

† Mean group differences and p-values are adjusted for age, sex, baseline weight, and baseline measure of the response variable.

Data are summarized as mean change from baseline and 95% confidence interval (CI). Multiple imputation was used to handle missing data. All P-values <0·05 were considered significant.

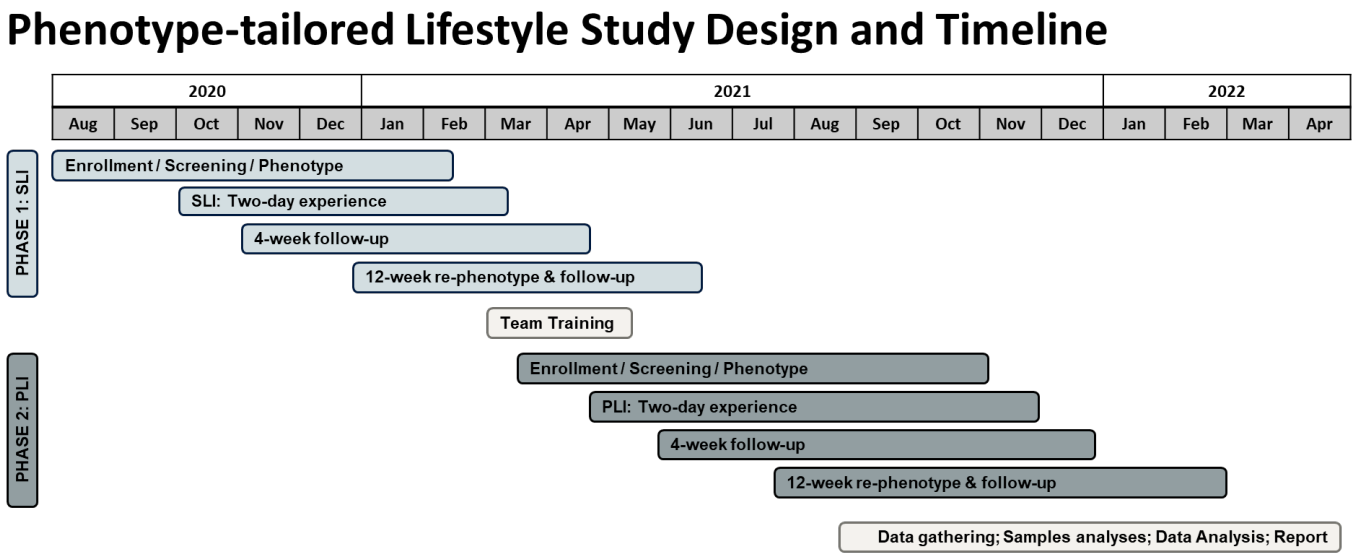
Abbreviations used: bpm, beats per minute; HbA1c, hemoglobin A1c; HDL, high density lipoprotein; HS, high sensitivity; LDL, low density lipoprotein.

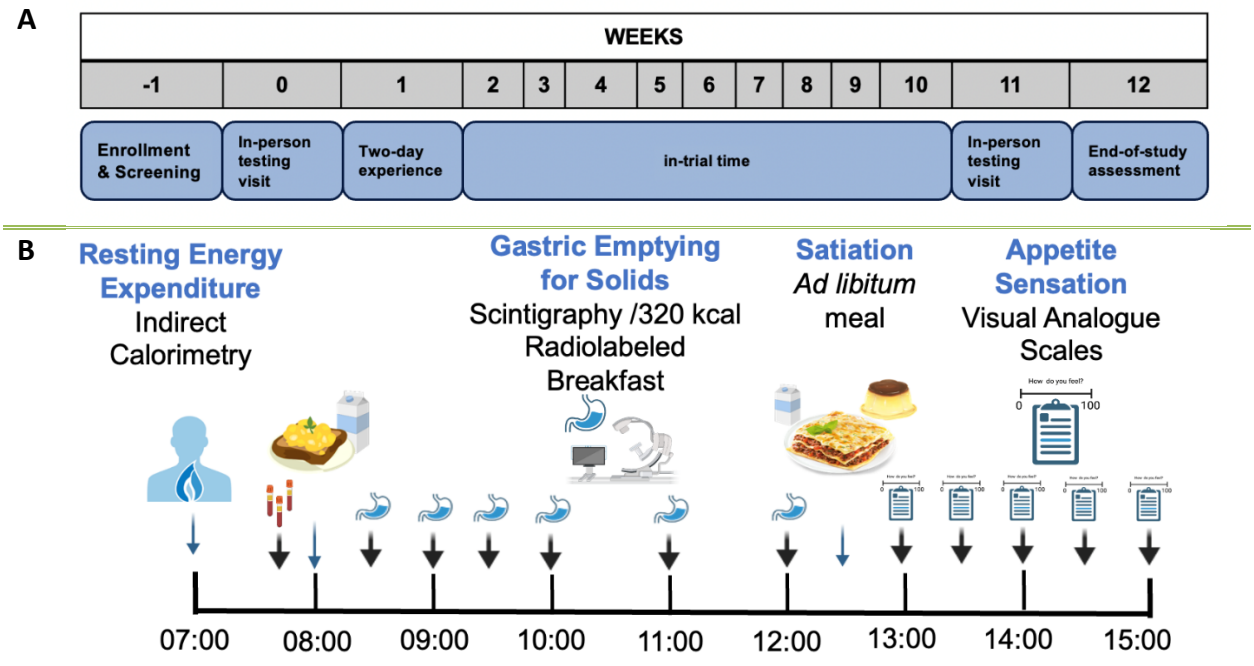
## Supplement Appendix Table 3: Changes in Physiological, Metabolic and Behavioral Variables between Baseline and Week 12.

| | Mean difference (95% confidence interval) | | | p-value† |
|---|---|---|---|---|
| | Standard Lifestyle Intervention | Phenotype-tailored Lifestyle Intervention | Adjusted† group differences (95% CI) | |
| **Food Intake** | | | | |
| Daily caloric intake, kcal | -197 (-477, 84) | -538 (-945, -131) | -143 (-536, 249) | 0.48 |
| *Ad libitum* meal, kcal | -94 (-202, 14) | -35 (-157, 87) | 25 (-109, 160) | 0.71 |
| **Gastric Function** | | | | |
| Gastric Emptied 120min, % | 1.4 (-3.9, 6.7) | -4.4 (-10.0, 1.1) | -3.8 (-10.4, 2.8) | 0.27 |
| Gastric Emptying $T_{1/2}$, min | 3.3 (-9.4, 16.0) | 12.6 (0.7, 24.6) | 7.5 (-6.9, 21.8) | 0.31 |
| **Appetite Sensations (VAS)** | | | | |
| Hunger pre-meal, mm | -0.9 (-8.1, 6.3) | 6.4 (-3.8, 16.6) | 2.1 (-9.8, 14.0) | 0.73 |
| **Resting Energy expenditure** | | | | |
| REE, kcal/day | -124 (-217, -31) | -96 (-184, -8) | 10 (-90, 110) | 0.85 |
| REE predicted/measure, % | -5.0 (-9.1, -1.0) | -1.8 (-6.3, 2.7) | 2.0 (-3.1, 7.0) | 0.45 |
| **Behavioral Questionnaires** | | | | |
| HADS Anxiety, score | 0.6 (-0.5, 1.7) | 0.1 (-1.4, 1.6) | -0.3 (-2.0, 1.4) | 0.75 |
| TFEQ, Emotional, score | -2.3 (-3.9, -0.6) | -1.7 (-4.2, 0.7) | 0.5 (-2.1, 3.0) | 0.73 |

† Mean group differences and p-values are adjusted for age, sex, baseline weight, and baseline measure of the response variable.
Data are summarized as mean change from baseline and 95% confidence interval (CI). Multiple imputation was used to handle missing data. All P-values <0·05 were considered significant.
Abbreviations used: CI, confidence interval; HADS, hospital anxiety and depression scale; REE, resting energy expenditure; VAS, visual analogue scale; TFEQ, three factor eating questionnaire.

