A. SPECIFIC AIMS

Caloric restriction (CR) from birth or during adult life in rodents and lower species prolongs life. Measurement of surrogate markers of longevity suggests that this may be the case in primates as well. The mechanism(s) responsible, and whether this is the case for humans is unknown. CR is associated with several well-known changes in metabolism, including lowering of the metabolic rate. It is unknown which, if any, of these adaptations might be responsible for extending maximum life span. One intriguing hypothesis is that CR lessens the oxidative damage and repair of vital tissues by reducing energy flux and metabolism. CR results in loss of weight and tissues, and lowers the rate of metabolism. A portion of this is the result of the reduced energy intake itself, and another portion is due to the decline in size of the metabolizing mass. Whether there is also a “metabolic adaptation,” defined here as a reduction of metabolic rate that is out of proportion to the decreased size of the respiring mass is a subject of continued debate.

We will test for this, and in addition, if the expected decline in metabolic rate (whether or not proportionate to the respiring mass) that follows CR is associated with reduced oxidative stress in tissues, and risk factors for age-related metabolic diseases, including cardiovascular disease and type 2 diabetes. In addition, we will test if combining physical activity (PA) and CR to produce the same caloric deficit alters the adaptations caused by CR alone. As part of these investigations, we will assess the expression of genes involved in energy metabolism and oxidative stress that are known to be associated with longevity in “lower” organisms.

This proposal is in response to an RFA to test if chronic CR, as it does in rodents and “lower” species and possibly in non-human primates, improves surrogate markers of longevity in humans, and therefore might extend the maximum life span. Thus, this is mainly a descriptive undertaking. However, we also propose to test several interesting hypotheses. These are stated below and listed in Table 1.

Hypothesis A. Chronic CR (resulting in loss of weight and maintenance of energy balance at a new lower body mass) is associated with several metabolic adaptations, including lower absolute and relative rates of energy expenditure, lower body temperature, and evidence of lower tissue oxidative stress.

Aims: Measure and compare for difference:
  A1) Energy expenditure (free living, sedentary 24-h, resting, exercise efficiency), and body temperature.
  A2) DNA, protein and lipid oxidative damage.

Hypothesis B. Chronic CR improves surrogate markers (risk factors) for chronic diseases, including cardiovascular disease and type-2 diabetes. These adaptations are the same whether the energy deficit is produced by combining PA and CR or by CR alone.

Aims: Measure and compare for difference:
  B1) CVD risk factors (BP, lipid profile, hemostasis factors, homocysteine, endothelial function, and markers of inflammation).
  B2) Type 2 diabetes risk factors. (Insulin action and secretion)

Hypothesis C. Chronic CR dampens the activity of the neuroendocrine axes, and lowers SNS activity, potentially by decreased leptin signaling. These adaptations are less pronounced when the same energy deficit is achieved by combining PA and CR.

Aims: Measure and compare for difference:
  C1) Hypothalamic neuroendocrine function (thyroid, adrenal, and somatotrophic axes), and diurnal rhythm of leptin.
  C2) SNS activity

Hypothesis D. Chronic CR is associated with adaptations in the expression of genes involved in aging, including those related to oxidative stress, energy metabolism (carbohydrate, lipid and protein), and longevity.

Aim D: Measure for differences in the expression of candidate genes in skeletal muscle and adipose tissue.

Hypothesis E. Psychophysiologic outcomes are improved when the energy deficit is produced by combining PA and CR compared to CR alone.

Aim E: Measure for differences in weight loss, compliance, rate of drop out, quality of life (QOL), mood, cognitive function, fitness & strength, reaction times, physical activity & food records, and risk of developing an eating disorder.
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title of Study: Metabolic Adaptations to Six Month Caloric Restriction

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?

Principal Investigators: Eric Ravussin, Ph.D. Tel: 225-763-3186

Medical Investigators: Frank Greenway, M.D.
Day Phone: 225-763-2576
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225-763-2576 (Weekdays 8:00a.m.-5:00 p.m.)
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Marlene Most-Windhauser, R.D., Ph.D.
Leonie Heilbronn, Ph.D.

