Caloric Restriction and Aging Revisited: The Need for a Geometric Analysis of the Nutritional Bases of Aging

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One of the most important findings in the field of the biology of aging has been the demonstration that modest dietary restriction extends lifespan in a wide range of taxonomically disparate organisms. There is currently a debate as to whether longevity is prolonged because of caloric restriction or due to more specific nutrient effects.

Recent advances in nutritional research, notably the development of state–space geometric models, the Geometric Framework (GF), offer new opportunities to disentangle the effects of calories and nutrients. We begin by introducing these models, then set out the four questions that must be addressed to establish the relationship between nutrition and aging and indicate how the GF might help in answering these. We next provide an exemplar experimental protocol and consider some practical challenges to implementing the GF. Our conclusion is that Drosophila provides the most suitable system for an initial study.

The view that dietary restriction without malnutrition prolongs life has become a central tenet in aging research. Since McCay and colleagues (1) published their seminal article on laboratory rats, numerous other organisms, including mice, dogs, fish, spiders, fleas, flies, worms, yeasts, and monkeys, have been found to live longer with modest dietary restriction [reviewed in (2–4)]. Some of these organisms (yeasts, fruit flies, nematode worms, and mice) have become model systems for studying the molecular and cellular mechanisms of aging, but it is not yet clear that common effects and mechanisms are involved in the responses of such disparate taxa to dietary restriction (5–9). Not surprisingly, there is considerable interest in the prospect that dietary restriction may prove a means of extending the quality of life in humans, although experimental data are lacking (10,11).

What does dietary restriction actually mean? It is widely held that the prolonging of life with dietary restriction arises from eating less energy (known as caloric restriction [CR]) (2,12,13). Recently, however, this view has been challenged by studies suggesting that restriction of particular nutrients, rather than energy, is responsible for increased life expectancy (14–16). A major impediment to disentangling the effects of calories and specific nutrients has been the lack of a suitable conceptual and experimental framework for studying nutrition. Recent advances in nutritional research offer a solution to this problem, notably development of the state–space modeling platform, known as the Geometric Framework (GF) (17–20). In this review, we first introduce the GF as a means to explore the nutrition of aging, and consider practical challenges for its implementation.

The Geometric Framework

The GF provides a means of analyzing multivariate metrics of nutrient requirements, the relative values of foods in relation to these requirements, the behavioral and post-ingestive responses of animals when feeding on foods (and combinations of foods) of varying composition, and the performance consequences of being restricted to particular dietary regimens.

In the GF, a model of an animal’s nutritional relationship with its environment is constructed around an n-dimensional nutrient space, where each dimension represents a nutrient. An animal’s current nutritional state is represented as a point within the nutrient space, as is the state that would optimize inclusive fitness (the intake target) and thus towards which the regulatory mechanisms controlling intake should have evolved. Foods are represented as vectors determined by the balance of the relevant components each food contains (nutritional rails). By eating, the animal changes its nutritional state along the vector coincident with the chosen food. One nutritional challenge for the consumer is to select a food the rail of which passes through the intake target (i.e., a nutritionally balanced food), so enabling it to reduce to zero any discrepancy between current state and optimal state.

A nutritionally imbalanced food, by contrast, does not enable the animal to satisfy its optimal requirements for all nutrients simultaneously, but forces it into a compromise between overingesting some nutrients and underingesting others, with associated costs. The animal can nonetheless achieve its regulatory target when eating a nutritionally imbalanced food, if it mixes its intake from this with a food...
containing a complementary imbalance of nutrients (i.e., one the rail of which falls on the opposite side in nutrient space of the intake target). In this case, the excess nutrient ingested from one food can be used to redress the deficits incurred on the other, and vice versa.

When neither nutritionally balanced nor complementary foods are available, the animal cannot balance its nutrient intake, but can nonetheless utilize the imbalanced food by selectively excreting ingested excesses. However, if the degree of imbalance exceeds the capacity of the animal to excrete ingested excesses, then it is constrained to accept excesses of some nutrients and/or deficits of others. The functional challenge in this case is to arrive at a balance between over- and underingestion that minimizes the costs of this predicament.

