Post-bariatric Surgery Diet

After the RYGB and VSG operations, patients were discharged home from the hospital and instructed to consume a full-liquid diet for 7-10 days as standard of care. Those in the Diet group remained on the CRC after the hyperinsulinemic-euglycemic clamp procedure on first study visit and consumed the post-bariatric surgery diet for 7-9 days; all meals were provided and prepared by the CRC Metabolic Kitchen. After the post-clamp meal, participants began a clear liquid diet that continued throughout the following day. The remaining days were a full-liquid diet of approximately 500-600 kcal per day and based on sample menus provided to the bariatric patients by the dieticians.

Hyperinsulinemic-euglycemic clamp

Participants were admitted to the CRC in the afternoon on the day prior to the clamp procedure and underwent a history and physical examination with vital signs, ECG, and safety labs. Participants were given a standardized meal and snack and restricted to water after 8:00 pm. The next morning, a catheter was inserted in an antecubital vein for infusions and a second catheter for blood drawing was placed either in a radial artery or heated, superficial vein to obtain arterialized-venous blood (1). After a baseline blood draw, an infusion of $[6,6\text{-}^2H_2]$-glucose was started with a priming dose of 22 μmol/kg and continued at a rate of 0.22 μmol/kg/min for 150 min for tracer equilibration and measurement of basal glucose kinetics. The hyperinsulinemic-euglycemic clamp procedure was then started with insulin infusion of 80 mU/m^2/min which was preceded by a priming dose of 320 mU/m^2/min for 5 min and 160 mU/m^2/min and continued for 150 min. Plasma glucose levels were maintained at 90-100 mg/dl with a variable infusion of 20% dextrose enriched with 2.5% $[6,6\text{-}^2H_2]$glucose, and the $[6,6\text{-}^2H_2]$-glucose infusion rate was reduced by 85% to account for the decrease in endogenous glucose production. Blood samples were obtained every 5 min during the last 30 min of the basal period and the clamp period for determination of tracer enrichment and plasma glucose levels, every 15 min for measurement of insulin, and once for measurement of free fatty acids (FFA), ghrelin, and adipokines. Participants were provided with a meal at the end of the clamp procedure.

Body composition

Total body fat mass (FM) and fat-free mass (FFM) were acquired by dual-energy x-ray absorptiometry with a Lunar iDXA whole-body scanner (GE Healthcare) and enCore 2007 software (version 11.4). Half body scans were performed from which whole-body tissue composition was extrapolated (2). Body composition was not obtained in one RYGB subject that exceeded the capacity of the scanner.

Adipose tissue microdialysis

After admission, the afternoon before the clamp procedure, a microdialysis catheter was inserted into the periumbilical subcutaneous adipose tissue using local anesthesia and sterile procedures. The microdialysis catheter consisted of a 30 mm semipermeable membrane with a 100 kDA cut-off to allow diffusion of large molecules such as cytokines (71 High Cut-Off Brain Microdialysis Catheter 60/30, M Dialysis). The catheter was connected to a micro vial to close the system overnight. The next morning the catheter was connected to a syringe containing T1 perfusion fluid (M Dialysis) supplemented with 4% Dextran-70 to avoid ultrafiltration that was placed in a 107 Microdialysis Pump (M Dialysis). At the start of the clamp procedure, the

microdialysis pump was started at a flow rate of 1 µl/min; after the first 60 min, the microdialysate was discarded and new vial used to collect a microdialysate sample over the next 90 min. Samples were collected on ice and immediately stored at -80°C until analysis. In vitro experiments determined that the recovery of analytes with microdialysis was 2-17%, consistent with the literature (3-5).

Hepatic magnetic resonance imaging (MRI)

