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Nutrition Modulation of Human Aging: The Calorie Restriction Paradigm

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Abstract

Globally, the aging population is growing rapidly, creating an urgent need to attenuate age-related health conditions, including metabolic disease and disability. A promising strategy for healthy aging based on consistently positive results from studies with a variety of species, including non-human primates (NHP), is calorie restriction (CR), or the restriction of energy intake while maintaining intake of essential nutrients. The burgeoning evidence for this approach in humans is reviewed and the major study to date to address this question, CALERIE (Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy), is described. CALERIE findings indicate the feasibility of CR in non-obese humans, confirm observations in NHP, and are consistent with improvements in disease risk reduction and potential anti-aging effects. Finally, the mechanisms of CR in humans are reviewed which sums up the fact that evolutionarily conserved mechanisms mediate the anti-aging effects of CR. Overall, the prospect for further research in both NHP and humans is highly encouraging.

Keywords

calorie restriction; aging; CALERIE; nutritional modulation

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1. Introduction

Aging is inevitable and an integral part of human life, and is associated with multiple physiological, metabolic, hormonal, immune and neurocognitive changes (Lamberts et al., 1997; Roberts and Rosenberg, 2006; Tosato et al., 2007) that collectively contribute to the development of age-related metabolic disease and physical disabilities. Remarkable improvements in life expectancy, in part the result of medical advances that have significantly decreased mortality from communicable diseases, have resulted in an unprecedented growth of the population of older adults (WHO, 2011). However, non-communicable diseases, including diabetes, heart disease, cancer, stroke, and Alzheimer's are now the leading cause of morbidity and mortality worldwide (WHO, 2011). A critical need is therefore to improve healthspan, i.e., to reduce illness, disability and dependency during the aging process. Interventions that have the potential to reduce disease risk and facilitate retention of health, productivity, independence, and quality of life through the aging years are therefore greatly sought after and merit scientific investigation.

2. The Calorie Restriction Paradigm

Over the past several decades, the anti-aging researchers have identified and tested several nutritional, life-style and pharmacological interventions known to improve health and longevity. Calorie restriction (CR) is the only nutrition intervention that, based on strong and consistent evidence in a variety of non-human species, is now widely recognized as a highly promising strategy to delay the onset and progression of age-related metabolic diseases (Roth et al., 2001) and extend lifespan (Fontana et al., 2010). CR is the restriction of habitual caloric intake without a reduction in essential nutrients. CR is prescribed or practiced with the primary aim of attenuating or delaying the onset of physiological and metabolic effects of aging and associated disabilities. McCay et al. were the first to show CR effects on age related disease risk reduction and extension of lifespan based on findings in rats (McCay et al., 1935). Similar beneficial effects have been subsequently demonstrated in various animal models, including yeast, flies, worms, fish, and in mammals (Anderson and Weindruch, 2012). However, human studies have been limited due to the feasibility issues of implementing an appropriately timed intervention with follow-up for the course of the lengthy human lifespan.

3. CR is a Relevant Delayed Aging Model in Primates

Non-human primates, owing to their high genetic and physiological similarity to humans, serve as an excellent model to test the translatability of CR in humans (Gibbs et al., 2007). In addition, nonhuman primate models also offer the convenience of tightly controlled experimental conditions and less complicated implementation of a uniform study protocol, not to mention the cost efficiency. Small-animal studies suggest that no single causal pathway or mechanism by which CR attenuates disease risk and improves markers of longevity, instead indicating that the benefits of CR are realized via a complex interplay of biological changes that are both synergistic and mutually non-exclusive. However, reduced metabolic rate, lower energy expenditure, and reduced core-body temperature have been implicated as responses to CR in both rodents and NHP (Lane et al., 1996). The net effect of

these physiologic changes is a reduction in oxidative stress-induced cellular damage and improvements in markers of inflammation and immune function; these outcomes are widely captured in CR studies and serve as markers for assessing the benefits of CR in humans. Newer mechanisms have been identified and are discussed in section 6 of this review.

The establishment of the first two prospective studies in NHPs, one at the National Institute on Aging (NIA) in 1987, and a second at the University of Wisconsin's National Primate Research Center (WNPRC) in 1989 marked an important milestone in laying the foundation for bridging animal and human CR research. The description of the NIA and WNPRC cohorts and major findings, including key similarities and differences between the two studies, have been detailed elsewhere (Cava and Fontana, 2013; Kemnitz, 2011; Mattison et al., 2017; Mattison et al., 2003). Most notably, compelling evidence for the beneficial effects of CR on healthspan has been clearly demonstrated in both of the NHP cohorts for a variety of physiological outcomes, including a lower body fat, favorable changes in lipid profile, improved insulin sensitivity and glucose tolerance, and lower incidence of cancer, type 2 diabetes and cardio-vascular disease (Anderson and Weindruch, 2012; Colman and Anderson, 2011; Colman et al., 2009; Colman et al., 2014; Colman et al., 1999; Colman et al., 1998; Edwards et al., 1998; Edwards et al., 2001; Gresl et al., 2001; Lane et al., 1992; Lane et al., 1999; Mattison et al., 2017; Mattison et al., 2003; Mattison et al., 2012; Ramsey et al., 2000; Roth et al., 2001; Roth et al., 2002b). Although lifespan effects are not entirely consistent in the two NHP cohorts due to the differences in study design and time of onset of CR (Mattison et al., 2017), the positive effects of CR on healthspan is largely consistent and highly encouraging. In summary, CR studies in NHP not only validated the findings from smaller animals, but also reinforced the scientific priority for conducting randomized controlled trials (RCT) in humans.

4. Population Studies Suggest that CR may be Conserved in Humans

In parallel to the emerging positive findings of the benefits of CR from ongoing studies in NHPs, findings from direct observation of human populations who fit the CR paradigm likewise suggest that CR may improve healthspan and promote longevity in humans. These data therefore provided evidence for the potential feasibility of conducting CR intervention studies in humans. Examples of such studies are described below.