Drs. Eric Ravussin and Donald Williamson direct this study, which is under the medical supervision of Drs. Frank Greenway and Steve Smith. We expect to enroll about 60 people in this study. The study will target volunteers age 25-50 for males and age 25 to
45 for females. The study will take place over a period of two years. Your expected time in this study will be slightly over six months. This study is part of ongoing research in the prevention and treatment of age-related chronic diseases at the Pennington Biomedical Research Center and is sponsored 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, at the Baton Rouge General Hospital and at the Neuromedical Imaging Center on Hennessy Blvd.

3- What is the purpose of this study?
It has been reported for many years that chronic calorie restriction without nutritional deficiencies increases the length of life and prevents 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 in response to a request from the National Institutes of Health to determine if long-term calorie restriction improves markers of age-related diseases in humans. We already know that weight loss improves insulin sensitivity and the lipid profile. Experiments performed in nature suggest that long term calorie restriction (with a healthy diet) may increase life span. This was observed in an island population in Japan that eats a healthy, low calorie diet and has the highest incidence of centenarians in the world.

4- Who is eligible to participate in the study? Who is ineligible?
You may qualify for the study if you meet the following criteria:
- Healthy men and women
- Women ages 25 to 45 (who are not menopausal)
- Men ages 25 to 50
- Men and women with a body mass index between 25 and 30 (a BMI over 25 means that you are mildly overweight)
- Are not travelling often and are planning to stay in the Baton Rouge area

You will not qualify for the study if any of the following applies to you:
- Personal history of cardiovascular disease or an elevated blood pressure (greater than 160/90 mmHg)
- Personal history of Diabetes
- Personal history of major psychiatric disorders
- Personal history of an eating disorder
- Post obese (must never have had a BMI greater than 32)
- Recent weight change (larger than 10 lbs. in the past 3 months)
- Smoking
- Exercising more than twice a week
- Regular use of medications, except oral contraceptives
- Individuals with alcoholism or other substance abuse
5- **What will happen to you if you take part in the study?**

You will have a series of three screening visits, each 1.5 to 2 hours in length. During these visits, the following will be done:

- Measurements will be taken for your height, weight and blood pressure.
- You will need to answer psychological questionnaires.
- You will have an interview with the psychology staff to determine potential obstacles to your participation in the study (i.e. work schedule, family responsibilities, travel schedule, driving distance or emotional problems).
- We will collect urine and blood samples from you.
- A discussion about your availability and your ability to provide dietary and physical activity records will take place.
- You will receive your dietary and physical activity questionnaires.
- A brief personal and family history will be taken.
- A physical exam and EKG will be performed.
- Your diet and physical activity diary will be reviewed (see explanation below)

Between screening sessions you will be asked to keep a food diary of all the foods you consume and all of the exercise you perform during the screening period. If all of the screening visits are completed successfully you will be invited to attend a final session to fully discuss the study requirements.

**Diet:**

You will then begin a 19-day baseline feeding period, the first 14 days of which you spend at home and 5 of which you will spend as an in-patient on the Pennington Metabolic Unit. The diet will consist of three meals a day and an evening snack. The metabolic kitchen of the Pennington Biomedical Research Center will provide all your meals to you. **You must not eat any other foods.** On weekdays you will be required to eat breakfast and dinner at the Pennington Center dining facility. Your lunches and a snack will be prepared for take-out. You will receive weekend meals and snacks on Friday to take home. Your personal likes and dislikes will be taken into account when preparing your meals. You will also be asked to complete a daily food diary. During this baseline feeding period a number of metabolic tests will be performed. These tests
are outlined in the flowchart and explained in more detail on pages 5-10. This testing period will be repeated after you have completed 3 months and again after you have completed 6 months of the study.

After the baseline period, you will be randomly assigned to one of four groups for the next 6 months. By “random” we mean that neither you nor any of the staff (including your doctor) can select the group that you will be in. Using a procedure like flipping a coin, a computer program assigns you to one of the groups.

1. **Group one**: The control group will be placed on a healthy weight maintenance diet based on the American Heart Association recommendations.
2. **Group two**: Group two will be placed on a calorie-restricted diet, with 25% less calories than their measured baseline energy requirements.
3. **Group three**: This group will have their calories slightly less restricted (12.5% of energy expenditure) but will also participate in physical activity, which will increase energy expenditure by 12.5%. The exercise requirements will be increased slowly to minimize the risk of muscle soreness and injury and you will have to perform this exercise at Pennington. By week 6 you will be exercising 5 days per week at a comfortable exercise intensity. After week 6, you will be given the option to exercise three days per week at the center and two days at home. If you exercise at home you may walk or run outside or ride a stationary bike inside.
4. **Group four**: Group four will be placed on a low calorie liquid diet until a 15% weight loss is achieved. Then volunteers will be progressively placed back on solid foods to maintain their new weight (15% weight lower than initial weight). During the liquid diet phase volunteers will need to come into the Clinic every 2 weeks for a metabolic checkup, including a blood test and an EKG.

