Review article

The diet restriction paradigm: a brief review of the effects of every-other-day feeding

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Abstract

It has been known since the early 1900s that restriction of dietary intake relative to the *ad libitum* (AL) level increases stress resistance, cancer resistance, and longevity in many species. Studies investigating these phenomena have used three paradigms for dietary restriction. In the first, the AL intake of a control group is measured, and an experimental group is fed less than that amount in a specified proportion, e.g., 40%. In the second, food is provided AL to both the control and experimental groups; however, the experimental group is subjected to periods of fasting. Recent studies using this paradigm provide food every other day (EOD). Both of these paradigms have been in use since the early 1900s. A third paradigm that combines them was developed in the early 1970s: one or more days of fasting separate the provision of a limited amount of food. It was assumed for many years that the physiological responses to these paradigms were due exclusively to a net decrease in energy intake. Recently, however, it was found that some species and strains of laboratory animals, when fed AL every other day, are capable of gorging so that their net weekly intake is not greatly decreased. Despite having only a small deficit in energy intake relative to control levels, however, these animals experience enhanced longevity and stress resistance in comparison to AL controls as much as in animals enduring daily restriction of diet. These observations warrant renewed interest in this paradigm and suggest that comparisons of the paradigms and their effects can be used to determine which factors are critical to the beneficial effects of caloric restriction.

Many species age more quickly with an *ad libitum* food intake than with one that is restricted, and the mechanism underlying this effect remains unidentified

The effects of dietary restriction [more commonly known as caloric restriction (CR)] on longevity and resistance to environmental stress were first noted in studies of cancer and malnutrition

The discovery that caloric intake strongly influences the rate of aging was not made in one step. It arose

gradually out of two independent fields of research: the influence of dietary intake on cancer and the effect of malnutrition early in life on growth, reproduction, and adult health. The work, which gave rise to what is now one of the most intense areas of interest in the field of biogerontology, was begun early in the 20th century. For example, one of the earliest studies on the role of dietary intake on cancer was led by Rous (1914), whose group published a study in 1914 which showed that reducing food intake reduced cancer incidence in rodents (Rous 1914). Enormous volumes of research have since supported and elaborated on this finding. Around the same time (1915), a study on juvenile malnutrition which influenced the biogerontological

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field was published by Osborne et al. (1915). In it, they described the resumption of growth after a long period of arrested development induced by a restricted food supply (Osborne et al. 1915). They concluded that CR during youth delayed maturation and extended life span. CR also slows the rate of aging even when begun after maturation is complete, but this was not completely proven until 1982 (Weindruch and Walford 1982).

The role of energy intake in the CR effect is accepted, but whether it is a direct or indirect role is controversial

The correlation between reduced food intake and increased life span did not demonstrate that *caloric* restriction was the causative factor. The initial hypotheses concerning the effects focused on individual macro- and micronutrients. However, evidence that decreased consumption of dietary energy was responsible, rather than decreased intake of any specific nutrient, began to accumulate. An early study of importance in this regard was conducted in 1937. Growth, life span, and body size were measured in rats in response to fat and carbohydrate intake. Restriction of either macronutrient led to life span extension (McCay et al. 1937). Several objections to these findings can be considered. Fat, for example, is hardly a homogeneous substance, and omega-6, trans-hydrogenated, or fully saturated fatty acids consumed in excess might well shorten the life span of a “control” group. The glycemic index of the carbohydrate content could be a variable as could food palatability if *ad libitum* (AL) controls ate enough to become obese. Throughout the ensuing years, each of these variables and others have been manipulated without changing the outcome of the experiment. Invariably, it has been the limitation of energy consumed that has been considered as the primary variable for the increased life span observed in these protocols. (For a brief but useful and interesting review, see Masoro 2003.)