The Okinawa Centenarian Study (<http://www.okicent.org/index.html>) is an ongoing population-based study of centenarians and select elderly (> 65 years of age) from the Japanese prefecture of Okinawa, whose caloric intake is historically documented as being lower than age matched elders in other regions of the world including mainland Japan (Willcox et al., 2007; Willcox et al., 2006a). These Okinawan elders reported consumption of a reduced calorie but nutrient dense diet since their younger ages. Compared to relevant reference elderly populations from around the world, the Okinawan elders exhibited less metabolic damage, better cardiovascular health (low plasma homocysteine levels), fewer hormone related cancers, a lower prevalence of osteoporosis and dementia, increased physical activity, fewer menopause-related problems, and higher sex hormone levels (Arakawa et al., 2005; Willcox et al., 2006b).

The Biosphere experiment in 1991, in which eight adults (four female and four male) were sealed inside a self-contained ecological space (Biosphere 2), accidentally resulted in reduced food production and therefore lower energy consumption by the crew members. While it was planned that they would produce and consume energy in excess of 2500 kcal per person per day for 2 years, during seven-eighths of the 2 year period the crew consumed only 1750–2100 kcal/d via a nutrient-rich diet of vegetables, fruits, nuts, grains, and legumes, with small amounts of dairy, eggs, and meat. They also consumed vitamin and mineral supplements consisting of vitamin B12, folic acid, vitamin D, Vitamin E and ascorbic acid (Walford et al., 2002). The men lost 19% of their body mass while the women lost 13%. Both men and women experienced decreases in systolic blood pressure of 25% and diastolic blood pressure of 22%. In addition, there were favorable changes in hematology (e.g. white blood cell count decreased by 31%), hormonal levels (e.g., insulin decreased by 42%; tri-iodothyronine (T₃) decreased by 19%), and biochemical parameters (e.g., blood sugar decreased by 21%; cholesterol decreased by 30%) (Walford et al., 2002).

There is also evidence of positive effects from a study of CR Society members (<http://www.crsociety.org/>), a group of voluntary practitioners of CR. A comparison of CR Society members with age- and gender-matched subjects eating typical Western diets found that CR members were consuming approximately 800 fewer kcal per day and had a significantly lower mean body fat mass, core body temperature, blood pressure, and T₃ level; as well as lower blood levels of inflammatory markers, namely, tumor necrosis factor alpha (TNF- α), high sensitivity C-reactive protein (hsCRP) and transforming growth factor-1-beta (TGF1- β). They also had lower total testosterone and free androgen indices but higher levels of sex hormone binding globulins (Cangemi et al., 2010; Meyer et al., 2006).

In sum, the collective evidence from available human CR observational and population studies strongly and consistently favors improvements in potential markers of health and longevity and supports the investigation of CR effects in humans using an RCT. Further, examining the benefits of CR on healthspan is a logical focus for human CR intervention studies.

5. Clinical Trials of CR in Humans

A CR study in humans that parallels the studies in NHP and are long enough to study survival effects would be both premature and impractical. A medium-term study of sustained CR that could provide valuable information of potential human effects including feasibility, safety, evidence for mechanistic parallels from animal studies, risk factors for age-related conditions and markers of longevity was considered as a rational first step and practical approach. Accordingly, the primary research questions were a) is CR feasible and safe in humans? b) does CR have the same beneficial effects in humans as in animal models, e.g., reduced rate of biological aging, enhanced immune function, reduced oxidative stress and improved insulin sensitivity? c) does CR have any unanticipated negative effects in humans that would preclude its widespread recommendation (e.g., any impairment of cognitive function or reduction in bone mineral density)?

5.1 CALERIE-Phase 1: Purpose, Approach, Findings and Implications for a Longer-term RCT

In response to the high scientific interest and the need for data in humans, in 2002 the NIA sponsored the first RCT of CR, the CALERIE (Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy) Study. CALERIE was implemented via a two-phased approach. Phase I (which will be referred to as CALERIE-1) was designed to examine the feasibility and best approach to CR in humans.

Several key design issues needed to be considered in planning the CALERIE-1 study. First, for the outcomes of in improving healthspan or expanding the lifespan of humans, it was not clear when to initiate CR. In animals, the degree of lifespan extension is influenced by the age of CR initiation, the magnitude of CR, and the strain/genetic background of the animal (Fontana and Klein, 2007). McCay et al (1935) (McCay et al., 1935) demonstrated that in rats, timing of the introduction of CR influenced median and maximum lifespan and the severity of chronic disease prevention or attenuation, with significantly greater effects observed when CR was initiated after puberty. In a study of mice, initiation of moderate CR (20–40%) at middle age resulted in positive effects on lifespan, albeit to a lesser magnitude than introduction at an earlier age (Weindruch et al., 2001). Similarly a trend for delay in age associated disease onset was observed in monkeys in whom CR was initiated at a younger age (1–14 years) vs. at an older age (16–23) (Mattison et al., 2012). However, in humans, initiating a CR intervention prior to adulthood imposes many practical, physiological, and ethical challenges, including consideration of the impact on growth and development. Therefore, introducing a CR intervention prior to adulthood is not an option in human studies.

