Beta Cell Restoration through Fat Mitigation

The BetaFat Study

A Participating Study in the

The Restoring Insulin Secretion (RISE) Consortium

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PROTOCOL

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CONFIDENTIAL

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1. EXECUTIVE SUMMARY

1.1 The RISE Consortium

The RISE Consortium is a collaborative effort involving investigators at 7 clinical centers who will determine the feasibility of different interventions in adults and children to preserve ß-cell function in individuals with prediabetes or mild, recently diagnosed type 2 diabetes. The Consortium involves three protocols:

- Three adult centers will perform a randomized, blinded, placebo-controlled trial comparing metformin, insulin glargine followed by metformin, or metformin plus liraglutide.
- Four pediatric centers will perform a randomized trial comparing metformin with metformin plus liraglutide.
- One adult center will perform a randomized trial comparing metformin with gastric band surgery.

To the greatest degree possible, the three protocols will use the same inclusion and exclusion criteria, study procedures, time line and outcome measures to allow for comparisons between groups and interventions. The protocol for the single adult center comparing metformin to gastric banding is presented here and is called the BetaFat Study

1.2 Specific Aims and Objectives

Type 2 diabetes (T2D) develops from progressive loss of pancreatic β-cell compensation for chronic insulin resistance. The loss of compensation occurs for years prior to the development of diabetes and continues after the diagnosis is made. Loss of compensation is caused by loss of β-cell mass and reduced function of individual β-cells. Several lines of evidence suggest that obesity and its adverse metabolic effects are primary drivers of falling β-cell compensation. First, obesity is a well-known risk factor for T2D. Second, weight gain is an important predictor of falling β-cell compensation, an effect explained in our prior studies by insulin resistance and separate effects of low-grade inflammation and falling adiponectin levels. Third, treatment of obesity with lifestyle modification reduces the risk of diabetes in a pattern that suggests slowing, but not arrest of the β-cell disease. Protection is limited by weight re-gain, even in the relatively controlled setting of clinical trials. Fourth, treatment with thiazolidindiones, which mitigate some of the metabolic effects of obesity, can slow or even stop the loss of β-cell compensation. The protective effect is closely related to “unloading” of β-cells through amelioration of insulin resistance; it occurs only in a subset of treated individuals. These four findings point to mitigation of excess adiposity as an important approach to arrest or reverse the β-cell disease that causes T2D. The findings also highlight important limitations in current behavioral and pharmacological approaches to β-cell preservation.

The present proposal seeks to expand clinical and scientific knowledge about β-cell preservation or restoration through mitigation of the metabolic effects of obesity. Specifically, we propose to compare weight loss induced by gastric banding to the standard pharmacological approach, metformin, for effects on β-cell function in pre- and mild T2D. Banding will reduce

adverse effects of obesity through weight loss that is more than can be sustained with lifestyle modification and which occurs without the potential confounding effect of direct stimulation of insulin secretion seen with operations that bypass the foregut. Metformin treatment will reduce insulin resistance and lower glucose levels. By comparing changes in β-cell function between treatment groups, we will compare the most commonly used medical monotherapy to a surgical approach for long-term effects on β-cell health. By examining changes in potential mediators of fat-induced β-cell deterioration, we may identify mechanisms for β-cell preservation that could serve as future therapeutic targets or markers of response to treatment. Based on data from weight loss and metformin in extant diabetes prevention trials and the greater weight loss expected with banding, **we hypothesize that gastric banding will be more effective than metformin treatment at preserving or restoring β-cell function.** We propose three specific aims to test this hypothesis and examine potential mechanisms for β-cell preservation or restoration.

AIM 1 – Clinical Study: We will conduct a prospective, randomized, 24-month study in obese individuals with pre-diabetes or mild T2D using two different approaches to mitigate the metabolic effects of obesity: (a) gastric banding and (b) metformin.

AIM 2 – Impact on β-cell Health: We will compare changes in markers of β-cell health, primarily β-cell compensation for insulin resistance, between groups. We predict that banding will improve β-cell health more than metformin will.

AIM 3 – Mechanisms Linking Fat to β-cell Health: We will measure body fat (total and regional by DEXA, abdominal distribution by MRI), along with potential circulating mediators of β-cell decline and use regression methods to identify mechanisms by which obesity may influence β-cell health and identify clinical markers thereof.

1.3 Overall Design and Study Interventions

BetaFat is a two arm, unblinded study in which volunteers with prediabetes or drug-naïve, recent onset, mild type 2 diabetes will be randomized to: (1) metformin or (2) gastric banding. The study will recruit patients over a 27-month period and follow each individual for a total of 24 months on study intervention. The primary outcomes will be β-cell compensation for insulin resistance, measured in two ways as defined below. The anticipated duration of the BetaFat study is five years, including pre-trial preparation and post-trial analysis and reporting.

ß-cell function, α-cell function, insulin sensitivity and glucose tolerance will be assessed using intravenous and oral tests. Both intravenous and oral tests will be obtained at baseline and after 12 and 24 months of therapy. Use of oral and intravenous tests will allow us to examine different aspects of β-cell function. Such use will also allow us to examine how well relatively simple measures of ß-cell function, α-cell function and insulin sensitivity (i.e., from fasting samples and oral glucose tolerance tests) correlate to more sophisticated measures (i.e., from glucose clamp studies) in the prediction of β-cell decline and/or response to treatment. Finally, a series of adipokines/inflammatory markers will be measured to determine whether they predict changes in ß- and α-cell function, insulin sensitivity and glucose tolerance without and with therapy.

Clinically, the project will serve as a test of concept for use of gastric banding relatively early in the spectrum of obesity and β-cell disease. We will derive information on the extent of weight loss necessary to fully arrest or reverse the β-cell disease, as well as potential clinical markers for successful treatment. Biologically, the results will provide crucial information on potential mediators of β-cell failure and its arrest or reversal in the context of obesity. Those mediators will guide the development of more effective strategies for prevention and early treatment of T2D with the aim of arresting or reversing the progressive β-cell disease.

2 BACKGROUND and SIGNIFIGANCE

2.1. Type 2 Diabetes: The Problem and its Importance

Type 2 diabetes is epidemic in developed and developing countries, where the disease affects approximately 5-10% of adults. Approximately twice as many people have impaired glucose levels and are at high risk for T2D. Prevalence and incidence rates of T2D and impaired glucose levels are on the rise, shadowing increasing rates of obesity. Projections place the number of people with diabetes in excess of 400 million by 2030 if nothing is done to stem these rising tides. Morbidity and mortality from acute and, especially chronic complications of T2D will follow. *Thus, development of effective strategies to prevent T2D and/or treat it once it develops is an extremely important public health issue across the globe*.

2.2 Disease Modification: Focus on Detrimental Effects of Obesity

The biology of T2D is characterized by progressive loss of pancreatic β-cell compensation for chronic insulin resistance (1-3). The progressive β-cell disease is present for years during the development of diabetes and it continues for many years after the diagnosis is made. The clinical face of this progressive disease is hyperglycemia that becomes harder and harder to treat. Many patients end up with severe insulin deficiency which, with their insulin resistance, makes it difficult or impossible to maintain low risk glucose levels. *Arrest or reversal of this progressive β-cell disease is fundamental to effective prevention and treatment of T2D*.

Toxicity from glucose and fatty acids (4), protein malfolding (5-7), and oxidative stress (8) are some processes that have been implicated in the loss of β-cell mass and function. In our own longitudinal studies of Hispanic women at high risk for T2D, declining β-cell compensation was most closely associated with weight gain (9). The association was explained statistically by covariate adjustment for three independent factors: increasing insulin resistance, rising levels of C-reactive protein and falling levels of adiponectin. *These findings suggest that obesity drives progressive β-cell decompensation both indirectly (through insulin resistance and β-cell loading) and directly (through circulating adipocytokines acting on β-cells).*

Based on these observations, we believe that mitigating the detrimental effects of obesity on β-cells is the most attractive strategy for arresting or reversing the progressive β-cell disease that causes and worsens T2D.

Theoretically, mitigation could be accomplished by reducing body fat or by ameliorating its adverse effects on β-cells. Clinical studies of prevention and early treatment of T2D support both approaches. Lifestyle modifications in the Finnish Diabetes Prevention Study (DPS) (10)

and the U.S. Diabetes Prevention Program (DPP) (11) achieved relatively modest weight loss that was partially sustained during 4-6 years of follow-up. The result was a 58% reduction in the diabetes incidence rate in each study. Protection from diabetes was more closely associated with weight loss than with increased physical activity (11). On average, the onset of diabetes was delayed ~2 years during the active treatment phase of the DPP (Fig 2 of REF (11)). It is not clear how many subjects actually stopped progressing toward diabetes, but clearly many did not. *Thus, modest weight loss may slow, but not arrest deterioration to T2D.*

We used thiazolidinediones to modify fat biology in the absence of weight loss. Subjects were Hispanic women with recent gestational diabetes. We observed a 55% reduction in diabetes rates over ~3.5 years (TRIPOD) (12). Importantly, β-cell compensation was fully stabilized in ~1/3 of the treated women. Protection from diabetes was very closely associated with β-cell "unloading", the term we have used to describe reductions in insulin secretory demands that occurred in response to insulin sensitization. The relationship between unloading and protection from diabetes was confirmed in a second study (PIOOD) (13). At least two other studies have demonstrated diabetes risk reductions of 62-72% with TZDs (14;15).

These studies demonstrate **three concepts that are fundamental to the present proposal.** First, reducing body fat or reducing its adverse metabolic effects in moderately obese individuals (average BMI was 30-35 in all studies) can slow rates of deterioration toward T2D. Second, the β-cell disease that causes T2D can be stopped through sufficient β-cell "unloading" or, possibly, related mechanisms. Third, neither modest weight loss nor TZD treatment is fully and uniformly effective in arresting progression toward and to T2D. There is great need to develop more potent approaches to β-cell preservation or restoration in pre- and mild T2D. The present proposal is designed as a test of concept for one such approach – weight loss induced by gastric banding – using metformin treatment as a comparator.