The GF was derived from studies of insects and has been used on a broad range of organisms in a variety of contexts, including studies on the dietary causes of human obesity (21) and the conservation of endangered species (22). A detailed guide to the use of the GF is provided elsewhere (23). Our aim in the present review article is to provide illustrations of how the GF might be used to address the nutritional bases of aging.

**FOUR KEY QUESTIONS**

Four questions must be answered to establish unequivocally whether CR or more specific nutritional responses to dietary restriction prolongs life span.

**Calorie Restriction Relative to What?**

Answering this question requires a measure—and appropriate concept—of baseline energy and nutrient intake. Although a fundamental concept, an animal’s nutrient requirements are seldom explicitly represented in nutritional or functional models other than the GF, in which the intake target provides the key referent for interpreting patterns of nutrient intake and utilization. Thus, it may be that animals live longer when their intake is restricted relative to animals fed a baseline diet either because eating less is life-extending, or because the laboratory baseline regimen is excessively rich in one or more nutrients (24).

One approach to locating the position of the intake target is to ‘ask the animal.’ The aim is to establish whether the animal has the capacity to regulate its intake of one or more nutrients in the face of challenges such as being provided with different pairings of complementary foods, having foods diluted with non-nutritional bulking agents, or being pretreated on an imbalanced diet and then offered the opportunity to redress the imbalance. Studies to date using these approaches have found that animals as diverse as insects, spiders, rodents, birds, and fish have the capacity to regulate independently their intake of protein and non-protein energy (18,19,25–28). Where such regulation to an intake target is found, the regulated point serves as the referent for measuring and interpreting the effects of nutritional perturbations on a range of response variables, including those associated with aging (20).

It is important to note that the position of the intake target changes over time, both throughout the life of the animal with growth, development, reproduction, senescence, environmental conditions, and levels of activity, and across generations through maternal effects and genetic evolution in response to persisting changes in the nutritional environment (18,29). Thus, the intake target is in fact a trajectory—a moving point, which needs to be tracked across varying time scales. The fact that different genotypes and life stages may not have the same intake target must be taken into account when quantifying the effects of dietary manipulations. For example, if the required amount and balance of nutrients changes over time, yet the laboratory diet is of fixed composition, animals will be forced to overeat some nutrients and undereat others to differing degrees as they grow, mature, and senesce. Even if a fixed diet composition is nutritionally balanced when integrated across the entire life span, changes in the intake target trajectory at a finer timescale will result in accumulated costs of short-term nutrient imbalances and will lead to animals fed any one diet performing less well than those able to track their changing intake target.

The expectation is that animals will have evolved regulatory responses that achieve maximal Darwinian fitness under conditions typical of their ancestral environment. However, when an animal is kept in the laboratory, with limited opportunities for exercise and a reduced need to expend energy for thermoregulation, its ad libitum intake, although regulated, may confer fitness costs. We will return to this point in Question 3, and also in discussion of an exemplar experimental protocol.

**Calories From What, and Are Those Calories Actually Available?**

To establish whether calories are important as such, or whether the source of those calories matters, the molecular substrates of calories must be partitioned. In the case of *Drosophila melanogaster*, carbohydrate and protein are the two key macronutrient dimensions (fat is a minor dietary component), whereas in omnivores and carnivores fat plays a larger role. Systematic exploration of both the concentration and ratio of these nutrients in the diet is required to generate a high-resolution protein–carbohydrate/fat intake “map,” onto which longevity, age-specific mortality rates, fecundity, and other relevant variables, including changes in molecular markers of aging, can be projected as response surfaces, either singly or in combinations (20). We fully concur with the recent critical discussion in the literature on the importance of distinguishing between the rate of aging, delay to the onset of aging, and changed susceptibility to mortality factors such as disease (13,30). Surfaces can be constructed over nutrient intake space for any chosen measure or combinations thereof.

