A Low Biologically Available Glucose and High Protein Diet for Treatment of Type 2 Diabetes Mellitus

Abbreviated Title: The LoBAG Diet and Type 2 Diabetes Mellitus
Alternate Abbreviated Title: **The Diet and Diabetes Study**

Alternate Title: A weight neutral, high protein, moderate carbohydrate diet for type 2 diabetes mellitus
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Project Summary

Diet is a cornerstone of type 2 diabetes mellitus (DM2) treatment. Current standard of care diet recommendations focus on caloric restriction and weight loss, goals which can be difficult for patients to achieve. A moderate carbohydrate, high protein diet has been designed specifically for people with DM2, and has shown promising results in preliminary studies. This diet, called the Low Biologically Available Glucose (LoBAG) Diet, does not require caloric restriction or weight loss to produce beneficial effects. Preliminary data are exciting but studies were done only in a small subject population and in a carefully controlled research setting that was not typical of life in the real world. The purpose of this project is to expand upon the preliminary studies and offer the LoBAG diet to a free-living population of people with DM2. In addition, as part of this project, we propose a pilot study to assess the changes in the gut microbiome that occur with this diet in people with DM2.

Subjects with DM2 will be enrolled in a randomized, parallel group trial of the LoBAG diet or a control diet over a 12 week intervention period. The subjects will be free-living and will do food preparation on their own. The main intervention will be instruction in either the LoBAG diet or the control diet. Measures of glycemic control will be collected before, during, and after the diet intervention. Stool samples will be used to collect preliminary data on gut microbiome changes. The diets will be isocaloric and subjects will not be asked to lose weight during the study. Our specific aims include: 1) to determine if the LoBAG diet improves hemoglobin A1c (HgbA1c) in a free-living population with DM2, 2) to investigate the effects of the LoBAG diet on fasting and postprandial glucose and insulin, fructosamine, and lipids, and 3) to determine the effects of the LoBAG diet on the composition of the gut microbiome. The hypothesis to be tested is that the LoBAG diet will produce a significant reduction in HgbA1c when compared to the control diet. The significance of this project will be to expand the generalizability of the LoBAG diet to a free-living population and to potentially improve dietary recommendations for the treatment and control of DM2.

1. Background

1.1. The LoBAG diet impressively improves glycemic control for subjects with DM2 in a controlled research setting. There remains a crucial need to translate these findings to a free-living population with DM2.

Diet has the potential to be a readily accessible and powerful tool for treatment of DM2. The specific components of the ideal diet, however, have not been clearly defined. According to the American Diabetes Association (ADA), there is no one ideal diet for treatment of DM2, and people with DM2 should focus on an eating pattern rich in nutrient dense foods in appropriate portion sizes (1). For those who are overweight or obese, caloric restriction and weight loss is recommended. Unfortunately, this goal is not usually achieved by patients.

There is both common perception and plentiful evidence that dietary alteration improves glycemic control in DM2 through caloric restriction and weight loss. Yet, studies of a moderate carbohydrate, high protein diet demonstrated impressive improvement in glycemic markers in subjects with DM2 *in the absence of caloric restriction or weight change* (2-5). This diet, the Low Biologically Available Glucose ($LoBAG_x$) diet (subscript indicates percent carbohydrate content in diet), was designed by Nuttall and Gannon for treatment of DM2 (6). The rationale for the design came from two observations made in their previous work: glucose in the diet is the major determinant of postprandial plasma glucose levels, and dietary protein stimulates insulin secretion (6). In a randomized, crossover trial of the $LoBAG_{20}$ diet (20% carbohydrate, 30% protein, 50% fat) compared to a control diet (55% carbohydrate, 15% protein, 30% fat) in 8 men with untreated DM2, glycated hemoglobin decreased from 9.8 ± 0.5% to 7.6 ± 0.3% ($p < 0.001$) over 5 weeks. In contrast, there was not a significant change in glycated hemoglobin with the control diet. In addition, both fasting and postprandial plasma glucose decreased with the $LoBAG_{20}$ diet, as did the net mean 24 hour integrated plasma glucose area response, which decreased by 77%. Subject weight remained stable on both the LoBAG and control diets (3).

