SUMMARY OF THE CALERIE PHASE 2 PROTOCOL

Specific Aims: The overall aim of CALERIE Phase 2 is to test the hypothesis that two years of sustained caloric restriction (CR), involving a reduction in energy intake to 75% of baseline (25% CR), in healthy men and women aged 25 to 45, will result in the same adaptive changes that occur in rodents subjected to CR. Particular emphasis on the adaptive responses thought to be involved in slowing the aging process and protecting against age-related disease processes. Primary outcomes include core body temperature and resting metabolic rate. An important secondary aim is to identify potential adverse effects of CR in humans. A number of exploratory aims will be assessed to evaluate the effect of CR on body composition, serum hormones, plasma growth factor concentrations, serum lipid and lipoprotein levels, skeletal muscle, adipose tissue and psychological factors. Consistency between the two sexes and across levels of body composition will be explored. In addition, biological samples will be stored in a biosample repository for future analysis.

Basic Study Design: The study will be conducted as a multi-center, parallel-group, randomized, controlled trial (RCT). A sample of 250 participants will be enrolled, and assigned to either the CR intervention or regular diet group (control). A 2:1 allocation ratio in favor of the CR intervention will be applied in order to maximize the number of subjects receiving the intervention of greater scientific interest. Participants in both treatment arms will be followed over a period of 24 months. A comprehensive set of evaluations will be performed prior to initiating the intervention, with follow-up evaluations at Months 1, 3, 6, 9, 12, 18 and 24 after randomization. It is expected that 10% of study subjects will drop-out in each of the two follow-up years, so that a sample of approximately 200 subjects is expected to complete the study.

Study Population and Eligibility Criteria: Participants must be between 25 and 45 years of age (inclusive), and body mass index must be greater than or equal to 22.0 and less than 28.0 kg/m_2. Otherwise, healthy individuals from both genders and all races are eligible to participate. Volunteers will be ineligible if there are significant medical conditions (e.g., history or clinical manifestation of cardiovascular disease, diabetes, cholelithiasis or cancer); abnormal laboratory markers (e.g., elevated potassium levels, hemoglobin or hematocrit below the lower limit of normal); psychiatric or behavioral problems (e.g., eating disorders or a history of drug and alcohol abuse); concomitant medications (e.g., steroids). Never-smokers of tobacco products or ex-smokers who quit completely at least 12 months ago are eligible. Breast-feeding or pregnant women (or those intending to become pregnant before the scheduled end of the intervention) and individuals performing any kind of heavy physical activity will be excluded. Volunteers will be screened out if they are unwilling or unable to adhere to the rigors of the CR intervention or the evaluation schedule over the entire two-year period.

Treatment Interventions: The active intervention will target a sustained 25% restriction in calorie intake. There will be no gradual ramping of CR, and the 25% energy reduction goal will be maintained for the entire 24 months. Control participants will be advised to continue their current diets on an *ad libitum* basis. The CR intervention will be implemented by a multi-disciplinary team including dietitians, psychologists, and physicians. No specific diet composition will be mandated. The approach will be tailored to the needs of the individual participant, with specific nutritional and behavioral strategies selected from an intervention “toolbox.” Examples include increasing dietary fiber, modifying recipes to decrease energy density, adding novel foods to relieve

boredom, strategies to avoid impulse eating, obtaining desired foods through home delivery or take-home meals, strategies for limiting caloric intake in public settings like restaurants, parties and work, and so on. Selections from the toolbox will be based on the participant's success in achieving adherence, and on problems arising at that point in time. The behavioral component will include group sessions and individual counseling, and during these sessions, the interventionist will provide specific and individualized dietary information to help the participant adhere to the CR regimen and meet his/her calorie target. No specific level of physical activity will be required or recommended. However, all participants will be advised of current recommendations from the Surgeon General (Centers for Disease Control) for minimum levels of dedicated physical activity. A complete daily vitamin and mineral supplement will be provided to intervention and control participants to ensure that they meet the current recommendations for these nutrients.

Recruitment and Screening: Participants will be recruited at the three CALERIE clinical centers using procedures that were successful in the Phase 1 studies. Recruitment will be continuous, and generally include media advertising, direct mail, health promotion events, databases, and referral sources. An effort will be made to recruit an ethnically diverse group based on the demographics of the three clinical sites. An initial telephone screening will record the volunteer's contact information as well as age, height, weight, and basic eligibility information. Volunteers who are clearly ineligible will be screened out at this point. Then, a staged screening process will be undertaken over a series of 3-4 visits. Exclusion criteria outlined above will be evaluated. Volunteers will meet with the study psychologist or a trained member of the behavioral team to assess any barriers to participation. A 14-day food record will be collected to assess the volunteer's ability to adhere and complete a food record continuously over a two-week period.

Randomization and Blinding: CALERIE participants will be assigned to intervention using a random process. A telephone-based, interactive voice-response system (IVRS) will be applied. Randomization will be stratified by sex and BMI within each clinical center, and within each stratum, subjects will be allocated in a 2:1 ratio in favor of the 25% CR intervention.

Outcome Determinations: A detailed series of evaluations will be performed on participants in both treatment arms at baseline and at periodic intervals during the study. They include the following: measures of energy metabolism, cardiovascular risk factors, glucose tolerance and insulin, immune function, endocrine response, qualify of life (QoL), psychological and cognitive functioning, physical activity measures, body height and weight, body composition, bone turnover, and nutrient intake. Biological material including blood, urine, muscle biopsy and abdominal fat biopsy will be collected and stored in a biosample repository for future analyses. A process is described for extending the protocol to incorporate advanced clinical endpoints as the opportunity arises.

Schedule of Evaluations: Evaluations will be performed with participants in both treatment arms at baseline and at periodic intervals during the study. Follow-up visits will be performed at Months 1, 3, 6, 9, 12, 18 and 24 following the start of the assigned intervention. The baseline visits and follow-up visits at Months 12 and 24 are the most elaborate and a complete set of evaluations will be performed. A smaller set of evaluations will be performed at Months 6 and 18, while an abbreviated follow-up will be performed at Months 1, 3, and 9.