Studies by Mair and colleagues (15) indicate a greater positive influence of dietary protein than carbohydrate deprivation on longevity (Figure 1, noting that yeast provides the only source of protein but also contains carbohydrate), but only four diets were tested, and intake was not measured. The importance of deriving as high a resolution map of nutrient intake space as practicable can be illustrated by reference to the paper on rats by Davis and colleagues (31). We are not claiming that this article is
representative of rodent literature (indeed, survival rates seem very low across the study relative to more recent work), but it does provide a useful illustration of the difficulty in drawing conclusions based on a restricted sampling of intake space. Rats were fed one of six diets. Three of these were isocaloric but varied in protein content and were provided ad libitum. The other three diets were manipulated such that the same amount of protein but only 2/3 of the total calories were eaten. Figure 2A plots mean kilojoules eaten per day of protein versus carbohydrate and fat on these 6 regimens at 12 and 18 months of age. Numbers associated with the points show the percentage of animals surviving for 2 years. Data were interpreted as indicating that CR, not protein restriction, prolonged life span, as fewer rats survived on the ad libitum regimens (along the upper dotted isocaloric line) than on the restricted regimens (lower dotted isocaloric line). However, the CR hypothesis would also predict that all three intake points along each of those dotted lines should be associated with the same mortality rate (because the data are plotted as energy, and a slope of $-1$ indicates equal caloric intake). This was clearly not the case: The difference within lines was as large as the difference between them. Figure 2B provides an alternative explanation: The points are part of a response surface that has a peak at around 75 kJ per day of protein and 150 kJ per day of nonprotein energy intake. Other, more convincing data from rats, which are used to support the primacy of calories in influencing longevity (31–33), are limited in their coverage of nutrient intake space and could also be open to alternative explanations (Figure 2C).

Another important aspect is whether ingested nutrients are actually available to the animal. For example, it has been found that methionine restriction extends life span in rodents (14). Recent data could, however, be interpreted to indicate that methionine restriction results in energy limitation through impairing carbohydrate utilization, as evidenced by depression in plasma glucose and insulin concentrations (34). Hence, the life-extending effects of eating less methionine may in fact equate to carbohydrate restriction. The GF provides a means of dealing explicitly with such interactions between nutrients and with the relationship between the intake and utilization of nutrients, by

Figure 1. Data from the study by Mair and colleagues (15) in which they separately manipulated yeast and sucrose concentrations in the diet of adult Drosophila melanogaster and measured effects on life span. Data indicated that yeast dilution prolonged life substantially more than did sucrose dilution. This is an important study, but various questions remain unanswered.

Figure 2. A, Data from the study on rats by Davis and colleagues (31), replotted on a bicoordinate intake plane. See text for explanation. Data were interpreted as indicating that caloric restriction (CR), not protein restriction, prolonged life span. B, Alternative explanation: The points are part of a response surface that has a peak at around 75 kJ per day of protein and 150 kJ per day of nonprotein energy intake. Other, more convincing data from rats, which are used to support the primacy of calories in influencing longevity (31–33), are limited in their coverage of nutrient intake space and could also be open to alternative explanations (Figure 2C). C, Data from the study on rats by Masoro and colleagues (33). Although supporting the CR hypothesis, there are too few points to draw a firm conclusion. P = protein; C = carbohydrate; F = fat.
been measured directly indicate that longevity (40). Short-term studies in which food intake has effects of dilution or lead to their own consequences for compensatory feeding responses (39), which may buffer the nutrient intake. This approach does not take into account rather, dietary dilution is used as a means of restricting measured throughout the duration of an aging experiment; and quantified, in D. melanogaster (41,42), as can other flies (43). It is important to measure compensation across a range of dilutions and nutrient ratios, as the capacity of animals to compensate varies with both of these (17,39,40). It is also important to measure food intake over prolonged periods, because it is well known that more concentrated foods are eaten in larger meals than diluted foods in the short term, but that this intake pattern reverses over prolonged exposure as nutritional feedback mechanisms operate (43).