Although the $LoBAG_{20}$ diet was effective, the significant limitation of carbohydrate content compared to a typical American diet was felt to reduce acceptability. Consequently, the $LoBAG_{30}$ diet (30% carbohydrate, 30% protein, and 40% fat) was designed with a goal of increasing acceptability of the diet for a general population of patients with DM2. The beneficial effect on glycated hemoglobin persisted. In a randomized, crossover trial of the $LoBAG_{30}$ diet in 8 men with untreated DM2 (2 completed only the test diet period) total glycated hemoglobin decreased from 10.8 ± 0.4% to 9.1 ± 0.5% ($p < 0.001$) after 5 weeks of the $LoBAG_{30}$ diet. Weight remained stable. There was a decrease in both fasting and postprandial plasma glucose concentrations with the $LoBAG_{30}$ diet, and a decrease in 24 hour plasma glucose area response (4). A second study of the $LoBAG_{30}$ diet in 8 men with untreated DM2 demonstrated that the decrease in glycated hemoglobin continued after 5 weeks, with a decrease in mean glycated hemoglobin from 10.0% at baseline to 8.7% at 5 weeks and 7.5% at 10 weeks (5). A main conclusion from the $LoBAG_{30}$ studies was that the diet was similarly effective to the $LoBAG_{20}$ diet in improving glycemic control in subjects with DM2, and contained a carbohydrate content that would be more acceptable to a broad range of patients.

Although these are exciting data, their main limitation was the delivery of the diet in a controlled research setting in a small, all male population. Thus, it remains unknown and is clinically important to determine whether these promising results can be replicated in a free-living population of men and women with DM2.

1.2. The gut microbiome may have an important role in the effectiveness of diet therapy for DM2, but its role is not yet understood.

Diet has important effects on the gut microbiome in mice and in healthy human subjects (7-11). It is increasingly recognized that the gut microbiome has a significant role in glucose metabolism (12), and that dysbiosis can lead to perturbations in normal metabolism (13; 14). It has been hypothesized that changes in bacterial metabolites, especially short chain fatty acids and secondary bile acids, is a mechanism by which the microbiome has an effect on metabolism (15-18). In human subjects with DM2, however, there is a knowledge gap. It remains unknown if the dietary recommendations given to patients with DM2 have an effect on the gut microbiome, and if so whether these changes confer metabolic benefit. Since the LoBAG diet alters macronutrient composition of the diet it would be expected to cause changes in the gut microbiome and bacterial metabolites. The importance of this is unknown.

1.3. Significance

The proposed study is significant because it will test the generalizability of the effects of the LoBAG diet in a general population of people with DM2. This could have important implications for DM2 treatment. The study is innovative in its use of a weight neutral, isocaloric, high protein and moderate carbohydrate diet in a free-living population of subjects. There are 29 million people with DM2 in the US (19), and so expanding and improving treatments to maintain glycemic control is an important public health goal. In addition, no study to our knowledge has investigated the gut microbiome before and after a dietary intervention for DM2. By contributing to the limited current knowledge about gut microbiome, diet, and diabetes, the study will provide pilot data for future studies. The study will hope to reshape effective dietary recommendations for the treatment of DM2.

2. Hypotheses

We hypothesize that the $LoBAG_{30}$ diet will produce a greater reduction in HgbA1c over 12 weeks in a free-living population of subjects with DM2 than a control diet consistent with ADA guidelines, without the need for weight loss. We have an additional exploratory hypothesis that the $LoBAG_{30}$ diet will induce potential beneficial changes in the gut microbiome, including increased species diversity, increased relative abundance of species associated with a healthy microbiome profile, and increases in short chain fatty acids and secondary bile acids.

3. Specific Aims to Test Hypotheses

3.1. Specific Aim #1: To compare the reduction in HgbA1c over 12 weeks in a free-living population of subjects with DM2 who consume either the $LoBAG_{30}$ diet or a control diet consistent with ADA guidelines.

The $LoBAG_{30}$ diet was chosen for study because of its high level of acceptability to subjects (5) and the likelihood that it could be used by a broad range of patients with DM2. The current ADA diet recommendations were used to develop the control arm of the trial because they represent the current standard of care in diabetes nutrition therapy. We hypothesize that, when compared to a control diet consistent with ADA guidelines, the $LoBAG_{30}$ diet will produce a better HgbA1c outcome.

3.2. Specific Aim #2: To investigate the effects of the $LoBAG_{30}$ diet compared to the control diet on fasting and postprandial glucose and insulin, fructosamine, and lipids in a free-living population of subjects with DM2.

Secondary endpoints to be assessed will include fasting plasma glucose, fasting serum insulin, postprandial glucose response, postprandial insulin response, fructosamine, and fasting serum lipids. It will be important to determine if the LoBAG diet reduces fasting and postprandial plasma glucose and affects serum insulin concentrations in this population. We also want to determine the effect of the LoBAG diet on fructosamine, which is a measure of glycemic control that reaches equilibrium more rapidly than does HgbA1c. Finally, we wish to be sure that the LoBAG diet does not have deleterious effects on the fasting lipid profile.