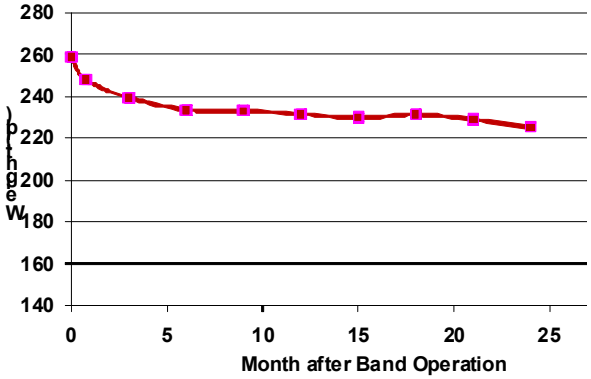
2.3 Rationale for Interventions

We considered a number of approaches. We rejected approaches based on stimulation of insulin secretion because our own data (12;13) and data from ADOPT (16) support the opposite approach. GLP-1 based therapies could have direct effects to enhance β-cell mass based on rodent studies (17), but available human data don't support such an effect in humans (18) . The limits of lifestyle interventions are apparent from the DPP and DPS (10;11). Thiazolidinediones (TZDs) have a strong effect to preserve β-cell function in pre-diabetes (12;13), but the one available TZD, pioglitazone, has recently been linked to an increase in bladder cancer (19). Finally, basal insulin treatment could reduce insulin secretory demands, but had only a small effect on diabetes risk reduction in the ORIGIN trial (20). Thus, we concluded that we could either take an approach that would combine two or more of these approaches in a mechanistically logical fashion, or we could take a bolder approach directed at what we believe to be a primary drive of β-cell decline – adverse metabolic effects of obesity. We chose the latter option and believe we can make important contributions to studies that will operate under the RISE Consortium.

We chose gastric banding as the primary experimental treatment because (a) it induces 2-3 times the weight loss that can be achieved and sustained with even aggressive lifestyle

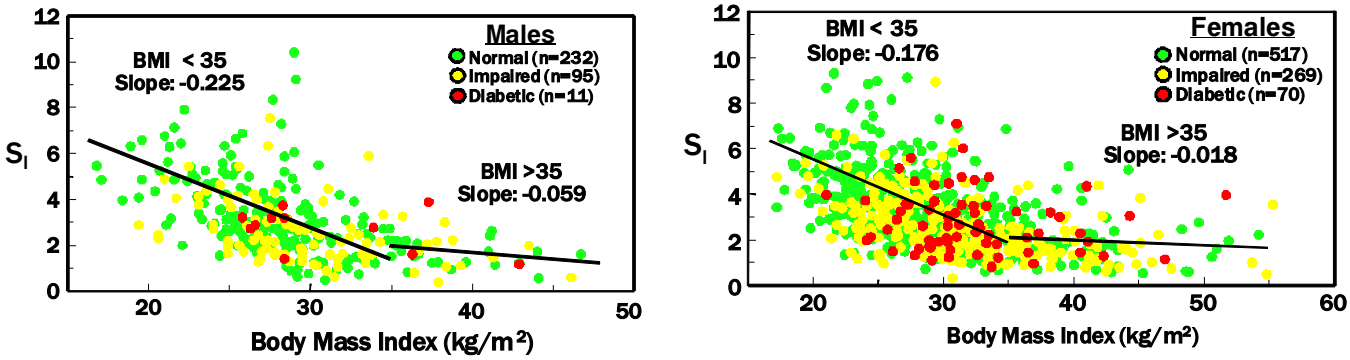
programs like the DPP ((21) and Figure 2.1); (b) It can reverse T2D (21); (c) it is not associated with the large stimulation of insulin secretion that appears to occur with foregut exclusion operations (22); (d) it is relatively non-invasive and safe compared to other bariatric procedures; and (e) the LapBand has recently been approved by the FDA for clinical use down to a BMI of 30 kg/m^2. The trend toward lower BMI thresholds for gastric banding provides both an opportunity and a need to study carefully the metabolic impact of the procedure in the presence of moderate obesity. We will apply banding to people with moderate obesity to maximize improvement in insulin sensitivity for any degree of weight loss (Figure 2.2).

Figure 2.1: Weight Loss in USC Gastric Band Program



Data are from 83 obese individuals with T2D who had follow-up for at least two years. Results were achieved without the intensive approach to band adjustment that we propose for this project.

Figure 2.2: Relationships between BMI and Insulin Sensitivity



Data are from 1221adults (mean age 35 years, mean BMI 29.6 kg/m^2). Insulin sensitivity (S_I) was measured with IVGTT-minimal model.

We chose metformin as the comparator for two main reasons. First, we will include people with mild T2D; standards of care dictate that they should receive medical therapy and metformin is the drug most often recommended in this context (23). Metformin is also the only drug that has been recommended for use in diabetes prevention (24). Thus, we will be comparing gastric banding to the current standard of medical monotherapy early in the course of T2D.

We will study people with pre- and mild T2D because we have observed a fairly linear decline in β-cell compensation across this range (9). We are also confident that we can assess insulin sensitivity and secretion across this range. Thus, we will be able to test for arrest or reversal of falling β-cell compensation. Overall, this approach and our expertise β-cell biology and preservation in humans put us in very strong position to make unique and important contributions to the RISE Consortium.

2.4 Rationale for Outcome Measures

We plan to employ technically complex "gold standard" measures for a high degree of scientific rigor in this relatively small study, together with simpler surrogate measures that could be applied in larger studies and in clinical settings. The complex measures will be made with intravenous stimuli (glucose, arginine). The simpler measures will be made in the fasting state (HOMA, biomarkers) and in response to oral glucose.

2.4.1 Intravenous-Based Tests of ß- and α-cell Function and Insulin Sensitivity

Glucose tolerance is dependent on ß-cell function and insulin sensitivity, and both of these can be quantified using the hyperglycemic clamp (25). Although α-cell function dysregulation occurs in type 2 diabetes, the exact role of α-cell function in determining glucose tolerance is not well defined.

The acute insulin response to intravenous glucose (AIRg), also known as the first-phase insulin response, occurs within the first ten minutes following glucose administration and is a sensitive marker of ß-cell function that decreases as the fasting glucose level rises. A potential limitation of this measure is that it becomes zero or negative when fasting glucose approaches the diabetic range (26). Since we will include people in this range, the AIRg is not the ideal primary outcome.

The second-phase insulin response to intravenous glucose begins at the time of glucose administration and continues for as long as the glucose level remains elevated. It is frequently quantified as part of a hyperglycemic clamp. Unlike AIRg, this particular response generally persists even when the fasting glucose concentration is in the diabetic range.

The prevailing glucose level modulates the ß-cell secretory response to non-glucose secretagogues such as peptides and amino acids. By performing hyperglycemic clamps with the addition of arginine, it is possible to quantify the maximum insulin response (AIRmax), which has been shown to correlate with β-cell mass in animal studies (27).

Normally, ß-cell responses are modulated by the prevailing insulin sensitivity. Thus, adjusting the proposed β-cell measures for the degree of insulin sensitivity (S_I) provides a measure of compensation for insulin resistance, a measure of β-cell function that declines consistently

during progression to and of T2D (3;28). The most widely used approach is calculation of a disposition index, a product of measures of insulin sensitivity and secretion based on demonstration of a hyperbolic relation between the two variables. AIRg (29;30), second phase insulin (31), and AIRmax (32;33) have been shown to have such relationships. Insulin sensitivity can be calculated from the hyperglycemic clamp as the ratio of steady state glucose infusion rates to steady state plasma insulin levels. We plan to examine relationships between β-cell measures and insulin sensitivity from the hyperglycemic clamp and use the disposition index or another, more appropriate method to adjust beta cell function for insulin sensitivity in primary and secondary analyses.

The reciprocal of glucose's ability to potentiate the ß-cell response is its ability to suppress the α-cell response. This function can also be measured using hyperglycemic clamps along with arginine. The minimum acute glucagon response at maximal glycemic suppression (AGRmin) is greater in people with type 2 diabetes, indicative of impaired α-cell regulation (30).

2.4.2 Oral-Based Tests of ß- and α-cell Function and Insulin Sensitivity

The oral glucose tolerance test (OGTT) also provides measures of islet function and has been used in large clinical trials. The early insulin response (insulinogenic index) is an important determinant of glucose tolerance (14). This response, when used in combination with a fasting measure of insulin sensitivity (1/fasting insulin, HOMA), provides another estimate of ß-cell function termed the oral disposition index (DIo) (20). Using modeling approaches such as those developed by Mari et al (63) and Cobelli et al (ref), it is possible to obtain a number of additional parameters of ß-cell function and insulin sensitivity. In addition, insulin secretion rates can be calculated from the OGTT (64). Finally, overall glucose tolerance can be calculated from the glucose excursion during the test (57).

Physiological glucagon suppression during an OGTT is an important determinant of glucose tolerance and provides an estimate of α-cell function. In individuals with type 2 diabetes, this response is increased compared to normal subjects (2; 26; 27).

These OGTT-based measures will be made in the study and will be used to determine their relationship to assessments made using intravenous tests both at baseline and with intervention. OGTT-derived measures can be evaluated for cross-sectional relationships with clamp-derived measures and for value as prospective predictors of therapeutic response.

