

Get back—calorie restriction: Dr. Masoro’s legacy

Yuji Ikeno^{a,b,c,*}, Lisa C. Flores^a

^a Barshop Institute for Longevity and Aging Studies, San Antonio, TX 78229, USA.

^b Department of Pathology, The University of Texas Health Science Center at San Antonio, San Antonio, TX 78229, USA.

^c Geriatric Research and Education Center, Audie L. Murphy VA Hospital, South Texas Veterans Health Care System, San Antonio, TX 78229, USA.

Abstract

After the initial discovery by MaCay and colleagues, which showed that reduced food intake extended the lifespan in rats, many aging researchers used calorie restriction (CR) as an experimental paradigm to seek the underlying mechanisms of aging and anti-aging actions of CR. Among those many researchers, one of the most intensive and systematic studies was carried out by Dr. Edward Masoro and his colleagues. Dr. Masoro’s approach with a strict animal maintenance protocol in a barrier facility along with using a defined semisynthetic diet allowed him to yield a large amount of data and very important information for the advancement of current aging research to areas such as reduced GH/IGF-1 actions, suppression of mTOR signaling, reduced senescent cell accumulation, enhanced insulin sensitivity and signaling, and others (Sirt1 and hormesis), *etc.* The interventions of a single mechanism/pathway have demonstrated an exciting and promising outcome for the translational aspect to humans. However, CR is still considered the most effective intervention of aging. The mammalian aging process seems to be far more complex than our initial prediction, and the anti-aging effects shown by CR require synergistic alterations of multiple mechanisms/pathways. Therefore, it may be time for us to get back and re-visit Dr. Masoro’s legacy by further examining the complexity of mechanisms of aging and the effects of CR. These further experiments are necessary to validate the successful implementation of CR to human aging (including lifespan, healthspan, and various age-related diseases) because of its diversity of genetic backgrounds, dietary compositions, lifestyle, and other factors that are different from the experimental animals in well-defined laboratory conditions.

Keywords: Calorie restriction, Fischer 344 rats, aging

Humans empirically noticed the health benefits of reduced calorie intake, which has been practiced by fasting in various cultures and countries [1]. In fact, the Japanese proverb, “eating moderately (20% less) keeps the doctors away,” has been shared among many generations from ancient times. Although the clinical importance of fasting for health has been advocated since the 1800s [1], the first scientific proof came from studies, in which anti-cancer and life-extending effects of calorie restriction (CR) were demonstrated with laboratory mice and rats in the early

1900s [2-4]. Since these very intriguing discoveries, CR has been extensively utilized for aging research by many investigators and groups.

Among the many studies testing the effects and seeking the underlying mechanisms of CR, a substantial amount of important information was yielded by Dr. Edward Masoro and his colleagues. The studies with Fischer 344 (F344) rats conducted over two decades (between mid-1970 to mid-1990s) by his group have shown that CR not only extends the mean, median, and maximum lifespans but also attenuates the age-related decline in many physiological processes and suppresses the occurrence of various age-related diseases [5]. Because of its broad anti-aging effects shown by Dr. Masoro, Dr. Walford, and others, CR is considered the “gold standard” for aging interventions in experimental gerontology.

A series of experiments with CR, conducted by Dr. Masoro, was carried out by feeding F344 rats 40% less compared to the food intake of their ad libitum-fed (AL) group. For the entire period, a semisynthetic diet was used

* Corresponding author: Yuji Ikeno, MD, PhD.

Mailing address: Barshop Institute for Longevity and Aging Studies, Department of Pathology, The University of Texas Health Science Center at San Antonio, 4939 Charles Katz Dr., San Antonio, TX 78229, USA.

Email: ikeno@uthscsa.edu

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because this diet had a defined macronutrient source that allows investigators to know the precise sources of the ingredients. Another notable fact is that the CR diet was supplemented with vitamins and minerals, which allowed the CR animals to consume the same amount of vitamins and minerals as the AL control group (Masoro diet). In addition, Dr. Masoro established a barrier facility, which had animal rooms with HEPA filtered air and followed a strict protocol for sterilization of items brought into the room by animal care staff, exclusively for the CR aging research colony. Having established this protocol and unique barrier facility, Dr. Masoro and colleagues conducted research to test the effects of 40% CR on aging and explore general hypotheses about the underlying mechanisms of aging and the benefits of CR.

The first survival study using this defined diet and barrier facility demonstrated that CR male F344 rats showed approximately a 50% increase in median lifespan and a similar increase in maximum lifespan compared to the AL group [6]. More importantly, another survival study conducted nearly four years after the initial study showed the survival curves from these independent studies are nearly superimposable [7]. Because of the extension of lifespan and its reproducibility of the CR study, 40% CR became an exceptionally useful paradigm for studying aging in both rat and mouse CR research.

In the second CR study, Dr. Masoro also examined whether the beneficial effects of CR were due mainly to restricting growth, which was hypothesized by McCay. Dr. Masoro critically evaluated this possibility by comparing three survival groups: 1) CR only during the period of rapid growth (6 weeks to 6 months); 2) CR started from near full growth (6 months) throughout the rest of life; and 3) CR started from after weaning (6 weeks) throughout the rest of life. Although all three CR groups showed the extension of lifespan compared to the AL group, the results clearly demonstrated that the magnitude of life-extending effects was larger in the group that CR started at 6 months and continued throughout their lives than the group with CR only in the period of rapid growth (6 weeks to 6 months). The life-extending effects were the greatest in the group with CR started after weaning (6 weeks) and continued throughout the rest of life [7]. These results strongly suggest that: 1) the length of CR plays a more important role than CR during the developmental period of life on the life-extending effect; and 2) CR may extend lifespan through different mechanisms during the developmental period and adult life [7].