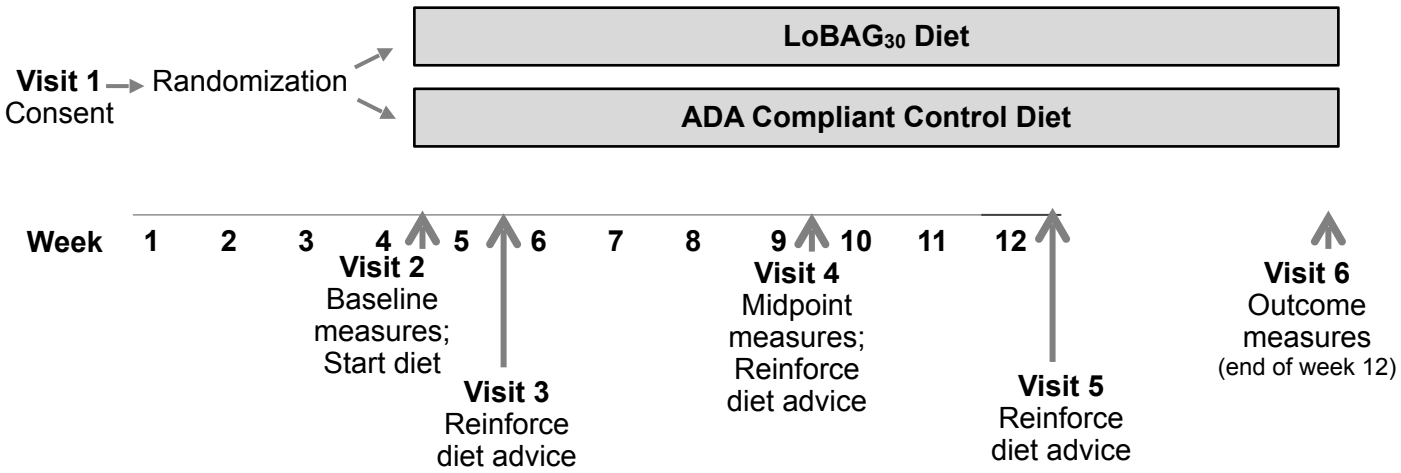
3.3. Specific Aim #3: To determine the effects of the LoBAG$_{30}$ diet compared to the control diet on the composition of the gut microbiome.

Stool samples collected before and after diet intervention will be used for species analysis of the gut microbiome. We hypothesize that there will be a change in the gut microbiome following the LoBAG$_{30}$ diet when compared to the control diet, and that this change will be associated with characteristics expected to provide metabolic benefit to subjects with DM2. These changes might include increased species diversity and increased relative abundance of species associated with a healthy microbiome profile. If funding can be secured, we will also analyze bacterial metabolites to determine if there are potentially beneficial increases in short chain fatty acids and secondary bile acids with the LoBAG$_{30}$ diet.

4. Research Plan

An overview of the proposed study design is shown in **Figure 1**. A detailed study timeline is included as an appendix to this document.

Figure 1. Overview of proposed study design.



4.1. Study cohort

Up to 70 men and women with DM2 will be recruited and enrolled (dependent on funding), for a goal of 44 participants completing the trial.

To be eligible, subjects must be ≥18 years of age, have a previous diagnosis of DM2 by ADA criteria (20) and have a HgbA1c of 7.0-9.5% while either taking no medications for diabetes or using metformin alone.

Exclusion criteria will be:

1) type 1 diabetes mellitus,
2) treatment with insulin,
3) BMI <25 kg/m^2,
4) lack of weight stability defined as a change in weight of more than 5 pounds in the prior 3 months,
5) estimated glomerular filtration rate < 60 ml/minute/1.73 m2,
6) urine albumin >300 mg/g creatinine,
7) pregnancy or immediate plans to become pregnant,
8) breast feeding,
9) dietary restriction(s) that would preclude consumption of study diets,
10) inability or unwillingness to prepare meals,
11) anemia, which would affect interpretation of HgbA1c,
12) presence of any disease which would make adherence to the study protocol difficult, and
13) use of antibiotics in the 3-month period prior to enrollment.

Subjects will be recruited from the University of Minnesota and surrounding communities. In addition, potential subjects will be identified from the Fairview patient population using Informatics Consulting Services (ICS) through the CTSI, and mailed letters informing them of possible study eligibility by the Fairview Research Administration.

4.2. Outcome Measures

The primary endpoint will be HgbA1c at the end of the 12 week diet intervention period. Secondary endpoints will be:

1) Weight
2) Fasting plasma glucose
3) Fasting serum insulin
4) Postprandial glucose response at +30, +60, +90, +120, +180 and +240 minutes following the start of a meal consistent with the assigned diet
5) Postprandial insulin response at +30, +60, +90, +120, +180 and +240 minutes following the start of a meal consistent with the assigned diet
6) Fructosamine
7) Fasting serum lipids
8) Stool samples for microbiome species analysis. Samples will also be saved for possible bacterial metabolite analysis.
9) Glucose variability as measured by continuous glucose monitoring (CGM).