2.4.3 Biomarkers

The detailed measures of β-cell function that we will be making provide an opportunity to identify biomarkers that can predict deterioration of β-cell function and/or response to

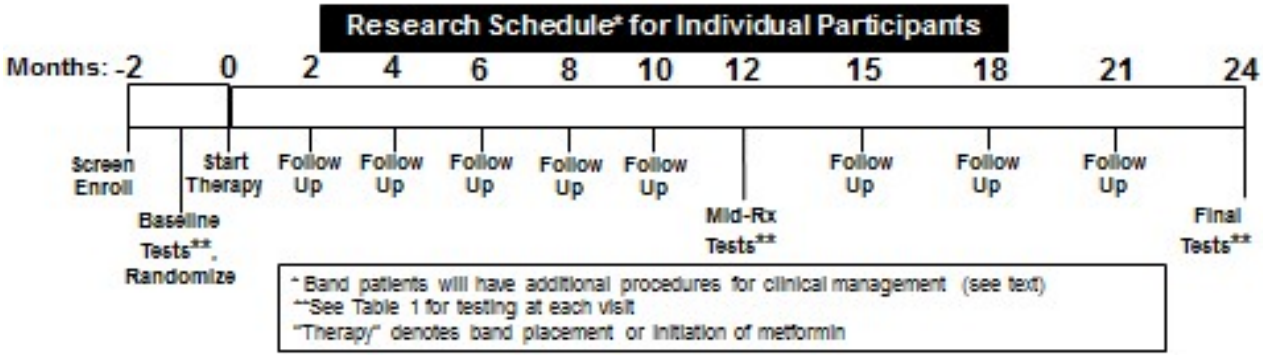
interventions that can mitigate such deterioration. The identification of such biomarkers is an important ancillary goal of the RISE Consortium. Because of budget constraints, the focus during this stage of the project will be on collecting patient specimens that can used in the future for identification of biomarkers once the main outcomes of the study are known. We will collect samples that can be used to examine previously described peptide markers of diabetes risk (e.g., adiponectin, ferritin, ApoB, C-reactive protein, IL-2 receptor A (34-37) and evolving markers of β-cell mass regulation (e.g., prolactin, DNA fragments and methylation, GLP-1, GIP, HSP90nucleic acids (38-42). We will also collect serum samples that can be analyzed using genomic, micro-RNA, proteomic and metabolomics approaches comparing for example responders to non-responders to the study interventions.

3 STUDY DESIGN

3.1 Overview and Rationale

The BetaFat study protocol described herein will involve 88 adults who are 20 – 65 years old and who have prediabetes or drug-naïve, recent onset and mild type 2 diabetes. Additional inclusion and exclusion criteria are defined below. Participants will be randomized over a 27 month period to either metformin or gastric banding in an open treatment format. The primary outcome will be rate of change in ß-cell compensation for insulin resistance, as defined below. Figure 3 provides an overview of the types and timing of main study procedures.

Figure 3



3.2 Primary Endpoints

The primary objective of the RISE Study is to evaluate the effects of randomized treatment on ß-cell function, measured by hyperglycemic clamp techniques. Given that insulin and C-peptide are both used in quantifying ß-cell function, each of them will be measured in certain or all tests in part to avoid the problem of collinearity when performing the statistical analyses. Two co-primary endpoints are specified:

- Change between baseline and end study in ß-cell function measured as the **second phase response (steady-state of the hyperglycemic clamp;** 100-120 minutes of glucose infusion). This will be calculated as mean C-peptide at steady state, adjusted for insulin sensitivity (ratio of the glucose infusion rate:plasma insulin concentration at steady state).

- Change between baseline and end study in ß-cell function measured as **AIRmax (arginine-induced acute insulin response at hyperglycemia glucose; ß-cell secretory capacity)** [30]. This will be calculated as the incremental area under the C-peptide curve from 0-10 minutes following arginine injection at a plasma glucose ≥450 mg/dl, adjusted for insulin sensitivity measured by the hyperglycemic clamp.

3.3 Secondary Endpoints

The following secondary endpoints are pre-specified for analysis.

3.3.1 Hyperglycemic clamp-derived ß-cell measures

a. First phase insulin response (incremental insulin area from 0-10 minutes after initial glucose bolus)
b. First phase C-peptide response (incremental C-peptide area from 0-10 minutes after initial glucose bolus)
c. Disposition index derived from a and b, using hyperglycemic clamp-derived measure of insulin sensitivity (glucose disposal rate/insulin: GDR/I)
d. Second phase insulin response, with adjustment for insulin sensitivity
e. AIRmax response to arginine calculated using insulin concentrations and adjusted for insulin sensitivity

3.3.2 OGTT-derived ß-cell measures

a. Early insulin response to oral glucose (insulinogenic index: Δ insulin from 0-30 minutes/Δ glucose from 0-30 minutes [ΔI/ΔG])
b. Early C-peptide response to oral glucose (analogous to (a))
c. Oral disposition index (DIo; insulinogenic index adjusted for insulin sensitivity)
d. Modeled parameters of ß-cell function (ß-cell glucose sensitivity, rate sensitivity and potentiation factor; static, dynamic, and total ß-cell glucose sensitivity)
e. Ratios of incremental insulin/glucose (iAUCins/iAUCg) and C-peptide/glucose (iAUCc-p/iAUCg) responses from 0-120 minutes
f. Incremental glucose (iAUCg) response as a measure of glucose tolerance
g. 2-hour glucose

h. GLP-1 total and incremental response to oral glucose load (samples will be collected, and measured for this analysis if funding allows)

3.3.3 Fasting measures of glycemia and β-cell function

a. Glucose
b. Proinsulin/insulin ratio
c. HOMA %B (using the Oxford calculator)
d. HbA1c

3.3.4 α-cell Function

a. Glucagon suppression in response to sustained hyperglycemia during the clamp (the decrement in glucagon concentration from baseline to nadir value at 100-120 minutes)
b. Acute glucagon response arginine (the incremental increase in glucagon concentration from pre-arginine baseline for the 10 minutes after the arginine injection)
c. OGTT-derived measures: integrated glucagon response from 0-120 minutes
d. Fasting sample-derived measures: glucagon

3.3.5 Insulin Sensitivity

a. glucose disposal rate at steady state (100-120 minutes) divided by ambient insulin concentrations during hyperglycemic clamp
b. Matsuda index and model-derived insulin sensitivity from OGTTs
c. 1/fasting insulin, HOMA-S (using the Oxford calculator), and QUICKI from fasting samples

3.3.6 Blood and Urine Biomarkers

a. Adipokines/inflammatory markers including C-reactive protein (CRP): adiponectin; others contingent on funding (see above in Section 2.4.3)
b. Non-esterified fatty acids
c. Urine albumin/creatinine ratio
d. DNA for future genetic analysis

3.3.7 Physical and Body Composition Measures

a. Physical measures: blood pressure, body weight, body mass index, waist circumference;
b. Total fat and lean mass; regional fat and lean mass by dual-energy X-ray absorptiometry (DEXA)
c. Visceral, subcutaneous, hepatic and pancreatic fat by abdominal magnetic resonance imaging (MRI)

3.4 Treatments

3.4.1 Metformin Arm

Metformin will be given at a dose of 2000 mg/d, titrated over four weeks. Study medication will be withheld the morning of each CTU visit and a new supply will be dispensed at the end of each visit. Medication compliance will be monitored by pill counts. Participants will be monitored for gastrointestinal side effects that are associated with metformin. Individuals who develop side effects will be given the option of reducing the dose. Individuals who continue to have intolerable side effects will be given the options to change to an extended release preparation at maximum tolerated dose or to discontinue metformin. Handling drop-outs and missing data is discussed under Data Analysis (section 9.6)

3.4.2 Gastric Band Arm

The LapBand will be placed and participants will be followed according to standard clinical practice under the supervision of Dr. Namir Katkhouda. Costs for these procedures will be paid by Allergan in the form of an unrestricted research grant. Pre-operative evaluation will include a psychological evaluation, a cardiac evaluation with EKG and stress testing, evaluation by an anesthesiologist, a chest X-ray and clinical lab testing (CBC, chemistries, UA, PT/PTT, hepatitis panel and, for women, pregnancy test). Participants also meet with the bariatric nutritionist for education on dietary restrictions that will apply once the band is in place.

Surgeries for placement of LapBands will be performed by Dr. Namir Katkhouda at the Keck Hospital of USC. The procedure is done laparoscopically on outpatients. Patients see Dr. Katkhouda 2 weeks after surgery for wound check and suture removal. Band adjustments are performed by Dr. Katkhouda, members of his bariatric surgical team, and/or Dr. Elizabeth Beale. Adjustments will commence ~4 weeks after surgery and will recur every 1-3 months at the time of regular research visits. Adjustments will be made based on specific criteria consisting of symptoms of satiety and hunger, dietary history, and weight loss. Patients will revert to liquid diet for 24-48 hours after band adjustments and advance diet over the next 3-5 days. The bariatric team has an on-call system to evaluate and treat patients who have symptoms related to their bands. Post-operative laboratory assessments include CBC; chemistries; vitamin B1, B6 and B12 levels; ferritin; and 25(OH) vitamin D (see Table 2 for testing schedule).

Although both groups will be on active treatment, it is possible that glycemic control could deteriorate to a point of needing additional therapies. We will be monitoring HbA1C at six month intervals. If a value is >8.0%, we will repeat it in one month and, if it remains >8.0% we

will consider the subject to have reached a safety endpoint for the trial. We will perform end-of study visits (scheduled for 24 months in Table 3) and refer the subject for care of his/her diabetes.

3.4.3 FDA Compliance

Participants will have BMI 30-40 kg/m^2 and either type 2 diabetes or impaired glucose levels. Metformin is approved by the FDA for use in people with type 2 diabetes regardless of BMI. The LapBand is approved by the FDA for use in individuals with BMI>30 kg/m2 and type 2 diabetes. An Investigational Device Exemption has been obtained from the FDA for inclusion of individuals with pre-diabetes.

3.5 Inclusion and Exclusion Criteria

3.5.1 Inclusion Criteria

1. Completion of at least two months in a diet, exercise and behavioral modification program within past two years
2. Fasting plasma glucose >90 mg/dl *plus* 2-hour glucose ≥140 mg/dl on 75 gm OGTT *plus* HbA1C ≤7.0%. There is no lower limit for the A1C and no upper limit for the OGTT 2-hour glucose based on prior studies that allow us to identify people with falling β-cell function.
3. Age 22-65 years
4. Body mass index (BMI) 30-40 kg/m^2
5. For participants with diabetes, known duration <1 year
6. No history of use of antidiabetic medications except during pregnancy

3.5.2 Exclusion criteria

1. Contraindications to LapBand(see Appendix 1)
2. Contraindication to MRI (claustrophobia; permanent metal objects such as pacemakers, prostheses, aneurysm clips)
3. Underlying disease(s) likely to (a) limit life span to less than study duration and/or (b) increase risk of intervention outside of the study and/or (c) limit ability to participate in outcomes assessment and/or (d) limit participation
4. An underlying disease known to have important effects on glucose metabolism
5. Active infections

6. Renal disease (serum creatinine ≥1.4 mg/dl for men; ≥1.3 mg/dl for women) or serum potassium abnormality (<3.4 or >5.5 mmol/l)
7. Anemia (hemoglobin <11g/dl in women, <12 g/dl in men) or known coagulopathy
8. Cardiovascular disease, including uncontrolled hypertension and symptomatic congestive heart failure. Participants must be able to safely tolerate administration of fluid/volume challenges during clamp studies.
9. Serum AST or ALT >3 times upper limit of normal in local clinical lab
10. Excessive alcohol intake
11. Suboptimally treated thyroid disease
12. Conditions or behaviors likely to affect the conduct of the BetaFat Study
    a. Unable or unwilling to give informed consent
    b. Unable to adequately communicate with clinic staff
    c. Another household member is a participant or staff member in BetaFat
    d. Current or anticipated participation in another intervention research project that would interfere with any of the interventions/outcomes in BetaFat
    e. Likely to move away from participating clinic in next 2 years
    f. Current (or anticipated) pregnancy and lactation.
    g. Major psychiatric disorder that, in the opinion of clinic staff, would impede the conduct of the BetaFat study
    h. Weight loss >7% in past two months for any reason except postpartum weight loss.
13. Additional conditions may serve as criteria for exclusion at the discretion of the local site.

