CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study: Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) Phase II – Study Consent

What you should know about a research study

- We give you this consent form so that you may read about the purpose, risks and benefits of this research study.
- The main goal of research studies is to gain knowledge that may help future patients.
- You have the right to refuse to take part, or agree to take part now and change your mind later on.
- Please review this consent form carefully and ask any questions before you make a decision.
- Your participation is voluntary.
- By signing this consent form, you agree to participate in the study as it is described.

1- Who is doing the study?

Investigator Information:

Principal Investigator:	Eric Ravussin, Ph.D. 225-763-3186
Medical Investigator:	Alok Gupta, M.D. Day Phone: 225-763-2656 24-hr. Emergency Phone Nos.: 225-763-2656 (Weekdays 7:00a.m.-4:30 p.m.) 225-765-4644 (After 4:30 p.m. and Weekends)
Co-Investigators:	Donald Williamson, Ph.D. Steven Smith, M.D. Corby Martin, Ph.D.

Dr. Eric Ravussin directs this study, which is under the medical supervision of Dr. Alok Gupta. We expect about 250 people from 3 sites (Pennington, Tufts University in Boston and Washington University in St. Louis) will be in this study. Pennington expects to enroll approximately 84 people at this site. The study will take place over a period of 6 years. Your expected time in this study will be approximately 2 ½ years. This study is part of ongoing research in the prevention and treatment of age-related diseases at the Pennington Center and is being funded by the National Institute on Aging.

2- Where is the study being conducted?

This study takes place at the Pennington Biomedical Research Center's inpatient and outpatient clinics and the Pennington Metabolic Kitchen.

3- What is the purpose of this study?

The overall purpose of this study is to gain knowledge about the effects of two years of food restriction in reducing the risk of disease associated with aging and in slowing of the aging process.

It has been reported for many years that sustained caloric restriction without nutritional deficiencies increases the length of life and prevents development and/or progression of some age-related chronic diseases such as cancer, diabetes and cardiovascular disease in a variety of animals. Whether this occurs in humans, is not known. This study is designed to determine the effects of sustained caloric restriction on physiology, metabolism, body composition, risk factors for age-related diseases, progression of age-related changes, and potential adverse effects in non-obese humans. It is already known that weight loss improves insulin sensitivity and the lipid profile.

4- Who is eligible to participate in the study? Who is ineligible?

You may qualify for this study if you meet the following criteria:

- Your BMI (your height to weight ratio) is $\geq$ 22 kg/m^2 and < 28 kg/m^2.
- You are a male age 21 to 50 or a female age 21 to 47.
- You are willing to postpone pregnancy for 2.5 years.

You will not qualify for the study if any of the following applies to you:

- You have a history of cardiovascular disease or an elevated blood pressure (greater than 140/90 mm Hg).
- You have an abnormal ECG.
- You have low bone mineral density as shown in your DEXA scan during the baseline testing.
- You are claustrophobic.
- You have a history of diabetes, gall stones or any other significant metabolic, hematologic, pulmonary, cardiovascular, gastrointestinal, neurologic, immune, hepatic, renal, urologic disorders or cancer.
- You have a history of stomach or intestinal surgery (except appendectomy) or major abdominal, thoracic or non-peripheral vascular surgery within one year prior to enrollment into the CALERIE study.
- You have any disease or condition that seriously affects body weight and/or body composition.
- Your potassium level is above the upper limit of normal at the screening visit and is confirmed by a repeated test within 2 weeks of the original.
- Your hemoglobin, hematocrit, red blood cell count or iron level is below the lower limit of normal at the screening visit and is confirmed by a repeat test within two weeks of the original test.
- You show evidence of active liver disease or liver enzyme levels above 1. 5 times the upper limit of normal.
- Your LDL Cholesterol is $\geq$ 190 mg/dl at the screening visit.
- You practice a vegan dietary lifestyle.
- You have a history of any eating disorder as determined by questionnaires and/or interviews you answer at the screening visits or at anytime during the study.

- You have a history of pharmacologic treatment for a psychiatric disorder within one year prior to the randomization date or a history of more than one episode of a pharmacologic treatment for a psychiatric disorder within your lifetime.
- You have a history of drug or alcohol abuse (up to 14 drinks a week are allowed) within the past two years.
- You scored ≥ 20 on the Beck Depression Inventory questionnaire at screening or baseline.
- You have any significant behavioral and/or clinical barrier to effective participation in the study as identified by the Barriers to Effective Intervention Interview.
- You have a history of short-term (less than a month) treatment with steroids within six months prior to your randomization into CALERIE Phase II.
- You have a history of treatment with steroids for more than a month within five years prior to your randomization into CALERIE Phase II.
- You require regular use of medications other than contraceptives.
- You participated in CALERIE Phase I studies.
- You smoke or quit smoking less than 12 months prior to screening.
- You have donated blood within 30 days prior to randomization into the study.
- You are participating in another intervention program.
- You are pregnant, breast feeding or planning to become pregnant before the end of the study.
- You engage in a regular physical fitness program involving some kind of heavy physical activity (e.g. jogging, running or riding fast on a bicycle for 30 minutes or more) five or more times per week over the past year.
- You are unwilling to be assigned at random to the CR or control intervention.
- You are unwilling or unable to adhere to the rigors of the CR intervention over the entire two-year period.
- You are unable or unwilling to discontinue dietary supplements or adhere to the alcohol consumption restrictions during the study.
- You are unwilling or unable to adhere to the rigors of the data collection and clinical evaluation schedule over the two-year follow-up period.
- You are female and unwilling to use an acceptable form of contraception (acceptable forms include: tubal ligation, partial or complete hysterectomy, barrier method, oral contraceptive, intrauterine device, implanted progesterone, contraceptive vaginal ring, spousal vasectomy, abstinence or natural family planning when other contraceptive methods are prohibited due to religious reasons) and continue to use such methods while enrolled in the study.