4.3. Intervention

A randomized, controlled, parallel group design will be used to compare the effects of the $LoBAG_{30}$ diet or a balanced diet consistent with current ADA guidelines on our outcomes. Dietary intervention periods will be 12 weeks long. Both the LoBAG and control diets will consist of common foods.

Subjects will be asked to keep their physical activity level constant during the study. Medications for diabetes will be continued without change during the study. Medications will be monitored, and if a participant's medications are altered this will be noted. If antibiotics are used during the study this will be recorded. Subjects will be instructed that they do not need to monitor blood sugars regularly at home.

After enrollment and consent subjects will be randomized in a 1:1 allocation ratio to the $LoBAG_{30}$ diet or a balanced diet consistent with ADA guidelines. Randomization will be stratified by diabetes treatment at enrollment (no glycemic medications or metformin) and by HgbA1c (7.0-8.5% or 8.5-9.5%), and implemented within stratum using randomly permuted block sizes of 2 and 4. If closely related subjects choose to enroll together (for example spouses or subjects that live together, and would typically share meals) they will be randomized to the same study arm. Baseline measures will be collected. Subjects will meet individually with the study dietitian, who will provide diet instruction, sample menus, and recipes consistent with the assigned diet. Dietitian consultation may be conducted

in person or by phone. During week 2 subjects will have a repeat consult with the dietitian to reinforce diet instruction. If weight has changed caloric intake will be adjusted as necessary for a goal of weight stability. A midpoint visit will be conducted at week 6, during which subjects will provide a blood sample, urine sample, and a stool sample, and will consult with the dietitian for reinforcement of diet advice. If weight has changed caloric intake will be adjusted to prevent further change. Midpoint blood and stool samples will be stored and analyzed in the future based on available funding. At week 9 subjects will consult with the study dietitian for reinforcement of diet advice and collection of a urine sample to assess compliance. The final study visit will be at the end of week 12. All outcome measures will be assessed at the final visit.

If funding is available continuous glucose monitoring (CGM) will be added at the beginning and end of the study intervention. Subjects will be asked to wear the Freestyle Libre Pro CGM system for 2 weeks following the screening visit (visit 1) and for 2 weeks between visit 5 and visit 6. The CGM sensor will be worn on the back of the arm and will monitor and record interstitial glucose continuously throughout periods of CGM use. Subjects will be blinded to CGM glucose data. Sensors will be returned to study staff by mail or in person at study visits. Subjects will be given the option to receive a copy of CGM data, and to review this data with study staff, at the final study visit.

4.4. Assessment of Compliance and Acceptability

At baseline, week 2, week 6, week 9 and week 12 urine samples will be collected for urine nitrogen and creatinine. The ratio of nitrogen/creatinine will be used as a marker of compliance with the high protein LoBAG$_{30}$ diet, as previously described (4). Subjects in both groups will complete surveys at week 1, week 6, and week 12 with questions about compliance, acceptability of the diets, and quality of life. A study investigator will contact subjects every two weeks during the diet intervention period to encourage diet compliance. Subjects will be asked to complete 3-day food diaries at the beginning, midpoint, and end of the 12 week study. If funding is available, an unannounced 24-hour diet recall will be conducted via telephone by the Nutrition Coordinating Center at the University of Minnesota. The recall will be administered by a trained interviewer and analyzed using Nutrition Data System for Research software.

4.5. Subject Compensation

Subjects will be provided a total stipend of $600 for completion of the study. This amount includes a weekly stipend to partially account for the increased price of required foods ($15 per week), compensation for time (average $15 per week), and a bonus for study completion ($240). Payments will be made at regular intervals during the study. If a subject withdraws from the study before completion, compensation will be provided for each week of participation up to the time of withdrawal. In addition to the above compensation, parking will be paid for at study visits.

Subjects who come for a screening visit only (do not qualify to go forward with the study) will be mailed a check with compensation ($30). Subjects who enroll in the study will be given the option of payment using pre-paid debit cards with the Greenphire ClinCard system. If a subject chooses this option, they will be given a handout with instruction on use of the card. If a subject prefers not to use this option, they will be paid through checks mailed following study visits. The payment schedule will be the same with either reimbursement option, except that check processing may delay reimbursement by a few weeks.

4.6 Data Collection

Data will be collected by study investigators in source documents and will be entered into a RedCap database. Data will be identified using indirect identifiers. Hard copies of source documents will be stored in a locked office. All computers used for this study will be password protected. In addition the

RedCap database will be password protected and will only be accessible to those study investigators and staff who require access.