For the first 3 months after randomization, no matter which group you are assigned, you will receive all your food from the Pennington and must come into the Center to eat breakfast and dinner every day (see flow chart). Your lunch and snacks will be provided to you to take-out. You will also receive weekly counseling sessions on a personal basis or with other members of your group to help you with any problems that make it difficult to complete the program. During the last 2.5 months of the study you will prepare your own food at home but will continue counseling each week.

Once per week, for the duration of the study, you will be weighed in the clinic in a hospital gown before breakfast and have your blood pressure measured. You will also have to provide a urine sample so that we can monitor your compliance to the diet. You will also be given a multi-vitamin to take each day to ensure that you are receiving adequate nutrition. We will provide you with a new supply each month and any unused vitamin pills must be returned to the clinic.
| Day 1-14 | Pregnancy test | Day 15 | RMR / FSIGTT CT Scan / MRS Exercise testing |
| Day 16 | Fat/Muscle Biopsy EKG Psychological Tests | Day 16 | Fat/Muscle Biopsy EKG Psychological Tests |
| Day 17 | 24 hr metabolic chamber | Day 17 | 24 hr metabolic chamber |
| Day 18 | Neuroendocrine Testing | Day 18 | Neuroendocrine Testing |
| Day 19 | TRH Test Discharge at 3PM | Day 19 | TRH Test Discharge at 3PM |

**Baseline: Breakfast & Dinner @ Pennington**

- Day 15
  - RMR / FSIGTT CT Scan / MRS Exercise testing
  - Fat/Muscle Biopsy EKG Psychological Tests
  - 24 hr metabolic chamber
  - Neuroendocrine Testing
  - TRH Test Discharge at 3PM

**Randomization**

- Group 3 ONLY
  - Exercise at Pennington with increasing freq. Up 5d/week after 6 weeks
  - Every two weeks Blood work & EKG

- Group 4 ONLY
  - Every two weeks Blood work & EKG

**Intervention: Breakfast & Dinner @ Pennington**

- Day 15
  - Pregnancy test DEXA (x2) Doubly labeled H2O test Brachial Artery test VO2 Max test
  - 1. counseling session pressure
  - 2. Heart rate monitor

- Day 16
  - Fat/Muscle Biopsy EKG Psychological Tests

- Day 17
  - 24 hr metabolic chamber

- Day 18
  - Neuroendocrine Testing

- Day 19
  - TRH Test Discharge at 3PM

**2.5 Months**

- Day 15
  - RMR / FSIGTT CT Scan / MRS Exercise testing

- Day 16
  - Fat/Muscle Biopsy EKG Psychological Tests

- Day 17
  - 24 hr metabolic chamber

- Day 18
  - Neuroendocrine Testing

- Day 19
  - TRH Test Discharge at 3PM

**Intervention: Breakfast & Dinner @ Pennington**

- Day 15
  - Pregnancy test DEXA (x2) Doubly labeled H2O test Brachial Artery test VO2 Max test
  - 1. counseling session pressure
  - 2. Heart rate monitor

- Day 16
  - Fat/Muscle Biopsy EKG Psychological Tests

- Day 17
  - 24 hr metabolic chamber

- Day 18
  - Neuroendocrine Testing

- Day 19
  - TRH Test Discharge at 3PM

**Intervention: Prepare Meals @ home**

- Day 15
  - Pregnancy test DEXA (x2) Doubly labeled H2O test Brachial Artery test VO2 Max test
  - 1. counseling session pressure
  - 2. Heart rate monitor

- Day 16
  - Fat/Muscle Biopsy EKG Psychological Tests

- Day 17
  - 24 hr metabolic chamber

- Day 18
  - Neuroendocrine Testing

- Day 19
  - TRH Test Discharge at 3PM

**Intervention: Breakfast & Dinner @ Pennington**

- Day 15
  - Pregnancy test DEXA (x2) Doubly labeled H2O test Brachial Artery test VO2 Max test
  - 1. counseling session pressure
  - 2. Heart rate monitor

