

The Effect of Bariatric Surgery on Metabolism,
the Metabolome and the Microbiome in
Patients With Type 2 Diabetes

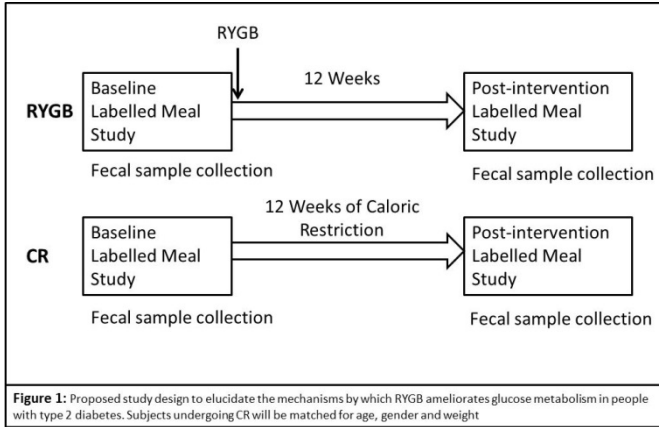
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Abbreviations: RYGB: Roux-en-Y Gastric Bypass; CR: Caloric Restriction; CRU: Clinical Research Unit; GLP-1: Glucagon-like peptide-1; DI: Disposition Index; ϕ : β -cell responsiveness; S: Insulin action; EGP: Endogenous Glucose Production; Meal Ra: Meal Appearance; Rd: Glucose Disappearance

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Bariatric surgery is associated with resolution of type 2 diabetes, and remission rates of ~84% have been observed after Roux-en-Y Gastric Bypass (RYGB), influenced by factors such as diabetes duration and treatment [1]. Improvements in glycemic control are seen well before any significant weight loss is produced and patients are often dismissed from the hospital with a marked decrease in their diabetes medications. The necessary calorie restriction that occurs as a result of post-surgical anatomical changes also results in better glycemic control [2]; however this does not explain the known superiority of RYGB over equivalent caloric restriction in improving metabolic parameters, particularly glucose metabolism (Figure 2). Although it is clear that over the

longer-term RYGB may be superior in terms of sustainable effects on fat loss and β -cell function, it remains uncertain to what extent CR contributes to the effect of bariatric surgery on glucose metabolism.

Recently, we have demonstrated that CR and RYGB are both associated with a decrease in fasting endogenous glucose production (EGP) in people with type 2 diabetes (Figure 2). It is unclear if the mechanism by which this is achieved is shared by the two interventions.

One newly established methodology to enable untargeted elucidation of the metabolic pathways that are altered by a given intervention is large-scale metabolomics. Metabolomics is used to profile the metabolome – defined as the range of endogenous small-molecule chemicals produced in an organism. Metabolomic profiling via mass spectroscopy in our comprehensive metabolomics center will enable quantitative assessment of the metabolomic changes that occur with CR and RYGB and elucidate changes that are shared or otherwise by these two interventions. Metabolomic profiling via mass spectroscopy is already enhancing the understanding of complex metabolic mechanisms and pathways involved in, for example, cardiovascular disease where it has aided with risk stratification and event prediction thus allowing care to be individualized [3]. The metabolomic changes produced by calorie restriction in overweight healthy volunteers have been shown to be associated with an improvement in insulin action [4]. RYGB also produces differences in the circulating metabolome, in particular decreases in branched chain amino acid and increases in certain metabolites which are of gut origin [5, 6]. In this application we provide preliminary data to show differences in the Amino Acid metabolome between RYGB and CR.

The microbiome, or gut host environment, consists of approximately 10^4 cells and has been the subject of much interest as it relates to energy homeostasis [7]. It changes in response to food intake and surgical intervention. There is compelling evidence to suggest that changes in the gut microbiome after RYGB are associated with improved insulin sensitivity [8, 9]. Using 16S rRNA-encoding gene sequences we will characterize the structure and dynamics of complex microbial communities, specifically how they change with intervention and then subsequently how the microbiome influences the circulating metabolome.

