Honoring Clive McCay and 75 Years of Calorie Restriction Research[1,2]

Roger B. McDonald[3]* and Jon J. Ramsey[4]

Departments of [3]Nutrition and [4]Molecular Biosciences, School of Veterinary Medicine, University of California, Davis, CA 95616

Introduction

This volume and issue of *The Journal of Nutrition* marks the 75th anniversary of the publication by McCay, Crowell, and Maynard entitled "The Effect of Retarded Growth upon the Length of Life Span and upon the Ultimate Body Size" (1). The data presented by McCay et al. described, for the first time, that the restriction of calories without malnutrition prolongs mean and maximal lifespan in rats compared with ad libitum feeding (**Fig. 1**).

While almost 50 years would pass before the importance of this work would be fully recognized as a viable research model for aging (2,3), the importance of this publication to research in nutrition and aging cannot be overstated. Calorie restriction (CR), also referred to as dietary restriction, remains the only nongenetic method that extends lifespan in every species studied, including yeast (4), worms (5), flies (6), and rodents (7). Data collected from nonhuman primates suggest that CR will have similar effects in this species (8,9) and data from the first human trials have been published (8). The ability of CR to extend life and delay the age-related functional decline has, arguably, contributed more than any other model to the overall understanding of the biological processes of aging and longevity. To honor and celebrate the 75-y legacy of the publication in *The Journal of Nutrition* of McCay, Crowell, and Maynard's pioneering work, we present this brief historical review on CR.

McCay et al. (1) state that: "The object of this study was to determine the effect of retarding growth upon the total length of life and to measure the effects of retarded growth upon the ultimate size of the animal's body. In the present study growth was retarded by limiting the calories."

The uniqueness of McCay's design compared with previous investigations was that retarded growth was achieved through the reduction of calories only. Increased longevity by retarding growth in prior investigations had been achieved by the use of nutrient deficiency along with food restriction (10–12). McCay thought that nutrient deficiency together with food restriction most likely caused metabolic problems beyond limiting growth and stated: "It is doubtful if such studies…test the hypothesis [life span and retarded growth], because the two groups, separated on the basis of growth, are not homogenous. The slower growing group tends to include the inferior individuals that die prematurely."

By this statement, McCay et al. established, long before organized aging research, a fundamental prerequisite for valid

longevity studies, i.e. all animals must be given the opportunity to achieve old age. Premature death during development is not considered normal aging and must be prevented to collect meaningful data on mean and maximal lifespan.

While McCay's interest in aging/longevity has been well documented (13), his 1935 publication also addressed the impact that undernutrition had on retarding growth. The title of the 1935 paper tells us that lifespan was used as an end point marker for the effect that undernutrition had on retarded growth. Documenting the effects of retarded growth on aging/longevity reflected the times in which these studies were being conducted. Mean lifespan had reached only 53 y of age in 1935. The 1920s and 1930s were still plagued by high infant mortality, childhood diseases that killed millions of children under the age of 10 y, and persistent bacterial infections that affected all age groups (widespread use of penicillin was still 7 years away in 1935). The short mean lifespan was the direct result of these medical realities. Moreover, rigorous basic research into the impact that nutrition and specific nutrients had on growth and the health of children had only just begun. Recall that most vitamins were discovered during the first 2 decades of the 20th century. CR's possible impact on the growth and health of children was an important topic in 1935. Mechanisms that underlie aging were not on the radar screen yet.

The purpose of this brief historical review is to recognize the 75 years of CR research that can draw its roots to the 1935 *The Journal of Nutrition* publication by McCay et al. (1). To this end, we will limit this historical review to general topics marking important steps in the development of the model or considerable changes to the fields of nutrition and/or aging caused by research using CR. Page limitations preclude a thorough discussion on the outcomes of research using CR and several excellent reviews accomplish this goal (7,14–17). Hundreds of investigators have contributed greatly to research in CR and we apologize for any failure to mention a particular investigator; the omission is not premeditated.

1935–1945: The forerunners of aging research

Research investigating the effect of retarded growth on lifespan and published between 1935 and 1945 were generally consistent with McCay's hypothesis that a reduction in energy rather than individual nutrients was the reason for increased longevity. Although no direct comparisons of lifespan of rodents restricted in energy or nutrients were made during this time, retarded growth by feeding rats diets inadequate in B vitamins, calcium, and/or protein decreased lifespan or caused significant mortality during development (18–20).