**Supplemental Appendix Figure 1: Phenotype-tailored Lifestyle Intervention: Study Design and Timeline.**






**Supplemental Appendix Figure 2:** A) The study consisted of a screening period, an in-person physiologic testing visit, and 12 weeks of treatment. Study visits occurred at screening (phenotype testing day), baseline [two-day experience at the Mayo Clinic Healthy Living Program (HLP); day 1-2], four weeks, and an end visit at week 12 (phenotype testing day). B) All participants in the study had physiologic in-person testing to measure energy expenditure, food intake, and appetite. Testing included resting energy expenditure measured by indirect calorimetry, gastric emptying for solids with a standardized radiolabeled 320 kcal breakfast after an 8-hour fasting, and satiation with an *ad libitum* meal test for lunch. Following lunch, participants rated their appetite sensations with visual analogue scales for fullness, hunger, satisfaction, and prospective desire to eat.

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# "Mayo Clinic Diet" Individualized Lifestyle Intervention for Obesity Management based on Obesity Phenotypes (PHENO-Diet trial)

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**IRB approved:** 19-007485

Conflicts of Interest: AA is stockholder of Phenomix Sciences, Gila Therapeutics, and Lipiquester and serves in Ad Board of Gila Therapeutics, Rhythm Pharmaceuticals, and General Mills. MC is stockholder of Phenomix Sciences and serves in the Ad Board of Kallyope.

Version 6.0

09/17/2021

# "Mayo Clinic Diet" Individualized Lifestyle Intervention for Obesity Management based on Obesity Phenotypes (PHENO-Diet trial)

## ABSTRACT

**Introduction:**

Obesity prevalence continues to increase worldwide. Estimated costs to the healthcare system are more than $550 billion annually. Obesity severity is associated with higher cardiovascular mortality. Weight loss with current treatments including medications, endoscopy and surgery is highly variable. The "responders" to each treatment achieve significant weight loss (>15% total body weight loss); this degree of weight loss reduces cardiovascular mortality and other obesity-related comorbidities. However, **it is unknown or unpredictable who will be the best responders to each treatment**. Thus, there is a *critical need to individualize obesity management, that is to identify the "responders" to each treatment – and move from a best guess approach to the **right intervention for the right patient**.* Based on a study of over 500 patients, we recently sub-classified obesity into different phenotypes based on dysfunctions in satiation, satiety, psychological, energy expenditure and other. These obesity-related phenotypes predict weight loss to obesity pharmacotherapy and devices. However, little is known about which the right lifestyle intervention for each obesity-related phenotype is.

**The Mayo Clinic Diet** is a lifestyle change approach to weight loss and not a 'diet' in the traditional sense of the word. The goal of The Mayo Clinic Diet is to help people achieve a healthier weight and lifestyle habits long-term. It is divided into 2 phases: LoseIt! and LiveIt! LoseIt! is a two week jump start phase in which people make sudden changes in 15 habits associated with diet and physical activity that promote safe and effective weight loss. In the LiveIt! phase, people take the changes in habits people made in LoseIt! and turn them into long-term lifestyle changes which, along with additional structured interventions, enables ongoing and sustained weight loss and improved health indefinitely. A primary dietary principle behind The Mayo Clinic Diet is consuming low energy-dense foods, such as vegetables and fruits, that help achieve satiety at a lower calorie intake. The recommended foods are also health-supporting independent of their energy content. The Mayo Clinic Diet also stresses key components of behavior change, such as finding your inner motivation to lose weight, setting achievable goals and handling setbacks.

While currently individuals can modify a diet to their own preferences, ***there is a critical need to identify unique diet and lifestyle characteristics and tailor them to the obesity-related phenotypes*** to achieve improved long-term weight loss and improved health. The Mayo Clinic Diet is flexible and macronutrient content and other characteristics can be modified. ***The Mayo Clinic Diet is ideal to determine lifestyle characteristics that increase weight loss among obesity-related phenotypes.*** **Hypothesis and Aims**

**Hypothesis:** *We hypothesize that obesity-related phenotypes may predict weight loss to an Individualized Lifestyle intervention based on a tailored Mayo Clinic Diet program.*
**Aim:** To test this hypothesis, we propose three aims: 1) to study the effect of the Mayo Clinic Diet in total body weight loss in the obesity related phenotypes; 2) to compare the effect on weight loss of the "modified" Mayo Clinic Diet tailored to each obesity-related phenotype vs. the "standard" Mayo Clinic diet control group; 3) Study the multi-omics profile of the best responders using machine learning tools.

**Specific aims**

1) to study the effect of the Mayo Clinic Diet in total body weight loss in the currently defined obesity-related phenotypes in 100 participants followed for 12 weeks. The primary end-point is 12-week total body weight loss.
2) to compare the effect on weight loss of a "modified" Mayo Clinic Diet tailored to each obesity-related phenotype in 100 participants followed for 12 weeks vs. the "standard" Mayo Clinic diet using the control group of the 100 participants from aim 1.
3) Study the deep-phenotype and the multi-omics profile (GWAS, proteomics, metabolomics, microbiome, exposome) of the best responders vs. poor responders in all groups using machine learning tools.

**Significance:** The purpose of this protocol is to define an "individualized diet" approach based on obesity related phenotypes (pathophysiology obesity classification), which would increase weight loss, adherence, and weight loss maintenance.

# "Mayo Clinic Diet" Individualized Lifestyle Intervention for Obesity Management based on Obesity Phenotypes (PHENO-Diet trial)

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## BACKGROUND

Obesity prevalence continues to increase worldwide(2) and, in the United States, 69% of adults are overweight or with obesity(3). Estimated costs to the healthcare system are more than $480 billion annually. Increased severity of obesity correlates with a higher prevalence of the associated co-morbidities. Likewise, obesity increases the risk of premature mortality (4). 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, physical activity, medications, endoscopy and surgery is highly variable (5). 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 (6). 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. Providers have to select anti-obesity pharmacotherapy based on potential side effect and patient comorbidities (7), 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 food intake and behavioral phenotypes *(1)*.

### 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 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 less healthy food ingredients like added sugars, sodium and alcohol. Additionally, guidelines recommend limiting or avoiding liquid calories (i.e. sodas, juices, alcohol, etc.). 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 weight loss or minimize weight regain long-term (>1 year) (8). Diet and physical activity recommendations can be in combination with a hospital/university or commercial behavior program; these are usually comprehensive lifestyle interventions that 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(9).