Whether the changes seen in the metabolome and microbiome are cause or consequence of the weight loss and resultant improvements in glucose homeostasis following Roux-en-Y gastric bypass has not been fully investigated and may reveal novel mechanisms in the study of glucose metabolism following weight loss surgery. The bioinformatics aspects of the Microbiome Program supported by the Mayo Clinic Center for Individualized Medicine (CIM) and the Metabolomic Core will be integral parts of this project.

Results of this proposal will be used to support an application for extramural funding at the end of this funding period.

Approach (Figure 1): Volunteers with type 2 diabetes and obesity who are scheduled to undergo RYGB as part of their clinical care will be studied immediately prior to surgery and at 12 weeks post-operatively using a labeled mixed meal to enable simultaneous measurement of glucose metabolism and insulin secretion and

action [10]. Blood samples for targeted metabolic profiling will also be drawn. A fecal sample to characterize the microbiome will be collected at both visits. A matched control group of subjects with type 2 diabetes and obesity who are not scheduled to undergo bariatric surgery will also be recruited and studied at baseline and 12 weeks following caloric restriction similar to that required post-operatively after RYGB. We will utilize weekly meetings with a dietician and a psychologist to ensure compliance with the prescribed diet.

The overall objective of this proposal is to form the basis of a line of investigation that will underpin my research career and future extramural funding. We will address the following specific aims: -

Specific Aim 1: Characterize the changes in the circulating metabolome that occur after Roux-en-Y Gastric Bypass in contrast to caloric restriction alone.

Hypothesis: *Metabolomic changes after Roux-en-Y gastric bypass surgery are associated with a decrease in endogenous glucose production in subjects with type 2 diabetes.*

Specific Aim 2: Characterize the changes in gut microbiome in subjects with type 2 diabetes after Roux-en-Y gastric bypass, in contrast to caloric restriction alone.

Hypothesis: *Gut microbiome changes unique to Roux-en-Y gastric bypass influence remission of type 2 diabetes.*

Preliminary Studies

A. Mathematical modelling of glucose turnover.

The meal minimal model can measure insulin secretion and action [11, 12] [13] and has been validated using hyperglycemic and euglycemic clamps as well as tracer infusions to directly measure glucose turnover. We have shown that insulin sensitivity (S_i) and β -cell responsiveness (ϕ) can be estimated from measured glucose and C-peptide concentrations [12, 14]. The appropriateness of insulin secretion for the prevailing degree of insulin action is assessed by calculating the total disposition index (DI_{total}), which equals the product of insulin secretion and insulin sensitivity ($\phi_{total} \times S_i$).

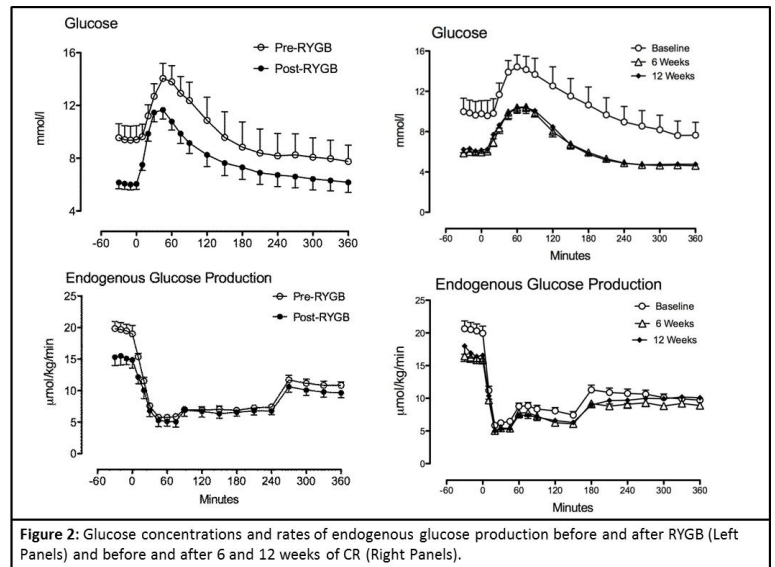


Figure 2: Glucose concentrations and rates of endogenous glucose production before and after RYGB (Left Panels) and before and after 6 and 12 weeks of CR (Right Panels).