McCay's group published 3 additional reports between 1939 and 1942 supporting their original work that energy alone was the factor inducing life extension in rats (21–23). A 1939
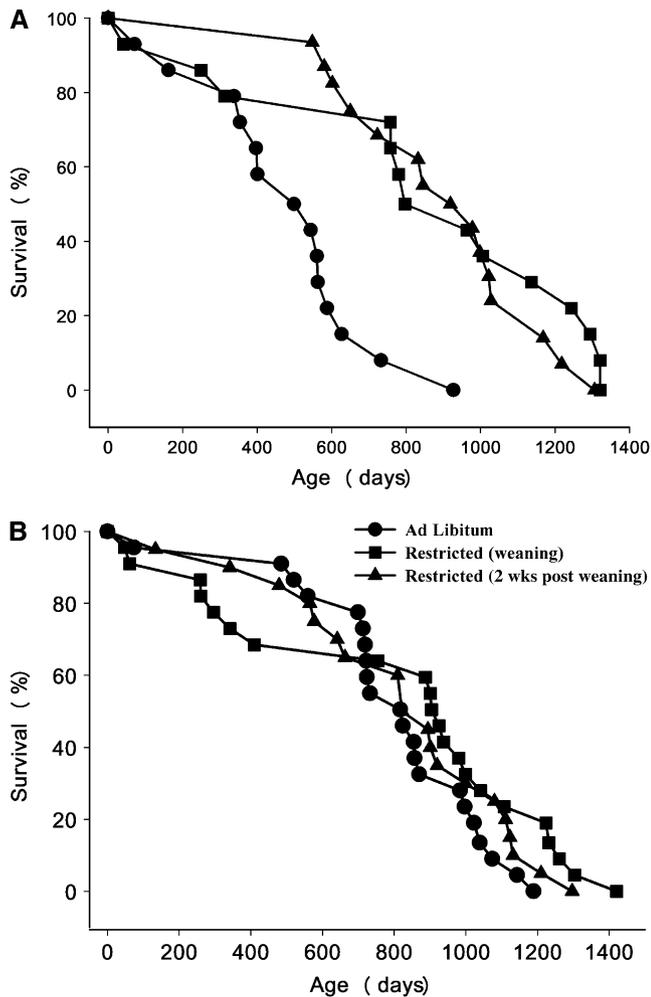
FIGURE 1 Survival curves generated from the data of McCay et al. (1) for male (*A*) and female (*A*) rats consuming ad libitum or CR.

reproduction are linked to longevity (see further explanation on this topic below).

1946–1955: Defining the experimental diet and feeding protocol

The reports published during this period and evaluating the effect of CR on development and lifespan focused primarily on defining the experimental diet ingredients and testing different restriction protocols. Prior to 1940, research in CR, including McCay's publications, used ill-defined "human" type food to provide optimal nutrition for the rats. Efficient methods for nutrient extraction from plants developed during WWII allowed for better definition of experimental diets used in nutrition research, including CR investigations. To this end, Risen et al. (29) were the first to report that energy-restricted rats fed a "synthetic" diet consisting of 48% sucrose, 32% casein, 6% corn oil, and defined mineral and salt mixes lived significantly longer than did rats consuming ad libitum the identical diet. Thus, the door was now open for subsequent investigations to manipulate the amount of specific nutrients to more precisely evaluate whether or not the restriction of energy or nutrients was the cause of extended lifespan.

One of the first publications to discuss the appropriateness of CR for humans appeared during this time period. Carlson and Hoelzel (30) speculated that the abundance of food presented to humans in modern society concomitant with our species' drive to eat would make daily CR difficult. These investigators suggested that a more realistic method of CR in humans would be to fast on a periodic schedule. The hypothesis was tested in rats using various feeding:food deprivation ratios. Intermittent fasting, whether done once every other day or 1 day in 4, significantly extended mean lifespan and reduced the incidence of spontaneous tumors compared with rats fed daily. While questions surrounding the effectiveness of CR in humans have yet to be answered, Carson and Hoelzel did establish a new method for CR, i.e. intermittent feeding, one that is currently being tested for use in humans (31,32).

1956–1975: Emphasis on nutrition and the application of scientific rigor to CR research

By 1956 the use of CR without malnutrition as a means to extend lifespan was beginning to gain acceptance as a viable research method, although its use remained, for the most part, within the confines of classic nutrition research. Research during this 20-y period and using CR focused primarily on how reducing food intake during development retarded growth that, in turn, effected an organism's life history. A few investigations during this time period described longevity as a primary focus of the research. This included investigators using CR to alter environmental conditions of insects and other nonmammalian species as a means to delay reproduction and extend lifespan (33,34). These data further supported the evolutionary principle first describe by Ingle et al. (26) using CR that genes are selected first and foremost for reproduction and that longevity is a by-product of that selection.