An additional challenge was the lack of consensus on the %CR that is both ideal and feasible in realizing maximal benefits for healthspan and lifespan extension. There is limited evidence suggesting that a reduction in ad libitum calorie intake of between 10–50% will result in proportional increases in lifespan in animals (Fontana et al., 2010; Rizza et al., 2014), and that CR beyond this limit will have harmful effects. There is similarly a lack of consensus on the macronutrient composition (ratio of carbohydrates, fat and protein) of an optimal CR diet. There have been studies in humans examining the effects of several energy restricted diets, varying in macronutrient composition, on healthspan expansion. Diets that have been studied include low fat/high protein, low fat/high carbohydrate and carbohydrate restricted diets (Volek et al., 2009). However, these studies were mainly conducted with overweight and obese participants with weight loss as the main focus. To our knowledge, no study has been conducted in non-obese humans that was specifically designed to determine the macronutrient composition that is most effective in producing the healthspan and lifespan expanding outcomes while avoiding any micronutrient deficiencies. Additionally, it is unknown whether different CR regimes, such as alternate day fasting (ADF), intermittent fasting (IF) or time restricted feeding (TRF) have differing effects on healthspan or lifespan (Patterson et al., 2015).

CALERIE-I was therefore designed to help identify the most feasible and effective approaches to CR in humans. To do this, short-term (6 months to 1 year) studies were conducted with non-obese humans at each of the 3 sites in the U.S. (Jean Mayer USDA

Human Nutrition Research Center on Aging (HNRC) at Tufts University, Boston MA; Pennington Bio-Medical Research Center (PBRC) in Baton Rouge, LA; and Washington State University in St. Louis, MO) (Ahmed et al., 2009; Anton et al., 2009; Civitarese et al., 2007; Das et al., 2007a; Das et al., 2007b; Das et al., 2009; Fontana et al., 2006; Fontana et al., 2007; Fontana et al., 2008a; Heilbronn et al., 2006; Larson-Meyer et al., 2006; Larson-Meyer et al., 2008; Lefevre et al., 2009; Martin et al., 2007a; Martin et al., 2007b; Meydani et al., 2011; Pittas et al., 2005; Pittas et al., 2006; Racette et al., 2008; Racette et al., 2006; Redman et al., 2007; Redman et al., 2008a; Redman et al., 2008b; Redman et al., 2010a; Riordan et al., 2008; Tam et al., 2012; Villareal et al., 2006; Weiss and Holloszy, 2007; Weiss et al., 2006). A summary of the approaches and key findings from the CALERIE-1 studies are summarized below and also presented in Tables 1&2.

The study conducted at the Tufts University site was designed to examine the feasibility of a 30% CR diet, and furthermore whether dietary glycemic load impacted adherence. In this study, 46 young (24–42 years of age) overweight (BMI, 25–29.9 kg/m²) men and women were recruited into a 1-year trial and randomized to either low (10% (N=12)) or moderate (30% (N=34) CR; and within each level of CR to diets of differing glycemic load (low-LG vs. high glycemic load - HG) (Das et al., 2007b; Das et al., 2009). Food was provided to participants for the first 6-month period and self-administered for the second 6 months. After the first 6 months, the individuals randomized to 30% CR experienced a significant reduction in weight and improvements in insulin sensitivity, fasting insulin concentration, first-phase acute insulin secretion, and lipid profile independent of the glycemic load (Das et al., 2007b; Pittas et al., 2006). Serum concentration of CRP was reduced in the 30% LG-CR group, but not in the 30% HG-CR group (Pittas et al., 2006). Moreover, both the 10% and 30% CR significantly improved T-cell function (i.e. delayed-type hypersensitivity response, proliferative response of T cells to T-cell mitogens) and prostaglandin E-2 production (Ahmed et al., 2009). In addition, favorable changes were observed in some measures of antioxidant defense and oxidative stress (increase in plasma glutathione peroxidase activity and decrease in plasma protein carbonyl levels) (Meydani et al., 2011). The findings from this study demonstrated that healthy diets with differing glycemic loads were equivalent in achieving adherence to %CR and decreases in body weight. However, the 30% CR was not sustained over the 1-year period.

The study conducted at PBRC was designed to examine the effects of 6 months of CR with or without exercise on changes in body composition, body temperature, energy expenditure, insulin and glucose, as well as markers of oxidative stress. In this study, 48 overweight (BMI 27.8 ± 0.7 kg/m²) participants (aged 36.8 ± 1.0 y) were randomized to one of four groups for 6-months: Controls had energy intakes at 100% of their energy requirements; the CR group consumed a diet at 25% CR; CR+ Exercise (EX) consumed a diet of 12.5% CR and also engaged in structured exercise to increase energy expenditure by 12.5%; and the low caloric diet (LCD) group consumed 890 kcal/d until 15% weight reduction was achieved, followed by weight maintenance. Findings indicate that CR (CR alone and CR+EX) resulted in significant reductions in body weight; metabolic rate was reduced beyond the level expected for reduced body size; and fasting insulin and body temperature (previously reported biomarkers of longevity) were reduced (Heilbronn et al., 2006; Martin et al., 2007b; Redman et al., 2007; Redman et al., 2009). The overall findings from this study support the theory

that CR improves markers of disease risk (a decrease in fat mass, and increase in insulin sensitivity) and is consistent with anti-aging effects (Civitarese et al., 2007; Larson-Meyer et al., 2006; Larson-Meyer et al., 2008; Lefevre et al., 2009; Redman et al., 2008a; Redman et al., 2008b; Redman et al., 2010b; Tam et al., 2012). However, since the energy deficit achieved at the end of 6 months, using CR alone vs. CR+EX, was not significantly different, support for using a combined (CR+EX) approach for achieving CR was weak.