5- What will happen to you if you take part in the study?

Once you have successfully completed the screening phase of the study, you will be asked to return for baseline testing. The first phase of the baseline period will be completed through 4 to 5 outpatient visits. The remainder of the baseline period will require a 2 night, 2.5 day stay at Pennington. The entire baseline process will occur over approximately 30 days.

Following successful completion of the baseline testing, you will be enrolled in the two-year CALERIE study. After enrollment into the study, you will be asked to come to the Pennington Center for follow-up evaluations at Months 1, 3, 9, 12, 18 and 24. Most study procedures will be completed in the outpatient clinic, however, for some of the baseline and follow-up testing you will be required to stay 1-2 nights at the Pennington Center's inpatient unit.

For enrollment into the study, you will be randomly assigned to one of two groups for the next 24 months. By "random", we mean that neither you nor any of the staff (including the doctor) can select the group you will be in. Using a procedure, like flipping a coin, a computer program assigns you to one of the groups. You will keep this group assignment for the remainder of the study. Participants will be assigned in a 2:1 ratio (meaning 2 people will be assigned to group 2 for every 1 person assigned to group 1).

1. Group One: The "regular diet" group will be instructed to continue to follow their regular diet as always and no modifications to their diet will be made. You have one in three chances to be assigned to this group.
2. Group Two: Group two will be the "calorie-restricted" group (CR). This group will be placed on a calorie-restricted diet, with 25% fewer calories then their measured baseline energy requirements. You have two in three chances to be assigned to this group.

If you are in the CR group, you will receive all your food for the first month after randomization. It will be fully prepared by the Pennington Center's Metabolic Kitchen. You will also receive weekly counseling sessions on a personal basis and one session with the other participants enrolled in the same group to help you with any problems that make it difficult to complete the program. During the remaining 23 months in the study, you will make your own selections and prepare your own food from home but will continue bi-weekly counseling on an individual basis and with other participants. You will be asked to fill out a food record every day and bring this to counseling sessions that will be scheduled every week during the initial phase of the study and every other week later in the study. The counseling sessions may be audio-taped. A random sample of counseling sessions will be audio-taped in order to certify that the staff is completing the session as they were trained to do. The frequency of counseling sessions will be tailored to assist adherence to the CR program. The following table explains the minimum frequency for counseling sessions:

Month	**Individual Session**	**Group Session**
Month 1	At least once per week	At least 1 group session
Month 2-6	At least twice per month	At least 2 group sessions per month
Month 7-12	At least twice per month	At least 1 group session per month
Month 13-24	At least once per month	At least 1 group session per month

If you are in the "regular diet group", you will be responsible for making your own food selections and preparing your own meals. You will not be required to return for frequent dietary counseling.

Whatever group you are assigned to, you will be requested to return for scheduled clinic and inpatient visits. In between the clinic visits, if you are in the calorie restricted group, you will be asked to weigh yourself at home weekly (daily during the doubly labeled water periods) on the scales provided to you by study personnel and keep an on-going record of your home weights. If you are in the "regular diet group", you will be requested to weigh yourself daily during each doubly labeled water period for 14 days. Whatever group you are assigned, these weight records should be brought in with you to your scheduled clinic visits.

All CALERIE participants are advised that current health recommendations from the Surgeon General (Centers for Disease Control) are for a minimum of thirty minutes per day of dedicated physical activity of at least a moderate level on a minimum of five days per week. Both groups will be encouraged to follow these physical activity guidelines. Between visits, both groups will

be requested to keep a log of any symptoms or health problems that may arise between visits and to bring this log to their clinic visits. If you are enrolled in the CR group, your log will be reviewed in person at least monthly and you may be asked questions about any information noted on the log. If you are enrolled in the “regular diet” group, your log will be reviewed monthly over the phone and in person at Months 1, 3, 6, 9, 12 18 and 24 visits. This log will be reviewed and you may be asked questions about any information noted on the log. Also, during clinic visits you will have your blood drawn, your resting blood pressure, pulse, heart rate and temperature taken and have an electrocardiogram (ECG) recorded. This is to ensure that you are monitored closely for health changes during the study. You may also be asked specific questions about how you are feeling.

The table below shows which procedures will be performed at each time-point:
* Caloric Restricted group only