**The Mayo Clinic Diet** is a lifestyle change approach to weight loss and not a 'diet' in the traditional sense of the word. (REF: Hensrud DD, ed., The Mayo Clinic Diet, 2nd ed. Rochester, MN, 2017) The goal of The Mayo Clinic Diet is to help people achieve a healthier weight and lifestyle habits long-term. It is divided into 2 phases: LoseIt! and LiveIt! LoseIt! is a two week jump start phase in which people make sudden changes in 15 habits associated with diet and physical activity that promote safe and effective weight loss. In the LiveIt! phase, people take the changes in habits people made in LoseIt! and turn them into long-term lifestyle changes which, along with additional structured interventions, enables ongoing and sustained weight loss and improved health indefinitely. A primary dietary principle behind The Mayo Clinic Diet is consuming low energy-dense foods, such as vegetables and fruits, that help achieve satiety at a lower calorie intake. The recommended foods are also health-supporting independent of their energy content. The Mayo Clinic Diet also stresses key components of behavior change, such as finding your inner motivation to lose weight, setting achievable goals and handling setbacks.

The Mayo Clinic Diet is flexible. Individuals can choose a diet higher in healthy carbohydrates and lower in fat, or one that is higher in healthy fats and lower in carbohydrates. While currently individuals can tailor the diet to their own preferences, there is a need to further refine the ability to tailor a diet to an individual based on specific characteristics to better achieve improved long-term weight loss and health.

## PRELIMINARY DATA

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 n satiation (21%), gastric capacity (15%), behavioral (13%), gastric sensorimotor (11%) factors and others (40%)(1). This obesity sub-classification may predict weight loss response to pharmacotherapy and bariatric endoscopy (1). Subsequently, we added energy expenditure to the characterization; with a final breakdown of obesity into: Satiation – hungry Brain(16%), Satiety – Hungry Gut(19%), Psychological – Emotional Hunger (20%), Energy expenditure - Slow Burn(16%), other - unknown (9%) and mix (20%) phenotype (Fig 5).

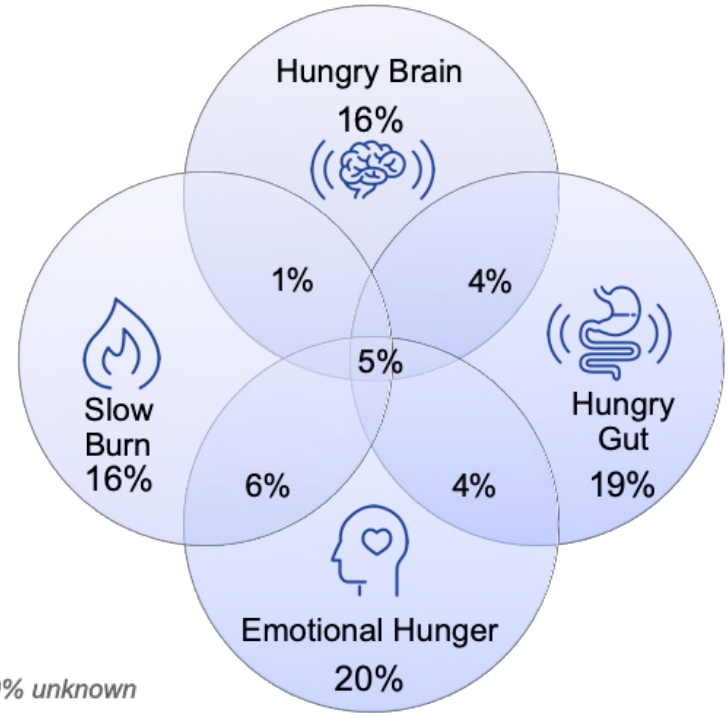



Figure 5: Obesity-related Phenotypes based on pathophysiology.

Obesity phenotypes to predict weight loss response: Thus far, we validated the applicability of obesity-related phenotypes in three randomized clinical trials (1, 10, 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 gastric emptying, satiation, satiety, and fasting and postprandial gut hormones in 24 obese adults using validated

assays. 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 ER was significantly associated with kcal intake at a prior satiation test. We concluded that prior satiation test predicts weight loss with PhenTop (Fig 2) (Figure 6) (1).

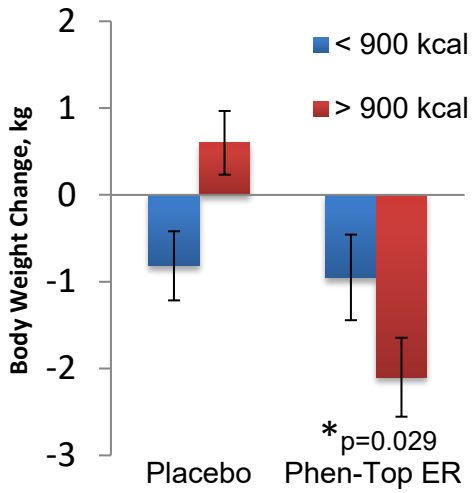



**Figure 6: *Body weight change by prior satiety test.*** *Association of change in body weight (in response to randomized treatment with placebo or PhenTop) and kcal intake at prior ad-libitum meal. This is shown (p=0.029) for the drug* treatment interaction.(1)*

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 (satiety) test predicts weight loss with exenatide (10)**.

Finally, in a prospective, randomized clinical trial with liraglutide, a long-acting GLP-1 receptor agonist, with the objective to compare effects of liraglutide and placebo over 16 weeks on gastric motor functions, 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. 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). At 5 and 16 weeks, GES $T_{1/2}$ correlated with Δ weight loss on liraglutide (p<0.02). Nausea was the most common adverse event in the liraglutide group (63.2%) compared to placebo (9.5%). Our results suggest that Liraglutide, 3.0mg, significantly

delays GES after 5 and 16 weeks' treatment; effects on weight loss are associated with absolute value of GES $T_{1/2}$ (satiety test) on liraglutide (11).

Furthermore, the findings from these three randomized clinical trials are supported by two case-controls studies. First, in a case-control prospective trial with data collected retrospectively, we applied a phenotype-guided pharmacotherapy [intervention group (n=55)] and compared to standard of care, physician selected pharmacotherapy [control group (n=175)] in patients with obesity managed at the Mayo Clinic Weight Management center. We showed that phenotype-guided pharmacotherapy doubles the weight loss at 12 months of treatment [Intervention group 12.9±1.9% total body weight loss (TBWL) compared to 6.7±1.2% TBWL in standard of care group, p<0.0025] (Fig 6a). Moreover, the intervention group had 74% responders (defined > 3% TBWL in 1st month) compared to 33% in controls (Fig 6b).