Blood, urine, and stool specimens will be collected by the study investigators or Clinical and Translational Science Institute (CTSI) or Clinical Research Unit (CRU) staff. All specimens will be identified using indirect identifiers and appropriate numbering and labeling for date, visit number, and time of collection. Specimens will be either transported directly to the lab for processing or will be stored in CTSI freezers.

The study PI will manage the study data and specimens, with assistance from the study coordinator. Records and frozen specimens will be stored indefinitely.

Protected health information will not be saved for potiential participants who are screened but do not come in for a study visit and thus do not give signed informed consent. At the conclusion of the screening survey potential participants will be asked if they are willing to be contacted about future studies for which they may be eligible. If yes, their name, age at time of screen, gender, reason for study ineligibility, method of finding out about the study (flier, letter, etc), and contact information will be saved in a password protected log. All other protected health information collected during the screening survey will be destroyed.

Following study enrollment all study participants will have a M Health medical record number (MRN) identified or created. The electronic health record (EPIC) may be accessed and used for scheduling purposes, as required by the clinical research unit (CRU).

4.7. Methods of Data Analysis

The primary outcome will be HgbA1c at the end of the 12-week study period. Results will be compared pre and post diet intervention (within an individual subject) using paired t-tests and between dietary interventions ($LoBAG_{30}$ versus control) using unpaired t-tests. Data will be analyzed at study completion.

Sample size was estimated a priori based on a two-tailed type I error of 0.05. For a difference of 1.5 points in HgbA1c and a power of 80%, 12 participants are required per intervention group. This number was increased by 30% to account for participant loss during screening, and an additional 30% to account for participant attrition, yielding 19 participants per intervention group (total 38 participants). An additional 12 participants have been enrolled using pilot funding from the University of Minnesota (of which 8 were eligible and 6 completed the entire study intervention).

This estimate uses preliminary data from existing $LoBAG_{30}$ studies which were collected in a carefully controlled research setting. This differs from the conditions planned for this study. There are no data on the performance of the $LoBAG_{30}$ diet in a free-living population of subjects with DM2. Thus, we will generate data that can be used to estimate effect size and variability for a power calculation for a future larger clinical trial.

With a goal of 38 plus 6 subjects completing the entire 12-week intervention, we anticipate recruiting up to 70 subjects in total.

If a subject withdraws from the study prior to study completion they will be invited to complete the final study visit prior to withdrawal, if possible. Any data collected from the subject prior to study withdrawal will be included in the final analysis. In the case of subject withdrawal an additional subject will be recruited to replace accordingly, dependent on funding.

4.8. Data and Safety Monitoring Plan

University of Minnesota Institutional Review Board (IRB) approval will be obtained prior to study initiation. Data and safety monitoring will be conducted by the IRB, the PI, and independently by qualified staff appointed by the CTSI. Monitoring by the CTSI will occur annually at a minimum. The PI will have primary responsibility for recording of events, follow up, and reporting to the IRB.

Any adverse events will be recorded immediately in the source document and in the appropriate study log for reporting to the IRB. The clinical course of any adverse event will be followed until resolution, stabilization, or until it has been determined that study participation is not the cause. Reports of all adverse events (including follow-up information) will be submitted to the IRB according to IRB requirements. Reports of any serious adverse events (defined as any event that is: 1) fatal 2) life threatening 3) requires or prolongs a hospital stay 4) results in persistent or significant disability or incapacity 5) results in a congenital anomaly or birth defect or 6) results in an important medical event) will be submitted to the IRB according to IRB requirements. Reports of any unanticipated problems involving risk to subjects or others (UPIRTSO) will be submitted to the IRB according to IRB requirements.

Subjects may discontinue participation in the study at any time, for any reason. Subjects will be withdrawn from the study in the case of a significant adverse event, consent withdrawal, or failure of the subject to adhere to protocol requirements. Failure to adhere to the protocol will be defined as compliance with <80% of requested study activities. In addition, any subject observed to have unacceptable responses to research procedures or to be unable to safely tolerate participation in the study as judged by the study investigator will be withdrawn.

The study will be stopped if >50% of subjects cannot meet compliance with >80% of study activities. This rule will be implemented after enrollment of the first 6 subjects.

4.9. Anticipated Problems and Proposed Solutions

Although a free-living study cohort was purposefully chosen to answer the central question of this study, it is anticipated that compliance with study diets in this cohort will be lower than that in previous LoBAG diet studies. To address this, measures of compliance have been included, and frequent phone calls added to the protocol to encourage diet compliance. Although the dietary interventions in this study are meant to be weight stable, we acknowledge that weight change during the study may influence our results. For this reason, we have included three predetermined points in the protocol when caloric advice can be adjusted if indicated. Finally, use of medications for diabetes could impact the size of the effect of the $LoBAG_{30}$ diet on HgbA1c, and so we have limited inclusion criteria to no glycemic medication or metformin only. We will also use stratified randomization to ensure equal distribution of medication use across study groups.