A subset of subjects (RYGB n=5, VSG n=9, or Diet n=6) underwent fat-water separated MRI collected on a 3 Tesla whole-body Achieva scanner (Philips Healthcare, Best, The Netherlands) using a 16-channel torso XL receive coil. To image the entirety of the liver, forty slices with slice thickness 7.5 mm were acquired as two stacks in a total of 8 expiration breath holds. Nominal scan time was 2 minutes 21 seconds requiring 18 second breath holds. Pauses between breath holds increased total data collection time to approximately 5 minutes. To achieve fat-water separation, 9 echoes with initial echo time (TE) = 1 ms and echo spacing 1.6 ms were collected after each RF excitation (flip angle = 12⁰) with a repetition time (TR) = 75 ms. Echoes were collected with "flyback" gradient so that all echoes experienced the same direction of chemical shift. In-plane field of view (FOV) was 408 mm and 520 mm in the anterior-posterior and right-light directions respectively. Acquired voxel size was $2 \times 2 \times 7.5$ mm^3. Real and imaginary parts of reconstructed complex images were saved for off-line processing. 3D fat-water separation based on a multiscale whole image optimization algorithm (6), implemented in C++ and wrapped with MATLAB (MathWorks, Inc., Natick, MA) was performed for the full image volume (7; 8). Fat was modeled using 9 peaks (9). Whole-body fat, water, shared transverse relaxation rate (R2*) and static magnetic field inhomogeneity maps (ΔB0) were estimated. The fat signal fraction (FSF) map also estimated as the fat image divided by (fat image + water image). Hepatic fat content was estimated from region of interest (ROI) measurements on the FSF maps. ROIs were placed to avoid hepatic vasculature.

Sample processing and analysis

Blood was collected in chilled ETDA vacutainer tubes and obtained plasma was immediately processed and frozen. For measurement of ghrelin, the EDTA tubes contained a final concentration of 8mM 4-(2-aminoethyl)-benzene sulfonyl fluoride (AEBSF) and 100 µl of 1 N HCl was added per ml of plasma. Acyl and desacyl ghrelin were separately measured using a two-site sandwich assays specific for the full-length peptides as previously described (10). Glucose was measured by the glucose oxidase method (YSI 2300 STAT Plus). Insulin was measured by RIA (Millipore, Burlington, MA). FFA were determined by the NEFA-HR(2) enzymatic colorimetric method (Wako Diagnostics, Richmond, VA). Serum FGF-19 and FGF-21 were measured by ELISA (R&D Systems, Minneapolis, MN). Plasma glucose tracer-to-tracee ratio was determined by using gas chromatography/mass spectroscopy. Adipokines (leptin, adiponectin, MCP-1, PAI-1, IL-8, IL-6, TNF-α, IL-1β) were measured with MILLIPLEX® MAP Magnetic Bead Panels with Luminex xMAP® detection using the following panels: plasma, HADK1MAG-61K and HAD2MAG-61K and microdialysate, HADCYMAG-61K with optimization to use one-half of the recommended per-well sample volume. TNFα and IL1-β were not consistently detectable in the microdialysate.

Calculations

The glucose rate of appearance (Ra) during the last 30 min of the basal and clamp periods were calculated as the tracer infusion rate divided by the tracer-to-tracee ratio using the Steele equation for steady-state conditions (11; 12). Basal endogenous glucose production (EGP, predominately hepatic) is reported as the basal Ra, and EGP during the clamp is reported as total Ra minus the rate of D20 infusion during the last 30 min of the insulin period. Hepatic insulin sensitivity index (HISI) was calculated as the inverse product of basal endogenous glucose production (EGP)(mg min^{-1}) and fasting insulin levels (µU/ml) x 1000 (13). Peripheral insulin sensitivity (predominately skeletal muscle) is reported as the tracer-calculated Rd during the last 30 min of the insulin infusion. The exogenous glucose infusion rate (M value), M relative to steady-state insulin levels (M/I), as well as percentage increase in Rd clamp over Rd basal are also reported. The metabolic clearance rate of insulin (MCR-I) was calculated as the insulin infusion rate during the clamp divided by the steady-state plasma insulin concentration (14). Adipose tissue insulin sensitivity was calculated as the product of fasting insulin and fasting FFA, which has good agreement with the gold-standard method for determination of lipolysis fluxes by tracer-dilution techniques during continuous intravenous insulin infusion (15).

Statistical Analyses

Data are mean ± SD in text and tables and mean ± SEM in graphs. One-way ANOVA with Tukey's test or Kruskal-Wallis test with Dunn's test, depending on the distribution of the data, and Chi square tests were used to test baseline demographics and weight loss among the groups. Repeated measures two-way ANOVA was used to test for the effect of time (pre- or post-intervention) and a group (RYGB, VSG, or Diet) by time interaction; in the case of a significant group by time interaction, a Bonferroni's multiple comparisons test was used for within group (pre vs. post) comparisons. A paired t-test was used to test for the effect of weight loss on hepatic fat. Significance was set at $P<0.05$. Analyses were performed in GraphPad Prism® version 8.0

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