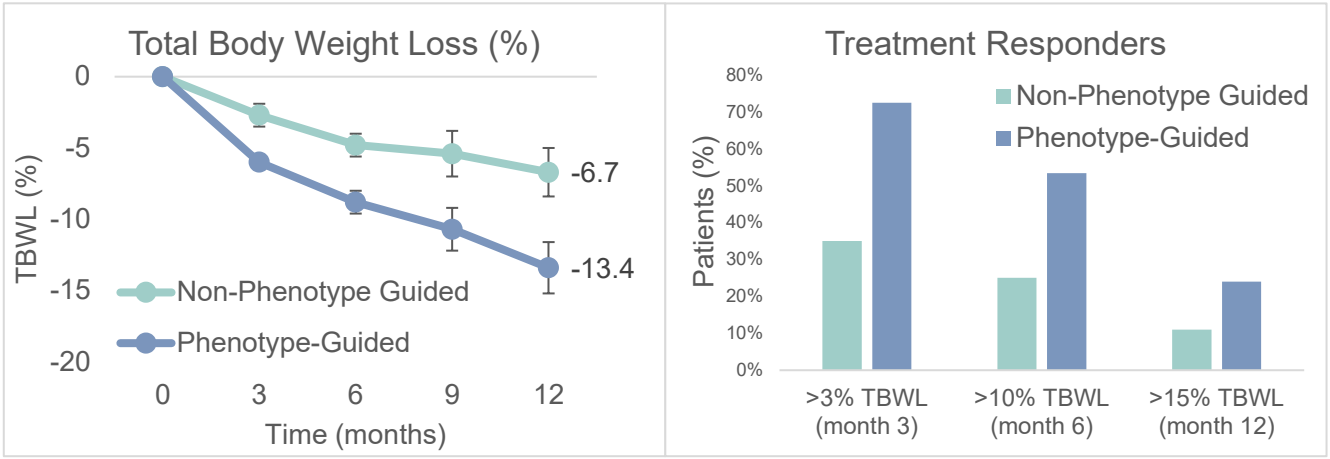



Figure 6: Case-Control Prospective Observation of Obesity Management with Anti-obesity Pharmacotherapy in a Multidisciplinary Weight Management Program comparing phenotype-guided pharmacotherapy to non-phenotype guided pharmacotherapy. A) total body weight loss, b) Percentage of treatment responders.

In another case-control study done in Hospital Sanchinarro in Madrid Spain, authors identified that an abnormal satiety test predicted response to the intragastric balloon therapy (p<0.001) and an abnormal satiation test predicted response to the endoscopy sleeve gastroplasty (P<0.01). These data externally confirm the applicability of an obesity-related phenotype algorithm to manage obesity.

Biomarker Discovery to predict Obesity Phenotypes: Obesity phenotypes are associated with higher BMI, distinguish obesity phenotypes, and may predict response to obesity pharmacotherapy and endoscopic devices (1). However, the tests are currently limited to a few research/academic centers. Thus, we have **developed (n=180) and validated (n=120) 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. 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 preliminary data, we derived Bayesian covariate predictors for abnormal satiation, abnormal satiety, behavioral eating, abnormal energy expenditure and mixed. These models yielded an ROC AUC of 0.9414, 0.9261, 0.9808, 0.9168 and 0.9852. These data suggested that the multi-omics test predict obesity phenotypes with a high sensitivity and specificity.

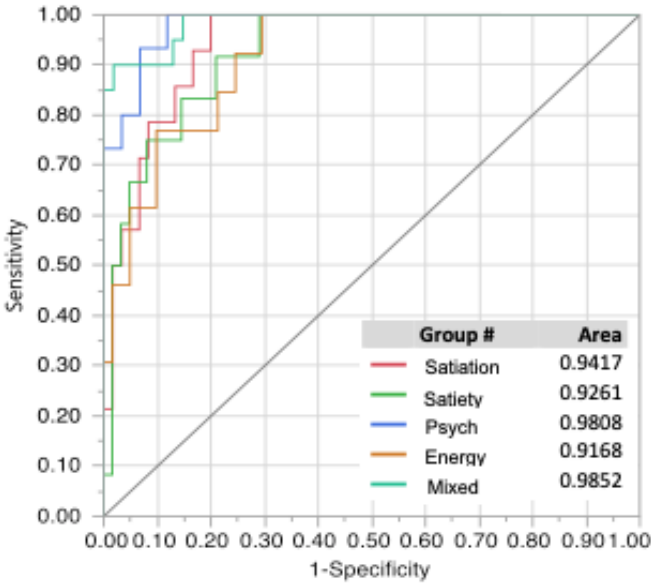


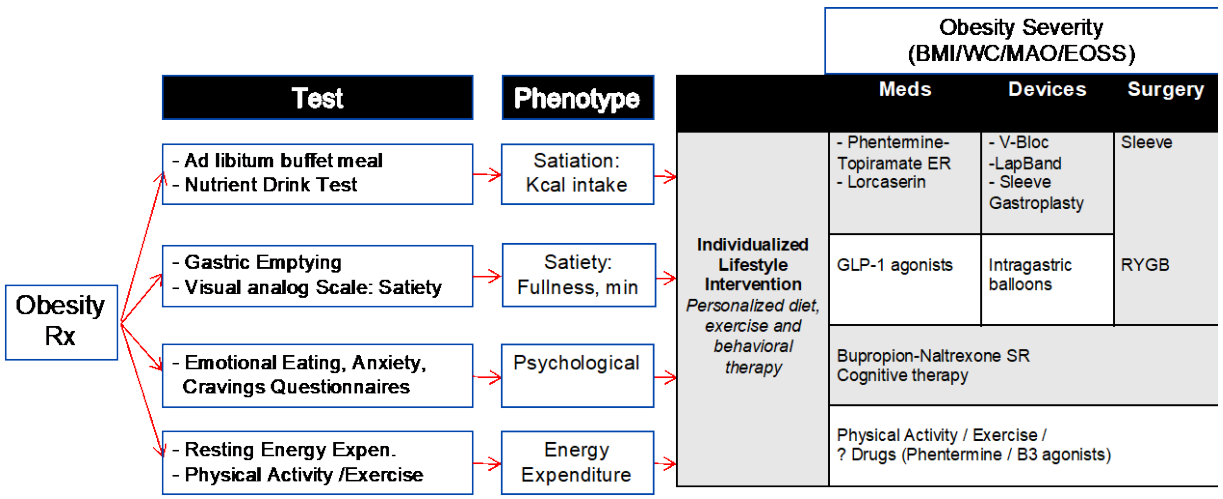

**Figure 7: Diagnostic test for Obesity Phenotypes** that predict weight loss response to interventions. ROC curves for each main group: Red line: abnormal satiation, Green line: abnormal satiety, Blue line: abnormal psych, Orange Line: abnormal energy expenditure and Turquoise line: mixed group (unpublished – confidential)

## Research Plan

Despite advances in understanding of aspects of obesity pathophysiology, weight loss with current treatments including diet, exercise, medications, endoscopy and surgery is highly variable (5). 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 obesity-related phenotypes (1, 10, 11). The novel obesity management algorithm (figure 8) was tested in two case-control studies at Mayo Clinic, Rochester MN and Sanchinaro Hospital, Madrid, Spain.

While currently individuals can modify a diet to their own preferences, ***there is a critical need to identify unique diet and lifestyle characteristics and tailor them to the obesity-related phenotypes*** to achieve improved long-term weight loss and improved health. The Mayo Clinic Diet is a lifestyle change approach to weight loss and not a 'diet' in the traditional sense of the word. The goal of The Mayo Clinic Diet is to help people achieve a healthier weight and lifestyle habits long-term. It is flexible and macronutrient content and other characteristics can be modified. ***The Mayo Clinic Diet is ideal to determine lifestyle characteristics that increase weight loss among obesity-related phenotypes.***

Figure 8: Novel obesity management algorithm using FDA-approved interventions.




