Effect of Roux-en-Y Gastric Bypass on Fasting and Postprandial Concentrations of Bile Acids and Fibroblast Growth Factor 19

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A. Study Purpose and Rationale

Bariatric surgery is currently the most effective method for attaining long-term weight reduction and has become a therapeutic option for the treatment of type 2 diabetes mellitus (T2DM) in obese patients.[iii] Growing evidence indicates that several changes in neurohormonal regulators of energy balance and glucose homeostasis following Roux-en-Y gastric bypass surgery (RYGB) contribute to long-term weight loss and improvement in glucose control in individuals with T2DM.[iiivv] RYGB restricts stomach volume and reroutes nutrient flow from the upper portion of the stomach into the mid-to distal jejunum through the alimentary loop. The proximal limb of the jejunum forms the biliopancreatic loop through which bile and pancreatic secretions are transmitted and is connected with the jejunum just before the terminal ileum. The alteration in nutrient flow is associated with changes in the secretory pattern of gastrointestinal hormones.

Bile acids (BAs) have long been recognized as critical for absorption of dietary lipid. More recently, their many metabolic effects via action on the nuclear Farnesoid X receptor (FXR) and the membrane G protein-coupled receptor TGR5 have been recognized. Through FXR stimulation, BAs decrease *de novo* lipogenesis and VLDL-TG export.[vi] Through TGR5, BAs increase skeletal muscle and brown adipose tissue energy expenditure and stimulate secretion of the incretin, glucagon-like peptide-1 (GLP-1), which promotes satiety and stimulates ß-cell insulin release[viiviiiix].

Through FXR, BAs stimulate secretion of fibroblast growth factor 19 (FGF19), a protein produced by the mucosal cells of the terminal ileum that has several metabolic effects including: 1) regulation of BA homeostasis via feedback inhibition of the hepatic bile acid synthesis rate-governing enzyme CYP7A1;[x] 2) regulation of gallbladder filling;[xi] 3) decreased gluconeogenesis; 4) increased glycogen and protein synthesis; 5) increased metabolic rate; and 6) decreased adiposity.[xiixiiixivxv] Indeed, FGF19 transgenic mice have decreased adiposity and are resistant to high-fat diet-induced weight gain. Infusion of recombinant FGF19 protein or transgenic FGF19 expression reduces hepatic lipid accumulation and improves insulin-sensitivity in leptin-deficient and diet-induced obese mice.[xvixvii]

The many positive metabolic effects of BAs and FGF19 raise the question of whether they play a role in weight loss and improved glucose homeostasis following RYGB.[xviii] Others have reported increased fasting BA and FGF19 levels in patients after RYGB.[xixxxxixxiixxiii] Increased postprandial levels of BAs have also been reported;[xxiv] these increases are specific to RYGB, and not weight loss per se, since they are not observed after laparoscopic adjustable gastric banding.[xxv] Nevertheless, T2DM patients were either excluded or not mandated by inclusion criteria in these studies. In T2DM patients, increases in fasting levels of FGF19 and BAs following RYGB are greater in those who experience remission of DM compared with diabetics who do not experience remission and nondiabetics.[xxvi] Yet despite the fact that FGF19 is secreted postprandially with peak levels occurring 1.5-2 hours following the postprandial rise

in BAs, no studies have thus far compared postprandial levels of these hormones following RYGB in T2DM patients.[xxvii]

The aim of this study is thus to quantify RYGB-induced changes in both fasting and postprandial levels of BAs and FGF19 in patients with T2DM. The hypothesis of this study is that levels of both hormones will increase predominantly in the postprandial, but also in the fasting, state following RYGB due to increased efficiency of enterohepatic circulation following intestinal rerouting. The clinical implications of this study include a possible role for direct FGF19 and/or bile acids administration to pharmacologically mimic RYGB and improve glucose homeostasis.

B. Study Design and Statistical Analysis

This investigation is an ancillary study to the Diabetes Surgery Study (DSS), a multicenter trial that randomized 120 patients with T2DM to intensive lifestyle and medical management with or without RYGB. The primary endpoint of the DSS study was a composite goal of HbA1C less than 7.0%, LDL cholesterol less than 100 mg/dL, and systolic blood pressure less than 130 mm Hg. The one-year results of this study have recently been published in JAMA.[28] For the purposes of this ancillary investigation, the first 15 subjects (10 from Columbia University Medical Center and 5 from the University of Minnesota) who underwent RYGB and from whom plasma is available will be studied.

The primary outcome of this study will be the change in plasma fasting and postprandial FGF19 and total bile acid levels after RYGB. Postprandial levels will be determined via area under the curve analysis, calculated from 0-120 minutes. Change in fasting and postprandial values will be determined by subtracting pre-bypass levels from post-bypass levels. Data will be presented as mean values ± SEM. Paired t tests will be performed to evaluate mean change from baseline to 1 year after RYGB. A two-sided p-value <0.05 will be considered statistically significant.

Power Analysis

Given that there is a fixed number of 15 subjects that will be included in this analysis, power analyses using a paired t-test reveal that the following smallest differences at a power of 80% and P=0.05 will lead to statistical significance:

Change in Fasting FGF19 Levels: Given the wide variability in reported FGF19 levels, assuming a standard deviation of change of 70 pg/ml, this study is powered to find an effect size of 54 pg/ml absolute change in fasting values.

Change in AUC FGF19 Levels: Given the wide variability in reported FGF19 levels, assuming a standard deviation of change of 15,000, this study is powered to find an effect size of 11,657 absolute change in AUC values.