Further details pertaining to exclusion criteria are specified in the Manual of Procedures.

4. RECRUITMENT AND RANDOMIZATION

4.1 Sources of Participants

There are two primary recruitment sources where we can access existing information on age, BMI and A1C or glucose levels to greatly enhance efficiency of screening. The first source is the clinical care database (EHR) of Kaiser Permanente Southern California (KPSC – Dr. Xiang). The second source is the Ambulatory Care Network (ACN) of Los Angeles County (Drs. Beale and Buchanan). These two sources provide access to thousands of individuals likely to meet recruitment criteria.

4.2 Recruitment

The primary recruitment technique is prescreening of electronic and clinical medical records to identify patients who appear to meet the age, BMI, A1C and medication criteria for the study to limit screen failure rates. In particular, for recruitment from the Ambulatory Care Network of Los Angeles County DHS, a search will be made of the LAC+USC Healthcare Network Affinity database by authorized query writers to identify patients who meet criteria for the BetaFat study as well as necessary contact information. On approval of this study by the USC HS IRB this list will be released to the BetaFat study team for storage on the secure, password protected study server. Although study data will be recorded on REDCap, the list of recruits from Affinity will not be uploaded to REDCap. Back-up recruitment techniques may include notices placed on bulletin boards at the medical centers, newspaper and radio advertisements and public service announcements, and referral from colleagues. Selection is based solely on the participant's ability to meet the criteria stated in the protocol and his/her willingness to participate in the study.

4.3 Informed Consent

Consent is obtained in a quiet setting prior to the initiation of any study procedures. The subject is provided adequate time to read and understand the written consent and ask questions as necessary. If they choose, they may take additional time to discuss the study with family and/or physicians outside the clinic setting. Subjects are not consented until they demonstrate adequate understanding of all aspects of the study and consent process. A copy of the consent is given to the subject. A model consent form appears in Appendix 2.

In the event a significant protocol change occurs, the informed consent will be adjusted appropriately and sites will submit the revised documents to their Institutional Review Board (IRB) for approval. Local IRBs will determine whether it is necessary to re-consent participants.

The clinical site for BetaFat will submit to the BetaFat Data Center stamped IRB approval letters and current copies of all consent forms prior to study initiation, and annually thereafter. These records will be maintained by the BetaFat Data Center as a local archive.

4.4 Screening

Screening will take place on the Clinical Trials Unit (CTU) at USC. Individuals will have a history and physical exam directed at study inclusion/exclusion criteria, will provide blood for clinical laboratory screening, and will have plasma glucose measured before and two hours after 75g oral glucose challenge. Qualified individuals will be asked to return for baseline testing and randomization.

4.5 Randomization

Individuals who complete baseline testing (described below, section 6.2) will be randomized 1:1 to banding or metformin using permuted block randomization stratified by gender, BMI (30-35 vs. 35-40 kg/m^2) and diabetes (no vs. yes when IDE approved) to minimize skewing. Individuals assigned to metformin will be given study medications and instructions at the end of the second baseline visit, to begin treatment the following day. Individuals assigned to gastric banding will be scheduled for pre-operative screening with the goal of having them undergo operation within two weeks.

4.6 Masking

No masking will be employed. The study team and each individual participant will be aware of his/her treatment allocation.

5. PARTICIPANT MANAGEMENT

5.1 Overview

Study treatment interventions are described above (Section 3.4). They will be provided free of charge to BetaFat participants. HbA1c will be monitored every 6 months. Procedures to be followed for rising HbA1c levels are described in Section 7.4.4. Beyond the treatment of the glucose-aspect of pre-diabetes and diabetes care, the research study will not assume responsibility for the medical care of the participant. Non-glucose aspects of diabetes care (e.g., blood pressure, lipids) and non-diabetes related medical problems will be referred to the participant's primary care provider. When a potential treatment could affect the study's outcome(s), the study's physician will discuss the choices with the participant's primary care provider. When a potential treatment could affect the study's outcome(s), the RISE physician may discuss the choices with the participant's primary care provider. RISE Study labs will be shared with the provider, with the participant's permission. For those participants who do not have a primary care provider, the study staff will help find appropriate medical care.

5.2 Drug Distribution

Metformin will be obtained from the same distributor used by other studies in the RISE consortium. The local research pharmacy of the USC Clinical Research Organization (CRO) will organize and maintain inventory. *S*ufficient medication is kept on hand to allow medication distribution for the next 3 month period. This would include medications required for the next 12 randomized enrollees (i.e., on average 3 initial distributions for each of the 4 treatment arms) plus all of the upcoming distributions to enrolled participants who will be returning within 3 months. Expiration dates of all medications will be double checked and documented

on arrival at the CRO pharmacy and at the time of distribution to participants to ensure that the provided supplies will not expire during the current treatment interval.

5.3 Adherence Measures

Adherence is documented, monitored, and addressed as part of participant care and management and to monitor whether procedures are being implemented and followed. Adherence refers to all study procedures – not only taking the prescribed amount of study drug, but also attending scheduled study visits and completing procedures at those visits. The goal is to maintain high levels of adherence in all participants. Primary adherence measures will address visit attendance and completion of study procedures and medication adherence. Participants will be instructed to bring all study medication (including empty bottles) to study visits for assessment of medication compliance.

5.4 Retention

Retention refers to efforts to prevent participant dropout or withdrawal from the study. It is critically important to successfully engage and retain participation over the course of the trial. For purposes of sample size estimation, investigators have predicted an annual withdrawal rate of 10% during the 24-month treatment periods and have adjusted sample size to meet study requirements. However, lower rates of attrition are desirable.

The Study Coordinator at each site is primarily responsible for monitoring participant attrition and initiating team conferences for retention strategies or dropout recovery rates. Subjects missing scheduled visits will be contacted within 24 hours of the scheduled visit to reschedule and discuss strategies to improve compliance. If the subject can't be contacted, a certified letter will be sent to encourage continuation in the trial even if they no longer wish to take the study medication. Study staff may implement additional efforts to personalize the study.

Attrition is monitored regularly by the Data Center and the RISE Consortium Recruitment and Retention Committee. An attempt is made to collect data on the reason for leaving the study in the case of a participant who withdraws. Studies in the RISE Consortium are encouraged to share their ideas and experiences via regular communication and conference calls for Study Coordinators. The BetaFat Data Center will prepare monthly reports on visit completion, compliance with the BetaFat Study protocol and participants on inactive follow-up.

Potential participants will be provided introductory material that will emphasize the importance of completing the study regardless of treatment assignment. We will emphasize this importance during the screening, enrollment and baseline testing procedures. Should dropout rates exceed the projected 10% per year, we will explore several options: (a) we may

intensify messaging about the importance of participation and identify reasons for drop-outs, targeting them for retention efforts; (b) we may increase overall enrollment if the difference between real and projected drop-outs is small (budgets limit large increases in enrollment); (c) we will examine projected completion in relation to power, noting that our current 80% power threshold is below the projected effect size, so that we may be able to tolerate some excess loss to follow-up; (d) if dropouts are occurring after 12 months, we may consider changing the primary outcomes to rates of change in the two primary beta cell measures, as we have used in prior observational studies with different durations of follow-up. Note that we plan to assess main outcomes at 12 and 24 months on treatment, allowing this approach.

6 DATA COLLECTION

6.1 Pre-screening and Screening Eligibility Measurements

Prescreening: Prescreening for potential eligibility is done using clinical databases at Kaiser Permanente Southern California and the Ambulatory Care Network of Los Angeles County. Participants are eligible to continue screening procedures if they have an A1C within six months that was 5.8-7.0% and are 20-65 years old with BMI 30-40 kg/m2. For Kaiser, letters are sent to potential participants describing the purpose of the study and providing individuals the opportunity to opt in to be contacted. For the ACN, potential participants are contacted directly. In both cases, a study recruiter interviews potential subjects by phone to determine potential eligibility and interest in the study.

Screening: Participants who meet pre-screening eligibility criteria are invited to the USC Clinical Trials Unit to provide informed consent and complete screening, including a directed history and physical exam, clinical laboratory tests, and a 2-hr, 75 g OGTT.

Table 6.1: Data Collection during Screening

STEP	TEST / PROCEDURE	COMMENTS
1) Pre-screening	HbA1C, BMI (EHR), age, ICD-9 codes	Contact if A1C 5.8-7.0%, age 20-65, BMI 30-40 kg/m^2
	Phone Screening questionnaire	Initial review of eligibility
2) Screening	fasting and 2-hr glucose (75 gram OGTT; measured at central lab), A1C	Eligibility: fasting glucose >90 mg/dl; 2-h glucose ≥140 mg/dl, AIC <7.0%
	Clinical eligibility labs (local clinical lab)*	Laboratory eligibility criteria
	Targeted history and physical exam, Beck Depression Inventory	Eligibility evaluation
* potassium, TSH, creatinine, ALT, AST, , hemoglobin, urine pregnancy test for women		

6.2 Main Study Procedures

The timing of major study procedures is illustrated in the Figure 3, Section 3 above. Data to determine main study outcomes using hyperglycemic clamp procedures will be obtained at baseline and study end, which will be defined as either the end of 24 months after initiation of metformin or placement of gastric band, or at the time of treatment failure (A1C >8.0% on two successive visits). Data for secondary analyses will be determined at other study visits. Blood and urine samples will be collected for planned measures and extra blood and urine samples will be stored for future analyses. Further details on these measures are provided below, and are summarized in Table 6.2.