B. Triple-tracer methodology for reliable

RYGB	Metabolite	Caloric restriction
↑		↑
↓		↓
*	Histidine	
*	Aspartic Acid	
*	alpha-Aminoadipic-acid	
*	beta-Aminoisobutyric-acid	
*	Cystathionine 1	
*	Methionine	
*	Asparagine	
*	Taurine	
*	Glycine	*
*	Alanine	*
*	Proline	
*	Ornithine	
*	Lysine	
*	Valine	
*	Tryptophan	

measurement of postprandial glucose fluxes. The dual-isotope method pioneered by Steele et al. enabled simultaneous measurement of both the systemic rate of appearance of ingested glucose ($R_{a\ meal}$) and postprandial endogenous glucose production [15]. We developed and validated a novel triple-tracer approach to minimize changes in both meal and endogenous plasma tracer-to-tracee ratios thereby allowing simultaneous measurement of $R_{a\ meal}$ and endogenous glucose production [10, 16]. We will use the validated triple-tracer method to measure glucose disappearance, endogenous glucose production and meal appearance in study subjects.

C. Differences in the metabolomic profile in subjects after RYGB vs. CR.

Targeted mass spectroscopy of the metabolomic profile in subjects after RYGB and CR showed clear differences in amino acid profiles between groups, despite comparable weight loss (Table 1). The changes shared by the two interventions were an increase in glycine and a decrease in alanine concentrations. Alanine is extracted by the liver and utilized in hepatic gluconeogenesis [17]; hence decreased concentrations may contribute to improvements in endogenous glucose production seen in both groups (Figure 2). Another interesting finding is the decrease in alpha amino adipic acid concentration unique to the RYGB group. An elevated amino adipic acid concentration is a robust biomarker for increased risk of developing diabetes [18].

Research and Methods

Study Subjects: Individuals aged 20-65 years seen for weight management in the Nutrition Clinic at the Mayo Clinic, will be recruited to the study prior to undergoing Roux-en-Y Gastric Bypass (RYGB). We will recruit 15 subjects who have a fasting glucose concentration of ≥ 126 mg/dl on two or more occasions or who have a history of type 2 diabetes or impaired fasting glucose treated with one or two oral agents other than thiazolidinediones. Potential participants will attend the Mayo Clinic Clinical Research Trial Unit (CRTU) for a screening visit. In addition, we will also recruit 15 subjects with type 2 diabetes or impaired fasting glucose who are **not** interested in pursuing surgical intervention but are willing to undergo supervised caloric restriction as detailed below. The 2 groups will be matched for age, gender and BMI and duration and severity (HbA_{1c} and no. of oral medications needed to achieve glycemic control) of diabetes.

Exclusion Criteria: Subjects <20 years of age will not be studied to minimize the possibility of type 1 diabetes. Subjects > 65 years of age will not be studied to minimize the potential confounding effects of age on glucose tolerance. Subjects on chronic antibiotic therapy will be excluded due to their influence on gut bacteria. Subjects will refrain from using antibiotics for 2 weeks prior to the screening visit until completion of the study, except for routine peri-operative use and as clinically indicated. Healthy status will indicate that the participant has no known active systemic illness and no active microvascular or macrovascular complications of their diabetes.