## Hypothesis and Aims

**Hypothesis:** *We hypothesize that obesity-related phenotypes may predict weight loss to an Individualized Lifestyle intervention based on a tailored Mayo Clinic Diet program.*

**Aim:** To test this hypothesis, we propose three aims: 1) to study the effect of the Mayo Clinic Diet in total body weight loss in the obesity related phenotypes; 2) to compare the effect on weight loss of the "modified" Mayo Clinic Diet tailored to each obesity-related phenotype vs. the "standard" Mayo Clinic diet control group; 3) Study the multi-omics profile of the best responders using machine learning tools.

## Common Methods

### Selection Participants

We plan to study a total of 223 patients with obesity (BMI>30 kg/$m^2$). Participants will be recruited from the community by standard IRB approved methods.

Inclusion criteria

- Adults with obesity (BMI >30Kg/$m^2$); these will be otherwise healthy individuals with no unstable psychiatric disease and uncontrolled life-threating comorbidities (i.e. unstable angina).
- Age: 18-65 years.
- Gender: Men or women.

Exclusion criteria

a) Weight change greater than 3% in the previous 3 months (weight stable).
b) History of bariatric surgery including lap band and bariatric endoscopy.
c) Significant untreated psychiatric dysfunction including binge eating disorders and bulimia.
d) Current use of anti-obesity pharmacotherapy.
e) A positive score on the AUDIT-C questionnaire as judged by an investigator.
f) Patient has a known history of any condition or factor judged by the investigator to preclude participation in the study or which might hinder study adherence
g) Pregnancy
h) Previously participated in the Mayo Clinic Diet experience program at the HLP.

### Anthropometrics and phenotype studies

Anthropometrics Measurements: will be taken of hip-waist ratio, height, weight, blood pressure, pulse at Visit 1, Visit 3, Visit 5 and Visit 7.

Phenotype studies at Visit 2 and 3 and Visit 6 and 7:

If participants have been previously phenotyped within the last 3 years, they will not need to complete the phenotype studies on Visit 2 and Visit 3 except for the DEXA body composition. Participants will report to the Mayo Clinic after an 8-hour fasting period, and the following validated quantitative traits (phenotypes) will be measured

a) Body composition will be measured by DEXA (dual energy x-ray absorptiometry).
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 $t_{1/2}$) (1, 12, 13). Images will be acquired at 0, 60, 120 and 240 minutes following the normal clinical gastric emptying testing protocol.
d) Appetite (hunger level) by visual analog score fasting and prior to the Satiation test (1).
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) (1).

f) Satiety by visual analog score postprandial after breakfast and the *ad*-libitum buffet meal test for every 30 minutes for 2 hours (1). Satiety will be measured in length of time of fullness.Permeability test is measured by urinary excretion of mannitol and lactulose after oral ingestion(14). We refined a previous HPLC-tandem mass spectrometry technique(15). We validated measurement of colonic permeability after oral delivery by direct instillation of sugars into the colon(16). We recently published the validation this test and showed significantly increased small bowel (P<.001) and colonic (P=0.10) permeability in IBS-D(17) We validated $^{13}$C-mannitol to avoid confounding by dietary $^{12}$C-mannitol (18). In this study, we will provide the $^{13}$C-mannitol (100 mg) with the 320 kcal breakfast.

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

h) Samples collection, handling and storage: Samples will be collected after an overnight fast (of at least 8 hours) in the morning. Plasma will be preserved following standard guidelines and protein degradation inhibitors, kalikrein and DPP-IV inhibitors will be added to preserve the samples. Plasma hormones, proteomics, metabolomics, blood DNA and stool will be stored at -80ºC in the PI's laboratory.
    a. Fasting Blood will be collected for basic metabolic panel, lipid panel, HbA1C, hsCRP
    b. Plasma hormones and proteomics (Total and active Ghrelin, GLP-1, CCK, PYY and bile acids) by radioimmunoassay and/or mass spectrometry measured fasting, and postprandial 15, 45, 90 and 240 min minutes, with the primary endpoint being the peak postprandial level (test should be done simultaneously to GE).
    c. Targeted Metabolomics: We will perform quantitative, targeted metabolomics of salient classes of compounds in plasma 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.

d. Blood DNA for GWAS
e. Stool will be collected and stored to study microbiome, short chain fatty acids and bile acids.
f. Urine will be collected to quantitate Excretion of Lactulose and $^{13}$C-Mannitol after Oral Ingestion of lactulose and $^{13}$C mannitol (5:1 ratio by mass) (47). The 0-2h urine most closely reflects small intestinal permeability and 6-24 urine reflects colonic permeability (46). A single urine collection will be collected once between the 6-8 hour time period. HPLC-tandem mass spectrometry will be used for detection of the sugars (Mayo Clinic CTSA Lab). Baseline and post treatment small bowel and colonic permeability will be performed for every patient. *Cumulative (Cum) excretion (0-2h and 6-24h)* = Concentration of sugar (µg/mL)] * total urine volume (mL). *Lactulose: mannitol ratio* (L:M R) is: 0.2 x (Cum excretion lactulose) / (Cum excretion **$^{13}$C-**mannitol).
g. Saliva: will be collected at fasting and postprandial 15, 45, 90 and 240 min minutes and stored for future testing of hormones, metabolomics and C13 mannitol.
h. Plasma glucose: will be measured fasting, and postprandial 15, 45, 90 and 240 minutes.

Participants will be asked to avoid of alcohol, sodas artificial sweeteners, NSAIDS, strenuous exercise (running > 3milles or equivalent) 48 hours before and during the permeability testing (Visits 2-6).

Questionnaires to Assess Behavioural Eating patterns and disorders

Participants will complete a series of questionnaires (all included in the APPENDIX)

a) AUDIT-C Alcoholism Screening Test (19) - 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 (20), 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) Three Factor eating questionnaire is 21-item questionnaire, validated, to assess for emotional eating disorders and food cravings.
d) Eating Behaviors - The Weight Efficacy Life-Style Questionnaire [WEL (21)] 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 (22)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 (22).
f) Exercise behavior- The Exercise Regulations Questionnaire (BREQ-3)*(23)* 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?(24).*
h) Hospital Anxiety and Depression Questionnaire
i) Mayo Clinic Habit Tracker – This brief binary questionnaire is used to determine adherence to the 15 habits in the LoseIt! phase of The Mayo Clinic Diet.
j) Diet assessment - Mayo Clinic Diet Assessment questionnaire, a food frequency questionnaire that evaluates the healthfulness of diet.
k) Mayo Clinic Diet Pyramid Tracker – a tool that evaluates adherence to the Mayo Clinic Diet.
l) 24 hour dietary recall – gold standard to document overall dietary habits and adherence.

Covid-19 Screening: Participants must have a negative COVID-19 PCR test to complete a treadmill stress test on Visit 3 at the Health Living Program. In order to determine the level of SARS-CoV-2 virus, patients will have a nasopharyngeal swab for SARS Coronavirus-2 PCR.