A summary of the various hypotheses proposed to explain the role of energy intake on life span was recently provided (Masoro 2003). Masoro lists the following: (1) the oxidative damage attenuation hypothesis, (2) the alteration of the glucose–insulin system hypothesis, (3) the alteration of the growth hormone-IGF-1 axis hypothesis, and (4) the hormesis hypothesis. The first postulates that oxidative damage has a major influence on the rate of aging, and that CR decreases the levels of such damage. In turn, these

decreases are proposed to be either a direct effect of decreased energy flow through the system, or an indirect effect due to alteration of metabolic pathways involved in repair and in free radical scavenging. The second is based on the strong correlation between decreases in average blood glucose and insulin levels with increases in life span in response to CR. This involvement of insulin and glucose in life span mediation has been hypothesized to be due to either direct changes in levels of damage (for example, lowered glucose would lead to decreased rates of spontaneous, nonenzymatic glycation of macromolecules) or to a programmed response to lowered insulin levels. The third hypothesis is based on the observation that genetic changes which decrease IGF-1 or growth hormone (GH) result in increased longevity, and that levels of these hormones are decreased in CR animals (although the fact that CR has an additive effect on life span extension when imposed on animals with genetically lowered IGF-1 or GH levels suggests the presence of separate mechanisms; Bartke et al. 2001). Here, a coordinated response to changes in hormone levels is thought to lead to changes in cell proliferation and death, as well as in other parameters influencing life span. Finally, the hormesis hypothesis suggests that a decrease in caloric intake induces a coordinated activation of pathways necessary to survival without food, thus leading to increased damage prevention and repair.

These hypothesized mechanisms underlying the effects of CR remain unproven. Despite their number and variety, they can be divided into two general classes. One class postulates that decreased energy availability leads to direct alterations in energy metabolism. The other class, in contrast, postulates that the difference between actual and desired intake, rather than the absolute caloric intake, leads to a programmed physiological response. Both postulates result in enhanced stress resistance, cancer resistance, and increased longevity.

Animals that gorge avoid a net energy deficit, but still benefit from intermittent fasting: this finding supports hypotheses postulating an indirect role for energy intake in the CR effect and suggests a new line of experimentation

Studies seeking to investigate the effects of CR can be classified according to the paradigm of restriction used. Three paradigms are in common use. In the first, the AL intake of a control group is measured,

and an experimental group is fed a fixed percentage of that amount. This paradigm is referred to as limited daily (LD) feeding. In the second paradigm, food is provided AL to both the control and experimental groups, but the experimental group is subjected to periods of intermittent fasting. This paradigm is referred to as either intermittent feeding or intermittent fasting (IF, in both cases). Recent studies using this paradigm provide food every other day (EOD). Both LD and IF paradigms have been in use since the early 1900s. A third paradigm was developed in the early 1970s by the Walford laboratory, and is a combination of the prior two: one or more days of fasting separate the provision of a limited amount of food (Walford et al. 1973).

In many animal models, eating rate is not influenced by food availability. Thus, food consumption is proportional to the duration of AL food availability. The intuitive prediction for a group of animals capable of maintaining caloric intake by increasing rate of food consumption during conditions of limited feeding duration is the lack of CR effect. Interestingly, this prediction has not been borne out. This somewhat surprising discovery was published in 2003 (Anson et al. 2003): even without a net decrease in weekly caloric consumption (Figure 1), C57BL/6J mice fed IF (EOD) exhibited much of the CR effect. In that study, the LD and EOD paradigms were compared. The outcomes examined were food intake, body weight, and fasting serum levels of insulin, of glucose, of beta-hydroxybutyrate, and of IGF-1. In addition, caloric restriction had been reported to provide protection against a neurotoxin (kainic acid), and so the effect of the two feeding paradigms on resistance to this agent was also determined. The results are summarized in Table 1. The mice fed EOD were able to gorge, and ate roughly 2 days' worth of food each day that food was provided, preventing any net caloric deficit. However, EOD feeding was even more protective against kainic acid-induced neurotoxicity than was LD feeding (Figure 2), suggesting that the protection provided by caloric restriction is mediated not by the caloric intake per se, but rather by periodic food deprivation. LD fed mice, like their EOD fed counterparts, spend a large fraction of their lives in a food-deprived state.

In addition to providing strong evidence in favor of the indirect-effect hypotheses previously discussed, these findings also suggest a means for separating physiological adaptations which play a mechanistic

role in the antiaging effects of CR from those which do not.