- Day 16
  - Fat/Muscle Biopsy EKG Psychological Tests

- Day 17
  - 24 hr metabolic chamber

- Day 18
  - Neuroendocrine Testing

- Day 19
  - TRH Test Discharge at 3PM

**Intervention: Breakfast & Dinner @ Pennington**

- Day 15
  - Pregnancy test DEXA (x2) Doubly labeled H2O test Brachial Artery test VO2 Max test
  - 1. counseling session pressure
  - 2. Heart rate monitor

- Day 16
  - Fat/Muscle Biopsy EKG Psychological Tests

- Day 17
  - 24 hr metabolic chamber

- Day 18
  - Neuroendocrine Testing

- Day 19
  - TRH Test Discharge at 3PM

* Test Order is representative only and may change if availability of equipment changes.

**Group 1**
- No Intervention - Weight Maintenance diet, No Calorie Restriction or exercise

**Group 2**
- Calorie restricted diet (25% less calories)

**Group 3**
- Diet-12.5% less calories, 12.5% increase in exercise
- 800 kCal/day liquid diet until 15% weight loss achieved, then weight maintenance diet

**Group 4**
- Fat/Muscle Biopsy EKG Psychological Tests
- 24 hr metabolic chamber
- Neuroendocrine Testing
All groups will be required to wear an accelerometer, except during sleeping and showering or other water based activities, for 1 week each month for the duration of the study. The accelerometer ties around your waist and your wrist and records all movements that you make. For one week each month you will also wear a portable heart rate monitor, which records your heart rate.

**Metabolic Tests:**
At baseline, 3 months and 6 months after randomization, metabolic tests will be performed over a 19-day period (see flow chart). From days 1-14, while you are living at home, your energy expenditure will be measured by doubly labeled water. On days 1 and 14 you will have DEXA measurements performed after an overnight fast. A brachial artery, macronutrient selection and VO2 max test will also be performed during this period.

**Days 1-14**
- Pregnancy test: 10 minutes.
  On day 1 all women will provide a urine sample to test for pregnancy. If you are pregnant then you will be withdrawn from the study and no further testing will be performed.

- Body composition and bone mineral density by DEXA: 20 minutes (Day 1 & 14).
  This test involves changing into a hospital gown, removing any metal objects and lying on a table called a DEXA scanner. The scanner uses low-dose X-rays to determine the amount of fat, bone and muscle in your body. We measure your body composition twice (Day 1 and Day 14) to assess changes in fat mass whilst we are assessing your energy expenditure by doubly labeled water. This tests your compliance to the diet you are assigned.

- Doubly-labeled water: 10 minutes per day for 14 days.
  This test is to measure your total energy expenditure over a 14-day period. After providing a fasting urine sample, you will drink a glass of water that contains 2 atoms found in normal water. However, for the purpose of our measurements, this water has been enriched with these two atoms which are called stable isotopes (non-radioactive). During the rest of the day, you will be asked to provide us with 2 more urine samples. You will then be asked to provide daily urine samples when you come to Pennington for breakfast during the next 14 days. Measures of the 2 atoms in your urine will tell us how many calories you burn during these 2 weeks and about your level of physical activity. This value and your measured metabolic rate will then be used to calculate daily calorie intake for the next six months (unless you are in group 4).

- Brachial Artery Test: 30 minutes.
  A brachial artery test will be performed one morning before breakfast. This test
measures how elastic your veins are. After resting on your back for 10 minutes an ultrasound is rubbed over your brachial artery (elbow area) on your non-dominant arm (the side you do NOT write with). After this baseline measurement a blood pressure cuff is placed around your forearm and inflated to reduce blood flow for 5 minutes. When the cuff is removed the ultrasound will be continued for 6 more minutes. You may feel a warm tingling on your elbow from the ultrasound.

- **VO2max test: 30 minutes.**
  Before breakfast on another morning your aerobic fitness level (VO2max) 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 get progressively harder each minute until you are winded and unable to continue. During the entire test, the volume of oxygen uptake and carbon dioxide production will be measured continuously using a metabolic cart. This requires that a mouthpiece and nose clips be in place during the entire exercise test. Heart rate will also be monitored continuously using a heart rate monitor.