### Mayo Clinic Diet Experience:

The Mayo Clinic Diet is not only a diet, it is a lifestyle change program. The Mayo Clinic Diet consists of two phases. LoseIt! is a two week phase that emphasizes sudden changes in 15 habits that are safe and have evidence supporting their effect on promoting weight loss. These habits include eating more vegetables and fruits, healthy fats, breakfast, and real (less processed) food. Physical acitivity habits include 30-60 minutes per day. Based on a pilot program, individuals who follow the LoseIt! program will lose, on average, eight pounds during the two weeks, and most people will lose six to ten pounds. In the LiveIt! phase, people take the changes in habits people made in LoseIt! and turn them into long-term lifestyle changes which, along with additional structured interventions, enables ongoing and sustained weight loss and improved health indefinitely. A primary dietary principle behind The Mayo Clinic Diet is consuming low energy-dense foods, such as vegetables and fruit, that help achieve satiety at a lower calorie intake. The recommended foods are also health-supporting independent of their energy content. The Mayo Clinic Diet also stresses key components of behavior change, such as finding your inner motivation to lose weight, setting achievable goals and handling setbacks.

## Standard of Care:

All participants will receive standard of care which consists of lifestyle intervention, behavioral evaluation and treatment, which will include:

a) Exercise capacity will be determined by a treadmill stress test using a standardized Bruce protocol.
b) Functional Movement Screen
c) Strength Testing

### *Lifestyle Intervention and Behavioral Treatment*

All the participants will meet the multidisciplinary team which consists of an Obesity Expert physician and a registered dietitian nutritionist as standard of care in clinical practice. All participants will guided to 1) Diet: Reduce dietary intake below that required for energy balance by consuming 1200 calories per day for women and 1400 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.).

## Specific aims

We propose three aims:

1) to study the effect of the Mayo Clinic Diet in total body weight loss in the currently defined obesity-related phenotypes in 100 participants followed for 12 weeks. The primary end-point is 12-week total body weight loss.
2) to compare the effect on weight loss of a "modified" Mayo Clinic Diet tailored to each obesity-related phenotype in 100 participants followed for 12 weeks vs. the "standard" Mayo Clinic diet using the control group of the 100 participants from aim 1.
3) Study the deep-phenotype and the multi-omics profile (GWAS, proteomics, metabolomics, microbiome, exposome) of the best responders vs. poor responders in all groups using machine learning tools.

Specific aim 1: To study the effect of the Mayo Clinic Diet in total body weight loss in the obesity related phenotypes.

Study design: To achieve this aim we propose to study the effect of the Mayo Clinic Diet in 12-weeks total body weight loss in the obesity related phenotypes in 100 participants who will participate in the Mayo Clinic Diet Experience in the Healthy Living Program (HLP)– Mayo Clinic and will be "deep" phenotype to identify the obesity-related phenotype. Participants and HLP staff will be blinded to the obesity phenotype until the end of the 12 weeks. All participants will follow as standard of care by their HLP team which includes wellness coach, dietitian and

exercise physiologist. Participants will have the following visits: Visit 1) screening and initial assessment; Visit 2) Gastric Emptying and Phenotype; Visit 3) Phenotype (if not done at Visit 2) and wellness testing; Visit 4) Counseling and management recommendations; Visit 5) 4 (+/- 1w) weeks after visit 4, meet coach and collect fasting samples; Visit 6) Gastric Emptying and repeat phenotype; Visit 7) 12 (+/-1w) weeks after visit 4: repeat phenotype (if not done at Visit 6) and meet coach. Participants will do 3 sets of questionnaires (HLP Daily tracker) with a 24-hour recall diet. Each set will include a weekday and a weekend, to be completed at baseline, 2-4 weeks and 10-12 weeks. The phenotype will include the following tests: Resting Energy Expenditure (by indirect Calorimetry); Body Composition (by DEXA); fasting blood collection (for PhenoTest which includes DNA, proteins, hormones and metabolites); Satiety tests: Gastric emptying (by scintigraphy), Postprandial blood collections (15, 45, 90 and 240 min); Permeability test, Saliva collection (fasting, and postprandial 15, 45, 90, 240 min), urine collection, Satiation test: Ad libitum Buffett Meal and VAS fullness; Behavioral questionnaires; exercise capacity and tolerance. Wellness assessment will include a treadmill stress test using the Bruce protocol, functional movement screen, and strength testing. In order to complete treadmill test, participants will require a negative COVID test result. Participants will also have weekly virtual wellness coaching sessions through the HLP. These virtual visits will begin the week after visit 4 and conclude the week prior to visit 7.

| **Merge – Standard Approach (aim 1)** | |
|---|---|
| Day One: | "we learn about you" (assessment) (visit day 2 and 5) |
| 7-8 am | Check-in / Biometric Measurements / REE |
| 8-8:15 | Keynote Introduction |
| 8:15-8:30 am | Breakfast / Brain-Gut physiology (includes images & labs)* |
| 8:30-9 am | DEXA |
| 9-10 am | Questionnaires - Behavioral / Food choices / Exercise / Activity |
| 10-11 am | Private Session with a Certified Wellness Coach |
| 11-12 pm | Physical Activity Exercise Assessment |
| 12 - 1 pm | Lunch (*ad libitum* buffet meal) |
| 1-2 pm | Cornerstones of The Mayo Clinic Diet |
| 2-3 pm | Private Session with a Nutrition and Weight Expert |
| 3-4 pm | Physical Activity Exercise Assessment |
| 4-5 pm | Assessment Review |
| 5-6:30 pm | Facility use, relaxation, Rejuvenate Spa |
| | |
| Day Two: | "you learn about you" (Wellness plan) (visit day 3) |
| 7:30-8 am | Healthy breakfast |
| 8-9 am | Building Your Personal Awareness for Change |
| 9-10 am | Exercise for the Mind, Body and Soul |
| 10-12pm | Guided Workout |
| 12-1:30 pm | Cooking Well (lunch included) |
| 1:30-2 pm | Enhance Resiliency and Manage Stress |
| 2-3 pm | Private Session with a Certified Wellness Coach |
| 3-4 pm | Design Your Activity Plan |
| 4-7 pm | Facility use, relaxation, Rejuvenate Spa |

[1]

[1] Schedule may vary.

| Study Procedures (Aim 1) | Visit 1 (Screening) | Visit 2 (Gastric Emptying) | Visit 3 (Phenotyping and Wellness Testing) | Visit 4 (Counseling and Wellness Recommendations) | Visit 5 (4 weeks +/- 1 week after visit 4) | Visit 6 (Gastric Emptying 12 weeks +/- 1 week after visit 4)) | Visit 7 (12 weeks +/- 1 week after visit 4) |
|---|---|---|---|---|---|---|---|
| Informed Consent | X | | | | | | |
| Medical History and Physical Examination | X | | | | | | |
| Vital Signs | X | | X | | X | | X |
| Pregnancy Test (urine) | | X[b] | | | | X[b] | |
| Obesity Phenotyping Testing (REE, DEXA, Buffet meal) | | X[d] | X[e] | | | X[d] | X[e] |
| Gastric Emptying | | X | | | | X | |
| Behavioral Questionnaires | X* | | X** | | | | X |
| Wellness Testing (Treadmill, functional movement screen, strength testing) | | | X | | | | |
| Nutritionist and wellness coaching meetings | | | X | X[c] | X | | X |
| Exercise education (guided workout) | | | | X | | | |
| Blood Draw (metabolic panel, lipid panel, HbA1C, hsCRP, GI hormones, metabolomics, Blood DNA) | | X | X[f] | | X*** | X[a] | X[a&f] |
| Virtual Coaching Sessions | | | | → | → | → | → |
| Stool Sample | | | X | | | | X |
| Saliva collection (fasting and postprandial) | | x | | | | x | |
| Urine sample | | x | | | | x | |
| 24-hour recall diet | | | X | | X | | X |
| COVID Screening | | | X[G] | | | | |

* HADS, Eating Disorder Questionnaire and Audit-C only

** TFEQ, WEL and physical active questionnaires only

*** Only fasting GI hormones and metabolomics at this visit

[a] Blood DNA not collected at this visit

[b] Urine pregnancy test may be completed up to 48 hours prior to GE test.

