

## **Longer Life Foundation Final Research Report**

**PI: Luigi Fontana, M.D., Ph. D.**

**Grant Title: Effects of Calorie Restriction on Markers of Aging and Longevity**

**Grant Dates: 00/00/00-00/00/00**

### **ABSTRACT**

In the near future, human aging and age-associated diseases will become one of the biggest challenges faced by developed and developing countries. Life expectancy has markedly increased in most developed countries in the last century, from about 45 years at the beginning of the 20th century to about 77 years today. This increase is due primarily to reduced deaths from infectious diseases and in infancy, but also to improved sanitation and working conditions, better nutrition and housing, organized sewage disposal, the development of antibiotics and vaccines, and better health care.

However, the overall increase in average life span is far greater than that for healthy life expectancy, as evidenced by the incremental burden of age-associated diseases, including coronary heart disease, stroke, heart failure, diabetes, hypertension and cancer. Cardiovascular disease, cancer, stroke and diabetes account for nearly 70% of the deaths in the United States and Europe. The financial burden caused by these age-associated chronic diseases is already overwhelming and, if present trends continue, is likely to become unbearable in the next few decades.

One of these trends involves the overconsumption of diets rich in empty calories and poor in nutrients and a sedentary lifestyle leading to a marked increase in age-associated chronic diseases. Another is the rapid increase in the proportion of older individuals, with the most dramatic increases in the number of adults over 65 years of age. In contrast to these harmful effects of overeating unhealthy foods, restriction of calorie intake with adequate intake of nutrients has a wide range of benefits. Moderate calorie restriction (CR) with optimal nutrition can prevent and reverse the harmful effects of obesity, type 2 diabetes, hypertension and other age-associated metabolic alterations and diseases. Studies on laboratory animals and preliminary studies on humans have shown that more severe CR without malnutrition has additional benefits on the aging process itself. Although it is currently not known if long-term CR with adequate nutrition extends maximal lifespan in humans, we do know that long-term CR without malnutrition results in some of the same metabolic and hormonal adaptations related to longevity in CR rodents. CR decreases insulin resistance, growth factors and inflammation, improves diastolic function, and alters neuroendocrine function. These are among the adaptations that have been hypothesized to mediate the slowing of aging and protection against cancer by CR in rodents. Additional studies are needed to identify the molecular and cellular mechanisms responsible for the therapeutic effects of CR and to identify reliable markers of aging to facilitate evaluating the effect of CR and other anti-aging interventions in randomized controlled clinical trials.

## **LAY SUMMARY**

The major goal of this proposal was to determine potential markers of aging and longevity by providing comprehensive evaluation of the effects of long-term calorie restriction (CR) in humans. We studied a unique group of healthy individuals who have been practicing CR for an average of nine years (range six to 18 years). These individuals eat high-quality diets that are high in protein and contain more than 100% of the RDA of all essential nutrients. We found that CR decreases insulin resistance, growth factors and inflammation, improves heart function and arterial stiffness. These are among the adaptations that have been hypothesized to mediate the slowing of aging and protection against cancer by CR in rodents. Finally, these data will provide the foundation for future grant applications involving longitudinal follow-up measurements on markers of prognosis that can be applied in the general population to persons of normal weight to predict health and longevity.

## **INTRODUCTION**

Calorie restriction (CR) without malnutrition slows aging and prevents or delays several age-related chronic diseases in many experimental animal models (1). The mechanisms through which this occurs are unclear, but likely involve a constellation of complex and interrelated metabolic factors, including neuroendocrine systems adaptations, prevention of inflammation, hormetic response, and protection against oxidative stress damage (2). Whether long-term CR with adequate nutrition slows aging in humans is unknown. We have found that long-term CR protects against obesity, type 2 diabetes, hypertension and atherosclerosis (3). However, little is known about the chronic effects of CR on the metabolic, neuroendocrine and cardiovascular adaptations that are associated with the anti-aging effect of CR in rodents. For example, data from studies conducted in laboratory rodents have found that CR without malnutrition and reduced function mutations in the insulin/IGF-I signaling pathway promote longevity in part by preventing or delaying the occurrence of several age-associated chronic diseases, and in part by slowing the rate of intrinsic aging (2, 4). Other important CR-mediated metabolic adaptations, that have been shown to play an important role in mediating the anti-aging effects of CR, are: 1) reduced levels of anabolic hormones (e.g. insulin, testosterone, leptin); 2) reduced levels of hormones that regulate thermogenesis and cellular metabolism (e.g. triiodothyronine, norepinephrine); and finally 3) increased levels of hormones that suppress inflammation (e.g. cortisol, adiponectin, ghrelin) (5-8).