- **Macronutrient Self Selection and Fat Preference: 45 minutes**
  For this test you will come to the clinic after an overnight fast and you will be given a selection of foods to eat at lunchtime and also a questionnaire to fill out. This test is performed only at baseline and is designed to tell us whether you prefer to eat foods that are high in sugar, fat or protein.

All other tests will be conducted from days 15-19 while you are an inpatient at Pennington (see flowchart). **You will therefore be required to live for 5 days at the Pennington Center.** You will be admitted to the metabolic unit on the afternoon of day 14. While in the metabolic unit, the following tests will be conducted: psychological testing, body composition testing, muscle strength and endurance testing, energy expenditure testing, and other testing as explained below.

**Day 15**
- **Resting metabolic rate testing (RMR): 40 minutes.**
  We will measure your metabolic rate early one morning (before breakfast) while resting in a bed at the Pennington Center. While you are lying comfortably and awake, we will put a clear plastic bubble over your head and seal it around your pillow. 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 (CO2) you breathe out. From these measures, we can calculate how many calories you burn at any moment during the 40 minutes.

- **Insulin sensitivity and secretion (IVGTT): 3.5 hours**
  In this test, we will measure how well your body produces insulin in response to a sugar challenge. Insulin is normally produced in your body during meals and helps your body use sugar. You will have 2 IV’s that will be inserted into a vein of each
arm. After withdrawing a baseline sample of your blood we will inject a sugar solution into one IV. We will then monitor your blood sugar and insulin from the other IV for 20 minutes. After 20 minutes insulin is injected into the IV and we measure your blood for another 3 hours. No more than 4 ounces of blood are drawn. This is approximately ¼ of the amount taken during a blood donation in a blood bank. You will be given a meal immediately following this test.

- **Body composition by CT scan:** 20 minutes
  The CT scan will be performed at the Baton Rouge General Medical Center on Bluebonnet. You will be asked to change into a hospital gown and remove any metal objects before lying on the CT scanner table. The radiology technician will take X-Ray pictures of your body at the level of your belly, your thigh and your calf.

- **Magnetic Resonance Spectroscopy (MRS):** 1 hour
  You will be asked to remove all jewelry or any other metal objects and lie on your back with your lower body within a large cylindrical magnet for up to 45 minutes while the chemical composition of the fat stored between and within your muscle fibers (extra and intramuscular lipids, respectively) in your calf are measured by MR Spectroscopy. A picture of your calf muscle will also be made using MRS Imaging. During the MRS imaging procedure, you may hear a loud banging noise. You will be provided earplugs and/or a head set with music to help decrease any discomfort from this noise.

- **Energy cost of exercise:** 30 minutes
  This test measures how much energy you burn during bicycling at 3 different levels of intensity by collecting the oxygen you breathe in and carbon dioxide you breathe out. This test requires you to wear a mouthpiece and a nose clip. Your heart rate will also be monitored continuously using a heart rate monitor.

- **Muscle strength and endurance testing:** 20 minutes
  Your strength and endurance will be measured while sitting in a special chair called a Cybex, using your dominant thigh for testing. Your dominant thigh is on the same side as the hand with which you write. Strength will be measured by extending and flexing your lower leg from the knee against a bar on the Cybex chair 5 times using your maximal effort. Endurance will be measured by extending and flexing your lower leg from the knee against a bar on the Cybex chair 20 or more times at a faster rate using a sub-maximal effort. This test will be done at our exercise facility.

**Day 16**

- **Fat and Muscle biopsies:** 1 hour
  *Fat:* This procedure is used to sample fat cells from underneath the skin of the abdomen and the thigh-buttocks area. 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 fat cells. About 1 gram (less than half a teaspoon)
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. 

Muscle: 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 150 milligrams (less than one eighth of a teaspoon) 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.

- EKG Test with meal: 45 minutes
  This test measures your autonomic nervous system. The autonomic nervous system is not under your control but is responsible for functions like your heart rate and blood pressure. In this test we will measure your heart rate by an EKG while you lie in a bed. You will be asked to breathe at a fixed rate of 15 breaths per minute. After you have done this for 5 minutes you will be fed a test meal. After this meal you will continue your breathing at 15 breaths per minute for another 30 minutes. A urine sample will also be taken.

- Psychological testing: 1 hour.
  You will be given several forms to complete. You will also be asked questions about your quality of life, mood, psychological well being and eating attitudes.