5.0 Retention of Study Records

Study records and data will be retained following completion of the study, in compliance with NIH and University of Minnesota requirements. Signed and dated HIPAA authorizations and consent documents will be maintained for at least 6 years after completion of the research. Other study records will be maintained for at least 3 years after completion of the research.

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Appendix 1: Timeline and Details of Study Visits

Screen. No visit needed. Will usually be done by telephone; may also be done by email or in person. (20 minutes)

Visit 1. Consent and Screening Visit. (1 hour)
- Review consent document and obtain informed consent.
- Collect baseline compliance survey, Diet Satisfaction Questionnaire, and Diabetes Treatment Satisfaction Questionnaire. Can alternatively be collected at visit 2.
- Measure weight and height.
- Non-fasting blood draw (for HgbA1c, basic metabolic panel, and complete blood count)
- Collect urine sample (for baseline urine nitrogen and urine creatinine, urine microalbumin, and pregnancy test if female and premenopausal).
- Send home with 3-day food intake diary (to be used by the study dietitian for caloric planning; the same food diary will also be collected at visit 4 and visit 6).
- If funding allows, place Abbott Freestyle Libre CGM. Provide supplies to return the CGM sensor (by mail or in person).
- Provide stool collection kit. Give instruction in stool sample collection.

Details for stool sample collection:
- Subject self-collects at home by sampling a stool with a sterile swab and putting the sample in a collection tube containing a preservative.
- This room-temperature-stable sample can be delivered to study investigators by the subject at study visits, or can be returned through the mail.
- Study staff will put the de-identified sample into the CTSI storage freezer.

Randomization. No visit needed.

NCC Diet Recall
- If funding allows, telephone call conducted by the Nutrition Coordinating Center (NCC) for an unannounced 24 hour diet recall (30 minutes; prior to visit 2/diet start).

Visit 2. Diet Instruction and Baseline Labs. (5 hours; beginning of Week 1)
- Subjects bring in stool sample or return by mail.
- If applicable, subjects bring in CGM sensor or return by mail.
- Collect weight, height, blood pressure.
- Fasting blood draw (for plasma glucose, serum insulin, lipid panel, fructosamine, and a stored sample).
- Provide a test meal consistent with the assigned diet (Control diet arm: 50% carbohydrate, 15% protein, 35% fat or LoBAG$_{30}$ diet arm: 30% carbohydrate, 30% protein, 40% fat). The composition of the control diet was chosen to be consistent with typical macronutrient intake in the United States (21) and consistent with ADA guidelines (1). In addition to the fasting samples, obtain plasma glucose and serum insulin at +30, +60, +90, +120, +180 and +240 minutes following the start of the meal.
- Collect 3-day food diary.
- Collect list of current medications.
- Collect baseline compliance survey, Diet Satisfaction Questionnaire, and Diabetes Treatment Satisfaction Questionnaire if not already administered at Visit 1.
- Meeting with study dietitian for diet instruction. Sample menus and recipes consistent with the assigned diet will be provided. Dietitian consult may be conducted by phone if needed to facilitate scheduling.
- If funding allows, provide Actigraph Activity Monitor for subject to wear between Visits 2 and Visit 3.

Visit 3. Diet Reinforcement. (1 hour; Week 2)
- If applicable, subjects return Actigraph Activity Monitor.
- Measure weight.

- Collect urine sample for urine nitrogen and urine creatinine.
- Meeting with study dietitian for reinforcement of diet advice. Adjust caloric intake if needed for goal of weight stability. Dietitian consult may be conducted by phone. If dietitian consult is conducted by phone the participant will be encouraged, but not required, to come to the study center for weight and urine sample collection (this exception applies to visit 3 only).

Visit 3.1. (5-10 minutes; Week 4)
- Telephone call conducted by study investigator or coordinator to encourage study compliance.

Visit 4. Midpoint Visit. (1 hour; Week 6)
- Subjects bring in stool sample or return by mail.
- Measure weight and blood pressure.
- Fasting or non-fasting blood draw for HgbA1c, fructosamine, and a stored sample.
- Collect urine sample for urine nitrogen and urine creatinine.
- Meeting with study dietitian for reinforcement of diet advice. Adjust caloric intake if needed for goal of weight stability. Dietitian consult may be conducted by phone.
- Collect 3-day food diary.
- Collect list of current medications.
- Collect compliance survey and Diabetes Treatment Satisfaction Questionnaire

Visit 4.1. (5-10 minutes; Week 8)
- Telephone call conducted by study investigator or coordinator to encourage study compliance.

