2. SPECIFIC AIMS AND OBJECTIVES / HYPOTHESES

The overall aim of CALERIE Phase 2 is to test the hypothesis that two years of sustained caloric restriction (CR), involving a reduction in energy intake to 75% of baseline (25% CR), in men and women aged 25 to 45 yr, will result in the same adaptive changes that occur in rodents subjected to CR, with particular emphasis on the adaptive responses thought to be involved in a) slowing aging, and b) protecting against age-related disease processes. A second aim is to identify potential adverse effects of CR in humans.

One reason for two years of CR intervention is that the one year in CALERIE Phase 1 was not sufficient to induce the adaptations in humans that are thought to increase longevity in rats. Many of these adaptations are also seen in humans practicing long-term severe CR. A second reason is to isolate the long term effects of CR (i.e., at weight stability) from the acute effects of weight loss.

2.1 Primary Specific Aims

Our primary specific aim is to test the hypotheses that CR in humans causes sustained (over two years) metabolic adaptation as defined by:

1. a reduction in core body temperature and
2. reduced resting metabolic rate (RMR) corrected for changes in body composition.

A reduction in metabolic rate has been proposed as one of the mechanisms by which CR slows aging, possibly by reducing oxidative damage. A lower resting metabolic rate, as measured in resting conditions and corrected for body composition, may be mediated by a reduction of serum triiodothyronine, decreased tissue conversion of T4 to T3 and/or a reduction in catecholamines (see Secondary Aim 1).

These aims (core body temperature and RMR) are sufficiently powered to provide a robust test of the hypothesis (see Protocol Section 17.2), address key unresolved issues in the field of CR, and are feasible as they build upon the experience of the Phase 1 CALERIE team as described in Protocol Section 4.

2.2 Secondary Specific Aims

Our secondary aims are to test the hypotheses that CR in humans:

1. Reduces serum triiodothyronine. Reduced triiodothyronine is a potential mediator of the predicted metabolic adaptation and will provide insight as to the mechanism of this hypothesized primary adaptation to CR. Decreased catecholamines are another potential mediator of the metabolic adaptation and will be explored as part of Exploratory Aim #2

2. Reduces inflammation as reflected in plasma concentrations of Tumor Necrosis Factor-α (TNF-α). Inflammation is one of the adaptive responses suggested as a mediator of the salutary effects of CR on the aging process in rodents. This measure will be interpreted in the context of additional inflammatory markers enumerated in Exploratory Aim #4.

3. An additional important secondary specific aim is to determine whether CR has adverse effects in humans and to evaluate their seriousness.
These secondary specific aims are sufficiently powered to provide a robust test of the hypotheses (see Protocol Section 17.2), address key unresolved issues in the field of human CR, and are feasible as they build upon the experience of the Phase 1 CALERIE team as described in Protocol Section 4.

2.3 Exploratory Aims

The rationale for these exploratory aims is to obtain information regarding the mechanisms by which CR mediates its effects at the structural, physiological, cellular and subcellular levels. Analyses and reporting of these tests will take into account the high potential for Type I errors resulting from the large number of comparisons and outcomes.

Exploratory aims are designed to test the hypotheses on the effects of two years of calorie restriction in humans as follows:

1. Changes body composition (fat mass, lean mass), including bone mineral density. Changes in caloric intake during CR impact body composition. As such, measuring body composition is important as a covariate for the primary aim (core temperature and RMR) in CALERIE phase 2. Intra abdominal fat is an important covariate for the interpretation of the atherosclerosis and Type 2 diabetes measures. Bone mineral density is important to assess the safety of CR.

2. Changes serum hormones, including DHEAS, cortisol, TSH, thyroid binding globulin, growth hormone, leptin, adiponectin, angiotension II, norepinephrine, sex hormones, and hormone-binding protein levels. Changes in hormone levels could provide clues regarding the mechanisms by which some of the effects of CR are mediated.

3. Lowers plasma growth factor concentrations, including Insulin-Like Growth Factor-1 (IGF-1), Platelet Derived Growth Factor-AB (PDGF-AB) and Transforming Growth Factor-\(\beta\) (TGF-\(\beta\)). A reduction in growth factor levels has been hypothesized to play an important role in mediating the effects of CR on longevity and protection against the development of malignancies.