Evaluation	Follow-Up Month									
	Baseline	**1**	**3**	**6**	**9**	**12**	**17**	**18**	**23**	**24**
General Examinations										
Medical and medication history	X									
Physical Exam	X					X				X
Vital Signs	X	X	X	X	X	X	X	X	X	X
Medications	X	X	X	X	X	X		X		X
Electrocardiogram (ECG)	X	X	X	X	X	X		X		X
Contraception Verification	X	X	X	X	X	X		X		X
Adverse Events	X	X	X	X	X	X		X		X
Safety Tests										
Hematology	X	X	X	X	X	X		X		X
Serum Chemistry	X	X	X	X	X	X		X		X
Urinalysis	X	X	X	X	X	X		X		X
Pregnancy Test (Females only)	X			X*		X	X	X*	X	X
Metabolic Testing										
Doubly Labeled Water (DLW)	XX			X*		X		X*		X
Resting Metabolic Rate (RMR)	XX			X*		X		X*		X
Core Temperature	X			X		X				X
Clinic Weight	X	X	X	X	X	X	X	X	X	X
Home Weights	XX			X		X		X		X
Cardiovascular										
Resting Blood Pressure	X	X	X	X	X	X	X	X	X	X
Bloodwork	X					X				X
Glucose Tolerance and Insulin										
Oral Glucose Tolerance Test (OGTT)	X					X				X
Blood work	X					X				X
Immune Function										
Delayed-type hypersensitivity (DTH)	X					X				X
Antibody response to vaccines	X						X	X	X	X
Endocrine Responses										
Blood work	X					X				X
Bone										
Markers of bone resumption and formation	X			X		X				X
Psychological Assessments										
Questionnaires (4)	X			X		X				X
Cognitive Function										
Testing Battery	X			X		X				X
Physical Activity										
7-Day Physical Activity Record	X XX X			XX*		XX		XX*		XX
Muscle Strength and Endurance										
Isokinetic Strength	X					X		X		X
Grip Strength	X					X		X		X
VO_2 max	X					X		X		X
Body Composition										
Waist Circumference	X	X	X	X	X	X		X		X
Fat Mass/Fat Free Mass (DEXA Scan)	XX			XX*		X		X*		X

The following is a summary of the procedures that will be completed throughout the study period (see schedule on page 6):

- Blood pressure, heart rate, temperature and respirations will be measured at baseline and months 1, 3, 6, 9, 12, 18 and 24.
- Pregnancy Test: (On Day 0, 14 and 28 of the baseline periods for both groups), Day 0 and Day 14 at Months 12 and 24 for regular diet group and Day 0 and Day 14 at Months 6, 12, 18 and 24 for the CR group. Pregnancy testing will also be performed during Months 17 and 23 visits when vaccination for immune testing will be provided. All women will provide a urine sample to test for pregnancy. If you are pregnant, you will be withdrawn from the study and no further testing will be performed.
- Contraception Verification: (Baseline, Months 1, 3, 6, 9, 12, 18 and 24) The clinic staff will verify female's current method of contraception.
- Body composition and bone mineral density by DEXA: (Day 0 and 28 at Baseline for both groups and Day 0 at Months 12 and 24 for controls; and Day 0 and 14 of Month 6, and Day 0 of Months 12, 18 and 24 for calorie restricted group) This test involves changing into a hospital gown, removing any metal objects and lying on a table called a DEXA scanner. This scanner uses low-dose x-rays to determine the amount of fat, bone and muscle in your body. Results of this test are used in the calculations of your adherence to the diet you are assigned.

  Precision assessments for whole body DEXA scans will be performed on the first 30 study participants at each site in the study by that sites's primary DEXA operator. After the first whole body scan is acquired, these participants will be asked to stand up and then be repositioned on the scanner table for a second whole body scan. The results of the repeated scans will be used by the central DEXA Reading Center to calculate appropriate measures of precision for the outcomes of interest, including fat mass, lean soft tissue mass, % fat and bone mineral content for the whole body and for subregions.

  Waist circumference measures will also be obtained at baseline and at months 1, 3, 6, 9, 12, 18 and 24.
- Doubly-Labeled Water (DLW): (Two separate doses at Baseline and one dose at Months 12 and 24; The CR group will also complete this procedure at Months 6 and 18) This test measures your total energy expenditure over a 14 day period through the collection of urine samples. Total energy expenditure is used to calculate adherence to your diet. At each DLW dose, you will be requested to provide a urine sample, before drinking a glass of water that is enriched with two atoms which are called stable isotopes (non-radioactive). The rest of the day, you will be asked to provide periodic urine samples. Measures of the 2 atoms in your urine will tell us how many calories you are burning. This value will be used to estimate your daily caloric intake over the next 2 years.
- Resting Metabolic Rate: (Twice during the baseline period and once at Months 12 and 24; the CR group will also complete the measure once at Months 6 and 18) Your resting metabolic rate is the number of calories you burn while resting in bed. This test will be performed after an overnight stay at the clinic, immediately after waking and involves lying quietly while breathing normally with a clear canopy (a large plastic hood) placed around your head for about 45 minutes to 1 hour. The hood is ventilated with fresh room air. This will permit the measurement of how much oxygen you breathe in and how much carbon dioxide (CO_2) you breathe out. From these measures, we can calculate how many calories you burn at rest.
- VO2 Max: (Baseline, Months 12 and 24) While fasting, your aerobic fitness level will be assessed while you run on a treadmill. Following a 5 minute warm-up, you will run at a

comfortable pace and grade that will progressively get harder each minute until you are winded and unable to continue. During the test, the volume of oxygen intake and carbon dioxide (CO_2) production will be measured continuously using a metabolic cart, a non-invasive equipment that is also used to measure your metabolic rate. The test requires a mouthpiece which you will breathe through and a nose clip during the exercise test. Your heart rate will also be monitored continuously using a heart rate monitor.