Visit 5. Diet Reinforcement. (1 hour, week 9-10)
- Measure weight.
- Collect urine sample for urine nitrogen and urine creatinine.
- Meeting with study dietitian for reinforcement of diet advice. Adjust caloric intake if needed for goal of weight stability. Dietitian consult may be conducted by phone.
- If funding allows, place Abbott Freestyle Libre CGM. Provide supplies to return the CGM sensor (by mail or in person).
- If funding allows, provide Actigraph Activity Monitor for subject to wear between Visits 5 and Visit 6.

Visit 5.1. (5-10 minutes; Week 11)
- Telephone call conducted by study investigator or coordinator to encourage study compliance.

NCC Diet Recall
- If funding allows, telephone call conducted by the Nutrition Coordinating Center (NCC) for an unannounced 24 hour diet recall (30 minutes; Week 9-12).

Visit 6. Final Visit (5 hours; end of Week 12)
- Subjects bring in stool sample or return by mail.
- If applicable, subjects return Actigraph Activity Monitor.
- If applicable, subjects bring in CGM sensor or return by mail.
- Collect weight and blood pressure.
- Collect urine sample for urine nitrogen and urine creatinine.
- Fasting blood draw (for HgbA1c, plasma glucose, serum insulin, lipid panel, fructosamine, and a stored sample).
- Provide a test meal consistent with the assigned diet (Control diet arm: 50% carbohydrate, 15% protein, 35% fat or LoBAG$_{30}$ diet arm: 30% carbohydrate, 30% protein, 40% fat). In addition to the fasting samples, obtain plasma glucose and serum insulin at +30, +60, +90, +120, +180 and +240 minutes following the start of the meal.
- Collect 3-day food diary.

- Collect list of current medications.
- Collect end of study compliance survey, Diet Satisfaction Questionnaire, and Diabetes Treatment Satisfaction Questionnaire**.**
- Meeting with study dietitian for debriefing and dietary advice for after study completion. Dietitian consult may be conducted by phone.

Appendix 2: Updated Procedures During COVID-19 Pandemic

Due to the risk of coronavirus infection and to protect study participants and staff, study procedures will be converted to virtual as much as possible during the COVID-19 pandemic. Revised procedures are detailed below. These procedures will be utilized through Sunrise phase 1 and 2, and may be continued beyond as appropriate according to University of Minnesota guidance.

Prior to each in-person study visit (the day before and day of) participants will be screened for exposure to or symptoms of COVID-19. If exposure or symptoms are reported, the in-person visit will be cancelled.

Screen. Will usually be done by telephone; may also be done by email or video conference.

- In addition to previously stated inclusion and exclusion criteria, during the COVID-19 pandemic participants must have ability to participate in virtual visits, using Zoom or a similar HIPAA compliant video conferencing platform. This will require participants to have a cell phone with camera or computer with camera and internet connection.

Visit 1. Consent and Screening Visit.

Part 1: Video conference (40 min).

- Review consent document and obtain informed consent using E-consent (delivered and signed via REDCap). Complete HIPAA form using same process. Note: If paper consent/ HIPAA is preferred by participant or E-consent is not possible, the consent form will be completed in person; no study procedures will take place until the consent form is signed.
- Collect list of current medications.
- If female, determine menopausal status and need for pregnancy test during in-person visit.
- Provide instructions for in-person portion of visit at CRU, including instruction to wear a mask and to practice social distancing as able. A mask will be provided if needed.
- Provide “COVID-19 and Research Participation Information Sheet” by mail or email at least 24 hours in advance of in-person visit.
- Provide Greenphire ClinCard FAQ sheet by mail or email.

Part 2: In person visit at CRU (20 min).

- Measure weight and height.
- Non-fasting blood draw (for HgbA1c, basic metabolic panel, and complete blood count).
- Collect urine sample (for baseline urine nitrogen and urine creatinine, urine microalbumin, and pregnancy test if female and premenopausal).
- Place Abbott Freestyle Libre CGM. Provide supplies to return the CGM sensor (by mail or in person at next visit).

Provide to participant in-person at visit, or by mail/email/Redcap after visit:

- Blank 3-day food intake diary

- Blank baseline surveys (compliance survey, Diet Satisfaction Questionnaire, and Diabetes Treatment Satisfaction Questionnaire)
- Stool collection kit and instructions for stool sample collection
- NCC food amounts booklet for Nutrition Coordinating Center (NCC) diet recall
- Actigraph activity monitor (or provide at visit 2)

Randomization. No visit needed.

NCC Diet Recall

- Telephone call conducted by the NCC to complete an unannounced 24-hour diet recall (30 minutes).

Visit 2. Diet Instruction and Baseline Labs (Beginning of Week 1).