Screening Visit: Subjects will provide written informed consent and undergo a history and physical examination along with blood collection for complete blood count, fasting glucose, HbA_{1c} and chemistry group; urine collection to exclude infection, proteinuria or pregnancy and ECG. Subjects will ensure that they have not taken any antibiotics for 2 weeks prior to the screening visit. The Minnesota Leisure-Time Physical Activity questionnaire will be administered to assess habitual activity levels [19]. They will be given instructions on managing their diabetes medications. Subjects will also complete the validated bowel disease questionnaire to ensure that there are no functional or organic bowel symptoms [20]. A receptacle to collect a baseline fecal sample prior to Study 1 will be provided to subjects.

Body Composition: Body composition will be measured at the screening visit using dual-energy X-ray absorptiometry (iDXA scanner; GE, Wauwatosa, WI) and abdominal CT Scan with cuts at L2/3 and T11/12 to determine percent body fat and visceral fat [21].

Experimental Design: Subjects will be studied on two occasions, the second study will occur approximately 12 weeks after Roux-en-Y gastric bypass or equivalent caloric restriction.

Study 1 (baseline): All subjects will be admitted to the Mayo Clinic CRTU at 17.00 hours the evening before the study. A fecal sample, produced within 48 hours of the start of the study, will be collected. Following ingestion of a standardized low calorie meal (197 Kcal: 40% carbohydrate, 30% fat, and 30% protein) subjects will fast overnight. The meal will occupy a volume that is tolerable to patients who have undergone restrictive upper gastrointestinal procedures. Approximately at 05:30 a cannula will be inserted retrogradely into a vein of the dorsum of the hand. This will be placed in a heated Plexiglas box maintained at 55°C to allow sampling of arterialized venous blood. A cannula will also be placed in the vein of the contralateral hand to allow infusion of tracers. Approximately at 06:30 hrs (-180 minutes) subjects will receive a primed continuous infusion of [6,6-²H₂] glucose. At the start of the study, approximately 09:30 hrs (0 minutes) subjects will consume a mixed meal consisting of one scrambled egg, 55g of ham), and Jell-O containing 35g of glucose enriched with [1-¹³C] glucose (to~4%) as previously described. An infusion of [6-³H] glucose will be started at the same time at a rate that mimics the anticipated glucose appearance of the [1-¹³C] glucose contained within the meal. At the same time, the rate of infusion of the [6,6-²H₂] glucose will be altered so as to approximate the anticipated pattern of fall in endogenous glucose production as previously described in detail [21-23]. Blood will be collected to allow measurement of glucose, tracer and hormone concentrations. At the end of the study (15:30, 360 minutes), the cannulae will be removed; participants will consume a late lunch and leave the CRTU.

Intervention: Subjects participating in both arms of this study will be asked to consume an identical diet that is controlled for caloric content and macronutrient composition (Table 2). This is identical to that consumed by patients undergoing Roux-en-Y gastric bypass at the Mayo Clinic. Subjects in the surgery arm will start the diet after surgery, while subjects in the caloric restriction arm will do so after the screening visit.

The diet consists of three small pureed meals (740kcal daily: 30% protein, 15% fat, 55% carbohydrate) per day for the first 4 weeks after surgery. In the group not undergoing surgery, compliance will be monitored in all participants by weekly weigh-ins and weekly meetings in the Division of Endocrinology or CRTU. A meeting with the dietician, to review the daily dietary record, will alternate with a meeting with the clinical psychologist to reinforce compliance techniques. Subjects will then progress to a soft diet consisting of 3 small meals of tender, easily-chewed pieces (each meal is approximately 1 cup of food), for an approximate daily intake of 875 kcal. We have previously been able to accomplish this in subjects with type 2 diabetes (see Figure 2).

