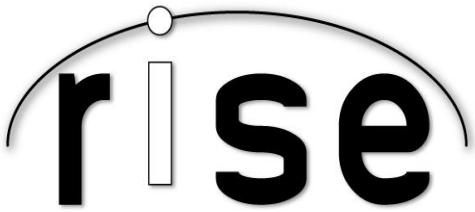
PROTOCOL

The Restoring Insulin Secretion (RISE) Study

RISE Adult Medication Study



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1 Executive Summary

A core defect in the pathogenesis of type 2 diabetes involves the ß-cell, which has been shown to be dysfunctional in both prediabetes, a high risk state that is antecedent to type 2 diabetes, and established type 2 diabetes [1]. In addition to ß-cell failure, insulin resistance and α-cell dysfunction are prominent features of the disease leading to diabetes onset [1, 2].

The possibility that improvements in ß-cell function in type 2 diabetes can be achieved and maintained for a period of time after withdrawal of a dietary or pharmacological intervention has been suggested by results from small studies in patients with varying degrees of hyperglycemia treated aggressively with insulin, oral hypoglycemic drugs and diet [3-5]. However, it is unclear whether any beneficial effect on ß-cell function can be maintained and the natural history of progressive loss of ß-cell function altered. Further, while these studies focused on the ß-cell, they did not consider the possibility that producing a sustained improvement in α-cell function may also be important in slowing progression of glucose intolerance.

Numerous medications, acting by different mechanisms, are available to treat type 2 diabetes and lower blood glucose. Metformin is thought to reduce hepatic glucose production and improve insulin resistance; it has been shown to prevent progression of prediabetes to diabetes [6]. Insulin lowers glucose while providing ß-cell “rest”. Insulin sensitizers may improve ß-cell function indirectly by reducing ß-cell stress [7, 8]. Sulfonylureas initially improve ß-cell function but may accelerate secondary failure [7, 8]. Glucagon-like peptide-1 receptor agonists (GLP-1RA) and other incretin-based therapies improve islet function [9, 10], but their ability to prevent diabetes and slow its progression is unknown.

The RISE (Restoring Insulin Secretion) Study consortium is comprised of seven clinical centers. Investigators at these centers will conduct three different protocols to assess the feasibility of different interventions in adults and children to preserve or improve ß-cell function in individuals with prediabetes or recent-onset type 2 diabetes. Three adult centers (the topic of research supported by this protocol) will together perform a randomized, partially blinded, placebo-controlled trial comparing three treatment regimens versus placebo. The three treatment regimens are: (1) metformin alone, (2) early intensive insulin treatment with insulin glargine (Lantus®) followed by metformin, or (3) the GLP-1RA liraglutide (Victoza®) plus metformin. These adult centers are located at the VA Puget Sound Health Care System / University of Washington, University of Chicago / Jesse Brown VA Medical Center, and Indiana University. In addition to this 3-center adult study, four centers will participate in a pediatric study performing a randomized trial comparing metformin with insulin glargine (Lantus®) followed by metformin. These centers are located at the University of Colorado, University of

Pittsburgh, Yale University and Indiana University. One additional adult center at the University of Southern California will perform a randomized trial comparing the effects of gastric band obesity surgery to metformin on ß-cell function. The three protocols will use many of the same inclusion and exclusion criteria, study procedures, timeline and outcome measures to allow for comparisons among groups and interventions. The overall goal of each active intervention is to reduce glucose levels to near normal during a 12-month treatment period and determine whether this slows deterioration or induces recovery of ß- and α-cell function and produces a prolonged remission, i.e. sustained improvement in islet function that is associated with improved glycemia after the intervention has been discontinued.

This protocol describes the study that will be performed at three centers in adults.

1.1 Specific Aims and Objectives

The primary objective of the RISE Adult Medication Study is to compare in a preliminary proof-of-principle trial the efficacy of three treatment strategies versus placebo on measures of ß-cell function after 3-months of post-treatment washout that will follow 12-months of active treatment. Participants will have prediabetes or untreated, recent-onset type 2 diabetes, defined by elevations in 2-hour post-challenge glucose levels along with elevations in hemoglobin A1c and/or fasting glucose concentrations. The hypothesis is that, in participants with prediabetes or recent-onset type 2 diabetes, one or more of the strategies aimed at lowering glucose will lead to preservation or recovery of islet function that will be sustained after withdrawal of treatment. To address this hypothesis, we have identified the following primary and secondary specific aims.

The primary specific aim is to determine whether:

- Functional improvements in ß-cell function following 12 months of active treatment with (1) metformin alone for 12 months, (2) insulin glargine short term (3 months) followed by metformin for 9 months, or (3) the combination of liraglutide plus metformin for 12 months can be maintained for at least 3 months following the withdrawal of therapy, compared to placebo.

The secondary specific aims are to determine in this same cohort whether:

- Treatment with the intervention therapies for 12 months improves ß-cell function compared to placebo,
- Functional improvements in ß-cell function are maintained for 9 months following the withdrawal of therapy, compared to placebo,

- Treatment with the intervention therapies for 12 months improves α-cell function compared to placebo, and whether this improvement is maintained after withdrawal of therapy,
- Measures of ß- and α-cell function from an oral glucose tolerance test (OGTT) and fasting samples made on and off therapy are sufficiently well correlated with more robust and technically demanding assessments, validating use of the simpler measures in large clinical trials, and
- Biomarkers obtained at baseline can predict parameters of ß- and α-cell function, insulin sensitivity and glucose tolerance and the response to an intervention.

1.2 Overall Design and Study Interventions

The RISE Adult Medication Study is a preliminary proof-of-principle, four-arm, partially double-blind study in which volunteers with prediabetes or drug-naïve, recent onset type 2 diabetes (HbA1c ≤7%, fasting glucose 95-125 mg/dl, and 2-hour glucose ≥140 mg/dl post 75 gram oral glucose challenge) are randomized to: (1) metformin alone, (2) short-duration insulin glargine followed by metformin, (3) liraglutide plus metformin, or (4) metformin placebo. The study will recruit participants over a three-year period and follow them for a total of 21 months from randomization. The primary outcome measure is ß-cell function as determined in two ways, defined below. The anticipated duration of the RISE Study is five years, including pre-trial planning 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, after 12 months of therapy and at 15 months (3 months after treatment has been stopped). In addition, oral tests will be done at 6 months on therapy, and 9 months off treatment. From these intravenous and oral tests as well as fasting measures, we will assess the sustainability of induced improvements and determine how well simpler measures of ß-cell function, α-cell function and insulin sensitivity correlate with more sophisticated measures. Finally, a series of biomarkers will be measured to determine whether they predict ß- and α-cell function, insulin sensitivity and glucose tolerance, and/or well as the response to therapy.

This study will provide critical information about the ability of early aggressive treatment approaches to lead to sustained recovery of islet function, and whether such approaches should be studied in larger clinical trials. Further, this study will provide valuable information regarding the potential use of simpler measures of glucose metabolism as well as biomarkers that can be used in future clinical studies and, ultimately, in routine clinical care.

2 Background and Significance

2.1 Prediabetes and Type 2 Diabetes: Epidemiology, Natural History and Pathogenesis

The increasing prevalence of obesity has been associated with a relentless rise in the prevalence of diabetes and prediabetes (impaired glucose tolerance [IGT] and/or impaired fasting glucose [IFG]). In the United States, there are currently nearly 26 million people, or 11.3% of the population aged 20 years or older, with diabetes; the incidence is estimated at 1.9 million new cases annually [11]. Over 90 percent of these cases are due to type 2 diabetes. In addition, approximately 79 million, or 35% of the same age group, have prediabetes [11]. While both diabetes and prediabetes are defined by differing levels of fasting and/or 2-hour plasma glucose [12] or HbA1c [13], one can consider the disorders a continuum of progressive dysglycemia. Individuals with prediabetes are at very high risk of developing diabetes [6, 14-16], while those with established diabetes exhibit a progressive loss of glucose control [17].

The pathogenesis of diabetes and prediabetes involves two major defects: insulin resistance and ß-cell dysfunction [1, 18]. Although improvements in insulin sensitivity can slow progression of both disorders, ultimately, progressive ß-cell dysfunction is critical for both the development [19] and progression [8] of type 2 diabetes. The ß-cell abnormality has been demonstrated to comprise abnormalities in both insulin release in response to glucose and non-glucose secretagogues, as well as a defect in the ability of the ß cell to efficiently process proinsulin to insulin [1, 20].

The progressive loss of the ability to release insulin determines the deterioration of glycemia [18, 21, 22], and thereby sets up a vicious cycle in which the metabolic derangements ("glucolipotoxicity") associated with type 2 diabetes beget further ß-cell dysfunction [23]. In addition to ß-cell dysfunction, ß-cell volume ("mass") is reduced in type 2 diabetes and possibly in those with prediabetes [24-26]. This loss of ß-cells may result from or be accelerated by glucolipotoxicity [23] and other abnormalities such as amyloid deposition that impact the islet [27].

Aside from the ß-cell lesion, a functional abnormality of the islet α-cell also contributes to hyperglycemia in type 2 diabetes [2, 28, 29]. While fasting glucagon levels may or may not be elevated [2], normal suppression of glucagon following oral nutrient ingestion does not occur; rather a paradoxical increase in glucagon levels can occur [2, 28, 29]. Further, during hyperglycemic clamps, glucagon release is greater in patients with type 2 diabetes than in healthy subjects [30]. Finally, α-cell dysfunction is present in subjects with IGT [31, 32] and first-

degree relatives of individuals with type 2 diabetes [33], both groups at increased risk of developing type 2 diabetes. Thus, α-cell dysregulation appears to also be an early feature of the islet lesion in patients with type 2 diabetes and those at high risk.

2.2 Obstructive Sleep Apnea (OSA) and Diabetes Risk

The alarming increase in overweight and obesity plays a pivotal role in the rise in diabetes prevalence. The obesity epidemic has in turn resulted in an increased prevalence of OSA, a treatable sleep disorder involving shallow and fragmented sleep as well as intermittent hypoxia. OSA is a well-documented risk factor for glucose intolerance, insulin resistance and diabetes, independently of body mass index [34, 35]. Further, studies have shown that OSA is a frequent co-morbidity of diabetes with a prevalence averaging 68% [36-38]. To date, however, clinical trials in individuals with prediabetes or diabetes have not taken the presence and severity of this co-morbidity into account. Thus, we will make assessments of sleep by questionnaire and examine whether disturbed sleep determines the progression of ß- and/or α-cell function over time or the impact of the glucose-lowering intervention.