- Subjects bring to visit, or return by mail or Redcap prior to visit: completed 3-day food diary, completed baseline surveys, stool sample, CGM sensor
- Provide Actigraph activity monitor if not already done

Part 1: In person visit at CRU (4-5 hours; face to face time with CRU staff 30 min total)

- Measure weight, height and blood pressure
- Place IV for use during blood draws
- Fasting blood draw (for plasma glucose, serum insulin, lipid panel, fructosamine, and a stored sample)
- Provide a test meal consistent with the assigned diet (control or LoBAG)
- In addition to the fasting samples, obtain plasma glucose and serum insulin at +30, +60, +90, +120, +180 and +240 minutes following the start of the meal
- Participants will follow CRU protocols for social distancing during this visit. Interactions with staff will be kept to a minimum. Participants will be asked to wear a mask during the visit, except when eating.
- Provide handouts for virtual dietitian visit (sample menus and recipes consistent with the assigned diet). Can alternatively be sent by mail/email.

Part 2: Virtual meeting with study dietitian for diet instruction (1 hour)

- Dietitian consult may be conducted by phone or by video conference. Meeting can be completed between blood draws while participant is at the CRU or from home later, depending on participant preference.

Visit 3. Diet Reinforcement (Week 2).

Part 1: In person visit at CRU (10 min). Option to skip in-person visit if participant and study staff agree this is not needed.

- Measure weight.
- Collect urine sample for urine nitrogen and urine creatinine.
- Return Actigraph activity monitor if not already done (or return by mail).

Part 2: Virtual meeting with study dietitian (30 min). Participant will be at home for this meeting.

- Reinforcement of diet advice. Adjust caloric intake if needed for goal of weight stability.

Visit 3.1. (Week 4)

- Telephone call or video conference conducted by study investigator or coordinator to encourage study compliance.

Visit 4. Midpoint Visit (Week 6)

Surrounding time of visit, provide to participant by mail/email/Redcap:

- Stool collection kit and instructions for stool sample collection
- Blank 3-day food diary
- Blank collection form for list of current medications
- Blank midpoint compliance survey and Diabetes Treatment Satisfaction Questionnaire
- Items can be returned in person at visit, by mail, or via Redcap

Part 1: In person visit at CRU (20 min).

- Measure weight and blood pressure.
- Fasting or non-fasting blood draw for HgbA1c, fructosamine, and a stored sample.
- Collect urine sample for urine nitrogen and urine creatinine.

Part 2: Virtual meeting with study dietitian (30 min). Participant will be at home for this meeting.

- Reinforcement of diet advice.

Visit 4.1 (Week 8)

- Telephone call or video conference conducted by study investigator or coordinator to encourage study compliance.

Visit 5. Diet Reinforcement (week 9-10)

Part 1: In person visit at CRU (20 min).

- Measure weight.
- Collect urine sample for urine nitrogen and urine creatinine.
- Place Abbott Freestyle Libre CGM. Provide supplies to return the CGM sensor by mail or in person at next visit
- Provide Actigraph activity monitor (or send by mail)

Part 2: Virtual meeting with study dietitian (30 min). Participant will be at home for this meeting.

- Reinforcement of diet advice.

Visit 5.1. (Week 11)

- Telephone call or video conference conducted by study investigator or coordinator to encourage study compliance.

NCC Diet Recall

- Telephone call in week 9-12 conducted by the NCC for an unannounced 24-hour diet recall (30 minutes).

Visit 6. Final Visit (end of Week 12)

Provide to participant for completion prior to visit 6:

- Blank 3-day food intake diary
- Blank final surveys (compliance survey, Diet Satisfaction Questionnaire, and Diabetes Treatment Satisfaction Questionnaire)
- Stool collection kit and instructions for stool sample collection
- Actigraph activity monitor
- Blank collection form for list of current medications
- Items can be returned in person at visit, by mail, or via Redcap

Part 1: In person visit at CRU (4-5 hours; face to face time with staff 30 min total)

- Measure weight and blood pressure
- Collect urine sample for urine nitrogen and urine creatinine.
- Place IV for use during blood draws
- Fasting blood draw (for HgbA1c, plasma glucose, serum insulin, lipid panel, fructosamine, and a stored sample).
- Provide a test meal consistent with the assigned diet (control or LoBAG)

- In addition to the fasting samples, obtain plasma glucose and serum insulin at +30, +60, +90, +120, +180 and +240 minutes following the start of the meal
- Participants will follow CRU protocols for social distancing during this visit. Interactions with staff will be kept to a minimum. Participants will be asked to wear a mask during the visit, except when eating.

Part 2: Optional virtual meeting with study dietitian for debriefing and dietary advice for after study completion (30 min).

- Dietitian consult may be conducted by phone or by video conference. Meeting can be completed between blood draws while participant is at the CRU or from home.

Part 3: Video or phone meeting with study investigator or coordinator for closeout, and to ensure all study procedures complete (10 min).

Study Completion