The study at Washington State University was designed to assess whether the effects of diet vs. exercise-induced reduction in energy expenditure improves gluco-regulation and insulin action (Weiss et al., 2006). In this study, non-obese men and women aged 50–60 years with a body mass index (kg/m^2) of 23.5–29.9 were randomly assigned to one of two 20% energy deficit-inducing interventions or to a control group: 12 months of exercise induced energy expenditure or caloric deficit (EX group; $n = 18$); or a 20% dietary CR (CR group; $n = 18$); or a healthy lifestyle (HL control group $n = 10$). The energy deficit achieved over the 1yr in the EX and CR groups were similar, as reflected in the changes in body weight and fat mass (Weiss and Holloszy, 2007). Improvements in insulin sensitivity were observed in both the EX and CR groups (insulin sensitivity index increased and the glucose and insulin areas under the curve decreased) but remained unchanged in the HL group, and did not differ significantly between the EX and CR groups (Weiss et al., 2006). In addition, small but significant increases in adiponectin and decreases in the ratio of tumor necrosis factor alpha (TNF- α) to adiponectin occurred in the EX and CR groups but not in the HL group. Significant reductions in mean blood pressure, total and low density lipoprotein (LDL) cholesterol, increase in high density lipoprotein (HDL) cholesterol, and reductions in triglycerides (TG) were observed (Fontana et al., 2007). T_3 levels decreased significantly in the CR group after 12 months, but not in the exercise or healthy lifestyle control group, but thyroid stimulating hormone (TSH), T_4 , and free T_4 did not change in any of the groups (Fontana et al., 2006). Taken together, the findings from this study were not strongly in favor of using exercise alone as a means for achieving a caloric deficit, due to the large volume of exercise required to achieve the energy deficit and challenges involved in overseeing compliance to the exercise regimen over a longer-term period.

5.2 Considerations for CALERIE Phase 2 Design

Several learnings from CALERIE-1 were instrumental in informing and refining the design of the second phase of CALERIE (CALERIE-2). First, there was general consensus that dietary energy restriction was the most feasible approach to achieving CR. Second, Phase 1 results also indicated that 25% CR was likely optimal goal, since 30% CR proved to be unsustainable. Third, since glycemic load did not affect adherence, within the framework of an overall healthy diet it would not be necessary to recommend a specific diet pattern. Metabolic rate, core-body temperature, and thyroid hormone changes were selected as primary outcomes due to their implicated role as anti-aging markers and confirmation of changes in these domains in CALERIE-1. A 2-year timeframe was selected since any observed changes in the first year could be attributed to weight loss, whereas those observed in the second year could be attributed to CR alone. In addition, since no safety concerns were apparent in the non-obese participants in Phase 1, and to further exclude confounding by weight loss effects, the higher range of the overweight BMI category was excluded.

Therefore, inclusion criteria for CALERIE-2 constituted BMIs in the lower range of the overweight and the upper range of normal weight.

5.3 CALERIE Phase 2, Purpose, Approach, and Key Findings

CALERIE-2 was a two-year, multi-center, single protocol (same study across the 3 sites, i.e. PBRC, Tufts University, and Washington University), RCT investigating the effects of sustained 25% calorie restriction (CR) in non-obese men (21–50 years of age) and women (21–47 years of age, to avoid confounding by peri-menopausal effects) (Rochon et al., 2011). The main objective was to examine whether CR would result in sustained metabolic adaptation, i.e., a reduction in core body temperature and reduced resting metabolic rate (RMR) adjusted for changes in body composition. Other key outcomes examined included energy metabolism, body composition, endocrine response and growth factors, cardiovascular measures, immune function, physical activity, safety panel and quality of life measures. CR participants were compared to ad-libitum (AL) controls.

Participants in this RCT were screened extensively prior to enrollment, using a 3 step process including a complete physical exam, to rule out significant medical conditions, abnormal lab values, psychiatric and behavioral problems, and other factors that may preclude them from being able to adhere to an intensive 2-year lifestyle intervention (Stewart et al., 2013). Baseline or habitual energy intake was determined, using two back-to-back measurements of energy expenditure with doubly labeled water (DLW), from which the individualized 25% CR prescriptions were derived (Ravussin et al., 2015; Rochon et al., 2011). Following baseline testing, participants were randomized to either CR or AL in a 2:1 ratio. Randomization was stratified by site, sex and BMI (normal weight, $22.0 \leq \text{BMI} < 25.0 \text{ kg/m}^2$ and overweight, $25.0 \leq \text{BMI} < 28.0 \text{ kg/m}^2$).

The CR intervention involved an immediate and sustained 25% restriction of energy intake (Redman et al., 2014; Rickman et al., 2011; Rochon et al., 2011). The intervention involved intensive nutritional and behavioral guidance via individual sessions with a behavioral counselor. Discussions were centered around a weekly weight loss graph depicting targeted weight loss trajectory for each participant over one year (Rickman et al., 2011). The targeted weight loss predictions were driven by a mathematical model based on Phase 1 data (Pieper et al., 2011) that predicted and accounted for weekly changes in body weight for each participant over one year and was also used as the primary tool to promote adherence during the intervention. (Rickman et al., 2011). Participants also received a structured curriculum and counseling delivered in a group format (Rickman et al., 2011). Control participants were advised to continue their current diets on a completely *ad libitum* basis. No specific level of physical activity was prescribed to participants in either group. A multivitamin and mineral supplement (Nature Made Multi Complete, Pharmavite LLC, Mission Hills, CA) and an additional calcium supplement (1000 mg/d, Douglas laboratories, Pittsburgh, PA) was provided to all participants to ensure current recommendations for micronutrients were met. Safety was monitored using a heightened surveillance protocol consisting of a panel of laboratory tests, bone mineral density measured by dual energy-Xray-absorptiometry (DEXA), and quality of life measures (Rochon et al., 2011; Romashkan et al., 2016).