2.3 Results of Type 2 Diabetes Prevention Studies

A number of studies have demonstrated the ability to slow the progression from prediabetes to diabetes. Overall, the greatest success has been achieved with the three thiazolidinediones (TZDs) which in TRIPOD (Troglitazone In the Prevention of Diabetes), DPP (Diabetes Prevention Program), DREAM (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) and ACT NOW (Actos Now for Prevention of Diabetes) reduced the short-to-intermediate risk of deterioration in glycemic control by 55-72% [15, 39-41], rates similar to or greater than the 58% risk reduction observed with lifestyle interventions in the DPP and FDS (Finnish Diabetes Prevention Study) [6, 14, 39]. The DPP also demonstrated that metformin reduced the risk of progression to diabetes by 31% over three years [39]. Other large studies examining the effects of medications showed that acarbose in STOP-NIDDM decreased the risk by 25% [42], but the ß-cell secretagogue nateglinide did not reduce the risk of progression to diabetes in NAVIGATOR (Nateglinide And Valsartan in Impaired Glucose Tolerance Outcomes Research) [43]. The ORIGIN (Outcome Reduction With Initial Glargine Intervention) study showed that insulin glargine reduced the risk of developing diabetes by 28% while participants were on therapy, and by 20% 100 days after withdrawal of therapy [44].

Medication combinations have had variable effects, with low dose rosiglitazone plus metformin in CANOE (Canadian Normoglycemia Outcomes Evaluation) producing a comparable effect to that reported in other studies where larger doses of a TZD were used [45]. Of relevance to RISE, no studies of diabetes prevention have yet been reported with incretin-related medications

alone or in combination with other approaches.

Follow up after discontinuation of the intervention, with the aim of assessing the durability of the intervention effects has only been undertaken in a few instances. In the FDS, withdrawal of the lifestyle intervention was associated with continued risk reduction three years later [46]. In DREAM On, after withdrawal of rosiglitazone, the annual incidence of diabetes was similar in those who were or were not randomized to receive the medication, while the cumulative incidence in those previously exposed to active medication remained lower; this observation suggests a delay but not a reversal of the underlying disease process [39, 47, 48]. To date there has not been a systematic evaluation to determine whether interventions can slow the progression to diabetes by changing the underlying pathophysiology, particularly that of decline in ß-cell function.

2.4 Long Term Intervention Studies in Patients with Early Type 2 Diabetes

Registration studies have examined the glucose-lowering effects of new medications for treating type 2 diabetes. These studies have been relatively short in duration (6-12 months) and follow up has typically been in the form of an open label extension lacking an active comparator. Importantly, with the exception of the UKPDS (United Kingdom Prospective Diabetes Study) and ADOPT (A Diabetes Outcome Progression Trial), long-term effects of diabetes treatments on ß-cell function have not been determined.

In the UKPDS, patients with recently diagnosed diabetes received “intensive” treatment with a sulfonylurea, insulin [17] or metformin [49] for an average of 10 years. After the first year, fasting glucose and HbA1c increased at similar rates in both the “intensive” and “conventional” treatment groups, firmly establishing the progressive nature of the disease [17, 49]. This deterioration in both groups was shown to be due to relentless ß-cell failure [50]. Why insulin did not prevent progression of ß-cell dysfunction is unclear, but may be because HbA1c was not reduced to near normal levels, reaching a nadir of about 6.4% at the end of the first year of treatment.

The findings in the UKPDS contrast with those of other studies in which short-term treatment with multiple daily insulin injections (MDI) or continuous subcutaneous insulin infusion (CSII) improved ß-cell function after fasting glucose was normalized [3-5]. The largest of these studies was performed in 382 Chinese adults with fasting plasma glucose levels of >126 mg/dl who were randomly assigned to therapy with insulin (MDI or CSII) or oral glucose-lowering medications in order to rapidly correct the elevated glucose and maintain it in the normal range for two weeks. Patients were subsequently prescribed diet and exercise alone and followed for

a year. More subjects in the two insulin groups achieved the target glycemic level and did so in less time than those using oral agents. Remission rates after one year were significantly higher in the insulin groups (51.1% in CSII and 44.9% in MDI) than in the group taking oral glucose lowering therapy (26.7%; p=0.0012). ß-cell function in the remission group, determined by HOMA B (homeostatic model assessment ß-cell index) and the acute insulin response to glucose, improved significantly after the intensive interventions, and was better sustained in the insulin groups after one year.

ADOPT compared rosiglitazone, metformin and glyburide in patients with recently diagnosed (<3 years) diabetes [7]. Over a median of four years, rosiglitazone reduced the progression of hyperglycemia more effectively than did glyburide, with metformin being intermediate in its effect. The glyburide-induced increase in secretory output was associated with a more rapid loss of ß-cell function than “resting” the ß-cell by decreasing secretory demand with rosiglitazone [7, 8]. Importantly, patients who progressed to monotherapy failure had higher fasting glucose and HbA1c levels and lower ß-cell function at baseline, and demonstrated the most marked decline in ß-cell function irrespective of treatment assignment [8]. The use of rosiglitazone has since been severely restricted by the U.S. Food and Drug Administration (FDA) because of concerns about associated cardiovascular events. The other TZD, pioglitazone, remains available but has been associated with bladder cancer [51, 52]. In addition, this class of drugs has been associated with weight gain, edema and fractures [7], side effects that further limit the attractiveness of these drugs in individuals early in the course of disease.

Incretin-based therapies hold new promise for promoting recovery and preservation of islet function. They not only increase insulin secretion and decrease glucagon release, but also preserve the morphology of cultured human islets [53] and increase ß-cell mass following partial pancreatectomy in rodents [54]. In humans, long-term intervention studies with these agents are limited. The only study examining the potential for durability compared the effect of three years of treatment with the GLP-1RA exenatide to insulin glargine in a small number of patients who were also taking metformin. HbA1c (7.4-7.6% at baseline) was reduced similarly in both groups to 6.7-6.8% after one year of therapy in 69 participants, with the exenatide group demonstrating improved ß-cell function while the insulin glargine group showed no change. Following one month of washout, ß-cell function was similar to pretreatment values in both groups [55]. Thereafter, therapy was restarted in a subgroup, and was continued to three years in 36 participants, at which point medication was withdrawn for one month. Better ß-cell function was observed in those treated with exenatide, in keeping with a benefit beyond the period of exposure [56]. The magnitude of improvement was small, however, and It is unclear whether the need for prolonged therapy to demonstrate a benefit was because subjects had reasonably long standing diabetes with poor ß-cell function at baseline and/or the attained

glucose level on therapy was not close enough to normal initially.

It may be that the greatest potential to induce prolonged remission will be with aggressive glucose lowering and in those with earlier disease and milder defects, possibly by reducing ß-cell burden to produce and release insulin. This is supported by the observations that (a) in ADOPT those who did not progress to monotherapy failure had lower glucose levels and better ß-cell function at baseline, (b) the greatest success in slowing progression in those with prediabetes and early diabetes occurred with TZDs, which reduce ß-cell secretory demand, and (c) the beneficial effect of exenatide was observed after prolonged therapy in individuals with well established diabetes in whom glucose levels were not near normal at baseline or during therapy.

Thus, we propose to study subjects with prediabetes or early diabetes who at baseline will have better ß-cell function than those with a longer duration of diabetes. We will use therapeutic approaches that aim to lower glucose to levels that are close to normal using approaches that will either increase ß-cell responsiveness or decrease secretory demand on the ß-cell and compare them to placebo.

2.5 Metformin

Metformin is a glucose lowering medication of the biguanide class that has been used in the management of type 2 diabetes for over 50 years. It reduces the excess hepatic glucose production that characterizes type 2 diabetes without increasing insulin secretion. In fact, in the DPP it was documented that the improvement in insulin sensitivity associated with metformin treatment resulted in a decrease in secretory demand and, thus, insulin release by the ß-cell [19]. In addition to its glucose lowering action, an added potential benefit of metformin is a modest weight loss, and some protection from weight gain with other diabetes treatments including insulin [57, 58]. Metformin is not currently approved for use in the prevention of type 2 diabetes. However, the DPP demonstrated that metformin is effective in persons with impaired glucose tolerance, reducing the development of diabetes by 31% [6].

The most common side effects associated with metformin are gastrointestinal. As many as 30% of persons report diarrhea, nausea, metallic taste, abdominal bloating, flatulence or anorexia. These symptoms are generally transient, resolve spontaneously and can be avoided by gradual escalation of dosage. About 4% of participants were unable to continue metformin in U.S. clinical trials. Metformin is not associated with hypoglycemia unless used in conjunction with other glucose-lowering medications (sulfonylurea or insulin). Metformin has been found to rarely cause lactic acidosis (about 0.03 cases per 1,000 person years), and then only when used

in persons with renal or hepatic insufficiency or during episodes of hypoxia or circulatory failure [57].

2.6 Insulin Glargine

Insulin glargine is a recombinant human insulin analogue that in most subjects is administered once daily, as it forms subcutaneous microprecipitates from which small amounts of this analogue are released, resulting in a relatively constant profile over 24 hours. It has been used since 2000 to treat patients with type 2 diabetes and has recently been reported to reduce the risk of developing type 2 diabetes in individuals with prediabetes by 28% [44]. Insulin glargine administered at night lowers plasma glucose by decreasing hepatic glucose production [59] and can be titrated in individuals with prediabetes and established diabetes to achieve fasting glucose concentrations of <95 mg/dl without a marked increase in recurrent or severe hypoglycemia [44].

The most common side effects observed with insulin glargine are hypoglycemia and weight gain. This insulin has been reported in some non-randomized, epidemiological studies to be associated with an increased risk of cancer [60]. However, ORIGIN, a randomized trial in which over 6,000 participants received insulin glargine for a median of 6.2 years, did not observe an association between insulin glargine and cancer [44].

