Role of Hepatic Glycogen on Nocturnal EGP IN T2D

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Protocol:
15 ND and 14 T2D subjects will be studied on two occasions (*glycogen loading vs. no glycogen loading*). Human Subjects: All studies and protocols will be approved by the UVA Institutional Review Board. These studies will be conducted in 15 T2D and 15 ND subjects (age 30 - 80 years, BMI 20 - 35 kg/m2) matched for age and degree of obesity. Menstruating female subjects will be studied during the follicular phase of their menstrual cycles Screen visit**:** Subjects will report to the UVA CRU after an overnight fast in the morning. After obtaining informed consent, a history and physical examination will be performed. Blood will be collected for complete blood count, serum creatinine, electrolytes, lipids, and HbA1c. A standard urinalysis will be performed. All women of childbearing potential will have a negative pregnancy test within 24 hours of the study visit. A dietary history will be taken at the time of screening to ensure adherence to a weight maintaining diet consisting of ~ 200 grams of carbohydrates per day and their diets met ADA guidelines for protein, fat, and carbohydrates. They will be instructed to adhere to the diet between screen and study visits. Body composition may be measured using measuring scale. Enrolled T2D subjects will withdraw oral hypoglycemic agents 2 weeks prior to study visit (medications will resume after each study visit) and will monitor their fingerstick glucoses. If three successive fingerstick glucose values ≥ 300 mg/dl, then they will resume their medication/s and will be withdrawn from study. Physical activity will be assessed with the Paffenbarger activity questionnaire and activity monitored by the subject with a Fitbit. Subjects will refrain from unusual / unaccustomed physical activity and should not change their body weight by >2% between screen and study visits. Pre-study visit protocols: All subjects will participate in both protocols outlined below in random order prior to their study visits. The study visits will be separated by 3-6 weeks *to allow for elimination of deuterated water* and will be identical except for the preceding glycogen loading or no glycogen loading protocols.
Glycogen loading (GL) protocol: Subjects will consume an isocaloric diet [60% carbs, 20% protein, 20% fat (33 kcal/kg/day)] prepared by the research dietician of the UVA CRU for 3 days prior to the overnight study 1. During this period, subjects will limit their daily activity to normal walking that will be monitored with Fitbit. Unusual physical activity will be discouraged.
No glycogen loading (NGL) protocol: Subjects will consume an isocaloric diet [40% carbs, 20% protein, 40% fat (33 kcal/kg/day)] prepared by the research dietician of the UVA CRU for 3 days prior to the overnight study 2. During this period, subjects will do their normal walking (monitored by Fitbit) but unusual physical activity will be discouraged.
Sample size estimation: The experimental design was configured with parallel cohorts (ND and T2D) with primary hypotheses of within cohort comparisons (i.e., glycogen loaded (GL) vs. non-glycogen loaded (NGL) meals). For sample size planning purposes, a paired t-test was utilized. At time of study design, no preliminary data existed on the effect of post prandial glucose uptake, so the effect size observed was utilizing a similar overnight study estimating GNG 3. For the studied T2D participants, the mean (SD) GNG (μmol/Kg/min) rate at 0700 hrs. was 12.9 (2.8) and 10.6 (3.0) under test and control conditions. This resulted in a change of 2.05 (1.68) between the two conditions. Assuming a similar magnitude of change of 2 units is clinically relevant

and a SD of the difference of 1.75 (to be conservative), a sample size of 9 was required to achieve 80% power at the alpha=0.05 level of significance. The protocol was written to study up to 15 participants per cohort to provide additional precision in the estimates and address the uncertainty in the glycogen loading effect. *Primary analysis.* A linear mixed model with compound symmetry variance structure was fit to the data that had repeated measurements at 0100, 0400 and 0700 hrs. This model specified parameters for study group (T2D vs. ND), time of night (0100, 0400 and 0700 hrs) and the glycogen loading treatment (GL vs NGL). This model estimated 12 (2 * 3 * 2) individual means for each outcome measure, with GNG planned as the primary endpoint at time of study planning. In order to summarize the effects of the model, model-based means were constructed to provide pooled estimates of the differences in means of interest. In some cases, several of the model-based means were averaged together (e.g., a “diabetes” effect was obtained by comparing the mean of the 0100, 0400 and 0700 hrs measures under both GL and NGL values averaged for each patient group). All p-values are unadjusted and reported in conjunction with estimates and 95% confidence intervals. Statistical analysis was performed using R version 4.1.2.

Reference

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