Perhaps more significantly, it has been demonstrated using the GF that insects and other animals have the capacity not only to compensate for differences in the overall concentration of the diet, but also to regulate their intake of specific nutrients, notably protein and carbohydrate (18,26,27).

Measuring intake in D. melanogaster is technically challenging. Techniques involving adding radiolabels (42) or dyes (41,44,45) to the food are not suitable for long-term studies, since they may have their own deleterious effects, and in the case of dyes are often strongly phagodeterrent when paired with diluted foods (Simpson S, Barton Browne L, unpublished observations, 1987). Inferences based on sampling the time flies spend with their proboscis extended (15) do not take into account the fact that differences in feeding rate on different diets greatly affects the volume ingested during a meal (43). Direct determination of consumption by measuring mass changes in the food is extremely challenging at such a small scale. Rather, we suggest using a modification of a volumetric technique developed for larger flies (43). Preliminary studies have indicated that such a measure is both practical and effective.

AN EXEMPLAR EXPERIMENTAL PROTOCOL

An example of a suitable experimental protocol is shown in Figure 4, using data for caterpillars taken from Simpson and colleagues (20). For present purposes we will consider a system in which protein and digestible carbohydrate are the two nutrients at issue (which was the case for caterpillars), but the same principles apply to any number of nutrients.

Step 1: Measure Where the Intake Target Lies

The first step is to allow animals to self-select between pairs of nutritionally complementary foods to allow the mechanisms regulating intake of protein and/or carbohydrate to be expressed. The aim is to discover to which point (integrated over a given time period) or trajectory (followed over time as they age) animals move towards in protein–carbohydrate intake space, and how well they regulate to this point or trajectory when challenged with different complementary foods. As an illustration, Figure 4A indicates the mean point reached over 5 days by caterpillars fed five different complementary food pairings. There was tight convergence to this point across all five treatments, proving that caterpillars have highly effective mechanisms of regulating intake of both nutrients, and that 150 mg of protein and 125 mg of carbohydrate is the bivariate point in nutrient space that they prioritize.

What is the Actual Intake of the Study Animals?

Intake is not always measured directly in studies of dietary restriction and aging. Although not a concern in most rodent studies, where intake is rigorously controlled and quantified, in D. melanogaster intake has yet to be measured throughout the duration of an aging experiment; rather, dietary dilution is used as a means of restricting nutrient intake. This approach does not take into account compensatory feeding responses (39), which may buffer the effects of dilution or lead to their own consequences for longevity (40). Short-term studies in which food intake has been measured directly indicate that D. melanogaster has the capacity to increase consumption in response to dietary dilution (41,42), as can other flies (43). It is important to measure compensation across a range of dilutions and nutrient ratios, as the capacity of animals to compensate quantifying how ingested nutrients are allocated to growth, storage, and metabolism (35,36).

Over What Range of CR Is Longevity Extended?

A full appreciation of the effects of diet on longevity requires measuring a dose-response across a range of nutrient intakes. Work on rodents indicates a near linear increase in survival down to 40% food restriction relative to nonrestricted controls (37). In Drosophila, life span increases as the standard diet decreases in concentration from 150% to 50%, and then falls at greater dilutions (38) (Figure 3). As discussed above, it is theoretically possible (if perhaps unlikely) that ad libitum–fed animals are eating a substantial and damaging excess of food relative to requirements, due either to being confined to a single food type that fails to match the animal’s changing needs, or to a mismatch between evolved regulatory responses and the laboratory environment. If this is not the case, rodents and flies live longer because of the benefits of dietary restriction rather than amelioration of the costs of overconsumption when feeding ad libitum. However, there is a need to incorporate more than one nutrient dimension into such experiments before the specific effects of nutrients can be disentangled from CR. Once again, this issue can be addressed using the GF by mapping performance landscapes onto a 2 (or more)-dimensional nutrient intake space.