2.7 Liraglutide

Liraglutide is an acylated human GLP-1RA with 97% amino acid sequence homology to the endogenous form of GLP-1(7-37). It was approved for use in type 2 diabetes in 2009. Liraglutide, like other GLP-1RAs, increases intracellular cyclic AMP in ß-cells leading to insulin release in the presence of elevated glucose concentrations; thus, insulin secretion subsides as blood glucose decreases and approaches euglycemia [10]. The clinical implication of this is that hypoglycemia is infrequent when liraglutide is combined with agents other than sulfonylureas or insulin. Liraglutide also decreases glucagon secretion in a glucose-dependent manner, but does not limit the α-cell's response to hypoglycemia. The mechanism of blood glucose lowering may also involve a delay in gastric emptying. Unlike native GLP-1, liraglutide is stable against metabolic degradation by dipeptidyl peptidase-4 (DPP-4) and neutral endopeptidases.

ß-cell function in patients with type 2 diabetes is enhanced by liraglutide therapy either alone or in combination with other glucose lowering agents [61]. In animal studies, beneficial effects on ß-cell mass have also been observed, but such an effect in humans has not been demonstrated. Liraglutide is also associated with reductions in body weight.

Liraglutide is associated with upper gastrointestinal side effects including nausea and vomiting, which frequently subside with continued treatment and can be reduced in severity by dose titration. GLP-1 based therapies, which would include liraglutide, have been reported to be associated with an increased risk of pancreatitis, the mechanism for which is unclear [62]. This adverse effect occurs on a background of an increased risk of pancreatitis in patients with type 2 diabetes [63]. In rodent studies, liraglutide has been found to cause thyroid C-cell tumors; however, it has not been established that this occurs in humans [62]. Given the above, liraglutide should not be used in patients with a history of pancreatitis, a personal or family history of medullary thyroid carcinoma or in patients with multiple endocrine neoplasia syndrome type 2.

2.8 Approaches to Measuring ß-cell Function, α-cell Function, Insulin Sensitivity and Glucose Tolerance

Glucose homeostasis is physiologically modulated under fasting and prandial conditions by a number of regulatory factors, most prominently insulin and glucagon. Measurements made under fasting conditions are generally proportional to direct measures of the dynamic responses, but the most rigorous measurement of ß-cell function (insulin and/or C-peptide responses to physiologic stimuli) comes from evaluation of dynamic tests. Dynamic testing can be accomplished with oral intake of glucose or mixed meals, or with intravenous infusion of glucose or other ß-cell stimulators. In RISE, the hyperglycemic clamp technique will be used to examine insulin sensitivity and ß-cell function, and the OGTT will be used as a complementary measure that more closely reflects the integrated physiologic response to ingested stimuli, including incretins.

2.8.1 Intravenous 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 [64]. The role of α-cell function in determining glucose tolerance is less well defined.

The acute insulin (C-peptide) response to intravenous glucose (AIRg or first phase insulin response) occurs within the first ten minutes following bolus glucose administration and is a sensitive marker of ß-cell function that decreases as the fasting glucose level rises. A potential "drawback" of this measure is that the first phase response drops sharply as the fasting glucose level approaches 115 mg/dl [65]. Above that level, the first-phase response is markedly diminished and frequently absent. Although this early response may respond to treatment interventions aimed at restoring or preserving ß-cell function in some patients, this defect, in the absence of medication, appears particularly resistant to reversal. The response is possibly

related to the type of intervention used to bring about improvement in ß-cell function. These difficulties with measuring early responses at baseline limit our ability to depend on this measure of ß-cell function as a primary outcome in RISE.

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 the acute insulin response to glucose, the second phase response persists and is measurable even when the fasting glucose concentration exceeds 115 mg/dl [66]. Moreover, it is proportional to overall ß-cell secretory capacity and can be measurably improved with treatment interventions targeting ß-cell function [67].

ß-cells also respond to non-glucose secretagogues, such as peptides and amino acids. The prevailing glucose level modulates the ß-cell secretory response to non-glucose secretagogues. By performing hyperglycemic clamps with the addition of a bolus intravenous arginine injection, it is possible to quantify the maximum insulin response (AIRmax), which provides an estimate of the maximum ß-cell secretory capacity at a given point in time. In the absence of techniques that provide a direct measure of ß-cell mass in living humans, this measure of maximal secretory capacity is currently taken as an indirect measure of ß-cell mass. AIRmax is decreased in patients with type 2 diabetes [30] and in those at risk of developing the disorder [68]. Again, unlike the first phase response, AIRmax responses are present and readily quantified in individuals with higher fasting glucose levels (>115 mg/dl). Effects of treatment interventions on AIRmax have not been extensively evaluated but are beginning to be studied [56].

ß-cell responses also are modulated by the prevailing insulin sensitivity. Therefore, expressing ß-cell function relative to insulin sensitivity provides a better estimate of how well the ß-cell is performing [69]. This approach of accounting for the effect of insulin sensitivity has been used extensively with AIRg derived from frequently sampled IV glucose tolerance tests, where the intravenous glucose tolerance test (IVGTT)-derived measure of insulin sensitivity (S_I) is used to produce the 'disposition index' (S_I x AIRg) [69]. This approach can also be applied to adjust other measures of ß-cell function, providing that the underlying assumption of a hyperbolic relationship between the insulin sensitivity variable and the ß-cell function variable is true. Likewise, AIRmax can be adjusted for insulin sensitivity (e.g. S_I x AIRmax; [69]). Insulin sensitivity-adjusted ß-cell function measures derived from second phase responses and AIRmax responses measured with hyperglycemic clamp methods have been selected as the primary outcome measurements for RISE.

The reciprocal of glucose's ability to potentiate the ß-cell response is its ability to suppress glucagon secretion from the α-cell. This function can also be measured using hyperglycemic clamps along with arginine [30, 70]. The ability of glucose to suppress the acute glucagon

response is impaired in people with type 2 diabetes and in those with prediabetes, indicative of impaired α-cell regulation [30, 71]. This parameter will be quantified using the hyperglycemic clamp with arginine injection (AGRmin, minimum acute glucagon response) and, if funding is available, will be analyzed as a secondary outcome in RISE.

2.8.2 Oral Tests of ß- and α-cell Function and Insulin Sensitivity

The OGTT provides measures of islet function and has been used in large clinical trials for this purpose. The early insulin response (insulinogenic index) is an important determinant of glucose tolerance [18]. This response, when used in combination with a fasting measure of insulin sensitivity (1/fasting insulin, HOMA, or QUICKI) provides another estimate of ß-cell function termed the "oral disposition index" (DIo) [22, 72]. However, the accuracy of HOMA in assessing changes in insulin sensitivity over time may not be as reproducible and accurate as clamp techniques [73]. Using modeling approaches such as those developed by Mari et al. [74] and Cobelli et al. [75], it is possible to obtain a number of additional parameters of ß-cell function and insulin sensitivity from the OGTT. In addition, using C-peptide levels and de-convolution techniques, insulin secretion rates can be calculated from the OGTT [74-76] . Finally, overall glucose tolerance can be calculated from the glucose excursion during the test [77], providing a measure that is directly interpretable and applicable in the clinical setting.

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, glucagon suppression is impaired [2, 28, 29], an abnormality that is improved with incretin-based therapy [10]. However, whether this change persists after withdrawal of therapy is unknown.

These OGTT-based measures will be performed in the study and their relationship with intravenous test-based measurements will be determined, both at baseline and following drug interventions.

2.9 Biomarkers of ß-cell Function, α-cell Function, Insulin Sensitivity and Glucose Tolerance

It is often the case that biologic phenomena of interest are both difficult and costly to measure directly. This can be true of physiologic states such as ß-cell function, but it is also true for time-dependent changes such as rates of progression to overt diabetes. In these instances we turn to biomarkers. An optimal biomarker is measured from a single biological sample (e.g., blood or urine) and is less costly, less labor intensive and highly correlated with the result from the process under evaluation. However, a biomarker might be the result of combinations of blood

tests, or measurements from other body fluids, or even measurements of other biological substances [78].

In RISE, treatments aimed at restoring or preserving ß-cell function are being implemented and their effects determined using dynamic measures of ß-cell function discussed above. This provides the opportunity to concurrently attempt to identify biomarkers to assess islet function in relation to the systemic metabolic state, and to predict how these change over time. The identification of such biomarkers is an important ancillary goal of the project since a practical application of strategies learned from this study will ultimately require a screening test to identify likely responders to the interventions.

2.9.1 Putative Diabetes 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 [79-82]) and evolving markers of ß-cell mass regulation (e.g., prolactin, DNA fragments and methylation, GLP-1, glucose-dependent insulinotropic peptide [GIP], HSP90nucleic acids [83-87]). 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

This document is specific for the RISE Adult Medication Protocol that three centers are performing together in individuals with prediabetes or recent onset type 2 diabetes. This preliminary proof-of-principle study is a randomized, partially blinded, placebo-controlled trial comparing metformin alone, insulin glargine followed by metformin, or liraglutide plus metformin to placebo. The primary outcome is ß-cell function, measured as defined below.

The RISE Adult Medication Protocol will involve 255 adults age 20-65 years old. Volunteers will be men and women with prediabetes or with drug-naïve, recent onset, mild type 2 diabetes who meet the following glucose-based parameters: HbA1c ≤7.0%, plus an OGTT fasting plasma

glucose 95-125 mg/dl and a 2-hour post challenge glucose ≥140 mg/dl. Participants will be randomized to: (1) metformin alone for 12 months, (2) 3-months basal insulin glargine titrated to normalize fasting glucose followed by 9 months metformin, (3) liraglutide plus metformin for 12 months, or (4) placebo for 12 months. Volunteers will be recruited over a three-year period and followed for a total of 21 months following randomization. After baseline assessments and the run-in period, participants will be treated with active medication or placebo for 12 months. To determine if treatment has persistent benefits on ß-cell function, subjects will be followed after discontinuation of treatment for a minimum of 3 months and up to 9 months if possible. Primary and secondary assessments will be performed at various time intervals while participants are receiving therapy and after medication discontinuation. The timing of the study, including interventions and testing, is shown in Figure 1.