[c] includes hands-on cooking class with nutritionist

[d] REE and Buffet Meal only

[e] Only DEXA if REE and Buffet Meal done at Visit 2

[f] If not done at Visit 2

[G] Done up to 7 days prior Visit 3

Specific aim 2: to compare the effect on weight loss of the modified" Mayo Clinic Diet tailored to each obesity-related phenotype vs. the "standard" Mayo Clinic diet control group in 100 participants followed for 12 weeks compared to participants from the standard Mayo Clinic Diet (aim 1). Participants and HLP staff will be informed of their obesity phenotype prior to initiation of the weight loss program. All participants will follow a "modified" Mayo Clinic Diet care by their HLP team which includes wellness coach, dietitian and exercise physiologist. Participants will have the following visits to the HLP: Visit 1) screening and initial assessment; Visit 2) Gastric Emptying; Visit 3) Phenotype and wellness testing; Visit 4) Counseling, phenotype explanation and management recommendations; Visit 5) 4 (+/- 1w) weeks after visit 4, meet coach and collect fasting samples; Visit 6) Gastric Emptying; Visit 7) 12 (+/-1w) weeks after visit 4: repeat phenotype and meet coach. Participants will do 3 sets of questionnaires with a 24-hour recall diet. Each set will include a weekday and a weekend, to be completed at baseline, 2-4 weeks and 10-12 weeks. The phenotype will include the following tests: Resting Energy Expenditure (by indirect Calorimetry); Body Composition (by DEXA); fasting blood collection (for PhenoTest which includes DNA, proteins, hormones and metabolites); Satiety tests: Gastric emptying (by scintigraphy), Visual Analog Scales for Appetite (VAS 100 mm charts), Postprandial blood collections (15, 45, 90, 240 min); Permeability test, Saliva collection (fasting, and postprandial 15, 45, 90, 240 min), urine collection, Satiation test: Ad libitum Buffett Meal and VAS fullness; Behavioral questionnaires; exercise capacity and tolerance. Wellness assessment will include a treadmill stress test using the Bruce protocol, a functional movement screen, and strength testing. Phenotype will be selected based on 75 percentile of abnormal trait (historical PHENOME registry database). Then, participants will be informed about their Phenotype and a tailored lifestyle intervention will be designed for them. The four potential lifestyle interventions will include: Mayo Clinic Hungry Brain diet, Mayo Clinic Hungry Gut diet, Mayo Clinic Emotional hunger diet, and Mayo Clinic Slow Burn diet. Each plan will include a nutritional, behavioral, physical activity and exercise plan. All participants will follow as standard of care by their HLP team which includes wellness coach, dietitian and exercise physiologist. See description below.

| **Phenotype** | **Plan** |
|---|---|
| **Hungry Brain / abnormal satiation** | • **1-2 large meals: low energy dense diet, volumetric diet, with vegetables and fruits for second serving during meals, minimize liquid calories,**<br>• Standard behavioral counseling<br>• Standard Physical Activity<br>• Standard Exercise |
| **Hungry Gut / abnormal satiety** | • **2-3 meals: high protein, with pre-meal protein snack, rich in fiber**<br>• Standard behavioral counseling<br>• Standard Physical Activity |

| | |
|---|---|
| | • Standard Exercise |
| **Emotional hunger / Hedonic eating behavior** | • **2-3 meals / no snacks, mindful eating.**<br>• **High Intensity behavioral counseling**<br>• Standard Physical Activity<br>• Standard Exercise |
| **Slow Burn** | • Standard low calorie diet<br>• Standard behavioral counseling<br>• Standard Physical Activity<br>• **Increased Intense Physical Activity with High Intensity Interval Training (HIIT) and Resistance Training** |
| **Standard of Care** | • Standard low calorie diet<br>• Standard behavioral counseling<br>• Standard Physical Activity<br>• Standard Exercise |

Additionally, each "intense lifestyle intervention will be tailored to comorbidities and participants desires". Participants will also have weekly virtual wellness coaching sessions through the HLP. These virtual visit will begin the week after visit 4 and conclude prior to visit 7.

| Day One: | "we learn about you" (assessment) (Visit day 2 and 5) |
|---|---|
| 7-8 am | Check-in / Biometric Measurements / REE |
| 8-8:15 | Keynote Introduction |
| 8:15-8:30 am | Breakfast / Gut physiology (includes images & labs)* |
| 8:30-9 am | DEXA |
| 9-10 am | Questionnaires - Behavioral / Food choices / Exercise / Activity |
| 10-11 am | Private Session with a Certified Wellness Coach |
| 11-12 pm | Physical Activity Exercise Assessment |
| 12 - 1 pm | Lunch (*ad libitum* buffet meal) |
| 1-2 pm | Cornerstones of The Mayo Clinic Diet |
| 2-3 pm | Private Session with a Nutrition and Weight Expert |
| 3-5 pm | Physical Activity Exercise Assessment |
| 5-6:30 pm | Facility use, relaxation, Rejuvenate Spa |
| | |
| Day Two: | "you learn about you" (Wellness plan) (visit day 3) |
| 7:30-8 am | Healthy breakfast |
| 8-9 am | You are Unique: Review assessment with a Nutrition and Weight Expert |
| 9-10 am | Private Session with a Certified Wellness Coach |
| 10-11am | Building Your Personal Awareness for Change |

| | |
|---|---|
| 11-12 pm | Guided Workout |
| 12-1:30 pm | Cooking Well (lunch included) |
| 1:30-5 pm | Tailoring the program to you |
| 5-7 pm | Facility use, relaxation, Rejuvenate Spa |
| | |
| not to disclose with guest: but potential plan from 1:30-5 pm | |
| HB | Mindfullness eating / meeting with dietitian |
| HG | Listening to your body / meeting with dietitian |
| EH | Enhance Resiliency and Manage Stress |
| SB | Strengthening with Suspension / Personal trainer |