- Cognitive testing: 1.5 hour.
  You will be given several forms to complete and some short tasks to do. You will be asked to provide word definitions, to 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.

Day 17
- Metabolic chamber: 23.5 hours
  The amount of calories you burn will be measured in a metabolic chamber. You will spend a day and a night in the chamber. At 7:00 AM, you will swallow a 10 x 20-mm silicone coated radio-capule. This capsule will send a signal to a recorder worn on your belt to record your core temperature. The metabolic chamber measures 10’x14’x8’, which is about the size of a small bedroom. It has two windows, a bed, a desk, a TV/VCR, a telephone and toilet facilities, motion sensors and a camera. A computer is available in case you would like to use one. While you are in the chamber you will follow your own schedule of quiet activities and sleeping. No exercise will be allowed. Meals will be served at fixed times and you will have to eat all food provided. You will be able to contact the chamber personnel by intercom, phone or pager at any time. Local phone calls can be made. You will have to collect all urine while in the chamber. Visual analogue scales will be filled at different time during the day to assess how hungry or full you are.
Days 18 & 19

- Neuroendocrine axes and TRH stimulation testing: 26 hours
  Blood will be removed periodically from the IV placed in your arm over a period of 24 hours. This blood will be used to test the concentration of different hormones during the course of the day, and markers for aging and cardiovascular disease. You will also be asked to chew a cotton swab for 60 seconds on 10 occasions throughout the day to measure your salivary cortisol levels. The following morning, we will inject a single dose of a naturally occurring hormone to measure the effect on thyroid hormones. The maximal amount of blood taken during this procedure is 9 ounces, or half the amount taken during a blood donation.

6- What are the possible risks and discomforts?
The risks associated with the procedures required for these studies include, but are not limited to:

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Risk</th>
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<tr>
<td>Hypocaloric diets</td>
<td>Calorie restriction and exercise require a diet adequate in minerals, vitamins and proteins in order to be safe. The dietitians 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. If you are on the liquid diet we will check your electrolytes every 2 weeks. We will also look for postural pulse and blood pressure changes which, if found, will be treated with salt supplementation. You may therefore sometimes feel weak or tired if you are in one of the caloric restriction groups.</td>
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Weight Loss: During weight loss there is a loss of calcium from the bones, but the heavier a person is, the heavier are their bones. When you lose calcium during weight loss the amount of calcium in your bone will remain normal for your new lower weight. Whilst malnutrition can interfere with your immunity, restriction of calories without malnutrition seems to help immunity. In fact, calorie restriction has been used as a treatment for diseases of the immune system like lupus. The risk of developing gall stones increases during rapid weight loss (such as could occur by liquid diet).
This is due, in part, to the gall bladder not contracting. You will be given 10 grams of fat per day during the liquid diet to cause your gall bladder to contract and minimize this risk. If you are on a food or exercise prescription it is unlikely that you will develop gallstones.

Blood pressure testing
You may experience temporary discomfort during blood pressure recording due to the pressure of the cuff on your arm.

DEXA
Minimal X-ray exposure. Example: 12 hours background radiation from the sun. Exposure to radiation can harm an unborn child, and pregnant women are not allowed to undergo these procedures.

Doubly labeled water
Measuring your energy expenditure by doubly labeled water carries no risk. The two natural atoms given in the water are not dangerous at all and are often given to small children or pregnant women.

Brachial Artery Test
There is no risk associated with low level ultrasound. Occlusion of the forearm also carries no risk but you may experience temporary discomfort when the blood pressure cuff is inflated. The cuff can be deflated immediately upon your request.

Treadmill testing
There is a possibility of certain changes that may occur during maximal or submaximal exercise. They include abnormal blood pressure, fainting, irregular, fast or slow heart rhythm, and in rare instances, heart attack, stroke or death. 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 deemed at risk. 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. Finally with any physical activity, there is a chance of muscle injury, ligament and tendon injury, as well as skeletal injury.

**Resting Metabolic Rate**

The measure of your resting metabolic rate using a ventilated hood carries no risk. The only adverse factor in this 30-minute 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. You may easily and rapidly remove the transparent hood, if necessary.