5.4 Does CR Impinge on Health in Humans

Main Findings from CALERIE-2—Results from CALERIE-2 (Tables 1&2) indicated that **CR is feasible** and sustainable at an average moderate level of 12% over two years (Ravussin et al., 2015). It should be noted that the planned 25% CR was not achieved, on average, over the two-year period. At this moderate level CR was generally **safe and well tolerated** in participating **non-obese** individuals (Romashkan et al., 2016) without adverse effects on quality of life (Martin et al., 2016), mood, cognition, hunger or sexual function (Ravussin et al., 2015). Core body temperature dropped slightly over the two years of CR, but the decrease was not significant in comparison to the change in the ad-libitum group. CR induced adaptive decreases in total daily energy expenditure and in T_3 levels (T_3 values were lower but within normal range at 24 months) (Ravussin et al., 2015). Resting metabolic rate was not reduced (Ravussin et al., 2015), however, the reduction in T_3 could be interpreted as early evidence of long-term metabolic adaptation and potentially favorable anti-aging benefits.

Healthspan Effects—Compared to AL control participants, CR participants had decreased body weight, percent body fat, and fat mass at 12 and 24 months (Ravussin et al., 2015). CR participants also experienced a 2 kilogram reduction in fat-free mass as a result of weight loss. However, this reduction in absolute fat-free mass is commensurate with the reduced body mass in younger adults; further, overall body composition in the CR group was favorable, with a higher percentage fat-free mass and lower percentage fat mass (72% fat-free and 28% fat mass), compared to body composition values prior to initiation of CR (67% fat-free and 33% fat mass) (Das et al., 2017). Most importantly, CR resulted in significant reductions in central adiposity, as determined by waist circumference, waist to hip ratio, and truncal fat measured by DEXA. Further, CR participants exhibited improvements in glucose control (as indicated by significantly greater decreases in the homeostatic model assessment of insulin resistance-HOMA-IR), reductions in blood lipids (total cholesterol and triglycerides), and mean blood pressure (Ravussin et al., 2015). Collectively, these outcomes suggest a beneficial effect of CR on cardiometabolic risk factors.

CR additionally induced changes that are suggestive of potential chemo-protective effects (Arcidiacono et al., 2012), including reductions in fat mass, increased insulin sensitivity, decreased systemic biomarkers of inflammation (significant reductions in levels of TNF- α and hs-CRP, total white blood cell and lymphocyte counts, intracellular adhesion molecule ICAM-1 and leptin) (Ravussin et al., 2015), and persistent and significant increase in insulin-like growth factor binding protein-1 (IGFBP-1) (Fontana et al., 2016). In the CR group, reductions in fat mass were accompanied by an increase in adiponectin (Villareal et al., 2016); adiponectin has demonstrated anti-inflammatory and anti-atherogenic properties (Chandran et al., 2003).

Combined, the results of Phase 2 (Tables 1&2) provide evidence for the beneficial effects of CR on the healthspan of humans. Importantly, these results were found in the absence of any adverse effects on quality of life.

6. Mechanisms of CR in Humans

Although our understanding on the precise mechanisms of CR is still incomplete, studies from humans, primates and other short-lived species suggest that CR impinges on multiple pathways regulating metabolism, oxidative stress, inflammation and autophagy to promote longevity and healthy aging (Lopez-Lluch and Navas, 2016). Among these, modulation of energy metabolism is one of the key effector pathways of CR (Anderson and Weindruch, 2010). CR induces metabolic reprogramming favoring energy efficient pathways as a response to reach a new state of equilibrium with reduced calorie intake (Martin-Montalvo and de Cabo, 2013). At the whole body level, this CR induced metabolic adaptation is manifested by decreased core body temperature, decreased resting metabolic rate and decreased energy expenditure in primates (Lane et al., 1996) and rodents (Duffy et al., 1990; Duffy et al., 1989). In humans, long term CR was associated with a mild ($\sim 0.2^{\circ}\text{C}$) but significant reduction in the 24hr mean core body temperature than their western diet counterparts (Soare et al., 2011). Results from CALERIE-I trials also support this phenomenon in humans (Heilbronn et al., 2006). However in the CALERIE-II trials, CR decreased total daily energy expenditure without any changes in the core body temperature or the resting metabolic rate (Ravussin et al., 2015).

Multiple hormones are involved in mediating this adaptive response to CR. T3 levels, which determines the basal metabolic rate, are consistently decreased with CR in humans (Fontana et al., 2006) (Ravussin et al., 2015), primates (Roth et al., 2002a) and rodents (Herlihy et al., 1990). CR is also associated with reduced IGF1 activity. CR decreases circulating IGF1 levels in rodents (Dunn et al., 1997; Harvey et al., 2014). On the other hand, CR does not alter IGF1 levels in humans (Fontana et al., 2008b), however there is an increase in serum IGF1 levels which decreases the bioavailability of circulating IGF1 leading to reduced IGF1 activity (Fontana et al., 2016). Inhibition of IGF-1 signaling has been implicated in improved insulin sensitivity, downregulation of growth pathways, decreased thermogenesis and increased resistance to stress, all of which promotes longevity (Junnala et al., 2013). In addition, CR increases serum adiponectin and cortisol levels in humans (Fontana et al., 2016; Lettieri-Barbato et al., 2016; Salehi-Abargouei et al., 2015; Yang et al., 2016), both known to have anti-inflammatory properties, while the former additionally possess insulin sensitizing properties. The fact that the hormonal signature associated with CR (reduced T3 and IGF1 activity, increased cortisol and increased adiponectin levels) is uniform across species suggests that evolutionarily conserved mechanisms are involved in CR's effect on longevity.