## References:

1. Weindruch R, Sohal RS. Caloric intake and aging. *N Eng J Med* 1997; 337:986-94.
2. Fontana L, Klein S. Aging, adiposity and calorie restriction. *JAMA* 2007;297(9):986-994.
3. Fontana L, Meyer TE, Klein S, Holloszy JO. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci U S A* 2004;101:6659–6663.
4. Tatar, M., A. Bartke, and A. Antebi. The endocrine regulation of aging by insulin-like signals. *Science* 2003; 299:1346-1351.
5. Straus DS. Nutritional regulation of hormones and growth factors that control mammalian growth. *FASEB J.* 1994;8:6-12.
6. Herlihy JT, Stacy C, Bertrand HA. Long-term food restriction depresses serum thyroid hormone concentrations in the rat. *Mech Ageing Dev.* 1990;53:9-16.
7. Howland BE. The influence of feed restriction and subsequent re-feeding on gonadotrophin secretion and serum testosterone levels in male rats. *J Reprod Fertil.* 1975;44(3):429-36
8. Sabatino F, Masoro EJ, McMahan CA, Kuhn RW. Assessment of the role of the glucocorticoid system in aging processes and in the action of food restriction. *J Gerontol.* 1991;46:B171-9.

## RESULTS

We evaluated the long-term effect of calorie restriction (CR), defined as CR with optimal nutrition, on potential markers of aging and longevity in 28 middle-aged volunteers who had been on a CR diet for an average of seven years (BMI  $19.6 \pm 1.8$  kg/m<sup>2</sup>,  $8.7 \pm 7.3\%$  body fat) and 28 age-matched healthy subjects consuming a typical Western diet (BMI  $25.9 \pm 3.2$  kg/m<sup>2</sup>,  $24.6 \pm 6.5\%$  body fat).

We found that long-term CR without malnutrition results in major improvements in indices of glucose tolerance and insulin action. Fasting glucose and insulin, the area under the curve (AUC) for insulin and the index of insulin sensitivity (ISI), calculated according to the method of Matsuda and DeFronzo, were all significantly lower in the CR group than in the age and sex-matched Western diet group.

Accordingly, plasma adiponectin concentration was higher and plasma leptin, IL-6 and TNF- $\alpha$ /adiponectin ratio were lower in the CR group than in the Western diet group. We also found that long-term CR in humans results in some of the same metabolic, hormonal and growth factors adaptations that are thought to be involved in mediating some of the anti-aging effects observed in CR rodents. In particular, we found that plasma testosterone, platelet derived growth factor, transforming growth factor  $\beta$ , total and free triiodothyronine concentration were lower, whereas plasma steroid hormone binding globulin and cortisol concentrations were higher in the CR group than in the Western diet group.

We didn't find any difference in plasma thyrotropin, thyroxine and dehydro-epiandrosterone sulfate concentration between the CR and the Western diet groups. Finally, we found that intima-media thickness of the common carotid arteries, pulse wave velocity (a marker of arterial stiffness) were lower, and left ventricular diastolic function was improved in the CR group.

### **DISCUSSION AND FUTURE PLANS**

In this study we demonstrated that the volunteers practicing CR undergo some of the same key metabolic and hormonal adaptations to CR that occur in rodents in which CR increases longevity. We found that CR provides a powerful protective effect against insulin resistance, inflammation, and age-associated growth factor and adipokine alterations. These data add relevant information to the growing research field of "healthy aging", and the identification of metabolic and hormonal factors that can improve organ function and, consequently, healthspan and lifespan in humans.