Investigations conducted during this time period and evaluating the nutritional aspect of CR on an organism's life history was led by Berg and Ross. The Berg laboratory in collaboration with Simms in the Department of Pathology at Columbia University confirmed in rats 2 important nutritional features of CR. First, a reduction in body weight brought about by CR prevented or delayed the onset of definable pathologies (35–37). Berg and Simms thought that excess body fat (obesity) was the cause of the early onset of disease and shortened lifespan of the

publication defined, for the first time, the aging research value of the CR model. Here McCay suggested that CR may be a model for studying aging: "Retardation of growth by diets, complete except for calories, affords a means of producing very old animals for studying aging" (21).

Several investigations published during this period described a reduction in specific pathologies such as spontaneous tumors (22,24) and nephritis (25) in rats and mice following long-term CR. These initial reports established the anticarcinogenetic effect of low-energy diets and most likely marked the beginning of experimental research into the relationship between nutrition and cancer. Indeed, decreased body weight and a reduction in the appearance of spontaneous tumors observed in calorie-restricted vs. ad libitum-fed mice led Tannenbaum in 1942 to state: "It follows that the avoidance of overweight through restriction of food intake may aid in the prevention of human cancer or at least delay its onset" (24).

Finally, the first use of CR in a nonmammalian species, *Daphnia longispina* (water fleas), was reported during this time period (26). These investigators described that a reduction in food given to juvenile water fleas delayed the start of reproduction, extended the reproductive period, and increased lifespan. In addition to showing that CR extends lifespan in nonmammalian species, Ingle et al. (27,28) described, for the first time, a result that would become a general principle in the evolutionary theory of longevity, i.e. genes selected for successful

ad libitum rat, although they did not test this hypothesis directly by conducting body composition analysis. Second, extended reproductive life of CR rats (38) confirmed in a mammalian model the link among diet, longevity, and reproductive success previously reported in protozoans (39) and insects (26,33).

Ross et al. (40–46) laid to rest any questions as to the impact that the protein concentration of the diet had on extended lifespan observed during CR. Although high vs. low levels of protein (casein) in the diet shorten the lifespan of restricted rats, a finding that would be confirmed during the 1980s (47), the effect was minor compared with the life-extension effect of low energy. There was no question now that McCay was correct in his original suggestion in 1935 that energy per se was the only nutritional factor causing life extension observed during long-term CR.

In our opinion, the most important contribution of Ross and colleagues to CR research was the application of scientific rigor to the experimental design. Ross's 1961 paper published in *The Journal of Nutrition* was the first in 25 y of CR experimentation to use standardized mortality analysis, a cohort life table, in determining the lifespan characteristics of his rodent population (41). The similarity of mortality rates among ad libitum- and CR-fed rats allowed Ross to conclude, where others had only speculated, that differences in life expectancy must be due to the rate of development. While this conclusion would be questioned in later years, rigorous statistical analysis would now be required for valid lifespan studies using CR.

1975–1985: The rise of CR as a tool in aging and longevity research

During the early 1970s, laboratories headed by Walford at UCLA and Masoro at the University of Texas Health Science Center became interested in CR as a method for evaluating mechanisms underlying aging. Walford had recently published a book outlining his thoughts on the relationship between immune function and biological aging entitled "The Immunologic Theory of Aging" (48). The proper testing of the Immunologic Theory of Aging would require aged mice of similar age but with different immunologic status. Previous studies, especially those by Ross, suggested that CR delayed or prevented some age-related immunological disorders. Thus, CR provided Walford with the model he needed and in 1975 his laboratory published their first report describing that "…the immune system [of calorie restricted mice] may mature less rapidly and stay "younger" longer than in the controls [ad libitum]." (49). The Walford group along with Weindruch, who was a graduate student at the time, spent the next several years validating CR as a model for aging research and its impact on age-related disease (14).