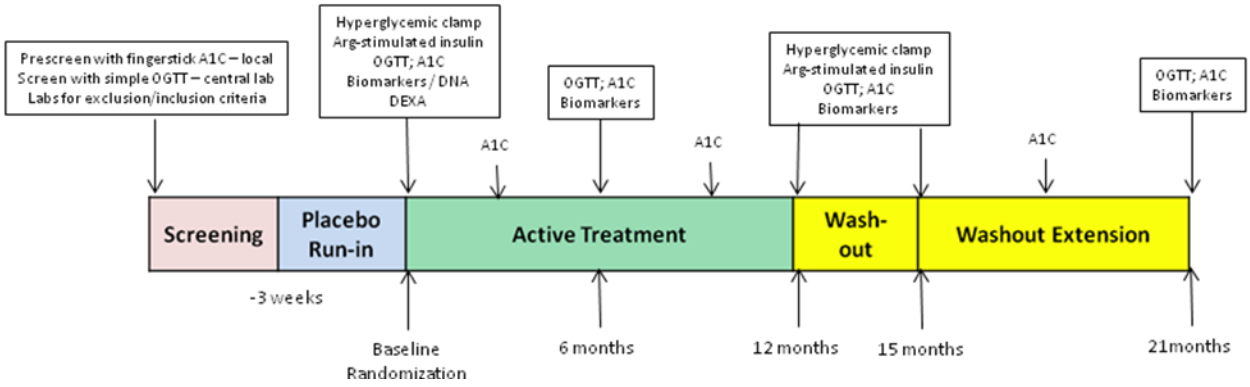

Figure 1: Timing of interventions and outcomes testing for RISE Adult Medication Protocol

The chosen interventions are intended to test whether "resting" the ß-cell (metformin alone or metformin following normalization of fasting glucose with long-acting insulin) or "improving" ß- and α-cell function (liraglutide) will retard the natural progression of prediabetes and type 2 diabetes. A placebo arm is included as a control group to determine the effects of the interventions relative to the natural history of the disease.

By including a 3-month washout period after which the primary outcomes will be assessed, the study will examine whether a beneficial effect of treatment persists beyond the period of active drug intervention. While shorter initial treatment intervals may be effective, the plan is first to determine the efficacy and durability of 12 months of treatment as a proof of principle. If no sustained effect is seen after 12 months of treatment, then it is very unlikely that a shorter treatment period would be efficacious. Complete end-of-study measures will be performed at the end of the 3-month washout period (15 months on study) and used for the primary analysis of the study. Where possible, ß-cell function will be measured using an OGTT at 9 months post therapy (21 months on study) to evaluate whether treatment effects persist beyond the initial

3-month washout period. Full evaluation of the length of any durable response beyond these time points will require future studies designed specifically to address that question.

3.2 Primary Outcomes

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 outcomes are specified:

- ß-cell function measured as the **second phase response (steady-state of the hyperglycemic clamp**) (100-120 minutes of glucose infusion) [64]. This will be calculated as mean C-peptide at steady state, adjusted for insulin sensitivity using the hyperglycemic clamp-derived measure of insulin sensitivity (glucose disposal rate/insulin: GDR/I).

- ß-cell function measured as **AIRmax (arginine-induced acute insulin response at hyperglycemic 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.

Overall, for primary and secondary outcomes we will use the insulin sensitivity measured during steady state of the hyperglycemic clamp (glucose disposal rate [derived from the steady state glucose infusion rate] divided by steady state plasma insulin concentration 100-120 min) for adjustment of ß-cell function measures [69].

The main analysis will evaluate the effect of study treatments to induce durable improvement in these primary outcome measures of ß-cell function, by comparing each treatment against placebo following a 3-month medication washout. The study will be analyzed under the intention-to-treat principle.

3.3 Secondary Outcomes

There are a number of pre-specified secondary outcomes. These include measures derived from the hyperglycemic clamp that are not specified as primary outcomes; measures derived from the OGTT; analyses related to treatment effect at the end of the 12 month active intervention period compared to pre-treatment baseline; and analyses related to various putative predictors of treatment outcome.

As presented in Figure 1, the timing of available secondary measures differs according to measurement methodology. Secondary outcomes will be evaluated by intention-to-treat analyses when evaluating effects of randomized study treatments.

3.3.1 ß-cell Function and Glucose Tolerance

(i) 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, adjusting for insulin sensitivity using the hyperglycemic clamp-derived measure of insulin sensitivity (glucose disposal rate/insulin: GDR/I)
   d. Second phase insulin response, with adjustment for insulin sensitivity using non-clamp-derived surrogate measures
   e. AIRmax response to arginine calculated using insulin concentrations and adjusted for insulin sensitivity

(ii) 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 (parallel 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)

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

3.3.2 α-cell Function (contingent on funding)

A. Hyperglycemic clamp-derived measures:
   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 to arginine (the incremental increase in glucagon concentration from pre-arginine baseline for the 10 minutes after the arginine injection)
B. OGTT-derived measures: integrated glucagon response from 0-120 minutes
C. Fasting measures: glucagon

3.3.3 Insulin Sensitivity

(i) Hyperglycemic clamp-derived measures: glucose disposal rate at steady state (100-120 minutes) divided by ambient insulin concentrations
(ii) OGTT-derived measures:
   a. Matsuda index
   b. Model-derived insulin sensitivity
(iii) Fasting measures:
   a. 1/fasting insulin,
   b. HOMA-S (using the Oxford calculator),
   c. QUICKI

3.3.4 Blood and Urine Biomarkers

(i) Glycemic control: HbA1c (baseline predictor, and as a time-dependent treatment outcome)
(ii) Adipokines/inflammatory markers: adiponectin; others contingent on funding (see list above in Section 2.8.1)
(iii) Non-esterified fatty acids
(iv) Renal/vascular: urinary albumin/creatinine ratio at baseline (contingent on funding)

3.3.5 Physical Measures

(i) Physical measures: blood pressure, body weight, body mass index, waist and hip circumference

3.4 Treatments

3.4.1 Three-week Placebo Run-in Phase

Prior to randomization, eligible volunteers will participate in a 3-week run-in period. They will be instructed by study staff in proper injection technique and will receive three weeks of both placebo injections and tablets. At the end of the placebo-run in period, they will return any unused medication which will be counted to assess adherence. Participants taking <80% of the medication will be excluded from further participation.

Those who have been compliant with medication use (≥80%) will be eligible to participate and will undergo the baseline study procedures. Once these are completed, and if willing to undergo these tests repeatedly as required by the study, they will be randomized to one of the treatment arms and will enter the active treatment phase. In addition, after completing the first set of study procedures, they will receive instruction in lifestyle modification, including individualized instruction on weight loss, exercise and diet.

3.4.2 Twelve-month Active Treatment Phase

Participants will be randomized to one of the four interventions. Participants will be seen every three months throughout the study and will be given three months of medication at a time during the active treatment phase. Study staff will monitor compliance by auditing returned medication every three months. Participants randomized to insulin will be required to perform home blood glucose monitoring.

Participants randomized to metformin monotherapy will not know whether they have been assigned to metformin or placebo. Double blinding of these two groups will be done to minimize dropout of those participants assigned to placebo.

The two groups receiving injectables (i.e., liraglutide and insulin glargine) will not be masked to the intervention.

All medications used in RISE are approved for treating type 2 diabetes and will be used according to the label; Investigational New Drug (IND) applications will be obtained for use of all medications in individuals with prediabetes. For study procedures performed during the active phase of treatment, study medications will be withheld on the morning of testing.

3.4.3 Metformin

Initiation and determination of maintenance dosing for metformin will be the same in all treatment groups, although the timing of undertaking these steps differs by treatment group.

To minimize non-compliance due to gastrointestinal side effects, participants will be titrated over four weeks from a starting dose of 500 mg once daily to a maximum dose of 1000 mg bid. Participants will remain on the maximum tolerated dose of metformin throughout the study. If participants are unable to tolerate metformin they will continue in the study but will not take the medication.

3.4.4 Liraglutide plus Metformin

Liraglutide will be started at a dose of 0.6 mg subcutaneously, once daily. This dose will be continued for one week, after which subjects will increase the daily dose to 1.2 mg if they have tolerated the 0.6 mg daily dose. Likewise, after one week at 1.2 mg daily, the dose will be increased to 1.8 mg daily, the maximal recommended daily dose in the treatment of diabetes. If participants cannot tolerate the higher dose, they will revert back to the previously tolerated dose and will remain on that maximal tolerated daily dose for the duration of the study. Once the maximal dose of liraglutide has been reached and is tolerated, metformin will be initiated and titrated as described above. If the combination of the medications produces intolerable adverse effects, liraglutide treatment will be retained in preference to metformin.

3.4.5 Insulin Glargine Followed by Metformin

Insulin will be titrated over a period of one month based on self blood glucose monitoring to achieve a fasting blood glucose of 80-90 mg/dl without hypoglycemia, and then continued with dose adjustment to maintain the fasting glucose in this range. Insulin glargine has been shown to be titratable in individuals with prediabetes and established diabetes to achieve fasting glucose concentrations of <95 mg/dl without a marked increase in recurrent or severe hypoglycemia [44]. Following 3-months of insulin treatment, insulin glargine will be discontinued and metformin will be commenced and titrated as above.

3.4.6 Placebo

Matching metformin placebo will be titrated equivalently to the active metformin. Adverse effects and final dosing achieved will be recorded in parallel with active metformin.

3.4.7 Medication Wash-out and Post-treatment Follow Up

Following the completion of the intensive re-assessments (hyperglycemic clamp and OGTT) after 12 months of the intervention, study medications will be stopped and the participants will continue to be followed off medications, for a minimum of 3 months and up to 9 months, depending on the time of randomization within the 5-year study period. At the end of the first 3 months off treatment (i.e., 15 months after randomization), the hyperglycemic clamp and OGTT will be repeated to determine the durability of the effects of the active interventions on ß- and

α-cell function. Although it is unlikely in this study population, participants may experience deterioration in ß-cell function during this 3-month wash out period, and require rescue therapy. Criteria for rescue, and protocol-specified rescue therapy, are described below (see Section 7.4). Participants who do not require rescue will undergo the final clamp and OGTT procedures at the end of the 3-month washout period. They will then continue to remain off study medications and retesting will occur 9-months following discontinuation of the intervention (i.e., 21 months after randomization). The rescue protocol will be utilized, as needed, during this last 6-month follow-up period. Following the complete 9-month washout period, patients will be referred to their primary care provider for further follow up.