Post-op (Wk)	Time	Liquid Intake	Food Intake
0-4	7:30 to 8 am		Scrambled egg (75kcal) ¼ cup cooked cereal (40kcal)
	8:30 am to noon	8 fl oz. skim milk (80kcal) + sugar-free instant breakfast mix (100kcal) 8 fl oz. water	
	12:30 to 1 pm		¼ cup pureed lean meat (77kcal) ¼ cup pureed vegetables (15kcal)
	1:30 to 5 pm	8 fl oz. skim milk (80kcal) 8 fl oz. water	
	5:30 to 6pm		¼ cup pureed lean meat (77kcal) ¼ cup pureed vegetables (15kcal)
	6:30 to 9:30pm	8 fl oz. skim milk (80kcal) + sugar-free instant breakfast mix (100kcal) 8 fl oz. water	
	Approximately 740kcal daily – 30% protein, 15% fat, 55% carbohydrate		
5-12	7:30 to 8 am		Scrambled egg (75kcal) ½ cup cooked or dry cereal unsweetened (80kcal) ¼ cup unsweetened canned fruit (30kcal)
	8:30 am to noon	8 fl oz. skim milk (80kcal) + sugar-free instant breakfast mix (100kcal) 8 fl oz. water	
	12:30 to 1 pm		½ cup cottage cheese low fat (110 kcal) ¼ cup cooked vegetables (15kcal) ¼ cup unsweetened canned fruit (30kcal)
	1:30 to 5 pm	8 fl oz. skim milk (80kcal) 8 fl oz. water	
	5:30 to 6pm		2 ounces of lean meat (well chewed) (110 kcal) ¼ cup mashed potatoes (40kcal) ¼ cup cooked vegetables (15kcal) ¼ cup sugar free “lite” yogurt (20kcal)
	6:30 to 9:30pm	8 fl oz. skim milk (80kcal) 8 fl oz. water	
	Approximately 875kcal daily – 30% protein, 15% fat, 55% carbohydrate		

Table 2: Post-RYGB surgery diet, weeks 0-12.

Study 2: This study will occur in the 12th postoperative (or equivalent) week to ensure that the participants have recovered, have adequate hemoglobin and are tolerating oral intake. The study will be identical to Study 1. The same meal will be consumed in both Study 1 and 2. Subjects will not resume antidiabetic medication in the 12 weeks after surgery. Body composition will be measured the week prior to study. Height and weight will be measured in the CRTU at the time of admission. **Analytic Techniques:** All analytic techniques for blood samples are either established in the applicant's mentor's laboratory or are routinely performed in the Mayo GCRC Mass Spectrometry, Immunochemical Core laboratories or GI imaging and Endoscopy core of the Mayo CTSA.

Exendin-(9,39) Amide Study:

Of the fifteen subjects undergoing Roux-en-Y Gastric Bypass (RYGB), we propose to study four of these subjects in the 4th postoperative week, to ensure that the participants have recovered, have adequate hemoglobin (as determined by PI) and are tolerating oral intake. Subjects will not resume antidiabetic medication in the 4 weeks after surgery. The study will be identical to Study 1 and 2. The same meal will be consumed. Body composition will be measured using dual-energy X-ray absorptiometry (iDXA scanner; GE, Wauwatosa, WI), the week prior to study. Height and weight will be measured in the CRTU at the time of admission. At approximately 0930 (0 minutes) an infusion of exendin-(9,39) amide, an inhibitor of active GLP-1 in humans, will be infused at 300pmol/kg/min in half of the study subjects ([24, 25]. The infusion will be continued till the end of the study, at 360 minutes. The remainder of the subjects will receive a saline infusion. Assignment of exendin-(9,39) amide or saline infusion will be in a randomized fashion (1:1).

Metabolomic analysis: A tandem mass spectrometer with stable isotope dilution for quantification will be used to define the metabolomic profile in both cohorts.