Table 6.2: Testing Schedule

	Baseline	2-mo	4-mo	6-mo	8-mo	10-mo	12-mo	15-mo	18-mo	21-mo	24 mo
Exam	X	X	X	X	X	X	X	X	X	X	X
OGTT	X						X				X
Hyperglycic Clamp	X						X				X
DEXA	X						X				X
MRI	X						X				X
A1C	X		X	X		X	X		X		X
Fasting/Biomarkers	X			X			X		X		X
Band Questionnaires	X			X			X		X		X
Sleep Questionnaires	X										
Clinical Labs-1	X			X			X		X		X
Clinical Labs-2				X			X		X		X

Fasting/Biomarkers are discussed in section 3.3. Clinical Labs-1 (CBC, UA, chemistries) are for all subjects; Clinical Labs-2 (B vitamins, ferritin, vitamin D) are for band group only (paid by Allergan).

6.2.1 Fasting Blood and Urine Samples (see Table 6.2)

HbA1c will be measured and fasting blood and urine samples will be collected every six months. Aliquots of fasting blood and urine will be stored for subsequent measurement of biomarkers. A blood sample for DNA will be collected at randomization. Routine chemistries will safety will be done at baseline and every 6 months.

6.2.2 Oral Glucose Tolerance Test (OGTT)

Participants will come to the clinical research testing facility in the morning following a 10-12 hour overnight fast. They will drink 75 grams of an oral glucose solution (centrally provided from a single manufacturer) over ≤5 minutes. Blood samples will be obtained at -10, -5, 10, 20, 30, 60, 90, 120, 150 and 180 min relative to glucose ingestion. OGTT procedures will be performed at baseline and 12 and 24 months after initiation of metformin or placement of gastric bands.

6.2.3 Hyperglycemic Clamp

Hyperglycemic clamps (at approximately 200 mg/dl) will be performed after a 10-12 hour overnight fast at baseline and at 12 and 24 months after initiation of metformin or placement of gastric bands. The clamp procedure will incorporate 3 main sets of blood sampling for the planned outcome measurements: first phase (0-10 minutes); second phase (100-120 minutes); and maximal stimulated responses (from 10 minutes before until 10 minutes after arginine injection once plasma glucose has been raised to~450 mg/dl). A priming glucose bolus will be used to stimulate first phase responses, a continuous dextrose infusion will be used to create steady state hyperglycemia at ~200 mg/dl for second phase and then raise glucose to ~450 mg/dl for maximum response to arginine. Arterialized venous blood samples will be obtained at 5-min intervals throughout for measurement of plasma glucose concentration to guide the glucose infusion.

For the OGTT and clamp measures, participants will be asked to refrain from vigorous exercise and tobacco use for 24 hours before the initiation of testing. Study medications will be withheld the morning of testing.

6.2.4 DEXA Scans

Patients lie flat for 10 min while DEXA is performed by a licensed technician using a Hologic Discovery A scanner. Body fat is calculated using software provided by the manufacturer. Results are transferred electronically to the study database to allow analysis of fat content by regions of interest. Bone mineral density will also be determined from the DEXAs

6.2.5 MRI Scans

Approximately 80 5mm axial slices are obtained from the top of the liver to the pelvis using a series of 10-15 second breath holds on USC's research dedicated GE Healthcare 3 Tesla magnet. The procedure takes approximately 30 minutes. Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL) is used to delineate subcutaneous and visceral fat depots and quantify organ fat. SliceOmatic (Tomovision, Inc.) is used for post-image processing to compute subcutaneous and visceral adipose tissue volume and fractional fat content of liver and pancreas.

6.2.6 Physical Measures

Height will be measured with a stadiometer. Weight will be measured with a calibrated electronic scale. Waist and hip circumferences will be measured at the mid-point between the iliac crest and the lowest rib (waist), and at the top of the femur (hip) with the participant standing upright. Body fat and regional fat distribution will be measured by DEXA. Abdominal subcutaneous and visceral fat and hepatic and pancreatic fat will be measured by magnetic resonance imaging (MRI).

6.2.7 Questionnaires

Participants will complete the Pittsburgh Sleep Questionnaire, Epworth Sleepiness Scale and Berlin Questionnaire at baseline for analysis of sleep-related measures as potential predictors of response to treatment. Dietary intake is assessed the Block Food Frequency Questionnaire. Quality of life is assessed using the RAND SF-36. Bariatric Analysis and Reporting Outcomes System (BAROS) is used to assess clinical outcomes related to gastric band patients. Visual Analog Scale is used to assess pain.

6.2.8 Safety Measures

Clinical labs for safety monitoring (local lab), including CBC, serum transaminase levels and creatinine, will be collected at baseline and every 6 months during treatment for all study participants. Additional measurements of vitamins B1, B6, B12; 1.25OH vitamin D; and ferritin will be made every six months during treatment for participants on the LAP-Band arm of the trial. Abnormal laboratory values that, in the estimation of the BetaFat clinician investigators, require medical attention will be provided to participants, who will be advised to follow-up the abnormal value(s) with his or her primary care provider. Additionally, the BetaFat team will communicate the abnormal laboratory value(s) directly to the primary provider. Adverse events (AE) will be surveyed at each visit using a standardized questionnaire. Pregnancy tests (local lab) will be performed in women with child-bearing potential before each DEXA scan and if indicated by symptoms and menstrual history prior to other study procedures.

6.3 Visit Windows

Follow-up visits are scheduled every two months after randomization during the first year and every three months during the second year. Each participant will have a visit calendar generated at the time of randomization and they will be encouraged to complete visits within 1 week before or after the scheduled date. However, it is likely that some visits will not be completed within this window. To maximize the amount of on-trial information available from each participant, we will define visit windows as (a) first year after randomization: from two weeks before to six weeks after a scheduled visit; and (b) second year after randomization: from one month before to two months after a scheduled visit. Visits that do not occur within these windows will be deemed missed. .

7 SAFETY / HUMAN SUBJECTS PROTECTION

This study will be conducted in compliance with the protocol and all applicable regulatory requirements. The participating sites have Federal-wide Assurance with the Office for Human Research Protections and they follow local HIPAA regulations. Prior to study initiation, the

protocol and the informed consent documents will be reviewed and approved by the IRB at each participating site and by an independent Data and Safety Monitoring Board (DSMB). Any amendments to the protocol or consent materials must be approved by the DSMB and the IRBs before implementation.

7.1 Data and Safety Monitoring Board (DSMB)

A DSMB consisting of appropriately qualified and conflict-free independent experts is appointed by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and provides input to the Institute. Board members are chosen by the NIDDK without consultation with the study investigators. The purpose of the Board is to assure independent review as to whether study participants are exposed to any unreasonable risk because of study participation, and to monitor study progress and integrity. In addition to safety monitoring, the Board can review unblinded study data and make recommendations for early termination of studies for achieving an end point or for futility. Before any of the studies in the RISE Consortium can begin, the DSMB, in addition to the local IRBs, must approve the protocol. Any subsequent major changes to the protocol must also be approved by the DSMB and IRBs.

Following each DSMB meeting a summary of AEs and DSMB recommendations is provided to the IRB of each participating clinical center and other institutional monitoring committees/boards as needed.

7.2 Data and Safety Monitoring Plan

7.2.1 Informed Consent

The informed consent process will be conducted by qualified study personnel. All participants must read, sign and date a consent form prior to participation in any stage of the study. The informed consent will be revised and participants re-consented whenever there is new, clinically significant information regarding the safety of the interventions, when a relevant protocol amendment is approved, or when any new information becomes available that may affect an individual's participation in the study.

7.2.2 Safety Review Plan and Monitoring

A. *Justification of Sample Size*: See Section 9.3.

B. *Stopping Rules*: The DSMB may suggest terminating a study arm at any time for safety or efficacy reasons. Details of interim analysis plans and stopping rules can be found in Sections 9.4 and 9.5.

C. *Safety Monitoring Committee*: The RISE Consortium will appoint an internal Safety Monitoring Committee which review AEs or other safety-related problems sent to them from the field. The RISE SMC will review AEs in a blinded manner. An external Safety Review Officer will evaluate any serious adverse events (SAE) where the SMC is not in agreement about whether the event is related to study drug as well as certain specified SAEs of concern (e.g., pancreatitis). The Safety Review Officer will not be blinded to study intervention.

D. *Safety Review Plan*: The DSMB will meet twice yearly to review study progress and safety as described in Section 7.1.

7.2.3 Confidentiality

A. *Protection of Participant Privacy*: Participants' names are linked to study IDs only on a single recruitment database and only certified project personnel are authorized to have access to this database. Only study IDs will be used in all other study databases (e.g., for tracking and merging and sharing with other sites and the NIH). NIH has obtained a certificate of confidentiality for the entire RISE Consortium, including the BetaFat Study

B. *Database Protection*: All hard-copy files are stored in locked cabinets within locked offices at the clinical centers. Primary electronic data are entered into and maintained on a secure USC server with two-level password protection against non-project personnel. The server is backed up daily and automatically. Data for analyses common to other studies in the RISE Consortium will be transmitted to a secure, password-protected website maintained by the RISE Coordinating Center (CoC) at George Washington University. The CoC holds no personal identifiers and stores the data on its enterprise server accessible only by approved staff.

   At the end of the study, all hard copy records will be kept in a secure locale for a period dictated by local IRB and Institutional policies, as well as FDA, regulations, whichever is longest.

C. *Confidentiality during AE Reporting*: AEs and SAEs are recorded on data collection forms and reported to the Coordinating Center without personal identifiers. Identifiers on accompanying documents (such as medical records) are removed before submission to the Coordinating Center and any subsequent transfer to the Safety Monitoring Committee and/or Safety Review Officer.

7.3 Expected Side Effects

The BetaFat Study will use two interventions: (a) metformin or (b) gastric banding with the LapBand system. The expected side effects are provided below.