| Study Procedures (Aim 2) | Visit 1 (Screening) | Visit 2 (Gastric Emptying) | Visit 3 (Phenotyping and Wellness Testing) | Visit 4 (Counseling, phenotype explanation and Wellness Recommendations) | Visit 5 (4 weeks +/- 1 week after visit 4) | Visit 6 (Gastric Emptying, 12 weeks +/- 1 week after visit 4)) | Visit 7 (12 weeks +/- 1 week after visit 4) |
|---|---|---|---|---|---|---|---|
| Informed Consent | X | | | | | | |
| Medical History and Physical Examination | X | | | | | | |
| Vital Signs | X | | X | | X | | X |
| Pregnancy Test (urine) | | X[b] | | | | X[b] | |
| Obesity Phenotyping Testing (REE, DEXA, Buffet meal) | | X[d] | X[e] | | | X[d] | X[e] |
| Gastric Emptying | | X | | | | X | |
| Behavioral Questionnaires | X* | | X** | | | | X |
| Wellness Testing (Treadmill, functional movement screen, strength testing) | | | X | | | | |
| Nutritionist and wellness coaching meetings (tailored to obesity phenotype) | | | X | X[c] | X | | X |
| Exercise education (guided workout tailored to obesity phenotype) | | | | X | | | |
| Blood Draw (metabolic panel, lipid panel, HbA1C, hsCRP, GI hormones, metabolomics, Blood DNA) | | X | X[f] | | X*** | X[a] | X[a&f] |
| Virtual Coaching Sessions | | | | → | → | → | → |
| Stool Sample | | | X | | | | X |
| Saliva collection (fasting and postprandial) | | | x | | | | x |
| Urine | | | X | | | | X |
| 24-hour recall diet | | | X | | X | | X |
| Covid screening | | | X[G] | | | | |

* HADS, Eating Disorder Questionnaire and Audit-C only
** TFEQ, WEL and physical active questionnaires only
*** Only fasting GI hormones and metabolomics at this visit
[a] Blood DNA not collected at this visit
[b] Urine pregnancy test may be completed up to 48 hours prior to GE test.
[c] includes hands-on cooking class with nutritionist
[d] REE and Buffet Meal only
[e] Only DEXA if REE and Buffet Meal done at Visit 2
[f] If not done at Visit 2
g Done up to 7 days prior Visit 3

Specific aim 3: Study the deep-phenotype and the multi-omics profile (GWAS, proteomics, metabolomics, microbiome, exposome) of the best responders vs. poor responders using machine learning tools.
Once aim 1 and 2 are completed, we plan to gather all the data from each participant into a secure, de-identified database. This database will be merge with a complete GWAS analysis, proteomics and peptidomics, targeted and untargeted metabolomics, microbiome and an "exposome" questionnaire. Then, using machine learning tools, we will identify the unique characteristics among the groups using clustering analysis and principal component analysis. Then, we would identify potential biomarkers that separate responders from non-responders. These data will facilitate the understanding of new unique phenotypes or deepen our understanding of the current phenotypes. Finally, we will identify unique management tools and recommendations that improve outcomes in each phenotype.

## Statistical Analysis Plan

Primary endpoint: Total Body Weight Loss in kg (defined as weight changed from baseline to 12 weeks) in the whole cohort in the Modified Healthy Living Program – Mayo Clinic Diet Experience compared with the standard Mayo Clinic diet program.

Secondary endpoints:

1. Total Body Weight Loss in kg (defined as weight changed from baseline to 12 weeks) in each obesity-related phenotype in the Modified Healthy Living Program – Mayo Clinic Diet Experience compared with the standard Mayo Clinic diet program.
2. Responder rate (>3% TBWL)
3. Change in waist circumference (cm),
4. Change in visceral adiposity (DEXA, %),
5. Change in phenotypes:
   a) total calories consumed in 24 hours for abnormal satiation
   b) calories to fullness in ad libitum meal for abnormal satiation
   c) gastric emptying t ½ in minutes for abnormal postprandial satiety
   d) gastric emptying % after 120 minutes abnormal postprandial satiety
   e) HADS-A score for abnormal emotional eating
   d) emotional eating score on the TFEQ for abnormal emotional eating
   e) body composition Fat free mass (%) for abnormal energy expenditure
   f) kcal/day measured by indirect calorimetry abnormal energy expenditure,
6. Adherence and compliance with program defined by the number of contacts with the team,
7. improvement in comorbidities in all cohort and per each phenotype.

**General Approach:** The primary analyses will be conducted on *primary analysis* cohorts (PA), defined as subjects who have at least one post-baseline assessment. Subjects who do not formally withdraw from the study, but do not have a final assessment at 12 weeks will be defined as *dropouts.* The primary approach for missing values due to drop-out or withdrawal in the *primary analysis* cohort will be to use last-value-carried-forward (LVCF). The *completers* cohort

consisting only of subjects who had an assessment at 12 weeks will be used for sensitivity analyses. All hypothesis tests will be 2-sided with a 0.05 significance level. All effect estimates will be reported with 95% confidence intervals.

**Primary Analysis:** The primary endpoint will be total body weight loss (TBWL), defined as the change in body weight from baseline to the 12-week time point. Weight loss will be assessed using ANCOVA models with sex, age, and weight at baseline as covariates in all patients. Mean TBWL and 95% confidence intervals will be reported overall and for each phenotype.

**Secondary Analyses:** Additional analyses will include analysis of secondary endpoints (e.g. responder rate, change in waist circumference, body composition via DEXA scan, change in phenotype, changes in phenotype defining measurements). Interactions will be used to test subgroup effects of modified diet within phenotype groups. Additionally, we will investigate whether the magnitude of change in phenotyping defining measures have a greater association with weight loss within the phenotype subgroup relative to other phenotypes. Linear regression models for the final weight at 12-weeks will be modeled with treatment, age, sex, baseline weight, and change in phenotype measurement plus an interaction between phenotype subgroup and change in phenotype measure. These analyses will explore the effectiveness of the modified diet on the phenotype defining measures within each phenotype after weight loss. The measures of specific interest for this analysis are total calories consumed in 24 hours and *ad libitum* meal caloric intake for abnormal satiation, HADS-A score and emotional eating score on the TFEQ for abnormal emotional eating, gastric emptying time and gastric emptying % after 120 minutes for abnormal satiety, measured versus predicted REE and lean mass percentage for abnormal resting energy expenditure.

**Exploratory analysis:** All other outcomes for aim 3 (hormones, proteomics, and metabolomics) measured at the baseline and 12-week clinical visits will be considered exploratory and analyzed using the methods described for the secondary outcomes, without penalization for multiple comparisons.

**Sample size assessment:** In our recent pilot study (with Liraglutide 3.0 mg vs. placebo), the SD for the overall weight change (pre-post) observed in placebo (lifestyle intervention alone) was 3kg at 3 months. Recently the DIETFITS trial observed a standard deviation of 10kg for weight loss at 12 months. Enrollment of 223 subjects total with a 15% drop out rate results in 95 subjects per group, which will provide 90% power (2-sided test of 0.05 Type I error rate) to detect a standardized difference of 0.47. Given our standard deviation estimates, we will be well powered to detect a mean difference of 1.4kg to 4.7kg between the groups.

## SIGNIFICANCE

The purpose of this protocol is to define an “individualized diet” approach based on obesity related phenotypes (pathophysiology obesity classification), which would increase weight loss, adherence and weight loss maintenance.

**Potential pitfalls, precautions taken, and alternative strategies:**

a. Feasibility - 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. 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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