Microbiome Laboratory. The microbiome lab in the Medical Sciences Building at Mayo Clinic houses 2 computers containing 32-core AMD processors and 64 GB of RAM on which the calculations and modeling proposed will be carried out. The DNA extraction and library preparation facility includes a FastPrep 24 beadbeater, 2 PCR machines, a qPCR machine, a biosafety cabinet, automated and manual gel electrophoresis, and all other equipment and reagents necessary for DNA extraction and 16S rDNA amplification. The Medical Genomics Facility houses 6 Illumina Hi-Seq, 2 Illumina Mi-Seq, Sanger, and Pac-Bio sequencers.

Calculations: Insulin sensitivity (S), β -cell responsivity indices and total disposition index (DI_{total}) will be calculated from plasma glucose and insulin concentrations using the "oral" glucose minimal model [26]. [6-³H] glucose will be used to trace the appearance of the ingested [1-¹³C] glucose and [6,6-²H₂] glucose will be used to trace endogenous glucose production. As previously described, meal appearance will be calculated by multiplying the systemic rate of appearance of [1-¹³C] glucose by the [1-¹³C] glucose in the meal [10]. In addition to measurement of peak hormone concentrations, we will calculate area above basal for the first 2 hours and for all 6 hours after meal ingestion using the trapezoidal rule as previously described [27]. Differences in the metabolomic profile between groups will be quantified. Differences in the microbiome profile between groups will be quantified.

We will subsequently explore correlations between changes in glucose metabolism and multiple metabolites. The metabolome and microbiome analysis will be undertaken in close collaboration with the bioinformatics core (Dr. Surendra Dasari, Ph.D.) to examine qualitative and quantitative differences as well as undertake pathway analysis to potentially identify specific pathways affected uniquely by CR or RYGB.

Statistical Analysis. We will examine between-group (RYGB vs. CR) differences from the baseline study to the post-intervention study (Study 2- Study 1). To do so we will utilize an analysis of covariance adjusting for relevant covariates (e.g. age, gender and change in BMI or lean body mass after surgical intervention) as previously described. We will primarily examine between group differences in:

1. **Endogenous Glucose Production:** Based on data from previous studies [27-29], 15 subjects per group would provide approximately 80% power to detect a 25% difference in nadir EGP values and 87% power to detect a 25% difference in integrated EGP values.
2. **Disposition Index:** 15 subjects per group would provide approximately 80% power to detect an association of intervention type corresponding to a 35% difference in mean DI values relative to the overall mean [28, 30].
3. **Alpha-Aminoadipic Acid:** 15 subjects per group would provide approximately 90% power to detect a 15% difference in mean values.

Interpretation. In the RYGB group, if the improvements in glucose metabolism are associated with a metabolomic signature that is unique, then we will accept our primary hypothesis that metabolomic changes after surgery are associated with a decrease in endogenous glucose production in subjects with type 2 diabetes. We will be able to differentiate between changes in insulin secretion (ϕ_{total}), insulin action (Si) and DI to determine the mechanistic basis for the observed improvements. If the improvements in glucose metabolism are associated with a unique change in the gut microbiome, then we will accept our secondary hypothesis that unique changes in the gut microbiome influence remission of type 2 diabetes. We will specifically be looking for metabolomic markers of the gut microbiome, which have been shown to influence insulin sensitivity [8, 9] and energy harvest, such as short-chain fatty acids [31].

Limitations. Changes in the gut microbiome and circulating metabolome are influenced by dietary intake. Our study design is such that both groups will have similar, but not identical macronutrient composition of their meals. We will minimize differences by utilizing a registered dietician as well as a clinical psychologist to help ensure compliance with the prescribed diet and discourage food consumption outside of what is provided by the study.

Future Directions. Identification of a unique metabolomic profile after RYGB will permit manipulation of concentrations of these substances in overweight subjects with type 2 diabetes to determine their effect on weight loss and glucose metabolism. I hope to use the results of this study to secure extramural funding at the end of this award and be able to continue working on the role of bariatric surgery in the amelioration of type 2 diabetes.