3.4.8 Rescue

The approach to rescuing a participant with severe hyperglycemia is described in Section 7.4.

3.5 Inclusion Criteria

1. Fasting plasma glucose 95-125 mg/dl *plus* 2-hour glucose ≥140 mg/dl on 75 gm OGTT *plus* HbA1c ≤7.0%. There is no upper limit for the 2-hour glucose on OGTT.
2. Age 20-65 years
3. Body mass index (BMI) ≥25 kg/m^2 (≥23 kg/m^2 in Asian Americans) but ≤50 kg/m^2
4. Self-reported diabetes <1 year in duration
5. Drug naïve (no prior to oral glucose lowering agent(s), insulin or other injectable glucose lowering agents)

3.6 Exclusion criteria

1. Underlying disease likely to limit life span and/or increase risk of intervention or an underlying condition that is likely to limit ability to participate in outcomes assessment
2. An underlying disease that affects glucose metabolism other than type 2 diabetes
3. Taking medications that affect glucose metabolism, or has an underlying condition that is likely to require such medications
4. Active infections
5. Renal disease (eGFR <45 mL/minute/1.73 m)
6. Anemia (hemoglobin <11 g/dl in women, <12 g/dl in men) or known coagulopathy

7. Cardiovascular disease, including uncontrolled hypertension. Participants must be able to safely tolerate administration of intravenous fluids required during clamp studies.
8. History of conditions that may be precipitated or exacerbated by a study drug:
    a. Pancreatitis
    b. Serum ALT more than 3 times the upper limit of normal
    c. Excessive alcohol intake
    d. Suboptimally treated thyroid disease
    e. Medullary carcinoma of the thyroid or MEN-2 (in participant or a family history)
    f. Hypertriglyceridemia (>400 mg/dl despite treatment)
9. Conditions or behaviors likely to affect the conduct of the RISE 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 RISE
    d. Current, recent or anticipated participation in another intervention research project that would interfere with any of the interventions/outcomes in RISE
    e. Weight loss of >5% in past three months for any reason other than post-partum weight loss. Participants taking weight loss drugs or using preparations taken for intended weight loss are excluded.
    f. Likely to move away from participating clinics in next two years
    g. Women of childbearing potential who are unwilling to use adequate contraception
    h. Current (or anticipated) pregnancy and lactation.
    i. Major psychiatric disorder that, in the opinion of clinic staff, would impede the conduct of RISE
10. 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.

3.7 Randomization

Randomization is stratified by clinical center and by prediabetes vs. diabetes status to ensure balance among the treatment groups with respect to anticipated differences in the participant populations. For each clinical center, the Coordinating Center (CoC) will generate two randomization schemes using a permuted block design. In this way, sample sizes across the four treatment arms will remain relatively equivalent as the trial progresses. The clinical center staff will use a computer-based system to input eligibility data and receive a random treatment assignment.

3.8 Masking

During the run-in period, participants will take metformin placebo and inject saline to assess compliance with possible study medications. After randomization, the metformin and placebo medications will be masked through a metformin-matched placebo. The insulin followed by metformin and liraglutide plus metformin arms will be unmasked.

Because the primary outcomes are based on a single set of measurements taken 3-months following medication withdrawal, the outcome measurements are inherently masked to both participants and investigators. Participants and clinical center staff will be masked to study results until after the final outcome measurements are complete.

4 RECRUITMENT

4.1 Recruitment

The primary source of participants for the RISE Study is the active patient population of the study sites and their usual referral sources. Participants will be recruited over a three year period. Recruitment techniques include notices placed on bulletin boards at the medical centers, newspaper and radio advertisements and public service announcements, and referral from colleagues. Prescreening of electronic and clinical medical records is strongly encouraged to reduce available screen failure rates. 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. Site-specific recruitment strategies are developed by each local study team.

The performance of the clinical centers in recruiting and retaining eligible RISE participants will be monitored by the CoC and the Recruitment and Retention Committee. Monthly recruitment summaries will be issued throughout the recruitment and randomization period detailing the number of potential participants screened, number randomized, and reasons for ineligibility or of refusal to participate by clinical center. The reasons for ineligibility or refusal to participate

will be compared among the clinical centers, and if large differences are found, explanations for these differences will be sought. If the randomization rate falls below the desired number of participants per month, data on reasons for ineligibility and refusal will be used to try to identify strategies that would increase the randomization rate. Site visits may be needed and assistance will be offered to clinical centers struggling with recruitment efforts.

4.2 Description/Justification of the Target Study Population

Preliminary screening will be done to enrich the yield of individuals who have prediabetes or type 2 diabetes. These individuals will be invited for initial field testing using HbA1c criteria. This testing will be performed on individuals who are highly predisposed to develop type 2 diabetes including, but not limited to, those with one or more of the following features:

- Known impaired glucose tolerance (IGT) and/or impaired fasting glucose (IFG)
- Overweight or obesity (BMI ≥25 kg/m^2 (≥23 kg/m^2 in Asian Americans) but ≤50 kg/m^2)
- Age >45 years
- First degree relative with type 2 diabetes
- Sedentary lifestyle
- Those with low HDL cholesterol or high triglycerides, hypertension (i.e., “the metabolic syndrome”)
- Certain racial and ethnic groups (e.g., Non-Hispanic Blacks, Hispanic/Latino Americans, Asian Americans and Pacific Islanders, and American Indians and Alaska Natives)
- Women who had gestational diabetes, or who have had a baby weighing 9 pounds or more at birth
- Women with polycystic ovary syndrome (PCOS)

The lifetime risk for type 2 diabetes among these groups is substantially higher than that seen in the general population. Further, the presence of more than one of these factors enhances the likelihood of development of type 2 diabetes to an even greater degree than does the presence of any single factor noted above. A recent evaluation of diabetes risk attempted to quantify the value of well known diabetes-associated risk factors [88].

The presence of risk factors/characteristics noted above will prompt screening these persons with a POC HbA1C; those who have a value of ≤7.2% will be asked to undergo a standard 75 gm OGTT along with a lab-based HPLC HbA1c. In this way, the proportion of subjects who will undergo an OGTT will be at high likelihood of having IGT or newly diagnosed type 2 diabetes by virtue of their risk status and elevated POC HbA1C. This approach is designed to increase the

yield of detecting IGT and type 2 diabetes while simultaneously maximizing the use of resources (both in terms of effort of personnel and use of testing supplies).

This strategy will yield individuals who will be identified as being at high risk for ß-cell dysfunction (risk group plus POC HbA1C ≤7.2%). Of those who undergo a 75 gram OGTT and the lab-based HPLC HbA1c, we project that approximately one-third of individuals will meet study enrollment criteria, i.e., will have a fasting plasma glucose 95-125 mg/dl *plus* a 2-hour glucose ≥140 mg/dl *plus* a laboratory-based HbA1c ≤7.0%. There is no upper limit for the 2-hour glucose on OGTT.

4.3 Informed Consent

The informed consent process will be performed in a quiet setting prior to the initiation of any study procedures. The subject will be 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. Consent documents are not signed until volunteers demonstrate adequate understanding of all aspects of the study and consent process. A copy of the consent will be given to the subject. There will be two consent forms – one for the Screening stage and one for the Intervention stage. Model consent forms are provided in Appendix A. Written informed consent will be obtained prior to initiation of any study-related activities.

In the event that a significant protocol change occurs, the informed consent documents 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.

Sites will submit to the CoC 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 CoC as a central archive.

4.4 Staged Screening and Randomization

Screening for enrollment in RISE will be conducted in a stepwise manner involving completion of an initial screening encounter, an OGTT screening visit and a 3-week run-in period at the end of which baseline measurements, including the OGTT and hyperglycemic clamp, will be performed. Screening proceeds from least invasive, most easily obtained criteria to the most demanding procedures. Once a participant is determined to be ineligible, the screening process stops.

The staged screening process is intended to accomplish the following:

- identify potentially eligible participants for RISE,
- verify eligibility of participants,
- accomplish the objectives of the informed consent process,
- complete a run-in period,
- establish a research cohort likely to complete the study and adhere to the medication schedule and the complex testing of ß-cell function and related measures, and
- randomize participants into RISE.

Complete definitions and procedures are in the Manual of Procedures.

All steps below will be conducted if and only if the individual provides written informed consent for participation in RISE. If for any reason the participant wishes not to participate in any of the activities and experimental procedures outlined in the informed consent, the consent form will not be signed and no further data collection or biological testing will be conducted. If the participant agrees to participate in RISE, he/she will provide written documentation by signing the consent form. If for any reason the participant wishes to withdraw from the study at any time, there will be no pressure upon the participant to continue and withdrawal will be documented.

Staged screening and enrollment includes the following steps:

1. *Initial Contact*: This contact aims to identify individuals who are predisposed to develop type 2 diabetes by virtue of having one or more risk factors (Section 4.2). Potential participants are seen and given detailed information about RISE, including research interventions, randomization, masking, test procedures, risks and benefits, and the eligibility process. If they give oral and/or written consent, they are first interviewed and records are reviewed for personal and medical history. If they pass these completely noninvasive eligibility criteria, they will have a POC HbA1c measurement. Should their value be ≤7.2%, they will be invited to attend the clinic for a full screening visit where a complete history will be taken and lab tests performed.
2. *Screening*: If the participant meets initial inclusion criteria, he/she will return for a physical examination, full eligibility testing, and instruction in taking medication for the placebo run-in phase. A fasting 75 gram OGTT will be performed. In order to qualify for further consideration, the fasting glucose must be between 95-125 mg/dl and the 2-hour glucose must be ≥140 mg/dl and the laboratory-based HbA1c must be≤7.0%.
3. *Run-in:* If the above criteria are met, the participant will be asked to undergo a 3-week run-in period. The run-in period (described in Section 3.4.1) allows staff to evaluate a patient's suitability for RISE. At the end of the run-in, clinic staff will assess the patient's

adherence to medication taking, assess final eligibility criteria, and determine whether the patient should be randomized to RISE.

