Data Collection – Supplementary Materials:

1) **Blood collection:** Approximately 50 ml of blood will be collected from the subjects at baseline, 6 and 12 months, and at extended follow-up. Samples will be collected after an overnight fast and in the early morning. Serum and plasma samples will be aliquoted in smaller vials, frozen at –80°C, and stored for later analysis of insulin, glucose, and CRP using standard techniques. Medication use will be assessed at each collection.

2) **Insulin and Insulin resistance measures:** As mentioned above, fasting blood samples will be collected, aliquoted and stored frozen at -80°C for analysis at study completion. Insulin resistance will be evaluated by the Homeostasis Assessment Model from fasting insulin and glucose [= fasting insulin (μU/mL) × fasting glucose (mmol/L)/22.5]; values > 2.61 will be considered insulin resistant [1]. Samples will be analyzed under the direction of Dr. Ngoc-Anh Le, Biomarker Core Laboratory at the Atlanta VA Medical Center. Glucose will be determined by colorimetric methods (Sekisui Diagnostics, Exton, PA), plasma insulin levels assessed using the immunoturbidometric method (Sekisui), and high sensitivity CRP analyzed via sandwich enzyme immunoassay (ALPCO). Samples will be grouped in random order for analysis, and a 10% blind quality control included. The CV for these analyses in the Le lab ranges between 3.9%-6.1%.

3) **Urine Collection:** 50 ml urine samples will be collected at baseline, 6 and 12 months and at extended follow-up in plastic containers by clean-catch technique, transported, and stored in the same way as fecal samples. Samples will be aliquoted into five containers/time point, coded by GCRC laboratory staff, and held at –80°C for future metabolome analysis led by Dr. O'Keefe [2].

4) **Fecal Collection:** Stool samples will be collected during the study. Subjects will be instructed in the use of a plastic device to cover the toilet seat and collect the stool. Two separate ~5 g samples will be taken for fecal microbiome analyses (mechanistic studies) and two additional samples for fecal SCFA and bile acid analyses (markers of compliance with HLD) at the time points specified in Table 1. All samples will be transported to the laboratory, coded by the research assistant, and held at –80° C for future DNA extraction and microbiome analysis by targeted and global approaches led by the O'Keefe laboratory.

5) Body composition: Body composition will be assessed using dual energy X-ray absorptiometry (DXA)[3] in the GCRC at Emory University. This method uses a whole-body scanner to measure total body composition and fat content with a high degree of precision. It is safe and noninvasive with little burden to the individual. Data from the DXA scans will be used to assess longitudinal changes in body fat that accompany weight loss. This data will also allow examination of changes in fat distribution at defined regions in the body. New software allows for the estimation of visceral fat. Women who could potentially become pregnant will be given a pregnancy test prior to a DXA.

References:

1. Monzillo LU, Hamdy O. Evaluation of insulin sensitivity in clinical practice and in research settings. *Nutr Rev*. 2003;61(12):397-412.
2. O'Keefe SJ, Li JV, Lahti L, et al. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun*. 2015;6:6342.
3. Lunar GH. X-ray bone densitometer with enCORE v17 software—user manual. *Madison: GE Healthcare Lunar*. 2016.