Human Subjects

Detailed Description: Suitable volunteers will be asked to visit the CRTU on a total of 14 occasions, including screening. During the first or screening visit they will meet with a member of the study team and undergo a history and physical examination to ensure that they fulfill entry criteria. If eligible, they will be asked to undergo determination of body composition. Subjects will be admitted on the eve of the study day. After an overnight fast, a cannula will be placed to allow IV infusion. Infusions will be started. In addition a retrograde hand vein for blood draws will be placed. This hand will be placed in a Perspex hot-box heated to 55degC. Subsequently they will ingest a mixed meal and blood will be sampled several times (via the retrograde hand vein) to obtain arterialized venous samples. The study will be repeated once at twelve week intervals.

Population: Subjects will be recruited from the Mayo Nutrition Clinic and from Olmsted County residents and surrounding counties, who have previously indicated a willingness to participate in research – and who meet entry criteria. The racial composition of the county is outlined in the table below (%) using data from the 2000 population census. No children or prisoners will be recruited.

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other	Total
Female	0.15	2.8	1.8	1.5	43.8	0	51.5
Male	0.15	2.8	1.8	1.5	43.7	0	48.5
Total	0.3	5.6	3.6	3.0	87.5	0	100.0

Research Materials: We will be obtaining blood samples to measure hormone and glucose concentrations in response to meal challenge.

Recruitment of Subjects: Subjects will be recruited by means of advertisements placed within our institution and from the nutrition clinic. Potentially eligible patients in the Mayo Nutrition Clinic will be identified by the physicians caring for the patient, and if interested in participating will contact the research team. If insufficient subjects are recruited by this approach, advertisements will be taken out in local newspapers until recruitment

is complete. Eligible subjects will be invited to participate in the study and to come to the CRTU for a screening visit.

The following are exclusion criteria;

- (a) Age < 20 (to avoid studying subjects who could have type 1 diabetes)
- (b) > 65 years of age will not be studied to minimize the potential confounding effects of age on glucose tolerance..
- (c) For female subjects: positive pregnancy test at the time of enrollment or study visits
- (d) Previous treatment with thiazolidinediones.
- (e) chronic antibiotic therapy
- (f) active systemic illness
- (g) active microvascular or macrovascular complications of their diabetes.

Potential Risks: Blood sampling. Blood samples are collected by venipuncture for this study. Bruising can occur with venipuncture, as can fainting, etc. Risk Monitoring / Risk Reduction: The samples are collected using aseptic technique in designated venipuncture areas of the CRTU where facilities are available should untoward reactions (fainting, etc.) occur. Given the aseptic nature of the sample collection and the small risk of bruising, the monitoring plan is focused on advising volunteers to call the investigators should they have unusual pain or discomfort from the venipuncture site. Blood drawn within an 12 week period will not exceed 550mL (one pint).

Vascular catheter placement. Catheter insertion, intravenous infusion and blood withdrawal are associated with a small risk of phlebitis. Risk Monitoring / Risk Reduction: This will be minimized by careful attention to sterile technique. If phlebitis occurs, it will be treated conservatively with heat and when appropriate, with antibiotics. The catheters will be cared for by experienced CRTU nurses in order to minimize the risk of these complications. In all protocols, “arterialized – venous” blood will be obtained by placing a hand in which a catheter has been inserted in a heated box during the study. The temperature inside the box is maintained at ~55°C. With prolonged exposure to continuous heat, there is a potential risk of local skin irritation or a minor burn. If this occurs, it will be treated appropriately. Catheter risks will be discussed with the volunteers prior to obtaining consent for the study.

Tracer and Hormone Infusion. Tracers infusions carry a risk of allergic reactions, bruising, discomfort at the site of infusion, and infection. Risk Monitoring / Risk Reduction: All infusates are prepared by trained personnel in a laminar flow cabinet using aseptic technique. Infusions take place on the CRTU where facilities are available should untoward reactions occur. Exendin-(9,39) (CS Bio, Menlo Park CA) will be prepared in a similar manner.