4. *Final screening visit and randomization:* If the participant is eligible, informed consent is obtained, baseline data are collected and the OGTT and hyperglycemic clamp are performed. If after completion of these studies the participant is willing to be randomized, the participant is randomly assigned to a treatment group.

If during the screening process the participant is deemed ineligible, but his/her condition changes during the recruitment period (e.g., the participant has uncontrolled hypertension that becomes adequately treated or an active infection that has resolved), he/she may be reconsidered for inclusion if he/she maintains eligibility for three months. Screening procedures are re-initiated and the participant must meet all eligibility criteria at the time of re-enrollment.

A minimal amount of data is recorded on participants who fail screening (such as age group, gender, race/ethnicity, diabetes duration) for purposes of comparing participants versus nonparticipants.

5 PATIENT MANAGEMENT

5.1 Description of RISE Research Facility Treatment(s)

Study treatment interventions are described in detail above (Section 3.4). Glucose management (including study medications) will be provided free of charge to RISE participants. In accordance with standards of care, HbA1c will be monitored every 3 months. Procedures to be followed for rising HbA1c levels are described in Section 7.4. Beyond the treatment of the glucose-aspect of prediabetes 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, obstructive sleep apnea) 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 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

Study medications will be distributed directly to individual study centers. Individual centers will organize and maintain inventory, in cooperation with their investigational or research pharmacy. Sites will ensure that sufficient medication is always on hand to allow medication

distribution for the next three month period. This includes medications required for the next 12 randomized enrollees (i.e., on average three initial distributions for each of the four treatment arms) plus all of the upcoming distributions to enrolled participants who will be returning within three months. Expiration dates of all medications will be double checked and documented on arrival at the study center and at the time of distribution to participants to ensure that the provided supplies will not expire during the current treatment interval.

Except for the placebos (metformin and liraglutide) distributed during the 3-week run-in period, medication will be dispensed to participants in 3-month quantities, and bottles/boxes/containers will be collected from participants at the quarterly visits to allow compliance assessment by pill counting or residual volume estimation for liquid medications. Subsequent treatment supplies will be provided with each quarterly visit.

Non-study medications will not be provided through the RISE study clinical centers.

5.3 Adherence Measures

Adherence will be documented, monitored, and addressed as part of participant care and management and to monitor whether procedures are being implemented and followed equivalently across all sites. 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 and across all sites. Primary adherence measures will address visit attendance and completion of study procedures, medication adherence, and adherence to recommended home glucose monitoring. Medication adherence will be assessed on a quarterly basis, by pill counting or by residual volume estimation of insulin glargine and liraglutide. Participants will be instructed to bring all study medications (including empty bottles/vials/pens) to study visits.

5.3.1 Placebo Run-in

Metformin placebo compliance will be assessed by pill counting. Liraglutide placebo compliance will be assessed by residual volume assessment.

5.3.2 Liraglutide

Liraglutide will not be blinded in RISE. Liraglutide pens contain 3 ml, and the amount that each participant should use is readily calculated based on their study dosing during and after titration. The expected amount consumed will be calculated and compared to residual amounts in the currently active pen, and in any residual unused pens.

5.3.3 Insulin

Insulin will not be blinded in RISE. Insulin use will be prescribed to assigned participants only for the first 3 months of their participation. The projected volume consumed cannot be generalized, but will need to be calculated for each participant based on their recorded dosing titration and their final dosing. The dosing administration record will be used to assess compliance, and confirmed with a residual volume assessment as with liraglutide pens.

5.3.4 Metformin/Placebo

Active or placebo metformin compliance will be assessed by counting of dispensed and returned tablets.

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, we have predicted withdrawal rates of 10% during the 15-months of primary data collection 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. Individual sites will develop strategies to enhance retention specific to their locale and specific population. Activities at both the national and local level will be coordinated to maximize retention and minimize attrition. Subjects missing scheduled visits will be contacted within 24 hours of the scheduled visit to reschedule and discuss strategies to improve compliance. If the site is unable to contact the subject, the site will send a certified letter to the participant to encourage continuation in the trial even if they no longer wish to take the study medication. At the local level, study staff will implement efforts to personalize the study (mailing out personal notes, birthday cards, etc.).

Attrition is monitored regularly by the CoC and the 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. Assistance is offered to any site with a higher than average attrition rate. Sites will also be encouraged to share their ideas and experiences via regular communication and conference calls for Study Coordinators. The CoC will prepare monthly reports on visit completion, compliance with the RISE Study protocol and participants on inactive follow-up.

6 DATA COLLECTION

6.1 Main Study Procedures

The timing of major study procedures during the different phases of the study is illustrated in Figure 1. Outcomes using hyperglycemic clamp procedures will be obtained at baseline, at the end of 12 months of treatment, and after a 3-month washout period (i.e., 15 months post randomization). Additional outcomes will be obtained from the OGTT concurrent with the hyperglycemic clamp procedures, and also at additional time points -- at 6 months on therapy, and at 9 months after stopping therapy (i.e., 21 months post randomization). Blood and urine samples for planned measures will be collected at these visits, along with extra blood and urine samples that will be stored for future analyses. Further details on these measures are provided below, and are summarized in Figure 1 above. All procedures and measures will be conducted by centrally trained and certified staff.

6.1.1 Fasting Blood and Urine Samples

HbA1c will be measured and fasting blood and urine samples will be collected at each 3-monthly study visit. Aliquots of fasting blood and urine will be stored for subsequent measurement of biomarkers. A blood sample for DNA will be collected at randomization for subsequent genetic analysis should funding become available. Routine chemistries for safety (examples include complete blood count, liver function tests, pregnancy tests) will be done at screening and at six months.

6.1.2 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 10, 20, 30, 60, 90, 120, 150 and 180 min after the glucose ingestion. OGTT procedures will be performed at baseline, at 6 and 12 months on active treatment, and 3 and 9 months after stopping treatment, i.e., 15 and 21 months after randomization.

6.1.3 Hyperglycemic Clamp

Hyperglycemic clamps (goal steady state glucose levels of 200 mg/dl and >450 mg/dl) will be performed at baseline, 12 months post randomization while on active treatment, and 15 months post randomization (i.e., after 3 months of washout). Participants will return to the testing facility to undergo the clamp after a 10-12 hour overnight fast. The clamp will be performed on a day separate from the OGTT.

The clamp procedure will incorporate 3 main sets of blood sampling for the planned outcome measures: first phase (0-10 minutes); second phase (100-120 minutes); and maximal stimulated responses (from 10 minutes before arginine until 10 minutes after arginine). To guide the clamp, a small volume of arterialized venous blood will be obtained at 5-min intervals throughout for clamp for measurement of plasma glucose concentration and titration of the dextrose infusion.

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

6.2 Eligibility Screening Measurements

Screening and run-in procedures are listed in Table 1.

Prescreening (initial contact): Prescreening for potential eligibility will be done using a single random capillary (fingerstick) blood sample for measurement of HbA1C with a POC instrument. Participants are eligible to continue screening procedures if the POC HbA1C is ≤7.2%% and screening questionnaire that includes a focused medical history, anthropometric and blood pressure measures indicate that they meet initial eligibility criteria. Subjects who are discovered to have HbA1c >7.2% will be excluded from participation and referred for formal diabetes assessment and treatment through their regular health care provider(s).

Screening: Participants who meet pre-screening eligibility criteria will present to the study center for formal screening to undergo a standard 2-hour 75 gram OGTT, with samples analyzed in the central laboratory. Only fasting and 2 hour post-load samples will be obtained (a laboratory-based HbA1c must be ≤7.0%, fasting glucose must be 95-125 mg/dl and 2 hour glucose must be ≥140 mg/dl for eligibility in RISE). Eligibility lab tests will also be performed, including electrolytes, TSH[†](when clinically indicated), triglycerides, creatinine, ALT, hemoglobin, and pregnancy test.

Placebo run-in: Participants meeting eligibility criteria will be instructed to begin the 3-week placebo run-in. Participants who have <80% adherence with study medications during this run-in phase will be withdrawn from the study and no further study procedures will be performed. Participants who are adherent with ≥80% of study medication during run-in will proceed to baseline study measurements. Participants will be asked demographic and medical history information.

Table 1: Data Collection During Screening and Run-In Procedures

STEP	TEST / PROCEDURE	COMMENTS
1) Pre-screening	Screening HbA1C (POC)	Continue screening if ≤7.2%
	Screening questionnaire	Initial review of eligibility
	Height, weight and BP	Initial screening for eligibility
2) Screening	HbA1c, 75 gram OGTT (central lab); glucose at 0 and 120 min	Eligibility HbA1c ≤7.0%, fasting glucose 95-125 mg/dl; 2-h glucose ≥140 mg/dl
	Eligibility labs*	Laboratory eligibility criteria
	Demographics, medical history, medications and physical examination	Eligibility evaluation
3) Run-in	Placebo dispensed	Initiate run-in
	Evaluate	Determine eligibility for randomization

*Eligibility labs: electrolytes, TSH[†], triglycerides, creatinine, ALT, hemoglobin

[†]*TSH should be assessed as part of trial screening procedures in the following settings: 1) reported use of anti-thyroid medication, 2) reported use of thyroid hormone, 3) reported history of thyroid disease or 4) clinical suspicion of thyroid disease on history and physical.*

6.3 Baseline and Post-Randomization Measures

Measurements to evaluate safety, adherence, and outcomes are summarized in Table 2. The outcomes, derived from hyperglycemic clamp procedures, will be measured at baseline, 12 months (end of treatment), and 15 months post randomization (end of washout). OGTT outcomes measurements and blood sampling for other outcomes will be performed at baseline, 6 and 12 months following randomization (on treatment) and then at 15 and 21 months (off treatment). Hyperglycemic clamp and OGTT outcomes measures are described in detail above. Other measurements are discussed below.