Figure 3. Results of the study by Chapman and Partridge (38) on Drosophila melanogaster, showing how life span varies with dietary dilution, and posing a series of issues related to the need to measure intake.
Step 2: Restrict Nutrient Intake

Next, animals are restricted to one of a range of intake points across nutrient space by varying the ratio and concentration of protein and carbohydrate in the diet. In the caterpillar example shown in Figure 4B, insects were provided with 1 of 35 foods containing 1 of 5 protein to carbohydrate (P:C) ratios and 1 of 7 levels of nutrient dilution. The resulting array of intake points achieved over 5 days is plotted (means ± standard errors). The array indicates the capacity of the animal to compensate for dietary dilution within a given P:C ratio. If compensation were complete, all 7 points along a nutrient rail (dotted lines) would have converged. There was only partial compensation for dilution, with the extent of compensation being greatest on diets closest to the intake target P:C ratio (40). There was an upper limit to nutrient intake that caterpillars would not exceed (dashed line).

Step 3: Map Response Surfaces Onto the Intake Array

The next stage is to map performance onto the intake array, fitting surfaces for chosen variables related to aging and reproduction (see above). Figure 4C shows a performance landscape fitted for caterpillars. The surface comprises a compound measure of performance, survival multiplied by growth rate, of which survivorship was the major determinant (20,24). Note that the summit on the performance landscape aligned with the regulated intake point. This need not be the case for two reasons (20). First, different performance measures will have different, and perhaps over certain regions, covarying, landscapes—as is expected for longevity and fecundity (6,46,47). Second, there may be a mismatch between the evolved regulatory systems of the animal and the current nutritional environment. For example, the regulated intake may have evolved to include a component for anticipated energetic needs (e.g., for foraging or thermoregulation) which, if not used under laboratory conditions, confers a cost. Hence, even if an animal regulates its intake precisely under ad libitum conditions in the laboratory, it may live less long than a food-restricted counterpart due to excessive fat deposition caused by a failure to expend energy at a rate that has been typical of life in its ancestral environment (20,48).

Logistical Challenges

Replicating an experiment such as that described above for caterpillars, but extended throughout the life of the study animal and replicated to a level that provides adequate survival data, presents substantial logistical challenges. In the case of adult *Drosophila*, brevity of life span is an advantage, but the main technical problem is measuring intake. As discussed earlier, we have overcome this problem by developing a system in which food is dispensed from

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Figure 4. Three-step protocol based on the Geometric Framework for exploring the nutritional bases of aging and its relationship to other life-history traits. Data come from an experiment undertaken on caterpillars of *Spodoptera littoralis* (20). See text for a detailed description. P = protein; C = carbohydrate.
micropipettes and consumption measured volumetrically. Rodents present another order of difficulty. To perform an exhaustive study of the magnitude that is readily achievable on insects would be expensive. However, the number of treatments required to sample intake space could be reduced using appropriate optimization techniques (49), bringing the numbers in line with a large conventional study. A further logistical issue is that dietary manipulations are best done using chemically defined diets, in which the levels and proportions of nutrients can be manipulated with precision. This is readily achievable in experiments on Drosophila and other insects using laboratory-made diets. It is, however, more of a challenge to get such manipulations made through commercial producers of rat chow, where considerations such as batch size and constraints on physical aspects of the diet need to be taken into account. One way forward would be to conduct an initial test of the use of the GF in aging research using Drosophila, and to use such a study to inform the design of rodent experiments.

CONCLUSION

Not surprisingly, the demonstration that dietary restriction prolongs life in organisms as diverse as single-cell yeasts and monkeys has been perceived to be of major importance. However, the specific nutritional bases of dietary restriction remain incompletely resolved. State–space models of nutrition (the GF) offer a solution to this problem. One benefit will be to make it considerably easier to establish whether the various metabolic and molecular pathways that have been implicated in aging can be reconciled within a common mechanistic framework that is shared across taxa (5–9). Another outcome will be to enable the effects of nutrient balance and nutrient dilution on patterns of gene expression to be disentangled and correlated with age-specific mortality, reproduction, and levels of compensatory feeding. The result will be a better-focused understanding of the general effects of diet on the schedule of aging, the trade-off between longevity and reproduction, and ultimately the underlying metabolic, molecular, and genetic mechanisms involved.

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