Several additional lines of evidence suggest that the relationship between energy intake and life span is indirect "Restricted" animals eat more per unit mass than AL-fed animals

Early research on CR was based on the assumption that restricted animals ate less per unit mass than AL-fed animals. Indeed, a carryover from this belief is evident in the use of diets supplemented with extra micronutrients for restricted animals in modern studies. When a full-grown organism is initially

Figure 1. Male C57BL/6 mice compensate for periods of fasting by increasing their food intake and gaining weight at rates similar to mice fed *ad libitum* (Anson et al. 2003). Average daily food intakes (calculated from 14-day intake) (a) and bodyweights (b) in mice maintained on one of four feeding regimens: *ad libitum* (AL), intermittent fasting (IF; alternate day feeding), pair-fed to the IF group (PF) and limited daily feeding (LDF; 40% reduction in calories relative to AL). IF bodyweights are postfeeding values. Values are the mean of measurements made in eight mice per diet group. At the end of the study, body weights and food intakes of group LDF were significantly lower than the body weights and food intakes of groups AL and IF ($P < 0.001$ in each case). Body weights and food intakes of groups AL and IF were not significantly different.

Table 1. Physiological responses to LD, PF or EOD feeding.

	EOD	PF	LD
Average food intake	↔ ^a	↔	↓↓
Body weight	↓	↓	↓↓
Body temperature	↓↓		↓
Tumorigenesis	↓↓		↓↓
Life span	↑↑		↑↑
Resistance to KA-induced hippocampal damage	↑↑	↑	↑
Resistance to tumor induction	↑ ^b		↑
Oxidative stress resistance	↑↑		↑↑
Fasting serum glucose	↓	↔	↓
Fasting serum insulin	↓↓	↔	↓↓
Fasting serum β-hydroxybutyrate	↑↑	↑	↓
Serum IGF-1	↑	↑	↓

Arrows indicate change relative to values of mice fed *ad libitum* (AL) ($P < 0.05$). EOD, animals fed *ad libitum* and fasted on alternate days; PF, animals fed daily an amount equivalent to the average daily intake of the EOD group; LD, animals fed daily an amount equivalent to 60% of the AL group's intake.

^aStrain-dependent.

^b48 hr fast/week. Data are taken from McCay et al. (1937), Anson et al. (2003), Carlson and Hoelzel (1946), Wan et al. (2003), Roth et al. (2002), Klurfeld et al. (1987), Yu (1996), and Radak et al. (2002).

switched to a restricted diet, the assumption is correct. At first, the animal is eating less per gram bodyweight (or per gram lean bodyweight, or per gram of adipose tissue) than it was immediately prior to the change in feeding regimen. However, when the CR regimen is maintained, or is started before growth is complete, the assumption is no longer valid. Bodyweight decreases to compensate for the decreased caloric intake. When equilibrium is reached, the CR fed animal will actually consume slightly more food per gram bodyweight or lean mass, not less (Masoro et al. 1982). The finding that bodyweight adjusts to match caloric intake is so robust that many investigators interpret differences in food consumption per gram bodyweight between groups as an indication of mistakes made in restricting food allotment or in the measurement of AL intake. It is energy intake per animal, rather than per unit of body mass, that differs between AL and CR animals: this supports an indirect relationship between energy intake and the CR effect.

Increased AL intake due to increased energy expenditure does not shorten life span

Further evidence demonstrating that the relationship between caloric intake and life span is not direct has been gained from experiments in which energy requirements are increased by exercise or by a decrease

in environmental temperature. In the former case, there is often not enough compensation in the exercised animals to give a clear increase in intake. In the latter, however, food intake can increase dramatically. In an elegant experiment by Holloszy and Smith (1986), a group of rats was trained to wade in room temperature water for several hours each day. This increased the daily loss of thermal energy in the group, and in an attempt to compensate they increased their food intake to 144% of control levels. Despite this increased consumption, their mean and maximum life spans did not differ from control values. The insignificant trend was toward a longer life span in the experimental group, not the shorter one expected if in fact energy intake were directly related to the rate of aging (Holloszy and Smith 1986).