Table 2: Timeline of Outcomes, Safety Testing, and Adherence Measures

	Baseline/ Pre random-ization	**Post Randomization Month**						
		3	**6**	**9**	**12**	**15**	**18**	**21**
Physical								
Height	x							

	Baseline/ Pre random-ization	Post Randomization Month						
		3	**6**	**9**	**12**	**15**	**18**	**21**
Weight	x	x	x	x	x	x	x	x
Waist / Hip circumference	x				x	x		x
Blood pressure	x	x	x	x	x	x	x	x
Outcomes								
Hyperglycemic clamp & AIR_{max}	x				x	x		
OGTT	x		x		x	x		x
HbA1C	x	x	x	x	x	x	x	x
Questionnaires	x							
Sleep (Pittsburgh Sleep Questionnaire, Epworth Sleepiness Scale, Berlin Questionnaire)	x							
Biomarkers	x		x		x	x		x
Stored Specimens								
DNA	x							
Plasma / Serum	x		x		x	x		x
Urine	x		x		x	x		x
Safety								
SMBG for those on insulin	x	x						
Hemoglobin or Hematocrit	x		x		x			
Blood chemistries**	x		x					
Adverse events	x	x	x	x	x	x	x	x
Pregnancy test^{+} (local measure)	x	x	x	x	x	x	x	x
Medication adherence	x	x	x	x	x			

** fasting serum ALT and creatinine; pregnancy tests as needed based on symptoms and menstrual history

6.3.1 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.

6.3.2 Sleep Assessments

During screening, participants will complete the Pittsburgh Sleep Questionnaire, Epworth Sleepiness Scale and Berlin Questionnaire for analysis of self-reported sleep-related measures as potential predictors of response to treatment.

6.3.3 Safety Measures

Acute treatment effects will be assessed using home glucose monitoring for those assigned to insulin therapy, plus scheduled clinic visits and telephone contact. The study will provide blood glucose monitors and strips to participants assigned to the insulin arm for use for the first 3 months of the intervention (baseline to 3 months). During the insulin treatment phase, participants will record fasting blood glucose once daily, in the morning, to guide dose adjustment for the insulin. Routine chemistries consisting of hemoglobin or hematocrit (local lab), serum liver enzymes (ALT) and creatinine will be collected for evaluation of liver and kidney function at screening and at six months during treatment. 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 prior to study procedures at each visit.

6.3.4 Participant Protocol Adherence

Pill counts will be performed at each visit from run-in to the end of the treatment period. Similarly, insulin or liraglutide pen residual volume will be determined. Participants will be asked to bring all medications to all visits. Self-monitoring blood glucose (SMBG) data will be reviewed at the 3 month study visit for subjects assigned to insulin treatment.

6.4 Visit Windows

Participants will be encouraged to complete visits within a 1-2 week window before or after the scheduled study visit. All participants will have a visit calendar generated at the time of randomization to assist in keeping visits scheduled as required.

7 SAFETY / HUMAN SUBJECTS PROTECTION

This trial will be conducted in compliance with the protocol and all applicable regulatory requirements. All participating clinical sites must have a Federal-wide Assurance with the Office for Human Research Protections. All sites will follow HIPAA regulations.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by the IRB at each participating clinical 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 comprised 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 RISE studies 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 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. At any time during the study, participants will have the option to withdraw from participation without repercussion. In the event that a participant withdraws, study medication will no longer be provided but the arm to which the participant was randomized will remain blinded, as the analysis will be one of intention-to-treat.

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 and Safety Review Officer – The study will appoint an internal Safety Monitoring Committee (SMC) which will 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 at least twice yearly either in person or by conference call to review study progress and safety as described in Section 7.1.

7.2.3 Confidentiality

A. Protection of subject privacy – Participants are identified within the study only by their RISE Participant ID. No confidential identifiers are used in any data collection.

B. Database Protection – All files are stored in a locked cabinet at the clinical centers, and data are entered into a secure, password-protected website maintained by the CoC. 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 records will be kept in a secure locale for a period dictated by local IRB and Institutional policies as well as federal regulations, whichever is longest.

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

7.3 Expected Side Effects

The RISE Study will use the following medications: metformin, insulin glargine and liraglutide.

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.

Insulin glargine: The side effects of insulin glargine include hypoglycemia and weight gain. The occurrence of hypoglycemia will lead to insulin dose adjustment at the discretion of the investigator. Participants will be counseled about the possibility of weight gain while on insulin therapy and given dietary counseling and resources. Participants on insulin will perform SMBG to minimize the risk of hypoglycemia.

Liraglutide: The most common adverse effects associated with liraglutide are gastrointestinal and include nausea, vomiting, and diarrhea. An increased risk of pancreatitis has also been reported. A history of previous pancreatitis is an exclusion criterion and an absolute contraindication to continuing liraglutide. In addition, increased levels of calcitonin, which are associated with medullary thyroid carcinoma, a rare form of thyroid cancer, have been reported in some animal and human studies. A history of medullary thyroid cancer or a family history of MEN-2 is an exclusion criterion in RISE.

7.4 Risk Management

Glucose management (including study medications) will be provided free of charge to RISE Study participants while participating in the study. Participants will receive training about the proper use of hypoglycemic medications as well as education about the symptoms, causes and response to episodes of hyper- and hypoglycemia. Participants who develop symptomatic hypo- or hyperglycemia, or experience any side effects of medications or study procedures will be instructed to call the clinical site. The following procedures will be implemented to minimize risk to participants:

- *Hyperglycemia:* Participants will be instructed regarding the signs and symptoms of hyperglycemia and are to call the clinic with any concerns. To minimize the risk of symptomatic hyperglycemia, the following procedures will be followed:
    - If HbA1c >7% at any routine quarterly visit, therapy will be invigorated by frequent telephone contact and/or visits to encourage optimal medication adherence and lifestyle choices. HbA1c will be obtained at the next quarterly visit. If HbA1c remains >7% but $\leq$8%, frequent contact will be maintained.
    - If HbA1c >8.0% at any routine quarterly visit, therapy will be invigorated and HbA1c will be repeated after 6 weeks. If it remains >8.0%, outcome assessment will be scheduled in 2 weeks (i.e., regardless of time since randomization).
    - If at any time – either a routine visit or when a participant is brought in due to symptoms – the HbA1c >9%, the HbA1c will be repeated within 2 weeks and, if confirmed, the participant will be scheduled for immediate outcome measurement.

- Metabolic decompensation: In the unlikely event that a study subject develops metabolic decompensation, defined as hyperglycemia (plasma glucose >300 mg/dl) accompanied by significant symptoms (e.g., vomiting, dehydration, lethargy) and/or moderate or large urinary ketones, the participant will be evaluated to determine if temporary use of insulin therapy is required. Once insulin is started, the site will attempt to wean insulin within 4 weeks. If insulin cannot be weaned off within 4 weeks, the patient will be censored at that time.
- If outcome measurements are performed prior to the scheduled time points of 12 or 15 months, once completed the participant will be referred back to their primary care provider for further care.

- *Gastrointestinal (GI) symptoms* are a common occurrence with metformin and liraglutide. However, these symptoms more commonly occur early in the course of treatment. All eligible participants will be slowly titrated to the maximal tolerated doses of metformin or liraglutide, in order to minimize gastrointestinal side effects. For metformin, the dose will be started at 500 mg daily and titrated slowly to 1000 mg bid. If GI side effects develop and are mild, the patient will be encouraged to remain on the study medication. If GI side effects are moderate or difficult to tolerate, metformin will be 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 will be reduced to the next step. If GI symptoms resolve, metformin will be re-escalated by 500 mg per day each week until reaching the previously tolerated dose. The participant will continue on the maximum tolerated dose. A similar procedure will be followed for liraglutide, with the relevant doses being 0.6, 1.2 and 1.8 mg daily.

- *Anemia* may be an adverse effect of metformin. It is defined as a hematocrit <30.0%, a hemoglobin <10 gm/dl, a decline in hematocrit by 4% from study entry, or a decline in hemoglobin by 2 gm/dl from study entry. If anemia persists for more than six months despite appropriate therapy, consideration will be given to discontinuing study medication.

- *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 after six months on therapy. Participants must have an eGFR of 45 mL/minute/1.73 m^2 or higher to be eligible. Metformin will be discontinued if eGFR is below 30 mL/minute/1.73 m^2 at the Month 6 visit. 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.

- *Mild hypoglycemia* is common in subjects with prediabetes or diabetes being treated with medications to lower glucose levels into or near the normal range. Participants will be educated to recognize and treat mild hypoglycemic episodes and to contact study staff to report hypoglycemic episodes or receive advice. Adjustments to study medication will be made when appropriate.

- *Severe hypoglycemia* is defined by the need to be treated with glucagon, the need for a third party to resolve a hypoglycemic episode, or loss of consciousness or seizure. Study medications will be adjusted downward as appropriate.

- *Metformin and its placebo are* temporarily discontinued 24 hours before, during, and for 48 hours after any of the following events to reduce the risk of lactic acidosis: 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.

- Other indications for temporary or permanent discontinuation of study medication include:
  - *Pregnancy:* Study medications will be discontinued if a participant becomes pregnant. The participant will be referred to a high risk obstetrics clinic for further care. Study medications will not be administered if a participant plans to become pregnant.
  - *Lactation:* No study medication will be administered to women who are nursing a baby.
  - *Lactic acidosis:* Any study participant who experiences a bout of lactic acidosis will have metformin and metformin placebo permanently discontinued.
  - *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.
  - *Pancreatitis:* Any study participant who is diagnosed with pancreatitis during the study will have liraglutide permanently discontinued.
  - *Medullary thyroid carcinoma:* Any study participant who is diagnosed with medullary thyroid carcinoma during the study will have liraglutide permanently discontinued.

- Adverse effects of the study procedures may occur very rarely and include:
  - *Glucose injection and infusion:* 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.

- *Arginine:* Overdosage, especially in children, can be fatal. To reduce this risk, the dose of arginine will be weight based (0.07 gm/kg in children) with a maximum dose of 5 gm per dose in both adults and children.