A talk given by Ross in 1973 on CR and longevity prompted Masoro to state in later years that "…the findings he [Ross] reported, which summarized the work of his group, were so compelling that I was led to spend most of my remaining research career on caloric restriction and aging" (50). Between 1977 and 1984, Masoro along with Yu and Burtrand published a series of papers describing the changes in lipid metabolism following long-term CR. However, it was the 1985 tandem papers published in *The Journal of Gerontology* (47,51) that established the Masoro group as leaders in the use of CR for aging and longevity research. While their findings that CR extends lifespan and delays age-related disease was a confirmation of several previous investigations, the details on husbandry and pathologic methodology in these 2 publications marked an important change in the way in which CR research was

conducted. These publications explained eloquently that meticulous husbandry, environmental testing for contamination, and reporting probable cause of death are critical to ensuring that diseases arising from environmental sources did not influence the lifespan results. Many of the methods described by Masoro's group, and to some extent those of Walford's group, remain today as standard procedures in the maintenance of longevity colonies.

The research prior to the publications of the Walford and Masoro groups assumed, for the most part, that lifespan extension with CR was the result of delayed development. This assumption was the reason that CR was being used primarily as a tool in nutrition research rather than a methodology for the exploration of the aging process. The switch in use of CR from a focus on development to aging occurred as a result of experiments conducted by the Walford and Masoro groups. Weindruch and Walford (52) using mice and Yu et al. (47) in rats showed that CR begun after the rodent had attained full development was effective in extending lifespan. Thus, it was unlikely that delayed development per se was the primary cause of lifespan extension with CR. This result led to the introduction of CR as a powerful tool in aging research and a reduced visibility of CR as a nutrition-only model.

1986–1995: Describing the effects of CR on aging

Research completed during 1986–1995 and using the CR model was characterized, for the most part, by investigations designed to describe how CR affected age-related declines in physiological systems. CR in rodents retarded the rate of aging in virtually every system evaluated as well as preventing or delaying the appearance of age-related damage. The results of studies using CR clearly showed that the cause of physiological aging was multifactoral. CR was turning out to be an extremely powerful tool in the study of aging. It was soon recognized that an expanded research program in CR would be needed to maximize efforts at identifying mechanisms underlying aging and longevity. To this end, the National Institute on Aging (NIA) established a colony of energy-restricted rats and mice that were then made available to researchers having NIH support. The NIA also began several sponsored programs during this time focusing specifically on the mechanisms of CR.

By end of 1985, many of the methodological questions in the use of CR had been answered. Reduction in energy was the major factor effecting lifespan, husbandry issues had been addressed, and the most effective age at which to begin CR was known. One question remained, however: "Does varying the level of CR have differential effects on lifespan and age-related disease?" This question was answered in part in a 1986 *The Journal of Nutrition* publication by Weindruch et al. (53). These researchers showed that maximal lifespan increased with increasing levels of CR (**Fig. 2**). Subsequent investigations in nonhuman primates have shown that modest levels of CR can significantly improve age-related disorders and decrease mortality rate (9,54). Although the greatest increases in lifespan have occurred with severe levels of CR in rodents, it is not likely that these levels of CR could be maintained over long periods of time in humans. Thus, there has been interest in determining the overall health benefits of more modest levels of CR in humans and other animals.

Throughout the 1980s and early 1990s, researchers began to discuss seriously the efficacy of using CR in humans. These discussions, although often collegially heated, led to the funding of CR studies in nonhuman primates. Two nonhuman primate studies on CR were established and remain ongoing, one at the
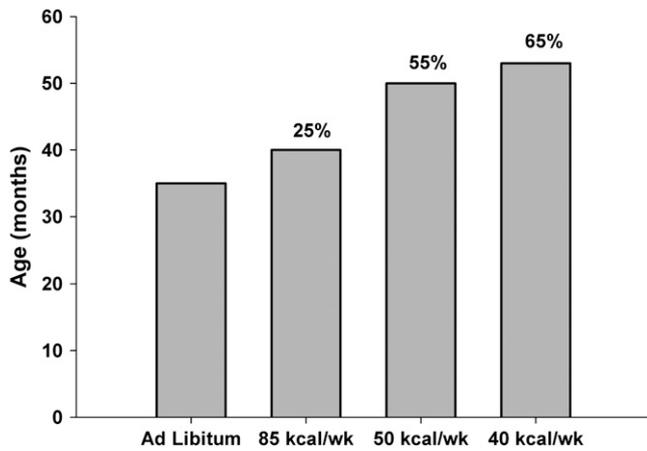
FIGURE 2 Maximal lifespan of male mice that consumed ad libitum and varying levels of CR. Values above bars are approximate percentages of CR. Adapted with permission from (53). 1 kcal = 4.186 kJ.

FIGURE 3 Number of original research reports using CR published between 1986 and 2009. Census was collected from a PubMed search using the key words "calorie restriction or dietary restriction and aging." Reviews, commentaries, and studies emphasizing obesity in humans were excluded from the count. Note that the number of publications in the 4-y period of 2006–2009 is just slightly less than the total number of papers published in the 15-y period between 1986 and 2000.