The nature of energy's role in the CR effect has practical implications for human health

Several laboratories are engaged in efforts to discover "CR mimetics," drugs providing the beneficial effects of dietary or caloric restriction without the need for hunger and deprivation. From the discussion above, the mechanism underlying the beneficial effects of CR determines which drugs will be effective. If the effects of CR are directly due to energy restriction, then appetite suppressants and drugs which decrease the efficiency of energy absorp-

Figure 2. Intermittent fasting was superior to (40%) caloric restriction in protecting hippocampal neurons against excitotoxic injury to an intrahippocampal injection of the excitotoxin kainate (KA); phosphate-buffered saline (PBS) was injected into the contralateral hippocampus of each mouse (Anson et al. 2003).

tion at the organismic or cellular level would be of great interest from a biogerontological perspective. If the programmed response hypotheses are correct, however, then such drugs are unlikely to have beneficial effects. Such hypotheses imply that the

organism must perceive the deficit in order to respond. If so, then it would follow that an energy deficit resulting from, for example, a decrease in appetite might not be sensed and in fact might not induce a beneficial physiological response. Instead, drugs that act on the systems which sense energy and respond to its availability would be more worthy of attention. Thus, the mechanism by which CR exerts its effects determines the direction of drug research intended to induce the response in humans, and discovering these mechanisms may have great impact on human health and well being. One purpose of this minireview is to argue that direct comparison of EOD and LD paradigms of CR will be of great utility in increasing our understanding of CR mechanisms.

A caveat: cross-study comparisons of absolute numbers are not valid

It is crucial to note that not all diets are nutritionally equivalent, and not all animal housing facilities are of equal quality. Furthermore, for rats in particular, the nomenclature used to refer to the animals may be imprecise, and the same term often refers to several

Figure 3. (A) Survival and (B) mean body weight for male C57BL/6J mice fed *ad libitum* (AL, dashed lines) or every other day (EOD, solid lines) from 1–2 months of age ($n = 40$ at start of experiment). Taken from Goodrick et al. (1990).

strains. Even assuming it is known precisely at the outset, the genotype of a given species and strain can drift and a cohort in use today may be quite different from one used many years ago. Thus, it is not unusual for the observed life spans in two studies using the same term to describe the animals used to differ dramatically. To help fully appreciate the differences that can arise between studies, several examples are in order. As examples, two studies involving Wistar rats can be considered. In one (Carlson and Hoelzel 1946), AL males reached a peak bodyweight of 275 g and 90% had died at 26 months. In the other (Everitt et al. 1980), AL males reached a peak bodyweight of 500 g and survival was not reduced to 10% of the original cohort until 33 months. Another example can be made with two studies that used male Sprague–Dawley rats. The control, AL animals in one study (Berg and Simms 1961) reached a peak bodyweight of 280 g and lived for 28 months. The control, AL animals in the other (Merry and Holehan 1981) reached a peak bodyweight of 950 g and lived 33 months. In the second study, the CR group weighed 400 g, 120 g more than the AL group in the first study!

Interpretation of these results then becomes a bit problematic. What hidden variable(s) differed between the two studies that caused the difference? Have the results been confounded entirely? This is not a clear-cut issue. Are any studies “perfect?” For a given species and strain, are we certain that we are using a nutrient mix that optimizes longevity, or might a better mix be discovered in the future? Does such a future finding invalidate current work? How do we take into account studies performed prior to the invention of air conditioning, when animal housing facilities were likely to reach high temperatures in the summer months? How do we consider studies that were conducted before barrier and disease surveillance systems were created to avoid introduction of pathogens to the colony? When the control animals in a study exhibit what seems to be an unusually short life span, should the assumption be made that the control group was experiencing an unknown insult, and that the experimental group was not, or should we instead assume that both groups experienced the insult but that the experimental group was resistant?

Ultimately, a case-by-case consideration is warranted, and we are forced to draw conclusions that are tentative and to rely on repetition by various laboratories to filter out random variables and provide

a clearer image of the true situation. The presence of a CR-induced benefit in one study is supported by its presence in other studies, and its absence in other studies negates it. Comparing the degree of benefit by absolute values or the actual life spans of the cohorts studied cannot be done. Cross-study comparisons of the *absolute* degree of life extension are, a priori, invalid. All that is possible is to note whether studies have observed life extension at all.