At the cellular level, one of the hallmark features of CR is improvement in mitochondrial metabolism, which could be mediated by increase in mitochondrial content and/or function (Martin-Montalvo and de Cabo, 2013). The effects of CR on mitochondrial biogenesis is controversial (Civitarese et al., 2007; Hancock et al., 2010; Nisoli et al., 2005), however it is well established that CR promotes mitochondrial efficiency where the mitochondria consumes less oxygen and generates less reactive oxygen species (ROS) while maintaining ATP production (Martin-Montalvo and de Cabo, 2013). CR also up regulates the gene expression of anti-oxidant genes like super oxide dismutase (SOD), catalase and glutathione peroxidase (Sreekumar et al., 2002) (Lee et al., 1999) (Lanza et al., 2012). As a result of

attenuation of oxidative stress, CR animals displayed less mtdeoxy nucleic acid (mtDNA) and nuclear DNA mutations with reduced oxidative damage in muscle (Bevilacqua et al., 2005; Lanza et al., 2012; Lee et al., 1998). Similarly in humans, findings from CALERIE trials suggest that CR subjects had less mtDNA damage and more mitochondrial content than their controls (Civitarese et al., 2007). In parallel, CR subjects also had higher transcript levels of genes involved in the regulation of mitochondrial function and biogenesis like peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC1 α), mitochondrial transcription factor A (TFAM), silent information regulator (SIRT1) and endothelial nitric oxide synthase (eNOS) in the skeletal muscle (Civitarese et al., 2007). Overall, CR has a profound effect on improving mitochondrial function, which ultimately reduces oxidative damage and the organism's rate of aging, as proposed in the 'mitochondrial free radical theory of aging'.

Aging is associated with dysfunction in cellular quality control processes like autophagy and proteostasis, which are involved in the removal of dysfunctional organelles and misfolded/aggregated proteins respectively (He et al., 2013) (Kaushik and Cuervo, 2015). On the other hand, CR attenuates aging induced decline in autophagy in multiple species (Cuervo, 2008). In rats, CR stimulates autophagy in aging hearts (Wohlgemuth et al., 2007) and prevents aging induced suppression of autophagy in the liver (Del Roso et al., 2003). The same is true in humans as reported in a recent study, where the transcript and protein levels of key mediators of autophagy like beclin-1 and microtubule-associated protein LC3 are higher in the skeletal muscle of subjects practicing CR for 3–15 years (Yang et al., 2016). The authors also report that CR increases the expression of a number of transcripts in the heat shock protein 70 (HSP70) pathway known to be involved in the regulation of proteostasis (Yang et al., 2016). Also, the longevity effects of pharmacological agents like resveratrol, spermidine and rapamycin are mediated, at least in part, by stimulating autophagy in rodents (Eisenberg et al.; Morselli et al., 2009) and non-human primates (Lelegren et al., 2016). Increase in the expression of mediators involved in autophagy and proteostasis was associated with decreased inflammation in CR subjects (Yang et al., 2016) but have not been examined within the framework of the CALERIE studies.

At the molecular level, activated protein kinase (AMPK), Insulin/IGF-1, mammalian target of rapamycin (mTOR) and SIRT1/PGC1 α are the nutrient sensing pathways that mediate CR's effect on longevity, as reviewed in detail elsewhere (Anderson and Weindruch, 2010; Lopez-Lluch and Navas, 2016). These pathways regulate mitochondrial function/biogenesis, cell growth and proliferation, inflammation and autophagy to promote healthspan and lifespan under calorie restriction. Although, there is accumulating evidence in humans confirming the physiological and hormonal response to CR as observed in other species, the molecular mechanisms behind these responses are yet to be elucidated in humans.

7. Conclusions and Comments

The scientific world has been intrigued by the potential of CR as nutritional modulator of aging and whether CR can be sustained in non-obese humans. Findings from CALERIE-2, confirm that moderate CR at the levels achieved in CALERIE –2 (12% on average) is feasible and well tolerated in non-obese individuals over a 2-year period. CR-induced

reductions in body weight, inflammatory and cardiometabolic risk factors, as well as adaptive decreases in daily energy expenditure and markers of aging, are collectively consistent with improvements in healthspan and potential anti-aging effects in the absence of adverse effects on quality of life. The striking parallels in the response to CR from the human studies to those identified in shorter lived species indicate that mechanisms relevant to human aging are likely conserved.

An average of 12% CR was sustained over the 2-year period in this non-obese population in CALERIE-2, and fell short of the targeted 25%. It must be noted however, that CALERIE-2 was distinctly different from weight loss studies in populations with obesity: a) due to the inclusion of a second year which allows for the separation of the effects of weight loss from that of CR, and mirrors the period when CR outcomes have been assessed in laboratory animals, b) and the examination of CR effects in a tightly controlled BMI range (non-obese) to remove confounding from the observations that may largely be attributable to weight loss in populations with obesity. The finding that this non-obese group was able to sustain 12% CR on average with benefits to healthspan suggest that the motivation to lead a healthier life rather than to lose weight which was highly emphasized in the CALERIE studies, and the substantial support that the CR group received throughout the 2-year period, are likely reasons for success.

However, it is important to note that CALERIE was not designed to determine where CR is advisable for the non-obese or for the population at large. That would necessitate longer, larger trials that specifically address how much and whether CR can be broadly recommended as a lifestyle practice across the varying subpopulations.

7.1 Future Directions: CALERIE As a Platform for Further Aging Research

CALERIE has helped open the scientific doorway to possible next steps for interventions to extend healthspan and attenuate age related changes in humans. Future research directed toward examining the longer-term effects of CR in humans are needed. It will also be important to conduct additional studies on the mechanisms by which CR modulates healthspan and possibly lifespan. The striking parallels in findings between NHP and humans suggest that use of NHP models is likely to help elucidate these mechanisms in humans. Research on mechanisms is likely to be further facilitated by technological advances in analytical methods as well as the diverse group of researchers now examining different aspects of the CR paradigm. To support these efforts, the CALERIE Research Network (CRN) provides resources and procedures for accessing a limited bio-repository of available tissue, samples and data from CALERIE-2 for investigators who wish to study additional biomarkers and cellular changes (see link for details, <https://calerie.duke.edu/apply-samples-and-data-analysis>).