- Oral Glucose Tolerance Test: (Baseline, Months 12 and 24) The oral glucose test will be done to measure your body's response to glucose (sugar). Before you eat breakfast you will drink a glucose beverage and have blood drawn every 30 minutes for 2 hours. An intravenous catheter (tube) will be temporally placed in your arm and blood will be collected through this tube. At 4 different time intervals for each testing period, approximately one milliliter (less than ½ teaspoon) of blood will be drawn. You will be asked to consume a high carbohydrate diet (at least 150 grams of carbohydrates) for approximately three days prior to this testing.
- Blood Tests: (Baseline, Months 1, 6, 17, 18, 23 and 24) Blood will be drawn for outcome measurements, antibody titers, hormones, inflammation and bone function. About 2 teaspoons of blood will be drawn during each testing period.
- Safety Tests: (Baseline, Months 1, 3, 6, 9, 12, 18 and 24) Blood will be drawn for hematology, chemistry and urinalysis for both groups. It is recommended that study participants not donate blood during participation in this study due to the total volume of blood taken. The total amount of blood withdrawn over the total of the 2 year period will be approximately what you would give for 3 standard blood donations.
- Muscle Strength Testing: (Baseline, Months 12 and 24) To test your leg strength, you will be asked to press against a bar as hard as possible and lifting as much weight as possible. Your grip strength will also be measured by squeezing a hand grip tool.
- Assessment of Physical Activity: (Twice at Baseline, Months 12 and 18 for controls; twice at Baseline, Months 6, 12, 18 and 24 for CR group) You will be asked to record your physical activity over the past 7 days so your level of activity can be estimated at different time points during the study.
- Psychological Testing: (Baseline, Months 6, 12 and 24) You will be given several forms to complete at your clinic visit. Some of the questionnaires ask explicit questions related to mood, eating behavior, weight control strategies, sexual functioning and other similar topics. You may either honestly answer these questions or you may choose to not answer the questions. You will also complete computerized tasks to determine how you feel about your body.
- Cognitive Testing: (Baseline, Months 6, 12 and 24) You will be given several forms to complete and short tasks to do. You will be asked to provide word definitions, repeat aloud numbers and object/animal names that are read to you, answer background information questions (i.e. age, occupation, highest education, level achieved, etc.) and other questions pertaining to your cognitive and emotional state. Additional cognitive tasks will be performed to determine how you process information.
- Core Temperature: (Baseline, Months 6, 12 and 24) During overnight stays, your internal body (core) temperature will be monitored by swallowing a small silicone coated radio capsule. This capsule will constantly send a radio signal to a recorder worn on your belt to record your core temperature for 24 hours. The capsule normally remains in the body for 24-72 hours and will be passed as you move your bowels.
- Immune Function Testing: (Baseline, Months 17, 18, 23 and 24) Immune status, an indicator of the body's ability to fight infection, will be assessed from measurements of

delayed type hypersensitivity response (DTH), examination of the blood cells and response to vaccines. The DTH test is similar to an allergy or tuberculosis skin test.

Delayed-type hypersensitivity response to three different antigens and a normal saline (salt) solution will be preformed. The antigens will be tiny amounts of protein, for example animal dander or pollen that stimulate a mild immune response. These will be injected into the skin (intradermally), like a tuberculosis skin test you had as a kid, on the inner surface of the arm at baseline, months 12 and 24. The diameter of the reddened skin around the antigen injection sites will be measured at 24 and 48 hours after administration and compared against the diameter of the reddened skin around the injection of the normal saline solution to gauge your response.

Three different vaccines (hepatitis A, pneumococcus, and tetanus/diphtheria) will be administered at Month 17. Participants who have received the Hepatitis A and/or pneumococcal vaccination prior to enrollment in the study will not receive the vaccine as part of the immune function testing. A booster shot of the Hepatitis A vaccine will be administered at 23 months. The booster will only be administered to those participants who received the vaccination at Month 17. Blood will be collected at baseline, months 17, 18, 23 and 24 for measurement of antibodies. Your body's antibody levels, in response to the vaccines, are another way to assess your immune status. If you are female it is recommended that you not be pregnant at the time of the Hepatitis A vaccinations, therefore urine pregnancy testing will be performed at Months 17 and 23 prior to these Hepatitis A vaccinations.

- Optional Testing

The following table shows the optional testing time-points:

- Biological Specimens (Optional Testing)

Evaluation	**Follow-Up Month**									
	Baseline	**1**	**3**	**6**	**9**	**12**	**17**	**18**	**23**	**24**
Archival Specimens										
Blood (includes WBC, serum and plasma)	X		X	X		X		X		X
Urine (24 hour collection)	X					X				X
Muscle (Quadriceps biopsy) (optional)	X					X				X
Fat (Abdominal Biopsy) (optional)	X					X				X

About using blood, urine and tissue for future research: CALERIE study will collect samples of blood for assays at baseline and Months 3, 6, 12, 18, and 24 visits. CALERIE study would like to collect urine collected over a 24 hour period at baseline, 12 and 24 months. CALERIE study would also like to collect muscle and fat tissue samples (optional) taken at baseline and Months 6, 12, and 24 visits. If you agree, the blood, urine and optional tissue samples will be kept by the CALERIE study and may be used in future research to learn more about effects of caloric restriction. The blood, urine and optional tissue samples will be given only to researchers approved by the CALERIE Steering Committee. Any research study using your samples must also be approved by an IRB. The research that is done with your blood, urine and optional tissue samples is not designed to specifically help you. It might help understand effects of caloric restriction in humans when new research techniques become available. Reports about research done with your samples will not be given to you or your doctor. These reports will not be put in your health records. The research using your blood, urine and optional tissue samples will not affect your care.

Things to think about: The choice to let the CALERIE study keep the blood, urine and optional tissue samples for future research is up to you. No matter what you decide to do, it will not affect your care. If you decide now that your blood, urine and optional tissue samples can be kept for research, you can change your mind at any time. Just contact your study investigator and let him or her know that you do not want CALERIE study to use your blood, urine and tissue samples, and they will no longer be used for research. Otherwise, they may be kept until they are used up, or until CALERIE Steering Committee decides to destroy them.