4. Decreases the intermediate risk factors that are predictive of developing atherosclerosis and Type 2 diabetes as evidenced by improvements in circulating inflammatory cytokines and CRP, serum lipid and lipoprotein levels, lowering serum insulin and glucose levels, reduction in abdominal fat, lowering of blood pressure, and reduction of 10-yr CHD risk using comprehensive population-based risk models. These adaptations are likely to be among the most important protective effects of CR against secondary aging.

5. Reduces oxidative stress, as reflected in urinary levels of isoprostanes, dinitrotyrosine and 2-deoxyguanosine levels. CR has been shown to lower oxidative stress in rodents, and this is thought to be one of the mechanisms by which CR slows aging.

6. Modulates immune function as reflected by a change in lymphocyte count, delayed-type hypersensitivity (DTH) and response to vaccine. Short-term CR has been shown to impact immune function. These studies are important because immune function impacts many other physiological and cellular
systems. In addition changes in the immune system may determine responses to environmental pathogens thus affecting mortality.

These measures capture the effects of CR at the structural and physiological levels. Many of the adaptations to CR occur at the cellular and subcellular levels. To explore the effects of CR on cellular structure and function, Phase 2 of CALERIE will collect and process tissue samples as a means of determining the cell structure, signaling, gene expression and pathways that mediate the beneficial effects of CR. This will occur for both candidate pathways (listed below in Exploratory Aims 7 and 8) and across the transcriptome, genome, and proteome.

7. Skeletal muscle. To explore the effects of CR to:
   - Increase the expression of SIRT1 and FOXO and decrease the expression of type II deiodinase.
   - Lower growth factor expression (IGF-1)
   - Reduce protein glycation
   - Alter the capacity of metabolic pathways (PDHK)

CR changes the expression of key genes, activation / de-activation of signaling systems. Taken together, these changes result in beneficial downstream changes in structure and function. These effects occur in animals and humans and in diverse tissues including skeletal muscle. The latter tissue is responsible for a large portion of the whole body energy consumption. Understanding the effects of CR in skeletal muscle will provide key contextual information for the interpretation of the primary aim of the study – metabolic adaptation.

8. Adipose tissue biopsies. To explore the effects of CR to:
   - Reduce adipocyte size and lower the adipose tissue content of inflammatory cytokines and markers of inflammation as measured by the gene expression (qRT-PCR) of amyloid A, IL-6, resistin, adiponectin, leptin, MCP1, CD68 and MAC2 and Immunohistochemistry to quantify adipose tissue macrophage infiltration content.
   - Change the levels of expression of mitochondrial and lipogenic genes.
   - Increase the capacity for free radical scavenging (SOD).

There is evidence that a major beneficial effect of CR is a reduction in fat mass, particularly visceral fat, and adipose tissue is now recognized as a contributor to whole body inflammation. These measures will explore the effects of long term CR on adipose tissue. Adipose tissue is important for the regulation of whole body energy partitioning (vis-à-vis metabolic and hormonal systems) and will provide key contextual information for the interpretation of the primary aim of the study – metabolic adaptation.

9. Psychological factors:
   - Quality of life.
   - Cognitive function and affective status.

The complex endocrine, physiological, and cellular changes that occur during CR theoretically may impact these two domains in both a positive and negative fashion.
As such, they represent important information to determine the safety and benefits of long-term CR.

   • VO₂max
   • Strength

Changes in caloric intake during CR impact body composition. How these changes impact function will determine the ultimate desirability of CR in humans and the overall interpretation of the data generated in phase 2 CALERIE.

Given the wealth of basic research in the biology of aging and the mechanisms by which CR enhances lifespan in pre-clinical models, these measures represent only a fraction of the potential scientific value of the phase 2 of CALERIE. As such, an important exploratory aim of phase 2 is:

11. To collect and archive biological specimens (plasma, biopsy samples, circulating cells, urine) and store them such that they can be used for testing of new hypotheses (under the parent protocol) and for ancillary studies (funded from a variety of sources).

A study of the size and scope of CALERIE is unlikely to be replicated in the near future. Many researchers, both basic and clinical, will be interested in testing established and new, novel hypotheses generated in the pre-clinical setting. To meet these demands, additional biological specimens will be collected and archived for this purpose and maintained by the Emerging Science Committee as described in Protocol Section 12.12.