Key highlights of the CRN and related resources:

- Website <https://calerie.duke.edu> with all resources
- Several CALERIE-related publications to date (also cited here)
- Three ancillary studies funded by the NIA Division of Aging Biology

- Database (physiologic and immune functions, psychologic outcomes, physical performance, dietary records, disease risk factors, blood chemistry and hematology)
- Specimen repository: serum, plasma, urine, buffy coat, muscle (vastus lateralis), and fat (subcutaneous abdominal), peripheral blood DNA and RNA
- Statistical Resources
- Needs and opportunities for studies on CR effects on aging-related mechanistic factors in humans, e.g., progenitor cell populations in muscle and fat, cell senescence, telomere length, circulating factors, blood gene expression, DNA methylation

Such studies could inform strategies to identify targets for new interventions influencing aging mechanisms and clarify mechanisms that mediate favorable effects in humans.

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Highlights

- Calorie restriction is a promising intervention for attenuating age-related changes
- Highlights from the first human calorie restriction studies are presented
- Moderate calorie restriction is feasible and well tolerated in non-obese humans
- Findings in humans are highly encouraging of further research

Table 1. Summary of design, methodology and findings of body composition and energy metabolism, of human CR studies

	Human Studies			
	Human Studies	CALERIE-1 Pennington Biomedical Research Centre (PBRC)	Washington University (WU)	CALERIE-2 Multicentre Trial, Single Protocol PBRC+Tufts+WU
Study Period	2002–2004	2002–2004	2002–2006	2007–2012
Study Design	RCT: Four arms	RCT: Four arms	RCT: Three arms	RCT: Two arms
Study Duration	1 year	6 months	1 year	2 years
CR	30% CR(HG) 30% CR(LG) 10% CR(HG) 10% CR(LG)	25% CR 12.5 %CR+12.5 %EX LCD Control	20% CR EX WD	25% CR Ad libitum
Number of Participants	46, M & F	48, M & F	58, M & F	220, M & F
Number of participants per arm	17 in each 30% CR arm 6 in each 10% CR arm	12 in each of the four arms	20% CR: 21 EX: 24 WD:13	25% CR:145 Ad libitum:75
Characteristics				
Age (yrs)	25–42 years	F: 25–45 M: 25–50	50–60	F: 21–47 M: 21–50
BMI (kg/m ²)	25 – 29.9	25 – 29.9	23.5 – 29.9	22 – < 28
Body Composition				
Body Weight	↓ Weight	↓ Weight	↓ Weight	↓ Weight ↓ BMI
FM	↓ FM	↓ FM ↓ Fat cell size ↓ TAT ↓ SSAT ↓ DSAT	↓ FM & % FM ↓ Trunk FM ↓ Abdominal FM	↓ FM & % Body fat
		↓ VFM ↓ IHL ↓ IMSL ↓ SAT ↓ FFM	↓ VFM ↓ SAT ↓ FFM	↓ FFM
FFM		→ BMD (total)	→ BMD (total)	→ BMD (total),
BMD		→ BMD (hip)	↓ BMD (regional - hip, intertrochanter, spine)	↓ BMD (regional-femoral neck, intertrochanter, trochanter, hip, lumbar spine)

	Human Studies			
	CALERIE-1 Pennington Biomedical Research Centre (PBRC)	Washington University (WU)	CALERIE-2 Multicentre Trial, Single Protocol PBRC+Tufts+WU	
Energy Metabolism	↑ CTX	→ CTX	→ CTX	
	↓ AP	→ AP	→ TRAP5B	
	→ NTX in CR		→ PINP	
	↑ NTX in LCD		↓ AP	
	→ Osteocalcin	→ Osteocalcin	↑ 25(OH)D	
			→ Parathyroid	
	↓ CBT		→ CBT	
	↓ 24 hr-EE	↓ Energy Intake	↓ TDEI	
	↓ Sleeping EE		↓ TDEE	
	→ RMR (CR)		↓ RMR	
	↓ RMR (CREX & LCD)			

AP, Alkaline phosphatase; BMC, Bone mineral content; BMD, Bone mineral density; BMI, Body mass index; CBT, Core body temperature; CR, Calorie restriction; CREX, Calorie restriction and exercise; CTX, Cross-linked C-telopeptide of type I collagen; DSAT, Deep subcutaneous abdominal adipose tissue; EE, Energy expenditure; F, Female; FFM, Fat free mass; FM, Fat mass; HG, High glycemic diet; IHL, Intra hepatic lipid; IMCL, Intramyocellular lipid; LCD, Low calorie diet; LG, Low glycemic diet; M, Male; NTX, Cross-linked N-telopeptide of type I collagen; PINP, Intact N terminal propeptide of type I procollagen; RCT, Randomized controlled trial; RMR, Resting metabolic rate; SAT, Subcutaneous adipose tissue; SSAT, Superficial subcutaneous abdominal adipose tissue; TAT, Total abdominal adipose tissue; TDEI, Total daily energy intake; TEE, Total energy expenditure; TRAP5b, Tartrate-resistant acid phosphatase; VFM, Visceral fat mass; WD, Western diet; **Source reference for CALERIE-1**(Ahmed et al., 2009; Anton et al., 2009; Civitarese et al., 2007; Das et al., 2007a; Das et al., 2007b; Fontana et al., 2006; Fontana et al., 2007; Fontana et al., 2008; Heilbronn et al., 2006; Larson-Meyer et al., 2006; Larson-Meyer et al., 2008; Lefevre et al., 2009; Martin et al., 2007a; Martin et al., 2007b; Mejdani et al., 2011; Pittas et al., 2005; Pittas et al., 2006; Racette et al., 2008; Racette et al., 2006; Redman et al., 2007; Redman et al., 2008; Redman et al., 2010; Riordan et al., 2008; Tam et al., 2012; Villareal et al., 2006; Weiss and Holloszy, 2007; Weiss et al., 2006)) and **CALERIE-2**(Das et al., 2017; Martin et al., 2016; Ravussin et al., 2015; Redman et al., 2014; Rickman et al., 2011; Rochon et al., 2016; Romashkan et al., 2011; Stewart et al., 2013).