IF feeding – EOD feeding in particular – has been shown to increase life span and to decrease cancer incidence

With the above caveat in mind, a review of some of the major EOD studies can be undertaken. One of the earliest reports to specifically examine IF diets was conducted using rats in 1945. Several patterns of fasting were examined, but unfortunately, several different diets were also tested on the same animals, preventing an unambiguous interpretation of the data. However, it is perhaps noteworthy that only one of eleven rats surviving more than 1,000 days (out of 136 at the start of the study) was in the control, AL group. In addition, the incidence of mammary tumors in females decreased from 37% in controls to a low of 7% in EOD fed rats (Carlson and Hoelzel 1946).

Maximum life span was increased by 18% in one study of male Wistar rats (200 vs. 169 weeks). The animals did not gorge, based on bodyweight data. At two years of age, the AL animals weighed 531 g, the EOD animals only 62% of this value (Beauchene et al. 1986). In another study using male Wistar rats, an 85% increase in maximum life span was observed (from ~100 to ~185 weeks based on Figure 1 in that paper), along with a 50% reduction in bodyweight (Goodrick et al. 1982). Other studies by the same group, using the same model, produced increases of 36% (from 110 to 150 weeks) and 20% (from 103 to 124 weeks) (Goodrick et al. 1983a, b). Here, the life span of the control animals in three studies remained nearly constant, but was shorter than the life spans usually seen for the Wistar rat. That of the restricted animals varied but was consistently longer than that of the controls. The presence of an unknown variable is suggested, but in each study the restricted group was either not exposed to the unknown variable or was resistant to its effects. This situation is not unique

to the EOD paradigm, since similar fluctuations are seen in comparisons of LD studies – see Table 2.4 in Weindruch and Walford (1988). (Aging has a stochastic component that presents itself in even the most careful and well-designed studies.)

A particularly informative study of the effects of EOD feeding on mice of different genotypes was published in 1990 by Goodrick et al. (1990). In that study it was reported that male A/J mice introduced as adults (6 or 10 months of age) to an EOD diet, did not decrease bodyweight and did not show any benefit in terms of longevity. In fact, those in the EOD group died early. In contrast, the maximum life span of male C57BL/6J mice begun on EOD at any age was increased. They also showed little change in bodyweight. (As noted elsewhere in this review, this is now known to be due to their ability to gorge when provided with food; Anson et al. 2003.) The F1 cross of the two strains responded well to EOD in terms of life span, but were unable to maintain bodyweight (Goodrick et al. 1990). In another study on the same mice, both EOD and LD groups were included. EOD feeding increased maximum life span by 56%, LD by 36% (Ingram and Reynolds 1987). In contrast to these rather dramatic life span extensions, a mere 11% increase was seen in an earlier study (Talan and Ingram 1985).

It has been mentioned several times that C57BL/6J mice maintain their bodyweight by gorging when food is available, but it is possible that this ability is gender specific. In a study of the effects of EOD feeding on female C57BL/6J mice, the EOD group exhibited a 25% decrease in bodyweight relative to the AL controls. Life span was not determined (Barrows and Kokkonen 1978).

One study attempted to evaluate the effect of EOD restriction in humans. It was performed in a retirement home in Spain with two groups of 60 healthy persons over the age of 75 (men and women) and lasted three years. The control diet was 2,300 kcal/individual, fed daily. The experimental diet did not involve an intermittent fast, but was sufficiently restricted to warrant mention in the present review. It involved alternating days of normal intake (2,300 kcal/individual) with days of limited intake (1 l of milk and 500 g of fresh fruit, which, depending on the fruit chosen, corresponds to roughly 1,000 kcal/day). In that time period, there were 44 illnesses and 13 deaths in the AL group, compared with 25 illnesses and six deaths in the restricted group (Arias-

Vallejo 1957). While there are complicating issues that prevent strong conclusions from being drawn (such as the possible role of extra fruit and milk in the diet), the possibility that an alternate day limited diet (without a complete fast) might have beneficial effects is intriguing.