7.3.1 Metformin

Known adverse effects associated with metformin are primarily gastrointestinal (diarrhea, nausea, vomiting, abdominal bloating, flatulence, anorexia), hematologic (reduced vitamin B12 levels and, rarely, megaloblastic anemia), and the rare possibility of lactic acidosis. The risk of lactic acidosis associated with metformin use will be minimized by (1) monitoring liver transaminases, (2) monitoring renal function, and (3) temporary discontinuation of metformin before radiologic studies involving the injection of contrast dye, surgical procedures requiring reduced fluid intake, and serious illness that might be associated with hypoxia, dehydration, or shock. To monitor for clinically significant vitamin B12 deficiency, we will follow guidelines in the metformin package insert and perform a complete blood count annually while participants are taking metformin.

7.3.2 Gastric Band

A variety of risks have been reported in the literature in association with gastric banding: erosion into stomach (2-9%), slippage (2-18%), leakage (1-4%), tubing or port malfunctions (1-10%), infection (1-5%), bleeding (<1%) and dyspepsia/ulceration (5-20%), and death (<0.1%). In Dr. Katkhouda's program at USC, there have been no deaths, erosions or perforations in nearly 400 operations (~200 with diabetes). The rate of slippage, leak or malfunction requiring revision is <3%.

7.4 Risk Management

7.4.1 Metformin

To minimize the risk of gastrointestinal side effects, metformin is given with meals, started at a dose of 500 mg/d, and titrated to the target does of 1000 mg bid over a four-week period. If GI side effects develop and are mild, the patient is encouraged to remain on the study medication. If GI side effects are moderate or difficult to tolerate, metformin is reduced to the next lowest dose (for example, 1000 mg bid to 1000 mg + 500 mg; 1000 mg + 500 mg to 500 mg bid; etc.). If symptoms persist, metformin is reduced to the next step. If GI symptoms resolve, metformin is re-escalated by 500 mg per day each week until reaching the previously tolerated dose. The participant will continue on the maximum tolerated dose.

Renal insufficiency increases the risk of lactic acidosis associated with metformin. To asses renal insufficiency, serum creatinine and estimated GFR (eGFR) will be calculated using the CKD-Epi equation (ref . Ann Intern Med. 2009;150(9):604-612.) at screening and every six months on therapy. Participants must have an eGFR of 45 mL/minute/1.73 m^2 or higher to be eligible for the BetaFat study. . Metformin will be discontinued if eGFR is below 30 mL/minute/1.73 m^2. If eGFR ≥30 and <45 mL/minute/1.73 m^2 then the RISE Safety Committee will review the case to assess the benefits and risks of continuing treatment, and make a recommendation to the clinical center investigators about whether to continue the participant on metformin.

Metformin will be discontinued temporarily 24 hours before, during, and for 48 hours after any of the following events: 1) procedure involving the injection of contrast dye; 2) surgery or other procedure requiring general anesthesia; 3) any illness that could be associated with hypoxia, circulatory failure, or dehydration; 4) hospitalization. Serum creatinine will be rechecked no sooner than 48 hours after the conclusion of the event and study medication recommenced if appropriate.

To monitor for clinically significant vitamin B12 deficiency, we will follow guidelines in the metformin package insert and perform a complete blood count annually while participants are taking metformin.

7.4.2 Gastric Band

Risks of the banding procedure will be minimized by (a) exclusion of people with standard clinical contraindications to the device (see list in the Appendix), (b) use of a single surgeon with an experienced surgical team and state-of-the art facilities; (c) pre-operative participant education and evaluation and careful post-operative care by a multidisciplinary team experienced in medical, surgical, nutritional and psychological aspects of bariatric surgery; (d) availability of an on-call physician to address symptoms and patient concerns; and (e) availability of the surgical team and an experienced radiologist to diagnose and treat potential complications.

7.4.3 DEXA Scans

Potentially fertile women have a urine pregnancy test immediately prior to DEXA, which is performed only if the pregnancy test is negative.

7.4.4 MRI Scans

Individuals with contraindications to MRI will be excluded from the study during Screening and enrollment. As an additional safety measure, all enrolled participants go through a safety

screening with the on-site MRI technician on the day of the exam to ensure that they are "MRI Safe". The scanner operates within FDA guidelines, particularly for RF power deposition (SAR) and gradient switching speed (dB/dt). During the exam, participants have intercom access to the operating technician. An emergency "squeeze ball" is also available to immediately stop the exam. The MRI procedure will be terminated immediately at the subject's wish. Participants will be informed that failure to complete the experiment will not result in any form of prejudice, or affect access to medical care.

7.4.5 Other indications for Interruption of Discontinuation of Medication

A. *Pregnancy:* Study medications will not be administered if a participant becomes pregnant. The participant is referred to a high risk obstetrics clinic for further care.
B. *Lactation:* No study medication will be administered to women who are nursing a baby.
C. *Lactic acidosis:* Any study participant who experiences a bout of lactic acidosis will have metformin permanently discontinued.
D. *Dermatological problems:* Any study participant who experiences severe dermatological problems, such as urticaria, bullous rashes, exfoliative dermatitis, Stevens-Johnson syndrome, thought to be related to study medication will have study medication permanently discontinued.

7.4.6 Hyperglycemia

Participants are instructed regarding the signs and symptoms of hyperglycemia and are to call the clinic with any concerns. HbA1C will be measured at six-month intervals. If a value exceeds 7%, therapy is invigorated by frequent telephone contact and/or visits to encourage optimal medication adherence and lifestyle choices. If a scheduled HbA1C exceeds 8.0% the test will be repeated one month later. If the value remains >8.0%, end-of study testing is performed and the participant completes participation in the study and is referred for standard care of diabetes.

7.4.7 Glucose and Arginine Infusions

Irritation of a vein resulting in phlebitis can occur with administration of concentrated glucose solutions. The risk will be decreased by minimizing the use of concentrated solutions and selecting large veins for infusion of solutions. Overdose of arginine can be fatal, especially in children. We will use a dose of 5 gm. in adults. This dose has been used safely in many studies.

7.5 HIPAA – Protection of Patient Information

All data collected in the process of pre-screening, screening, and conducting the proposed research will be stored and maintained locally and centrally in compliance with HIPAA regulations. Access to study data will be secure with limited, password-protected access. Only study personnel who are IRB approved will have access to data collected as part of these studies. Data collected locally for the BetaFat study will be stored on a server that is maintained with the highest stringency of protection. Similarly, data transferred to the RISE CoC will be stored on a server that is maintained with the highest stringency of protection.

8 ADVERSE EVENT REPORTING

An adverse event is defined as any medical problem experienced by a BetaFat participant whether or not considered intervention-related by the clinical center staff. The timely and complete reporting of adverse events is a critical requirement in the conduct of this trial. This trial will be conducted under an IDE for participants without diabetes and will comply with all FDA reporting requirements.

8.1 Definition of Serious Adverse Events

a. The event results in an inpatient hospitalization (any overnight stay associated with an admission).
b. The event results in the prolongation of a hospital stay.
c. The event results in permanent or severe disability.
d. The event results in death.
e. A pregnancy results in a congenital anomaly.
f. The event results from an overdose (either accidental or experimental) of the study medication.
g. The event is life-threatening.
h. Treatment is required to prevent a serious event, as defined above.

8.2 Non-serious Adverse Events

Non-serious are all AEs which do not meet the above criteria for “serious”.

8.3 Reporting Adverse Events

AEs will be ascertained in an unbiased manner using standard questions that are identical and administered identically to participants in both treatment arms. To accomplish this, AEs will be reported on a standard form that is completed by the study staff at each regular study visit. Targeted non-serious AEs (e.g., abdominal pain) are ascertained by asking questions relating to

specific events of import in prediabetic or diabetic participants as well as side effects that may be associated with any of the study treatment arms. AEs also include any significantly abnormal physical finding identified on examination and any significantly abnormal laboratory result obtained on the patient between visits or at the time of the visit.

In order to facilitate timely reporting of SAEs, the clinical center staff who learn of an SAE must enter the completed RISE Consortium Serious Adverse Event Report prior to the close of the following business day.

8.4 Tracking Adverse Events

- *Serious Adverse Events:* SAEs will be monitored by the RISE Safety Monitoring Committee, which will remain blinded to treatment arm. The external DSMB will monitor SAEs by treatment arm. It is important to note that all serious and unexpected AEs must be reported to the CoC, regardless of the drug-related assessment. For example, a patient struck by lightning requires a report, even though this is not likely to be a drug related event.

- *Non-serious adverse events*: Non-serious AEs are tabulated by the CoC in periodic reports in the same format as is done for the DSMB. Summaries of the AEs are provided to the Safety Monitoring Committee, which reviews this summary during one of its regularly scheduled meetings.

- *Safety Monitoring Committee:* The Safety Monitoring Committee reviews AEs in a blinded fashion. The committee considers whether changes in the protocol (monitoring, consent process, etc.) are indicated based on the occurrence, frequency, or severity of AEs. The committee also evaluates whether there is any clustering of AEs by clinic. Any concerns on the part of the Safety Monitoring Committee may be referred to the Safety Review Officer and/or DSMB, which can review SAEs in an unblinded manner.

- *Safety Review Officer:* The Safety Review Officer will review all SAEs in an unblinded fashion and will confirm or reject the causality determination of the SAE to study intervention. As deemed necessary, he/she may contact the local site PI to obtain additional information about the SAE. Any concerns on the part of the Safety Review Officer may be referred to the DSMB.

8.5 Emergency Unmasking

Treatments will not be masked in the BetaFat study, so emergency unmasking is not relevant to this study.

9 STATISTICAL CONSIDERATIONS

All analyses will be conducted under the intention-to-treat principle using the treatment as assigned to each subject, and using all available data from all subjects. We will assess the distributional characteristics of all variables and will adjust the analysis accordingly by employing transformations or robust (model free) or nonparametric (distribution free) methods.

9.1 Primary Outcomes and Analyses

Two primary outcomes, each measuring ß-cell function, will be used to compare the two intervention groups at the end of the treatment period. ANCOVA will be the primary approach used for the analysis. We will employ White's robust (information sandwich) estimate of the covariance matrix of the model coefficients as the basis for inferences. This approach preserves power and protects the type 1 error probability when the homoscedastic normal errors assumptions are violated. All analyses will be adjusted for the baseline value of each endpoint. Both primary outcomes will be analyzed separately with a total type I error probability of 0.05 for each, i.e. without an adjustment for 2 separate outcomes.