Summary findings of cardio-metabolic health, hormonal, immunology, oxidative stress and quality of life related findings from the human CR studies

Table 2.

	Human Studies			
	CALERIE-1		CALERIE-2	
	Tufts University	Pennington Biomedical Research Centre (PBRC)	Washington University (WU)	Multicentre Trial, Single Protocol PBRC+Tufts+WU
Cardio-Metabolic				
Blood Pressure		→ SBP → DBP	→ SBP → DBP	↓ Mean blood pressure
CVS Structure		↓ % Factor VIIc		
Bio Markers		→ Fibrinogen → Homocysteine	↓ Global ventricular stiffness	
Lipid Profile	↓ TAG ↓ Total Cholesterol ↓ LDL ↑ HDL		↓ TAG ↓ Total Cholesterol ↓ LDL → HDL-C ↓ Total Cholesterol: HDL ratio → FFA	↓ TAG ↓ Total Cholesterol
Hormonal				
Thyroid		↓ T ₃ ↓ T ₄	↓ T ₃ → Reverse T ₃ → Free T ₄ → Total T ₄ → TSH	↓ T ₃
Insulin / Glucose	↓ Insulin ↓ Glucose	↓ Acute insulin response ↓ Fasting insulin ↑ Insulin sensitivity → Fasting glucose ↓ Leptin → Adiponectin → GH in CR ↑ GH in LCD & CREX	↓ Fasting insulin ↑ Insulin sensitivity ↓ HOMA-IR → Fasting glucose ↓ Leptin ↑ Adiponectin → IGF-I → IGF-I :IGFBP-3 ratio	→ TSH ↓ Insulin ↓ HOMA-IR
Leptin / Adiponectin				↓ Leptin ↑ Adiponectin → IGF-I ↑ IGFBP-1
GH /IGF- 1				

Human Studies				
	CALERIE-1 Pennington Biomedical Research Centre (PBRC)	Washington University (WU)	CALERIE-2 Multicentre Trial, Single Protocol PBRC+Tufts+WU	
Sex Hormones	↑ GH half life in CR		→ IGFBP-3	
	→ GH half life in LCD & CREX		→ IGF-1 :IGFBP-3 ratio	
	→ IGF-1 in CR		↓ IGF-1 :IGFBP-1 ratio	
	↑ IGF-1 in LCD & CREX		→ Total Testosterone	
Cortisol		↓ Estradiol	→ Free Testosterone	
	→ DHEA	→ Cortisol	↑ SHBG	
			→ FSH	
			→ LH	
Immune System & Oxidative Stress			→ Cortisol	
		→ TNF α	↓ TNF α	
	↑ IL-6 in CR	↓ TNF- α : adiponectin ratio	↓ CRP	
	→ IL-6 in LCD & CREX		↓ PDGE-AB	
Antioxidant Defence and Oxidative Stress	→ CRP	→ hs CRP	↓ TGF- β 1	
	→ Gene expression for inflammatory markers			
	↓ DNA damage			
	→ Protein Carbonyl			
Physical Activity	↑ Expression of mitochondrial function related genes			
	↑ Mitochondrial DNA			
	→ Activity of mitochondrial enzymes			
	↓ DNA damage			
Behavior and Quality of Life	→ PA	→ PA	↓ PA	
	→ Cognitive test performance		→ Grip strength	

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Human Studies			
CALERIE-1		CALERIE-2	
Tufts University	Pennington Biomedical Research Centre (PBRC)	Washington University (WU)	Multicentre Trial, Single Protocol PBRC+Tufts+WU
→ Mood			
→ Vitality, mental health, bodily pain			
↑ General health)			
→ Sleep quality			
→ Perceived stress			
→ Reported sexual function			

BP, Blood pressure; CR, Calorie restriction; CREX, Calorie restriction and exercise; DBP, Diastolic blood pressure; DHEA, Dehydroepiandrosterone; DTH-Delayed type hypersensitivity; F, Female; FFA, Free fatty acid; FSH, Follicle stimulating hormone; GH, Growth hormone; HDL, High density lipoprotein; HOMA-IR, Homeostatic model assessment insulin resistance; CRP, C reactive protein; IGF-1, Insulin like growth factor-1; IGFBP-1, Insulin-like growth factor-binding protein 1; IGFBP-3, Insulin-like growth factor-binding protein 3; IL-6, Interleukin 6; LCD, Low calorie diet; LDL, Low density lipoprotein; LH, Luteinizing hormone; M, Male; PA, Physical activity; PDGE-AB, Platelet derived growth factor AB; PGE2, Prostaglandin E2; SBP, Systolic blood pressure; SHBG, Sex hormone binding globulin; TAG, Triacylglycerides; TGF-β1, Transforming growth factor beta 1; TNFα, Tumor necrosis factor alpha; TSH, Thyroid stimulating hormone; **CALERIE-1**(Ahmed et al., 2009, Anton et al., 2009, Civitarese et al., 2007, Das et al., 2007a, Das et al., 2007b, Das et al., 2009, Fontana et al., 2006, Fontana et al., 2007, Fontana et al., 2011, Pittas et al., 2005, Pittas et al., 2006, Larson-Meyer et al., 2006, Larson-Meyer et al., 2008, Lefevre et al., 2009, Martin et al., 2007a, Martin et al., 2007b, Martin et al., 2010, Riordan et al., 2008, Tam et al., 2012, Villareal et al., 2006, Weiss et al., 2006, Weiss et al., 2013), **CALERIE-2**(Martin et al., 2016, Ravussin et al., 2015, Redman et al., 2014, Rickman et al., 2011, Rochon et al., 2011, Romashkan et al., 2016, Stewart et al., 2013).