In the future, people who do research with your blood, urine and tissue samples and people who do other types of health-related research may need to know more about your health. While CALERIE investigators may give these researchers reports about your health, they will not give them your name, address, phone number, or any other information that will let the researchers know who you are.

Sometimes blood, urine and tissue samples are used for genetic research (about diseases that are passed on in families). Even if your blood, urine and tissue samples are used for this kind of research, the results will not be told to you and will not be put in your health records.

Your blood, urine and tissue samples will only be used for research and will not be sold. The research done with your samples may help to develop new products in the future, but you will not be paid.

Benefits and risks: The possible benefits of research from your blood, urine and tissue include learning more about what effects caloric restriction may have on aging and aging-related diseases, how to prevent them, and how to treat them.

The greatest risk to you is the release of information from your health records. CALERIE investigators will protect your records so that your name, address, phone number, or any other information that may easily identify you will be kept private. The chance that this information will be given to someone else is very small.

Making your choices

Please read each question below and think about your choice. After reading each question, circle “yes” or “no.” If you have questions, please talk to your investigator or their staff member.

Remember, no matter what you decide about the collection and use of the blood and urine samples, and optional tissue samples in this research study, you may still take part in CALERIE study.

1. I agree to participate in the optional testing for muscle and fat tissue collection at baseline, 12 and 24 months.

YES NO Subject’s Initials_____

2. My blood, urine and optional tissue samples may be kept by CALERIE study for use in future research to learn more about effects that caloric restriction may have on aging and aging-related diseases, and on how to prevent them, and how to treat them.

YES NO Subject’s Initials_____

(If you answer “NO” to question 2, please also answer question 3)

3. My blood, urine and optional tissue samples may be kept by CALERIE study for use only by CALERIE investigators in future research to learn more about effects that caloric restriction may have on aging and aging-related diseases, and on how to prevent them, and how to treat them. My blood, urine and tissue samples cannot be shared with the researchers outside CALERIE study.

YES NO Subject's Initials_____

- Follow-Up Questionnaire: There is a possibility a follow-up questionnaire will be sent after your completion of the study. The purpose of this questionnaire is to gather information on your long term eating habits and weight changes after study completion.

Summary of all testing periods:
Most testing periods will be completed in more than one clinic visit (either outpatient or inpatient). Below is a description of the number of visits for each testing period.

- Baseline: 5 outpatient visits and a 2 night/3 day inpatient stay
- Month 1: 1 outpatient visit
- Month 3: 1 outpatient visit
- Month 6: CR Group – 2 outpatient visits and a 1 night/1 day inpatient stay
  Regular diet group – 1 night/1.5 day inpatient stay
- Month 9: 1 outpatient visit
- Month 12: 2 outpatient visits and a 2 night/2.5 day inpatient stay
- Month 18: CR Group – 2 outpatient visits and a 1 night/1day inpatient stay
  Regular diet group – 1 outpatient visit
- Month 24: 2 outpatient visits and a 2 night/2.5 day inpatient stay

All participants should agree to avoid taking any new non-prescription medications, vitamins or nutritional supplements during the study without informing the study physician and agree to inform the study personnel of any changes in any medications prescribed by a personal physician. A daily multivitamin/mineral and calcium supplement will be provided to both groups by the study. If you are female and change your method of contraception while enrolled in the study, then you should notify the study staff immediately.

You cannot participate in this study if you are or plan to become pregnant during the study, or if you are breast feeding. It is not known if caloric restriction can cause harm to an unborn child.

Except for complete abstinence (no sexual intercourse), no method of birth control is always 100% reliable. Although the risk of becoming pregnant is low with many methods, unplanned pregnancies occur with all of them. If you are a woman of child bearing potential, you must use adequate contraception while in the study. Your study doctor will discuss the acceptable methods of birth control to reduce your risks of getting pregnant. You should immediately contact your study doctor if there is a change in your contraception method.

If you become pregnant, suspect pregnancy or if you missed your period or it is late, of if you have a change in your usual menstrual cycle (i.e. heavier bleeding during your period or bleeding between periods), you should immediately contact your study doctor.

6- What are the possible risks and discomforts?

There are certain risks and discomforts that may be associated with the study. They include the following:

Calorie Restricted Diet Leading to Weight Loss Calorie restriction requires a diet adequate in minerals, vitamins, and proteins in order to be safe. The dieticians involved in this study are trained in creating diets containing adequate nutrition. You will be seen frequently in an effort to quickly discover any trend leading to a nutrition problem. As an extra precaution, all participants in the study will be provided a daily multi-vitamin and mineral supplement. We will look for dietary deficiencies and watch for signs such as faintness, postural pulse and blood pressure changes which, if found, will be treated. You may/may not feel weak or tired at times if you are in the "calorie restricted" group

Excessive weight loss is associated with some possible health risks which include loss of bone density, electrolyte disturbances, constipation, gall and kidney stones, cognitive dysfunction (difficulty concentrating) and sex hormone abnormalities (including disturbances of the menstrual cycle for females and loss of libido for males). Too rapid weight loss may increase the risk of these problems as well as the risk of heart rhythm abnormalities. You will be monitored closely for the development of these events if you are assigned to the "calorie restricted" group through more frequent collection of blood, urine and electrocardiograms. Cognitive testing and questionnaires will be reviewed for any undesirable changes. If you experience any of these problems you need to report such problems to your study investigator or a member of the research staff as soon as possible. If weight loss is thought to be too rapid, the investigators may increase your daily food intake by providing more food or suggesting you eat more food (depending on the phase of the study). If you are in the "calorie restricted" group, you will be monitored closely regarding your weight loss, if it continues to be too rapid or falls below the normal range, you may be asked to discontinue the study. You will be provided contact numbers in case of an emergency and you should inform the investigators and/or nursing staff of any changes in your health.