**Insulin sensitivity/secreton**

You may experience some bruising at the site of needle insertion in veins. The major risk of this test is that your blood sugar may fall due to insulin. Low blood sugar can make you feel sick to your stomach, sweaty, irritable and sometimes confused. We will check your blood sugar after we inject insulin every ten minutes. After the study is finished you will be given fruit juice with sugar and a high carbohydrate meal.

**CT scan**

The CT scan will expose you to an equivalent of two chest X-rays series. Exposure to radiation can harm an unborn child, and pregnant women are not allowed to undergo this procedure.

**Magnetic Resonance Spectroscopy**

There are no significant risks associated with MR spectroscopy and imaging. There is a small chance of claustrophobia or muscle-
skeletal discomfort from lying partially in the magnet for up to 45 minutes. During the imaging measurement, you may hear loud banging that may be somewhat unpleasant. Earplugs and/or a head set are provided to mute this banging. Although the long-term risk of exposure to magnetic fields is not known, the possibility of any long-term risk is extremely low in view of the information accumulated over the past 10 years.

Muscle strength and endurance testing
Flexing and extending your knee while sitting in a chair even with maximal effort is considered to be moderate physical activity and no more dangerous than 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 would prevent you from participating in this test.

Fat and muscle biopsies
These biopsies carry the risk of local infections and small scars. For muscle biopsy about one in 70 will have damage to the nerve to the skin. This goes away within 6-7 weeks. This does not affect muscle or joint function.

Autonomic Nervous system (EKG)
There are no risks associated with this test.

Psychological testing
There are no anticipated risks during participation in this testing. If signs of minor stress are apparent, the session will be discontinued immediately.

Cognitive functioning
There are no anticipated risks during participation in this testing. If signs of minor stress are apparent, the session will be discontinued immediately.

Metabolic chamber
You may experience some level of discomfort (e.g. stress or anxiety) by staying
overnight in the metabolic chamber. However, you will not be locked in and will be able to open the door at anytime. You may feel somewhat uncomfortable to continuously be monitored by a camera. However, the camera has been installed for your own safety, and, apart from the chamber personnel, no one is allowed to enter the room with the monitor without your authorization.

Neuroendocrine tests
This 24-hour test carries the risk of bruises at the IV site. There is no risk associated with the bolus injection of TRH, a naturally occurring hormone.

Venipuncture (blood draw)
You will undergo needle sticks during visits where blood samples are collected, i.e. for a screening blood sample, the insulin sensitivity/secretion test, and the neuroendocrine testing. You may have pain, lightheadedness, 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 for these unwanted effects. You may feel hungry or weak during the times you are required to fast. The total amount of blood withdrawn over the 4 days of testing is no more than 13 ounces. This amount is approximately what you would give for a blood donation.

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.

7- What are the possible benefits?
We cannot promise any benefits from your being in the study. However, benefits include gaining information about your current health, and receiving diet and exercise advice at no charge. You may loose weight and become normal weight. Your cardiovascular health and your glucose metabolism are likely to be improved.

8- If you do not want to take part in the study, are there other choices?
You have the choice at any time and for any reason not to participate in this research study.
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 at 763-3186. If you think you have a research-related injury or medical illness, you should call Dr. Frank Greenway at 225-763-2576 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 Food and Drug Administration, the National Institutes of Health, or the Pennington Biomedical Research Center 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.

11- Can your taking part in the study end early?
Drs. Ravussin or Williams 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 procedures.

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?
There are no charges for you to pay to participate in the study.

14- What payment will you receive?
If you agree to take part, we will pay you $10/day for 180 days (6 months) plus $1000 for each of the 3 fully completed testing blocks, for a total of $4,800. If you are or have been an employee of LSU within the current calendar year, the normal employee payroll deductions will be withheld.

15- Will you be compensated for a study-related injury or medical illness?
No form of compensation for medical treatment 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.
16- 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 copy of the consent form.

__________________________________                              _____________
Signature of Volunteer         Date

__________________________________                              _____________
Social Security No. of Volunteer

__________________________________                              _____________
Signature of Person Administering Informed Consent              Date

__________________________________                             _____________
Eric Ravussin, Ph.D.                                   Date
Principal Investigator

__________________________________                             _____________
Frank Greenway, M.D.                               Date
Medical Investigator

The study volunteer has indicated to me that the volunteer is unable to read. I certify that I have read this consent form to the volunteer and explained that by completing the signature line above the volunteer has agreed to participate.

_________________________________                                             ____________
Signature of Reader                                                                              Date