Radiation. Subjects will be exposed to radiation in this study. Radioactive tracers will be used in the proposed studies. Lean body mass, percent body fat, and visceral adiposity will be measured at the time of screening using DEXA (dual energy x-ray absorptiometry) and CT-scan. Risk Monitoring / Risk Reduction: The lowest dose of radioactive tracers that can be reliably counted in plasma will be used. In all instances, the amount of radiation that a volunteer will receive will be well below levels that result in significant risk of harmful effects. Proposed radiation exposure will be reviewed by the Mayo Clinic Radiation Safety Board prior to initiation of any study. Women who could become pregnant will be required to have a negative pregnancy test prior to participation in each study utilizing radioactive tracers.

Confidentiality. All studies expose participants to the psychosocial risks arising from any breach in confidentiality. The risks include anxiety, confusion, damage to family relationships or a compromised ability to obtain insurance or employment. These risks may not be confined to the individual but may extend to other family members. Risk Monitoring/Risk Reduction: The nature of the information obtained will be explained in detail to each participant. All information will be stored anonymously in the database and only the PI or one of

his designates will have access to the data.

Data Safety and Monitoring Plan. The ultimate goal of this application is to further our understanding the role of different therapies in the management of diabetes. The DSMP utilized will adhere to the protocol approved by the Mayo Clinic IRB. We propose the following plan: -

Data quality and management: The principal investigator will review all data collection forms on an three-monthly basis for completeness and accuracy of the data as well as protocol compliance.

Adverse events grading: The common grading scale listed below will be used to grade AEs:

- 0 No adverse event or within normal limits or not clinical significant
- 1 Mild AE, did not require treatment
- 2 Moderate AE, resolved with treatment
- 3 Severe AE, resulted in inability to carry on normal activities and required professional medical attention
- 4 Life threatening or disabling AE
- 5 Fatal AE

Attribution scale: An adverse event includes both, an expected side effect that is of a serious nature, or an unexpected side effect/ event regardless of severity. All events will be graded as to their attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol). Any event that is reported to either the principal investigator or his designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such.

Data Monitoring. The majority of data generated from these protocols will be from analyses performed in our laboratory or the immunochemical core laboratory. Standard quality control procedures are in place for each assay. Genotyping results are examined for consistency between repeated genotypes of a given sample chosen at random. The frequency of data review for this study differs according to the type of data and can be summarized in the following table:

Data type	Frequency of review
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	three-monthly basis
Adverse event/safety rates (injuries)	Weekly
Study data	three-monthly basis
Annual report	Yearly for CRTU, IRB

Informed Consent. Written informed consent will be obtained from all individuals who participate in the study. The principal investigator or member of study team, meet with each participant, review the consent form in detail and confirm the subjects understanding of the study. They answer all questions posed by the participants and when convinced that the subject verbally demonstrates understanding of the protocol obtains a signed consent. Only designated staff are authorized to obtain informed consent.

Benefits: This study exposes subjects to risks detailed above. However, as detailed it will advance our knowledge of how to treat diabetes and help make clinicians make rational therapeutic choices in the future.

VI. Gender/Minority Mix

The majority of residents in Rochester, Minnesota and surrounding counties are White, recent population estimates from over the past decade have indicated that minorities make up a significantly larger segment of our communities. According to census area data such cultural groups as the Somalis, Hispanic/ Latinos, and South East Asians have increased the percentage of minorities living in Rochester and the surrounding counties from 3% in 1990 to nearly 10% in 2000. Despite the increases in the number of minorities within rural Rochester, Minnesota, it is likely that recruitment will fall short in the area of minority participation. However, we are actively working with the minority outreach specialist in the Center for Patient Oriented Research and Tribal Elders from the local minority populations, to develop complementary community-based strategies for recruitment of minorities at Mayo Clinic Rochester.

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