The risk of developing gall stones increases during rapid weight loss. This is due, in-part to the gall bladder not contracting. This is unlikely to happen because you will have at least 10 grams of fat per day as part of your daily dietary intake if you are in the "calorie restricted" group.

There may be unknown risks to you, the embryo, fetus or nursing infant if you are or become pregnant during the study or are breast feeding during the study.

Blood Pressure Testing You may experience temporary discomfort during blood pressure recordings due to the pressure of the cuff on your arm.

Dual Energy X-ray Absorptiometry (DEXA) DEXA is used to measure your body composition (fat and lean mass content) and provides minimal x-ray exposure, about the same amount you would receive from 12 hours background radiation from the sun. Exposure to radiation can harm an unborn child therefore pregnant women are not allowed to participate in this study and undergo this procedure.

Precision assessments for whole body DEXA scans will be performed on the first 30 study participants at each site in the study by that sites's primary DEXA operator. After the first whole

body scan is acquired, these participants will be asked to stand up and then be repositioned on the scanner table for a second whole body scan. The results of the repeated scans will be used by the central DEXA Reading Center to calculate appropriate measures of precision for the outcomes of interest, including fat mass, lean soft tissue mass, % fat and bone mineral content for the whole body and for subregions.

Doubly Labeled Water (DLW) Measuring your energy expenditure by DLW carries no risk. The two natural atoms given in the water are not dangerous at all and are often given to small children and pregnant women. The water you drink during this test is prepared under sterile techniques.

Resting Metabolic Rate (RMR) The measure of your RMR using a ventilated hood carries no risk. The only adverse factor about this testing may be a feeling of claustrophobia. A person will be at the bedside at all times and will check to see that you are comfortable and awake. The transparent hood can easily be removed, if necessary.

Muscle Strength and Endurance Testing Flexing and extending your knee while sitting in a chair even with minimal effort is considered to be moderate physical activity and no more dangerous then the activities that you experience in your every day life. As with any new activity, you could experience some soreness in the muscles tested on the following day. In addition to the risks listed above, you may experience a previously unknown risk or side effect. You will be asked in advance about any previous injury, which could prevent you from participating in this test.

VO2 Max Testing There is a possibility of certain changes that may occur during maximal or sub-maximal exercise. They include abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instances, heart attack, stroke or death (about a 2 in 10,000 chance). The increase in this risk is very low ($<$0.01%) and similar to when you exercise during your daily life. You may experience “shortness of breath” or become “dizzy” or “lightheaded” during maximal exercise testing and high intensity cardiovascular exercise. These feelings are normal and transient in nature. If these feelings are prolonged and increase in intensity after the end of exercise, notify the exercise physiologist or Principal Investigator immediately. A doctor will be present during treadmill testing for volunteers who are at risk according to ACSM guidelines. Exercise that is not commonplace or routine may also cause muscle soreness and stiffness. This is normal at the beginning of an exercise program and should subside with time. However, if undue soreness or stiffness continues, or if more than slight swelling occurs, please notify the Principal Investigator. Peak VO2 testing for endurance does include the risk of sudden death (about 2 in 10,0000). Finally with any physical activity, there is a chance of muscle injury, ligament and tendon injury, as well as skeletal injury.

Electrocardiogram (ECG) There are no risks associated with this test. There is a small possibility there may be some redness or itching if you happen to be allergic to the electrodes’ adhesive.

Psychological Testing There are no anticipated risks during participation in this testing. If signs of minor stress or fatigue are apparent, you will be given time to take a break from testing. The questions contained in some of the questionnaires may make you feel uncomfortable. Your responses to the research questions will be coded to protect your confidentiality. Some questionnaires ask explicit questions related to mood, eating behaviors, weight control

strategies, sexual functioning and other similar topics. You may either honestly answer these questions or you may choose to not answer the questions.

Cognitive Functioning There are no anticipated risks during participation in this testing. If signs of minor stress or fatigue are apparent, you will be given time to take a break from testing. During cognitive testing, you will complete a number of tasks that measure your attention and concentration, memory, processing speed and the way you process information. The examiner will encourage you to do the best that you can on these tasks.

Interview Assessments Interviews could cause fatigue and possible anxiety. Fatigue will be kept to a minimum by keeping the interview sessions brief and taking breaks. You may also refuse to answer questions which cause you anxiety.

Immune Function Your immune function will be tested on various occasions during the study by a delayed hypersensitivity skin test (similar to an allergy or TB test). Each intradermal injection contains a small amount of protein that stimulates the body's ability to fight infection. The risks associated with these skin tests include redness, swelling and/or discomfort at the test site. Allergic reactions to the antigens are possible and might occur. Systemic allergic reactions, including anaphylactic shock, may occur in some people; anaphylaxis is a severe allergic reaction that can result in hives, asthma symptoms, swelling of the mouth and throat area, difficulty breathing, and for some participants could be life-threatening. If such reactions occur, epinephrine will be administered by the medical or nursing staff. Epinephrine might cause temporary sweating, headache or shaking.