Change in Fasting Total Bile Acid Levels: Given the wide variability in reported BA levels, assuming a standard deviation of change of 2.2 µM, this study is powered to find an effect size of 1.7 µM absolute change in fasting values.

Change in AUC Totale Bile Acid Levles: Given the wide variability in reported BA levels, assuming a standard deviation of change of 500, this study is powered to find an effect size of 389 absolute change in AUC values.

Study Protocol

RYGB consists of creation of a 20 ml pouch, a 100-centimeter biliopancreatic limb, and a 150-centimeter Roux limb. Subjects will be studied before and 12 months after surgery. Venous blood samples are drawn in the fasted state and 15, 30, 60, 90 and 120 min after a liquid meal of Ensure (237 ml, 250 kcal, 6 g fat, 40 g carbohydrate, 9 g protein).

The 0 and 12 month samples from each subject will be run in the same assay. FGF19 levels will be determined by ELISA (R&D Systems, Minneapolis, MN) with a minimum detection limit of 1.2 pg/ml and run in duplicate. BA measurements will be determined by LC-MS (Waters Quattro Micro with Waters 2795 Alliance HPLC), with an assay sensitivity of 0.01 µM.

D. Study Drugs

There will be no use of drugs as part of this study.

E. Medical Devices

There will be no use of a medical device in this study.

F. Study Questionnaire

There will be no study questionnaires used in this study.

G. Study Subjects

Detailed enrollment criteria, methods and 12-month clinical outcomes have recently been reported for the DSS.[xxviiixxix] Briefly, key inclusion criteria are as follows:

1. Age 30-67 years
2. Minimum 6 month history of T2DM and active medical care from a doctor
3. BMI 30 – 39.9 kg/m^2
4. HbA1c 8%- 14%
5. Serum C-peptide level > 1.0 ng/ml 90 minutes after a liquid meal of Ensure (237 ml, 250 kcal, 6 g fat, 40 g carbohydrate, 9 g protein)
6. Willingness to accept randomization
7. Written informed consent.

Key exclusion criteria are:

1. Any medical or psychiatric condition that would contraindicate surgery, including:
    a) Cardiovascular event (myocardial infarction, acute coronary syndrome, coronary artery angioplasty or bypass, stroke) in the past six months.
    b) Current evidence of congestive heart failure, angina pectoris, or symptomatic peripheral vascular disease.
    c) Cardiac stress test indicating that surgery or IMM would not be safe.
    d) Pulmonary embolus or thrombophlebitis in the past six months.
    e) ·Cancer of any kind (except basal cell skin cancer or cancer in situ) unless documented to be disease-free for five years.

f) Significant anemia (hemoglobin 1.0 g or more below normal range) or history of coagulopathy.
g) Serum creatinine ≥ 1.5 mg/dl.

2. A history of alcohol or drug dependency (unless alcohol or drug free for five years)
3. Being pregnant, nursing, or planning to become pregnant in the next two years.

H. Recruitment of Subjects

Between February 2008 and December 2011, 120 patients were recruited for the DSS at four study sites (Columbia University, University of Minnesota, Mayo Clinic, and Taiwan) via referrals, mass media, and direct mail. Detailed recruitment strategies have recently been published.[xxx] Given that this is an ancillary investigation of the DSS that will use previously collected samples from subjects at two study sites (CUMC and University of Minnesota), no further recruitment of subjects is necessary.

I. Confidentiality of Study Data

All participants have received a unique study code number. Personal identifying information, including hospital unit numbers, social security numbers, subject names/ initials, phone numbers, and addresses have been removed. Study data will be maintained only on password-protected and/or encrypted computers and accessible only to the investigators.

J. Potential Conflict of Interest

Funding for this project has been provided by grants from Covidien and the NIH.

K. Location of the Study

As previously mentioned, plasma samples of the 15 subjects to be analyzed were obtained from patients at both CUMC and The University of Minnesota. Analysis of FGF19 and Bile Acid levels will be conducted at CUMC, in the lab of Dr. Judith Korner (principal investigator), located on the 9th floor of CUMC's Black Building. IRB approval from the University of Minnesota will also be obtained.

L. Potential Risks

Given that samples have already been collected and stored from all 15 subjects, no additional risks will be incurred

M. Potential Benefits

There will be no additional immediate benefit to subjects participating in this particular analysis beyond those obtained from enrollment in the DSS. On a societal level, this analysis may provide evidence for the development of pharmacologic treatments to mimic the beneficial effects of RYGB, thereby evading the morbidity of this surgical intervention while duplicating its positive effect on glucose homeostasis.

N. Alternative Therapies

Medical management is an alternative therapy to RYGB for the treatment of T2DM, both of which were studied by the DSS. Despite bringing about a greater improvement in A1C and cardiovascular risk factors than intensive medical management, RYGB is associated with many risks, including perioperative and postoperative complications such as dumping syndrome in

addition to a higher prevalence of nutritional deficiencies. This ancillary investigation will not study any additional experimental investigations beyond those assessed by the DSS.

O. Compensation to Subjects

All subjects involved in the DSS received RYGB surgeries, medical care and medications related to hyperglycemia and cardiovascular disease risk factors, and 38-visits of intensive lifestyle intervention free of charge. There will be no additional compensation for those subjects involved in this ancillary investigation.

P. Costs to Subjects

The subjects will not incur any additional costs as a result of participating in the study.

Q. Minors as Research Subjects

This study does not involve participation of minors.

R. Radiation or Radioactive Substances

This study does not involve radiation or radioactive substances.

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