NIA in Maryland and the other at the University of Wisconsin. Although complete lifespan data are still years away, noninvasive measurements such as glucose tolerance, body composition, etc. are finding a similar delay in the rate of aging as that observed in rodents (54). The University of Wisconsin group reported this year that 80% of the CR-fed monkeys were alive at a time point when 50% of the ad libitum diet nonhuman primates were dead (9). It appears that the life extension properties of CR observed in other mammalian species will be verified in nonhuman primates.

1996–Present: The search for mechanisms
General biological aging research being conducted during the 1990s led to the most important change in direction for CR research since McCay's original publication. Several investigators were reporting that the manipulation of individual genes could alter the lifespan of *Caenorhabditis elegans* (55,56), *Saccharomyces cerevisiae* (4), and *Drosophila melanogaster* (6,57). The newly discovered longevity genes expressed proteins linking energy balance and reproduction to longevity. Moreover, the evolutionary problem of longevity and aging was solved. Evolutionary theorists had mathematically and empirically demonstrated that longevity was genetically determined from genes that were selected for a reproductive advantage (27,28). Age-related decline in function was due to random events occurring during development and the reproductive phase (58). The descriptive phase of biological aging had ended and the mechanistic phase had begun in earnest. Importantly, CR in invertebrates would provide the method necessary to probe the molecular mechanisms underlying longevity and aging.

The use of invertebrates in longevity and aging research greatly increased the number of researchers using CR as a tool in their investigations. The increased use of CR in invertebrates most likely reflected 2 practical matters. First, the savings of time and cost in using short-lived invertebrates for aging research opened the door to many investigators who had previously been unwilling to invest resources into the slow pace of longevity studies using long-lived vertebrate species. Second, the cell and molecular biologist as well as the geneticist who traditionally used invertebrate models could now apply their techniques to the identification of the mechanism underlying CR's effects on longevity. The end result was a surge in reports being published
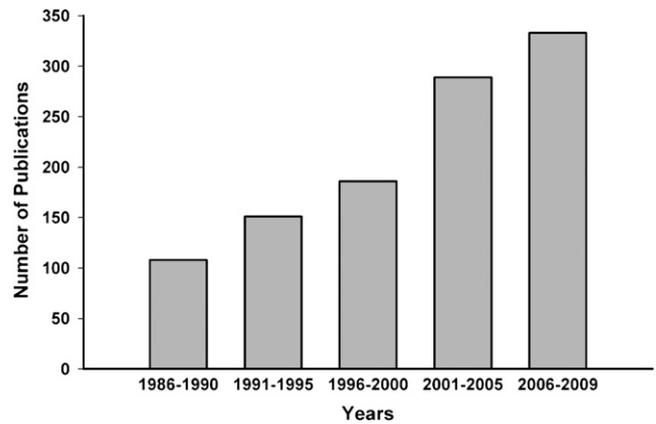
after the year 2000 using CR (**Fig. 3**), the majority of which involved invertebrate species. A series of recent reviews have described important findings from research involving CR in invertebrates (59–61).

2010–Future
The rapid advancements in biotechnology make predicting the direction for CR research difficult. The current focus on mechanisms underlying the life extension properties of CR will assuredly continue as will finding appropriate methods for use of CR in humans. We think that 3 general areas show great promise in this respect. First, the data from invertebrate species linking neural regulation of food intake (energy) to reproduction and longevity have clearly established a neuroendrocine basis for the effects of CR. Evaluation of the effect of CR on neural and endocrine signaling pathways at the cellular and molecular level within more complex eukaryotic species should prove valuable. Second, the fact that energy per se is the nutritional factor that imparts the life prolongation effect of CR suggests strongly an alteration to cellular energetics. Evaluation of the biophysical basis of CR including membrane mechanics, proton conductance, and mathematical models should help to identify how cell architecture affects aging. Finally, identifying the mechanisms that underlie life prolongation of CR should lead to testing of possible CR mimetics. Mimetics such as pharmaceuticals and gene therapies will be necessary if the health benefits of CR are to be applied generally to humans.

Regardless of where CR takes research in the coming years, we owe a great deal of gratitude to Clive McCay and his publication on CR that appeared in *The Journal of Nutrition* 75 years ago. Thank you, Dr. McCay, from the authors and the hundreds of other researchers that you have inspired to carry out CR research.

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