Other immunological measurements will be performed using blood samples drawn at baseline, Months 12, and 24. Hepatitis A, pneumococcus and tetanus vaccination testing will be performed at Month 17 and a Hepatitis A booster will be performed at Month 23. The vaccine antibody responses to hepatitis A, pneumococcus and tetanus/diphtheria will be measured at baseline, Month 17 prior to the vaccinations, one month after vaccination at Month 18, prior to the Hepatitis A booster at Month 23 and one month after the booster at Month 24 for all three vaccination responses. If you have been previously (prior to enrollment in the study) vaccinated with Hepatitis A and/or pneumococcus , then you will not complete this portion of the immune function testing. There is possible risk these vaccinations may cause allergic reactions in some people that would be allergic to the antigen. All these tests are routinely used for monitoring immune status by physicians.

Venipuncture (blood drawn) You will undergo needle sticks during visits where blood samples are collected, i.e. for a baseline blood sample, follow-up blood samples and the neuroendocrine testing. You may have pain, light-headedness, infection, bleeding or bruising at the site of injection; however the staff will use proper technique while taking blood samples in order to reduce the risk of these unwanted effects. You may feel hungry or weak during the times you are required to fast. For some tests on IV catheter, will be inserted to withdraw your blood. Precautions will be followed by staff to reduce unwanted effects. The total amount of blood withdrawn over the total 2 year period is about 1500 milliliters (approximately 100 tablespoons). This amount is approximately what you would give for 3 standard blood donations.

Muscle Biopsy (Optional Testing) This procedure is used to sample muscle cells from underneath the skin of the leg. After cleaning the skin with iodine and using a local anesthetic, the doctor will make a small incision in the skin and introduce a needle under the skin to remove muscle cells. About 200-750 milligrams (less than a teaspoon size) of muscle will be removed.

After the biopsy is completed, the skin will be held closed with a sterile adhesive bandage and an antibiotic ointment will be applied.

Biopsies carry the risk of bruising, pain, local infections, and small scars. The biopsy will be performed while you are an in-patient on the metabolic ward and you will be asked to check the biopsy site(s) for signs of infection for the first 48 hours and report any problems to the research staff. The plastic dressing will be removed by nursing staff two days after the procedure.

For muscle biopsy, about one in 70 will have temporary damage to the upper thigh nerve of their skin. This loss of skin sensation may be temporary (3 months) or in some cases permanent. However, it generally goes away within 6-7 weeks and does not affect muscle or joint function.

Fat Biopsy (Optional Testing) This procedure is used to sample fat cells from underneath the abdominal skin after cleansing the skin with iodine and using a local anesthetic. After cleansing the doctor will make a small incision in the skin and introduce a needle under the skin to remove fat cells. About 1 gram (less than half a teaspoon size) of fat will be removed. After the biopsy is completed, the skin will be held closed with a sterile adhesive bandage and an antibiotic ointment will be applied.

Biopsies carry the risk of bruising, pain, local infections, and small scars. The biopsy will be performed while you are an in-patient on the metabolic ward and you will be asked to check the biopsy site(s) for signs of infection on a daily basis for the first 48 hours and report any problems to the research staff. The plastic dressing will be removed by nursing staff two days after the procedure.

Others In addition to the risks listed above, you may experience a previously unknown risk or side effect. Continuous monitoring by the investigators will minimize all potential risks and discomforts. If you are a woman, and miss a menstrual cycle (period) during the study, you should notify the investigator(s). If you are pregnant, or intend to become pregnant during the study, you should not participate in the study and notify the investigator(s) immediately if you learn you've become pregnant during the study.

The investigator is willing to discuss any questions you might have about the severity, frequency and the duration of these risks and discomforts.

7- What are the possible benefits?

We cannot promise any benefits to you for your participation in the study. However, possible benefits include gaining information about your current health and receiving dietary advice at no charge. You may lose weight if enrolled in the "calorie restricted" group of the study. Weight loss has secondary benefits for you and society; such as loss of body fat, decreased risk of diabetes, lowering blood pressure, lowering of blood cholesterol and improvements in cardiovascular function.

8- If you do not want to take part in the study, are there other choices?

You have the choice at any time not to participate in this research study. If you choose not to participate, any health benefits to which you are entitled will not be affected in any way.

9- If you have any questions or problems, whom can you call?

If you have any questions about your rights as a research volunteer, you should call the Institutional Review Board Office at 225/763-2693 or Dr. Claude Bouchard, Executive Director of PBRC at 225/763-2513. If you have any questions about the research study, contact Dr. Eric Ravussin (PI) at 225/763-3186. If you think you have a research-related injury or medical illness, you should call Dr. Alok Gupta at 225/763-2656 during regular working hours. After working hours and on weekends you should call the answering service at 225/765-4644. The on-call physician will respond to your call.

10- What information will be kept private?

Every effort will be made to maintain the confidentiality of your study records. However, someone from the National Institutes of Health, National Institute on Aging, the Pennington Biomedical Research Center, and the Coordinating Center at Duke University may inspect and/or copy the medical records related to the study. Results of the study may be published; however, we will keep your name and other identifying information private. Other than as set forth above, your identity will remain confidential unless disclosure is required by law.

Since the study is sponsored, a representative of the funding agency (NIA) may inspect these research records. As representatives of the NIA, the CALERIE Coordinating Center, Duke Clinical Research Institute (DCRI) may inspect your research records. All study related data will be transferred into a centralized database at the DCRI. Only authorized personnel at the DCRI will have access to the data files containing these data. Security will be accessed through user logon IDs, passwords and appropriate access privileges. All study participants will be identified by their study ID number and no personal identifying information, such as name, address, telephone numbers, etc., will be entered into the Coordinating Center's database. Any participants' specific data reported to study leadership will be identified only by the study ID number.