Direct comparison of the IF and LD paradigms provides a means for separating physiological adaptations which play a mechanistic role in the antiaging effects of CR from those which do not

Many variables that change in response to age or caloric intake are difficult or impossible to manipulate independently, and no direct evidence for their ability to influence life span has been presented. As a result, much of the published data regarding factors controlling the rate of aging and factors mediating the influence of caloric intake on aging rate, are correlative. For example, it has been known for many years that free radical damage in mitochondria increases with age, and a causal relationship was hypothesized based on this correlation: only recently, however, have transgenic mice with elevated levels of such damage been developed for direct evaluation of this hypothesis (Van Remmen et al. 2003). The fact that no acceleration of aging was observed in those mice highlights a danger learned by every beginning student of statistics: “correlation does not equal causation.”

There is a tremendous need for methods which separate events that are merely coincidental from those that are critical to obtaining an effect of interest. CR, for example, causes weight loss, a fact that will surprise no one. The amount of weight gained or maintained is a function of the amount of food eaten. (There is a bit of variability in terms of the utilization of the nutrient intake—activity and heat vs. structure—but this variability is much smaller than the gross changes in intake observed in CR experiments.) In addition to weight loss, there is another effect of CR, the primary focus of this paper: in many cases, CR decreases the rate of aging. How are these two facts related? Is low bodyweight necessary to extended longevity? An entire review could be written on this topic alone and a firm conclusion would remain elusive.

It is possible to hypothesize that one of any two correlated events is cause and the other effect, but

unless a situation can be found in which one of the two events occurs independently of the other, it is impossible to test such a hypothesis. Until recently, events such as weight loss and increased longevity, as well as many other responses to caloric restriction, have all been inseparable, and so no causal relationships could be demonstrated or denied. However, investigations into the failure of some strains of mice to decrease bodyweight when fed EOD has led to one of the first reports of major differences between the EOD and LD paradigms. Originally, the maintenance of bodyweight was thought to occur in spite of a net decrease in caloric intake (Goodrick et al. 1990). However, this was later reevaluated in light of the now well-known constancy of food intake per gram bodyweight. Recent studies in which food intake was carefully measured revealed that the maintenance of bodyweight in some strains when they are fed EOD is due simply to their ability to gorge when food is available (Anson et al. 2003). Since there is a mild (~5%–10%) decrease in bodyweight even in these strains, the answer regarding the role of bodyweight awaits a study in which a group of animals fed a diet isocaloric to that of the EOD group is included. Fortunately, the ability to answer this question, and many others, now lies in our grasp.

Summary and conclusions

The study of caloric restriction began over a century ago, when researchers in the fields of cancer and nutrition noticed that periods of restricted food intake had beneficial effects in the animals they used as models. Years of investigation to determine the identity of the responsible nutrient led to the conclusion that it was energy intake, rather than any particular nutrient, that mediated the effect. The nature of this mediation, however, is still controversial. The effect may be direct (for example, decreased intake could cause alterations in the redox state of the electron transport chain during oxidative phosphorylation) or indirect (mediated by the neuroendocrine system). Even so, there is already ample evidence that EOD feeding can extend life span and, like LD feeding, delay at least some age-related diseases. Additionally, it was recently discovered that at least some of these effects are induced by EOD feeding without a net reduction in caloric intake. A life span study comparing the effects of an isocaloric LD and EOD diet

remains to be done. There is at present no reason to postulate that the two feeding paradigms exert their effects through independent mechanisms: Occam's Razor leads to the hypothesis that they share a common mechanism. This hypothesis leads to the prediction that these paradigms will cause similar changes in the mortality rate doubling time (MRDT) for a given model. When age-related changes continue at the normal rate in an experimental group but fatality due to those changes is decreased relative to controls, as was recently reported to occur in response to dietary restriction in *Drosophila* (Mair et al. 2003), then the MRDT does not change. In contrast, when the rate of aging itself is slowed, MRDT increases. Thus, if one feeding paradigm is acting to slow aging while the other is altering the risk inherent in age-related changes, then this difference will be observed in their effects on MRDT.

Assuming for now that the hypothesis that the paradigms are operating via a single mechanism is correct, the fact that they do not completely overlap in terms of their induced physiological adaptations provides a means for separating adaptations which play a mechanistic role in the antiaging effects of CR from those which do not.

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