7.5 Potential Benefits

There is potential benefit of participation by individuals with either prediabetes or type 2 diabetes. Prediabetic participants are at increased risk of developing diabetes and may benefit from the frequent monitoring and testing that will allow earlier recognition should their condition progress; treatment is also predicted to slow progression from prediabetes to type 2 diabetes. Participants with type 2 diabetes will benefit through the close monitoring and the improvement in glucose control that should occur in all subjects. In addition, they have the potential to receive insulin or liraglutide that should further improve glucose control in them and potentially slow progression of their disease. Further, liraglutide could help with weight loss. As all subjects have a condition that is becoming more prevalent and carries with it the risk of additional complications, participation will also benefit the individual, the researchers and society through the gaining of new knowledge.

7.6 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 entered into the central database will be stored at the CoC on a server that is maintained with the highest stringency of protection. Similarly, data entered into local databases will be stored at the clinical center 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 RISE 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. In 2013 the FDA issued an IND exemption for the RISE Study. Therefore, the study group and NIDDK are not required to report any study adverse events to the FDA.

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.

8.2 Non-serious Adverse Events

Non-serious adverse events 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 identically administered to participants in all 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 following commencement of study-related activities. 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 laboratory result obtained on the patient between visits or at the time of the visit.

The clinical center staff who learn of an SAE must data enter the completed RISE Serious Adverse Event Report prior to the close of the following business day. Upon request, all SAEs will be reported within the prescribed period to the relevant pharmaceutical sponsors.

All pregnancies occurring during the study must be reported by the clinical center staff to the CoC. This reporting must occur prior to the close of the business day that follows the clinical center becoming aware of the pregnancy. Further, the outcome of the pregnancy must be determined and reported by clinical center staff as soon as possible after the pregnancy is completed. All this information will be reported within the prescribed period to the relevant pharmaceutical sponsors.

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. Summaries of the AEs, tabulated by clinic, 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 unmasked manner.

- *Safety Review Officer:* The Safety Review Officer will review any SAEs 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 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

The Safety Monitoring Committee remains blinded to treatment group. If a SAE warrants unblinding, an on-call member of the Safety Monitoring Committee is available for consultation at all times. Unblinding will be performed by a selected member of the study group in combination with the CoC.

9 STATISTICAL CONSIDERATIONS

All analyses will be conducted under the intention-to-treat principle using the treatment as assigned to each participant, and using all available data from all participants. Analyses of study data will be conducted to address the primary and secondary objectives of the trial, other stated objectives, and other interrelationships among elements of study data of interest to the investigators and of relevance to the objectives of the study.

9.1 Primary Outcome and Analyses

Two primary outcomes each measuring ß-cell function after 3-months of washout will be assessed for this trial. In order to maintain a study-wide α=0.05, a closed testing procedure will be used to assess the primary outcome [89]. The closed testing procedure is a method of hierarchical testing which tests higher-order comparisons before allowing lower-level comparisons, thus controlling the type I error and preserving power. First, ß-cell function will be compared across the four treatment groups using an analysis of covariance model adjusted for baseline ß-cell function. From this model, the overall test of equality across the four treatment groups will be computed. If that overall test is significant at the α=0.05 level, then each of the four possible sets of three interventions will be compared in four separate analysis of covariance models. The final significance testing of any set of two treatment groups is only undertaken if the p-values for each of the two 3-intervention tests which include a particular two intervention group are both p<0.05. For example, for interventions I_1, I_2, I_3 and I_4, the first analysis of variance test I_{1234} assesses whether there are any differences among the four groups. If the overall test across the four groups is not significant, testing concludes and no treatment group is declared different from any other. Alternately, if that initial 4-group test is significant at the α=0.05 level, four separate analysis of covariance models with combinations I_{123}, I_{124}, I_{134} and I_{234} are tested. The comparison I_{12} is only tested if both I_{123} and I_{124} are significant at p<0.05 and so forth. The closed testing procedure is chosen as the primary outcome analysis to maintain an overall study-wide α=0.05 while preserving power and allowing each set of interventions to be compared under pre-specified circumstances. The two primary outcomes will be analyzed separately with a total type I error probability of 0.05 for each, i.e. without an adjustment for two separate outcomes.

Each ANCOVA model will employ White's robust (information sandwich) estimate of the covariance matrix of the model coefficients as the basis for inference [90]. This approach preserves power and protects the type 1 error probability when the homoscedastic normal errors assumptions are violated.

9.2 Secondary Outcomes and Analyses

Comparisons of the primary study outcomes will be made for those with vs. without diabetes 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 three intervention groups and the placebo group for the primary measures of ß-cell function at the end of the active intervention phase (12-months) and 9-months post-washout as well as for additional continuous variables of

interest at the end of the intervention phase (12-months) and at 3-months and 9-months post-washout. As for the primary outcomes, all analyses will be adjusted for the baseline value of each outcome and will use the closed testing procedure described above to preserve an overall α=0.05 for those outcomes as well.

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. Correlations will be assessed for measures made both on and off therapy.

Finally, 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.

9.3 Sample Size/Power Calculations

There is little preliminary data on the expected differences in the primary outcomes in previously drug-naïve patients following a washout. Several unpublished analyses provided ranges of possible differences between treated and placebo patients, as well as expected correlations between baseline and follow up measures. Therefore, sample size was based on a minimum effect size (i.e., absolute difference between any two groups divided by the standard deviation (SD)) using a two-sided significance level of α=0.05, and a conservative correlation of 0.57 between baseline and follow up measures. A sample size of 56 per arm (224 total) at the end of the washout provides ≥80% power to detect a minimum effect size of 0.60 between any two treatment groups in either of the primary outcome measurements (see Table 3) for the closed-testing procedure as described above using a baseline-adjusted analysis of covariance. A higher effect size will result in significantly increased power. A total of 255 participants will be recruited to allow for a 10% loss to follow-up over the course of the study.

Table 3: Power for a sample size of 224 at end of study (56/treatment arm)

Minimum Power	Adjusted Effect Size	Correlation
84%	0.62	0.60
81%	0.60	0.57
77%	0.57	0.50

9.4 Interim Analysis Plans

The CoC will provide interim analyses of primary and major secondary trial outcomes to the DSMB on a predefined schedule. These results will not be shared with the study group unless the DSMB recommends early stopping of the trial.

9.5 Stopping Rules

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.

9.6 Handling of Missing Data

It is anticipated that a small number of participants will require immediate rescue therapy and be unable to have the 3-month post-washout evaluation. In these cases, the primary outcome will be missing. A sensitivity analysis which includes these participants will use a conservative approach by assuming that these participants have worse function than the participants who were measured. This is implemented by ranking the observed function values from the participants who completed the washout evaluation, and assigning a lower value (or worst rank) to those with missing data [89]. The groups are then compared using a Wilcoxon rank test.

10 DATA PROCESSING AND MANAGEMENT

10.1 Data Management System

Data are entered by the clinical centers using a web data entry application that directly enters study data into the CoC's study database. The web application guides the clinical site staff member through the data entry process. If an invalid response is entered, the website signals and provides a message about the error and how to solve it. At any point during entry, the staff member can make an electronic note concerning a particular response. Valid individual responses are saved as soon as they are entered. The system includes programmed skip patterns as required by the case report forms, and also includes quality control checks such as lists of valid values for multiple choice items. The system provides automated consistency checking so the study staff can resolve inconsistencies quickly without a lengthy communication with the CoC. The same checking is also run on the central database at the CoC to verify that centers are resolving consistency checks.

10.2 Data Transfer

Newly entered clinical center data are received by the CoC immediately upon data entry. The CoC merges newly received data with the accumulated data in a SAS database. Data transfer from central units such as the central laboratory will be 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 centers for correction or verification. 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

The CoC adheres to the George Washington University Biostatistics Center's data backup and security policies to ensure the safety and confidentiality of the data. 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. George Washington University 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 CoC, 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 clinical center, gender, and race/ethnicity
- tables describing adherence to the study protocol (attendance at scheduled study visits, study intervention compliance)
- database inventory
- number of data edit queries generated and outstanding, by clinical center
- progress of analysis and manuscripts

10.6 Archiving and Study Close-out

At the end of the study, after all data have been received and edited, the database will be archived in computer readable format, including 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, with shared procedures and data collection approaches 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

The major organizational components and their responsibilities are described:

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

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

- The *Coordinating Center (CoC)* located at the George Washington University Biostatistics Center operates under subcontract to the Seattle Institute for Biomedical and Clinical Research (SIBCR). The CoC has responsibility for coordinating the study, including production and distribution of materials and documents, set-up and administration of the data management system, maintenance of the central database, analysis of primary study results, distribution of final study data to the clinical centers and NIDDK repository, and reporting of results in collaboration with the other investigators.

- 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 the tests and assays, for performing quality control, for providing patient-level reports to clinical centers, and for transferring results to the CoC.

- 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.

11.2 Central Laboratories and Reading Centers

In collaboration with the CoC 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 and NIH policy 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 held by the study group. The CoC staff and selected clinical center staff with expertise in various study components will provide instruction in all aspects of the study. The purpose of the training workshop 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, physical, metabolic 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, and supplying the CoC with an IRB approval letter and stamped informed consent forms.

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 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 Study 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, and 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 Study 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, NIDDK Program Official and study group member) determines (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 Executive 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 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. Major factors in consideration of ancillary studies will include:

- Clinical importance and scientific validity;
- Compatibility of goals with those of RISE; and
- Burden on study subjects and staff, including those at the CoC

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 study.

11.9 Protocol Amendments

Adoption of protocol amendments requires two-thirds majority approval by voting members of the RISE Steering Committee and approval by the DSMB. The amended protocol will be resubmitted to the IRB, and the participant consent will be revised appropriately.

11.10 Repository for Storage and Distribution of RISE Data and Samples

At the end of the study, de-identified research data and samples of blood and urine 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 or postpone the onset of obesity and/or diabetes.

As a component of the informed consent process, 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 allows for recruitment over the first 3 years of the study, which commences after all approvals have been obtained (DSMB, FDA, IRBs, CTAs or CRADAs), central study training has been performed and all study medications and supplies have been procured. Intervention and washout phases occur through the middle of year 5 and presentations and publications throughout the period of the study.

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