DLW urine samples will be sent to Baylor College for Medicine to be analyzed and blood and biopsy samples will be sent to University of Vermont and Esoterix to be analyzed. The University of Vermont Laboratory will be reserving some sampling for storage in a repository for CALERIE investigators as well as other researchers applying for CALERIE ancillary studies will have access to your data and/or biopsy and archive blood/urine sample data. Your identity will not be known and the samples will only be identified by the study ID number and specimen visit date. DEXA scans will be sent to a central reading center at the University of San Francisco for interpretation and reading results will be sent to the DCRI database. Food records will be sent to a central reading center at the University of Cincinnati for data entry; this data will then be transferred and merged into the database at DCRI. Other researchers applying for ancillary studies will have access to your data and/or biopsy and archived blood/urine sample data. However, this data will be de-identified, through use of a study ID number with your permission.

Audiotapes with recordings of the counseling sessions will be sent to the Pennington Biomedical Research Center where the designated psychologist will listen to them and score them to evaluate whether your psychologists and dieticians presented you with all necessary information as required for this study. Your identity will not be known and the tapes will only be identified by the study ID number and session date. Tapes will be destroyed after they are scored.

Finally, participants will not be identified in any reports or publications, nor will the data be presented in such a way that the identity of the individual participants can be identified. All participants' identifiers will be stripped off any study results analysis files.

The research team will use and share your information until 5 years after closure of the study. At that time afterwards, the research information in your records at the coordinating center will be destroyed or information identifying you will be removed, making it impossible to link you to the study. Your participation is voluntary and you may choose not to participate in this research study or you may withdraw at anytime. Your choice will not affect the commitment of your health care providers to administer care and there will be no penalty or loss of benefits to which you are otherwise entitled. Also, your study physician also has the right to discontinue you from the study at any time if your health or safety could be harmed, if you continued in the study. While in the study, you will also be told about any new information that might make you change your mind about participating in the study.

If you decide to discontinue your participation in the study before your two-year commitment is over and you do not want your information used in study analyses or shared with other centers as stated above, then you must provide this in writing to Dr. Eric Ravussin at Pennington.

11- Can your taking part in the study end early?

Drs. Eric Ravussin and Alok Gupta or the study sponsor can withdraw you from the study for any reason or for no reason. You may withdraw from the study at any time without penalty. Possible reasons for withdrawal include inability or unwillingness to continue participation and adherence to the required screening procedures. The sponsor of the study may end the study early.

12- What if information becomes available that might affect your decision to stay in the study?

During the course of this study there may be new findings from this or other research which may affect your willingness to continue participation. Information concerning any such new findings will be provided to you.

13- What charges will you have to pay?

None

14- What payment will you receive?

You will receive $5000 for completing the CALERIE study. This reimbursement will be provided in installments throughout the study according to your time and effort. Your study doctor or coordinator will give you more information on the reimbursement schedule. If you do not complete the study, your reimbursement will be prorated for the visits which have been completed. Your check will be requested from the LSU payroll department when you complete the study or at the appropriate milestone if you are compensated during the course of the study. It usually takes about 2-3 weeks for it to arrive at Pennington.

15- Will you be compensated for a study-related injury or medical illness?

No form of compensation for medical treatment or for other damages (i.e., lost wages, time lost from work, etc.) is available from the Pennington Biomedical Research Center. In the event of injury or medical illness resulting from the research procedures in which you participate, you will be referred to a treatment facility. Medical treatment may be provided at your expense or at the expense of your health care insurer (e.g., Medicare, Medicaid, Blue Cross-Blue Shield, Dental Insurer, etc.) which may or may not provide coverage. The Pennington Biomedical Research Center is a research facility and provides medical treatment only as part of research protocols. Should you require ongoing medical treatments, they must be provided by community physicians and hospitals.

This research is not intended for the purpose of diagnosing or treating any medical problems, not specifically stated in the purpose of the research. Participation in a research study does not take the place of routine physical examinations or visits to your personal physician.

16- HIPAA

Records that you give us permission to keep, and identify you will be kept confidential as required by law. Federal Privacy Regulations provide safeguards for privacy, security, and authorized access. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in records disclosed outside of Pennington Biomedical Research Center (PBRC). For records disclosed outside of PBRC, you will be assigned a unique code number.

Protected Health Information (PHI) is any health information through which you can be identified. PHI is protected by federal law under HIPAA (the Health Insurance Portability and Accountability Act). A decision to participate in this research means you agree to allow the research team to use and share your PHI for the study explained above. The research team will look at your hospital/medical records (in and out-patient), laboratory, pathology and/or radiology sample results, questionnaires/interviews, and other pertinent medical information. and record such information needed for the study in the research file. Your research file will contain information such as your initials, subject ID # and date of birth. PHI may be shared with individuals involved in the research study.

17- Signatures

The study has been discussed with me and all my questions have been answered. I understand that additional questions regarding the study should be directed to the study investigators. I agree with the terms above and acknowledge that I have been given a signed copy of the consent form.

With my signature, I also acknowledge that I have been given either today or in the past a copy of the Notice of Privacy Practices for Protected Health Information.

_____ _____
Signature of Volunteer Date

_____
Date of Birth of Volunteer

_____ _____
Signature of Person Administering Informed Consent Date

Eric Ravussin, Ph. D.
Principal Investigator

Alok Gupta, M.D.
Medical Investigator