9.2 Secondary Outcomes and Analyses

Comparisons of the primary study outcomes will be made for those with vs. without diabetes at study entry, BMI <35 kg/m2 vs. ≥35 kg/m2 at study entry, by race/ethnicity and sex, by baseline HbA1c and ß-cell function, and by categorical groups of various biomarkers.

Analysis of covariance will be used to compare the metformin and gastric band groups for additional continuous variables of interest at both the 12-month and 24-month time points relative to the start of the interventions. As for the primary outcomes, all analyses will be adjusted for the baseline value of each endpoint.

An important secondary analysis of this study is to determine whether ß- and α-cell function from OGTT and fasting samples are sufficiently well correlated with more robust assessments of ß- and α-cell function obtained from the hyperglycemic clamp to validate their use in large clinical trials. For these analyses, Pearson and non-parametric Spearman correlations of simple versus sophisticated measures of ß- and α-cell function will be computed.

To examine biomarker associations with glucose metabolism, Pearson and non-parametric Spearman correlations of each biomarker with ß- and α- cell function, insulin sensitivity and glucose tolerance measures will be determined. In addition, multiple linear regression models

will be developed to predict measures of glucose metabolism with the most informative biomarker sets.

Other planned secondary analyses will examine relationships between exposure variables such as body weight and fat, insulin resistance; circulating adipokines and fat metabolites, and glucose levels and primary and secondary β-cell outcomes within and across treatment groups.

9.3 Sample Size/Power Calculations

We calculated sample size and power based on practical and statistical considerations. Practically, we can perform surgery on 44 subjects within the time frame and budget of the NIH U01 grant and associated support from Allergan. Allowing loss to follow-up rate of 10% per year, this will give us ~35 subjects per group to complete 24-months follow-up. Statistically, with 35 subjects/group, by using two-group t-test we can detect a minimum effect size of 0.68 in difference between the two intervention groups with type I error of 0.05, 2-sided t-test, and 80% power. Adjustment for baseline value using analysis of covariance will give us 89% power to detect the minimal effect size of 0.68, or give us 80% power to detect a minimal effect size of 0.59 when the baseline and follow-up are correlated with a correlation coefficient of 0.5.

Feasibility of observing this effect size is supported by data from diabetes prevention studies using weight loss, TZDs or metformin, as follows:

- The diabetes risk reduction associated with a mean 12 lb. weight loss in the DPP was 58%, similar to the 55% risk reduction in TRIPOD, where we have direct measures of beta cell function. Those measures revealed an effect size of 0.33 for β-cell compensation between placebo and troglitazone at two years.
- Given the similarity of diabetes risk reduction between TRIPOD and the DPP and assuming mitigation of adverse effects of obesity as a primary mechanism for diabetes prevention in both studies, we estimate an effect size for β-cell compensation of 0.33 for weight loss of ~12 lbs. vs. placebo.
- Gastric band patients at USC had an average weight loss of 35 lbs. at 24-months (Figure 1, above), nearly three times the average weight loss seen over the course of the DPP. Thus, we may see an effect size of 3 x 0.33 = 0.99 for β-cell function in gastric banding compared to no treatment.
- Diabetes risk reduction with metformin in the DPP was 31%, approximately 53% of the reduction seen with lifestyle induced weight loss. If all of the protection were due to preservation of β-cell compensation, this would represent and effect size of ~0.18 vs. placebo.

The differential in projected effect sizes between band-induced weight loss (~0.99) and metformin (~0.18) is ~0.80, well above the 0.68 or the 0.59 minimal effect sizes that we have the power to detect with our study design.

9.4 Interim Analysis Plans

The DSMB will monitor trial outcomes as they emerge for futility with specific rules outlined in the DSMB Monitoring Plan. This trial will not be stopped for lack of efficacy, as it is a proof-of-principle study with small numbers of participants in each treatment arm.

9.5 Handling of Missing Data

It is anticipated that a small number of participants may reach HbA1C >8.0% on two visits and, thus, have primary outcomes completed prior to 24-months. If follow-up time between baseline and end-of-trial differ between the two intervention groups, we will use rate of change (defined as (baseline-end-of-trial)/follow-up time) as the outcome in the analysis to take into account of differential follow-up time.

10 DATA PROCESSING AND MANAGEMENT

10.1 Data Management System

We will use REDCap (http://project-redcap.org) as our primary tool for data capture. REDCap is a secure, web-based application that is free through the CTSA consortium. It provides: 1) an intuitive interface for data entry (with data validation); 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for data downloads to common statistical packages such as SAS; 4) procedures for importing data from external sources (e.g., DEXA data). REDCap has been adopted by many CTSAs across the country. All BetaFat study personnel at KPSC have access to REDCap through CTSA consortium. A data dictionary and web-based data entry system will be developed which includes data validation algorithms according to case report forms (CRFs). Data from non-CRFs such as laboratory and imaging will be merged by unique study IDs for data analyses.

10.2 Data Transfer

Newly entered and imported clinical center data on REDCAP are monitored and managed by the BetaFat data center. Those data will be transferred to George Washington University CoC through the CoC data management system.

10.3 Quality Control

Range checks, inter-item checks, cross-table checks, and double data entry verification are used where appropriate to ensure accurate data entry. Specific quality control procedures are run to check for missing, incorrect, and questionable values immediately after they are entered. Reports with the necessary patient identifying information and the problem values are printed

and sent to the clinical research coordinator for correction. When returned, corrected values are entered and checked again for consistency with other items. The goals are to make quality control a continuous process, to make the turnaround time between error detection and correction as short as possible, and to document any changes made to the database.

10.4 Back-up, Data Security and Confidentiality

To assure participant confidentiality, names are linked to study IDs only on a single recruitment database and only limited project personnel are authorized to have access to this database. Only study IDs will be used in all other study databases (e.g., for tracking and merging). All data are maintained on a secure USC server with two-level password protection against non-project personnel. Servers are automatically backed up daily. Backup procedures include: twice-weekly system backup, daily incremental backup, and off-site disaster recovery backup. Security procedures include: logon and link password protection, and for internet access, separate Web servers which use SSL and encryption algorithms. Virus and malware protection software is used on all computers and is updated on an hourly basis. All portable computers employ full disk encryption. Both USC and KPSC computing facilities provide support in the event of a disaster. Access to the server and databases is secured by use of login user accounts and passwords. Remote access is granted only to authorized users and is accomplished using a secure virtual private network (VPN). Appropriate filtering/firewall setup is used to prevent unauthorized access.

10.5 Tracking Study Progress

The purpose of tracking reports is to keep the collaborative group informed of study progress, and to report special problems and resolutions. Reports will be produced regularly by the BetaFat Data Center, as directed by the Steering Committee. These reports will be distributed to the study group through the study website.

Tracking reports include the following types of information:

- screening and enrollment (versus goal), by gender, BMI and HbA1C
- tables describing adherence to the study protocol (attendance, intervention compliance)
- database inventory
- progress of analysis and manuscripts

10.6 Archiving and Study Close-out Including Repository

At the end of the study, after all data have been received and edited, the database is archived in computer readable format, including readme documentation files, files of study documents (such as forms annotated with variable names, protocols, and manuals of procedures), data

files in the form of SAS transport files and input statements, data dictionaries, and program code documenting any derived variables. After the study is complete, all data will be available to RISE study investigators and to investigators from outside the study in a manner consistent with the NIDDK's data distribution procedures. Data will be stored at a readily accessible, password-protected website.

11 STUDY ADMINISTRATION

This randomized clinical trial is one of three studies conducted by the RISE Study consortium. RISE is a collaborative study group funded by the NIDDK of the National Institutes of Health (NIH) under a cooperative agreement mechanism. The goal of the RISE Study is to test different interventions to preserve ß-cell function in separate trials, while allowing for the ability to compare data across studies and combine data from individual studies by harmonizing study outcomes and data collection. The RISE Study investigators will be conducting three separate clinical trials. The investigators for each trial are responsible for recruiting participants and implementing their respective protocols. In addition, the RISE Study investigators participate in consortia activities, as described below.

11.1 Organization (Including Sponsors)

The major organizational components and their responsibilities are described:

- The *RISE Steering Committee*, composed of the principal investigators of the clinical centers, the coordinating center, and the NIDDK project office, is the primary decision making body for the RISE Consortium with overall responsibility for the design and conduct of the common elements of the three study protocols.

- The *NIDDK project office* participates in all decision-making activities and selects and oversees the activities of the DSMB.

- The *BetaFat Data Center*, located at Kaiser Permanente Southern California has responsibility for coordinating the BetaFat study, including production and distribution of materials and documents, set-up and administration of the data management system, maintenance of the local study database, analysis of primary study results, and distribution of final study data to the Coordinating Center.

- The *Coordinating Center* located at the George Washington University Biostatistics Center is responsible for maintenance of the central database and website, for preparing reports for the monitoring committees and NIDDK, and for reporting of results in collaboration with the other studies in the RISE Consortium, and for transmission of study data to the NIDDK repository.

- The *Central Blood Laboratory (CBL)* operates under subcontract to the Seattle Institute for Biomedical and Clinical Research. The CBL is responsible for providing procedures for the handling, storage, and shipment of blood specimens, for performing specified tests and assays common to the three RISE Consortium studies, for performing quality control, for providing patient-level reports to clinical centers, and for transferring results to the coordinating center.

- The *DSMB* is composed of outside experts in the design and conduct of clinical trials, and in type 2 diabetes. The board is responsible for reviewing the study documents, monitoring study progress, and monitoring patient safety.

- *Working committees* include Outcomes, Recruitment and Retention, Safety and Monitoring, Publications and Presentations, Ancillary Studies, Lab Quality Control, and Program Coordinators. Committees can be discontinued and additional committees can be created as required.

- *Allergan Corporation* provides funding but has no other role in the study.

11.2 Central Laboratories and Reading Centers

In collaboration with the coordinating center and study investigators, central laboratories and reading centers perform the following tasks:

1. Establish procedures and standards for training staff involved in the measurement, collection, preparation, handling, transfer, and all other procedures and processes.
2. Conduct training sessions and contribute training materials to the study manuals of procedures.
3. Provide or facilitate the acquisition of equipment and materials, including specifying brands, sizes, and suppliers as applicable.
4. Establish procedures for data entry and transfer of data to CoC.
5. Develop procedures for the internal as well as external quality control, and provide periodic reports on the quality control surveillance.
6. Provide long-term storage of reserve specimens or materials as directed by the Steering Committee for use in ancillary or future studies.