Another survival group as a part of the second CR study was to address the question of whether the restriction of protein only without CR was the contributing factor to the life-extending effect. To accomplish this experiment, using a semisynthetic diet has a great advantage. A diet with 40% restricted protein (casein) without any changes in caloric intake was fed to F344 rats ad libitum (protein restriction). The protein restriction extended lifespan, however, the magnitude of life extension was not as robust as 40% CR and had no notable effects on age-related physiological changes. The pathological examination suggested

that suppression of kidney pathology by protein restriction may be the causal factor to the relatively modest (approximately 16%) life-extending effect by protein restriction without reduced calorie intake [8]. This possibility was further examined later by comparing the survival and kidney pathology of groups fed a diet with two protein sources (casein versus soy protein) [9]. In this study, replacing the protein source from casein by soy protein without changes in calorie intake extended lifespan (approximately 16%), which is similar to protein restriction. The soy protein-fed group also showed that kidney pathology was markedly retarded compared to the casein-fed group. Therefore, attenuating one of the major and possible fatal pathologies, i.e., chronic nephropathy, by protein restriction or soy protein diet has some benefits on longevity. However, it is reduced calorie intake that has a maximum impact on the life-extended effects by CR.

The results of the protein restriction study also led Dr. Masoro to test the impact of other macronutrients on longevity. To evaluate this, the diet was made with a reduction in either the fat or the mineral component to 60% of the control diet without changes in calories by increasing the dextrin content of the diet. The results showed that, unlike protein restriction, restricting fat or mineral components had no effect on lifespan [10]. Thus, restriction of individual macronutrients, i.e., protein, fat, and minerals, showed a somewhat modest (approximately 16%) increase in longevity possibly attenuating major and potentially fatal disease (kidney pathology) by protein restriction or no effect on longevity by fat and minerals restriction. Once again, these results indicate that total calorie intake is the most important contributing factor to the life-extending effects by CR.

Since the feeding pattern differs between AL and CR animals, there is a possibility the changes in circadian rhythms by different feeding patterns could be one of the important factors to the extended longevity by CR. To address this intriguing question, a survival study was conducted using: 1) AL fed group; 2) 60% CR group with a single daily meal at 1500h; and 3) 60% CR group with two daily meals at 0700h and 1500h. Both CR groups showed significantly extended lifespans compared to the AL-fed group, however, there were no differences in longevity and pathology between those CR groups that had different feeding patterns [11]. Therefore, the changes in circadian rhythms by CR under this experimental protocol do not significantly affect the anti-aging effects of CR.

As stated at the beginning, humans empirically noticed the health benefits of reduced calorie intake and the Japanese proverb shared by many generations suggested a modest (20% less) reduction of calorie intake. If we advocate this calorie restriction paradigm to society, an important question is how much CR is practical to have a meaningful impact to improve human health and extend healthspan, and possibly extend lifespan? Although the assumption is that less CR could be less effective on longevity compared to 40% CR, there is not much information regarding the effects of less (lower than 40%) CR on aging. In the last CR project, Drs. Masoro and McCarter tested the effects

of 10% CR on aging and age-related diseases. Contrary to initial expectations, the 10% CR group showed an extended lifespan, which was almost similar to the 40% CR group, although the effects on age-related pathology were greater in the 40% CR than in the 10% CR group [12]. The efficacy of different levels of CR (e.g., 5, 10, 15, and 20%) has to be further examined because this could be greatly beneficial for humans to determine a practical level of CR to extend health span and lifespan.

The vast amount of data obtained from studies using CR has led current aging research to uncover the underlying mechanisms involved in the slowing aging process and the life-extending effects by CR, e.g., reduced GH/IGF-1 actions, suppression of mTOR signaling, reduced senescent cell accumulation, enhanced insulin sensitivity and signaling, and others (Sirt1 and hormesis), *etc.* Each of these possible underlying mechanisms of aging and the effects of CR have been proven in its roles using genetic and pharmacological interventions in various animal models. Although an intervention of a single mechanism/pathway shows an exciting and promising outcome, it seems that CR is still the most effective intervention of aging. This is possibly due to the fact that the aging process, especially in mammals, is far more complex than our initial prediction, and multiple mechanisms seem to be simultaneously altered to have anti-aging effects shown by CR. This very simple manipulation, i.e., reducing calorie intake, is a potentially ideal intervention for humans because of little negative effects on pathophysiology. To further examine the translational implications to dietary intervention of human aging, it is essential to address the complexity of the mechanisms of aging and the effects of CR. Therefore, it may be time for us to “get back to where we once belonged” and re-visit Dr. Masoro’s legacy by further examining: 1) the efficacy of different levels of CR (e.g., 5, 10, 15, and 20%); 2) effects of CR on physiological changes (both benefits and disadvantages) in different stages of life, e.g. growing phase, adulthood, and old age; 3) effects of restriction of individual dietary components (restriction of a single component or combination of some); 4) potential interactions between CR and circadian rhythm; and 5) interactions between CR and genetic backgrounds. These further experiments are necessary to validate the successful implementation of CR to human aging (including lifespan, healthspan, and various age-related diseases) because of its diversity of genetic backgrounds, dietary compositions, lifestyle, and other factors that are different from the experimental animals in well-defined laboratory conditions.

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