Each director represents the laboratory or center at RISE Steering Committee meetings, on Steering Committee conference calls, and on other conference calls where the director's participation is deemed necessary.

11.3 Training and Certification

During the start-up period, clinic staff will receive central training and certification at a training workshop or other mechanisms held by the study group. The CoC staff and selected staff from other study components will provide instruction in all aspects of the study. The purpose of the training workshops and other training venues is to provide training for study staff in order to insure that the study is conducted in a standardized manner across all participating centers. The training, based on the study manual of procedures, includes review of study design, focus on eligibility criteria, subject follow-up schedule and assessments, metabolic and physical measurements, processing and shipment of specimens, use of data entry software and electronic forms, transferring data to the CoC, maintaining patient and data confidentiality, and patient treatment guidelines.

Prior to being allowed to recruit participants, each clinical center must pass certification criteria, including satisfactory participation in training as above, supplying the coordinating center with an IRB approval letter and stamped informed consent forms, and completion of conflict of interest policy by all investigators.

Throughout the study, new staff will be trained at the appropriate clinical center and by CoC staff where appropriate. Clinic staff will re-certify annually on identified procedures. Records of certifications and training will be maintained at the clinical sites.

11.4 Site Visits

The Steering Committee will be responsible for ongoing remote monitoring of the performance of study components.

There are two types of site visits that can be conducted: (1) scheduled monitoring and (2) as needed to address specific problems. The RISE Consortium does not anticipate scheduled monitoring site visits. If necessary, site visits will be conducted to address specific problems at a clinical center. Depending on the specific issues, site visits will be attended by the study chair or designee, the NIDDK project office, the CoC, clinical center coordinators, and others as needed. Each visit will follow a predetermined format and site visitors will complete a checklist to record findings. The site visit team will review study procedures and compare data collection records to listings from the central database. A formal site visit report will be provided to the clinical center and to the RISE Executive Committee to aid in correcting any issues found. A follow up report from the clinical center should go to the CoC and executive committee reporting on the steps taken to rectify any significant problems identified. A follow-up site visit will be conducted if necessary.

11.5 Study Website

The CoC maintains the study website, which is a secure site requiring a user ID and password combination for access. The web server utilizes the Secure Socket Layer (SSL) protocol that encrypts all traffic to and from the server. Investigators, coordinators, consultants, and other study staff who would benefit from access to the information on the website are each given a unique user ID and password, which identifies the user to the web server and can be used to restrict access to particular web pages if desired.

The website contains study documents such as the protocol, manual of procedures, and forms, study calendar, directory, meeting and conference call information, links to other sites, tracking reports, minutes, and agendas.

11.6 Conflict of Interest Policy

The RISE Consortium investigators have adopted a conflict of interest policy similar to that used by other NIDDK collaborative groups. On an annual basis or whenever there is a significant change in status, RISE collaborators are required to disclose any financial or related interest that could present an actual conflict of interest or be perceived to present a conflict of interest. Disclosure is required to protect each individual's reputation and career from potentially embarrassing or harmful allegations of inappropriate behavior, and to protect the integrity of RISE Study research. Conflict of interest forms are kept on file at the CoC, and all conflicts are declared at each study group meeting.

The RISE Ethics Committee (NIDDK Project Scientist and NIDDK Project Officer) determine (1) if the disclosed interests could directly and significantly affect the performance of study responsibilities and (2) the actions needed for management, reduction, or elimination of the conflict. In addition to complying with the RISE conflict of interest policies, collaborators must certify to the Ethics Committee that they have complied with all of their local and institutional requirements regarding conflict of interest and disclosure. This is accomplished by supplying the CoC with copies of the local IRB letter of approval and stamped informed consent form(s). Should an institution make a determination regarding an investigator's potential conflict of interest that could affect participation in RISE, the local clinic should report this to the RISE Ethics Committee.

11.7 Publications and Presentations Policy

The Publications and Presentations Subcommittee (PPS) will coordinate, monitor, review, and assume responsibility for overseeing the preparation of all study-wide communications (press releases, interviews, presentations (oral and posters), and publications) relating to the scientific

aspects of the study. There will be no publication or presentation of study-wide plans or results, including ancillary studies, which have not been reviewed and approved by a majority of the PPS, and for some types of communications, a majority of the Steering Committee.

With respect to publications and presentations from the RISE Study consortium, the goals of the PPS are to:

1. Ensure accurate, uniform, timely, and high quality reporting of RISE activities and results;
2. Preserve the scientific integrity of the study;
3. Safeguard the rights and confidentiality of participants;
4. Assure that the timing of publications and presentations serves the right of the public to know the results of the program without jeopardizing its conduct; and
5. Ensure that appropriate credit is acknowledged for people responsible for RISE and the data being reported and for the funding organizations.

Detailed policies are found in the Manual of Operations.

11.8 Ancillary Studies Policy

The Ancillary Studies Subcommittee will evaluate all proposals for studies that involve RISE participants and/or data that are not a part of the protocol. These studies may be done in all RISE participants or only on a subset of participants in RISE. Ancillary studies may make use of data and/or samples already collected from RISE participants or may involve the collection of new data and/or samples. The RISE Ancillary Studies Subcommittee must review all proposed ancillary studies and these must receive final approval by both the Steering Committee and DSMB before initiation. Major factors in consideration of ancillary studies will include:

- Clinical importance and scientific validity;
- Compatibility of goals with those of RISE; and
- Amount of burden on study subjects and staff, including those at the Coordinating Center

Approved ancillary studies will be reviewed by the DSMB. Detailed policies are found in the Manual of Operations. Ancillary studies will have to obtain funding from outside the RISE study.

11.9 Protocol Amendments

Adoption of protocol amendments for elements that are shared between BetaFat and other studies in the RISE Consortium requires two-thirds majority approval by voting members of the RISE Steering Committee and approval by the DSMB. Amendments that involve protocol elements specific to BetaFat require only approval of the local BetaFat investigators and the DSMB. The amended protocol is resubmitted to the local IRB, and the participant consent is revised appropriately.

11.10 Repository for Storage and Distribution of Data and Samples

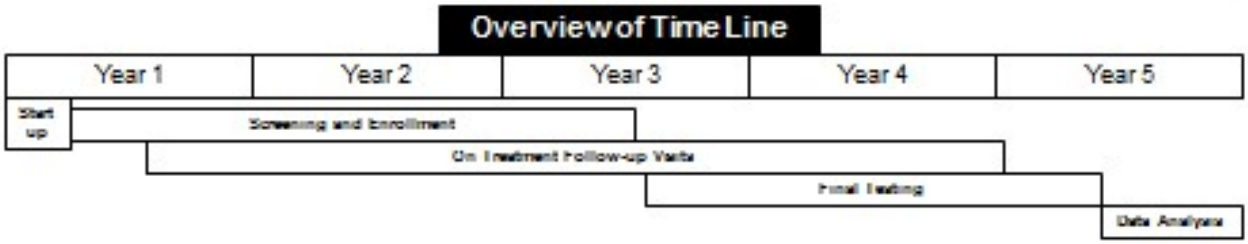
At the end of the study, de-identified research data and samples of blood will be provided to the NIDDK Central Repositories, a research resource supported by the National Institutes of Health. The Repository collects, stores, and distributes biological samples and associated data from people with many kinds of disorders, from unaffected family members, and from other healthy people. The purpose of this collection is to make samples available for use in research for the study of obesity and diabetes and related complications after the RISE Study is completed. Sending samples to the Repository may give scientists valuable research material that can help them to develop new diagnostic tests, new treatments, and new ways to prevent obesity and/or diabetes.

All participants will be asked to provide specific informed consent for release of data and samples to the NIDDK Repositories. Before the RISE Study investigators send data or samples to the Repository, each sample and data record will be given a code number and the data will be de-identified according to HIPAA requirements. Participants may choose to participate in RISE but not provide consent to have their samples and/or data transferred to the NIDDK Repository.

12 STUDY TIMELINE

The study timeline appears Figure 12.1. Recruitment will commence after IRB and DMSB approvals have been obtained, central study training has been performed, and all study medications and supplies have been procured. Screening and enrollment will begin with people with diabetes, for which an IDE/IND is not required. Recruitment of people with pre-diabetes will begin when FDA approval is obtained. Recruitment and screening are projected to occur over 27 months in years 1-3. On-trial visits are projected to occur over 42 months in years 1-4. Final testing is projected to occur over 24 months in years 3-5. Primary data analysis is projected to occur in year 5. Presentations and publications are projected to occur throughout the period of the study.

Figure 12.1


Overview of Time Line
Year 1
Year 2
Year 3
Year 4
Year 5
Start up
Screening and Enrollment
On Treatment Follow-up Visits
Final Testing
Data Analysis

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Appendix 1: Contraindications to Gastric Banding and MRI

1. Contraindications to Gastric Banding

- Inflammatory bowel disease
- Cardiac and/or pulmonary disease that imparts increased surgical risk: Angina pectoris, prior myocardial infarction or revascularization procedure, congestive heart failure, chronic bronchitis or emphysema
- Conditions associated with risk of gastrointestinal bleeding, including cirrhosis with portal hypertension, peptic ulcer disease, espohagitis, esophageal or gastric telangiectasia or arterio-venous malformation
- Conditions associated with narrowed esophagus, such and congenital narrowing or scarring
- Conditions associated with portal hypertension, including cirrhosis
- Congenital or acquired GI tract stenosis or atresia
- Prior intra-operative gastric injury, such as a gastric perforation at or near the location of the intended band placement
- Chronic pancreatitis.
- Drug or alcohol addiction
- Active infection anywhere in the body that could contaminate the surgical area
- Current or recently completed (within three months) glucocorticoid treatment at supraphysiological doses
- Inability or unwillingness to comply with the required dietary restrictions
- Allergy to the materials in the LapBand
- Personal or family history of an autoimmune connective tissue disease such as systemic lupus erythematosus or scleroderma
- Pregnancy