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Beyond calorie restriction: aging as a biological target for nutrient therapies

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Abstract

Arguably, the most important discovery in the biology of aging to date was that simply reducing food intake extended life and improved many aspects of health in a diversity of animal species. The conventional wisdom that emerged from first 50 years of rodent food restriction studies included (1) that the longevity impact of restriction was greater the longer restriction was imposed, and (2) that restricting calories rather than any specific macronutrient was critical to its health and longevity benefits. However these assumptions began to crumble as more and more restriction research was performed on other species besides laboratory rodents. Recent investigations of flies, rodents, monkeys, and increasingly humans, has begun to parse how calorie restriction, protein restriction, intermittent fasting, and the temporal pattern of eating all impact the health benefits of food restriction. Fly research continues to inform, as it has repeatedly shown that genotype, age, sex, duration, and tempo restriction all affect the health impact. Ultimately, optimizing human diets will require a personalized approach using omics approaches.

Introduction

Arguably, the most important discovery in the biology of aging to date – and also one of the oldest discoveries – was that simply reducing food intake extended life and improved many aspects of health in a diversity of animal species [1,2]. The field of what was most often called food restriction or dietary restriction (DR) was dominated for many decades by a relatively small group of researchers using laboratory rodents in long-term survival studies. Following McCay's original research paradigm using rats [3], one key research assumption was that the health and longevity effects of food restriction, which we will call the DR effect, required chronic, long-term restriction. Another assumption based on rodent studies was that restricting calories, rather than specific macronutrients was responsible for the DR effect [1,4]. As a consequence, DR was more and more frequently called *caloric restriction*. In the late 20th century as invertebrate models such as *Drosophila melanogaster* flies and *Caenorhabditis elegans* nematodes became more and more influential in aging studies, researchers using those models did not automatically accept these assumptions. Largely because of research initiated in invertebrate models, these assumptions have begun to crumble. Also, as research attention has shifted from focusing mainly on *lifespan* to focusing

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Conflict of interest statement

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on *healthspan*, and as humans have become more eager for evidence-based nutritional advice on healthy diets for themselves, macronutrient composition of the diet, as well as the length and timing of restriction have emerged as important contributors to the healthful impact of DR. In this review, we will highlight some of these recent variations on an old paradigm (Figure 1).

Translation, short-term fasting and protein restriction

Two long-term DR studies in nonhuman primates revealed that the translation of DR rodent studies to humans was anything but straightforward. Laboratory rodents spend their lives in cages only a few times bigger than their body length so have little opportunity for physical activity. They have food available 24 hours per day, and not surprisingly are significantly more obese than rodents living in the field [5]. When we restrict their diets are we returning them to a body composition more like they evolved with, or are we reducing them from a 'normal' weight to an emaciated but healthier state? The two nonhuman primate studies illustrate the translational issue this poses. Both studies used rhesus monkeys. Both restricted their animals' calories by 30% from control levels. The University of Wisconsin study allowed their control animals to eat *ad libitum* and become obese as captive monkeys invariably do. They found 30% DR improved multiple aspects of health and significantly increased survival [6]. The National Institute of Aging study fed their control animals a regulated amount to achieve a 'healthy body weight' and restricted from there. Although they found modest improvements in several aspects of health, there was no vestige of a DR effect on longevity [7]. Either of these studies may be relevant to different subgroups of modern humans. Short-term human studies have also been of both types. The CALERIE study was a controlled clinical trial on healthy young to middle-age non-obese people with a target of 25% reduction in energy intake over two years. Confirming that most humans are not capable of sustained major reduction in energy intake, the achieved level of energy reduction over the two years was 11.7 ± 0.7% (mean ± standard error) with a 10.4 ± 0.6% weight loss, less than half the targeted value. However, this modest reduction of Body Mass Index (BMI: kg/m^2) from marginally overweight 25.1 (range: 21.9–28.0 kg/m^2) at the beginning of the study to 22.5 ± 0.1%, the middle of WHO's 'healthy weight' range, was attended by improvements in multiple cardiovascular risk factors [8]. A more extreme, although less controlled, food restriction regime is practiced by members of the Calorie Restriction Optimal Nutrition Society, or CRONies) [9], whose level of motivation inspired by the prospect of longer life has allowed them to maintain a high level of self-imposed restriction for years. The CRONies studied by Holloszy and Fontana had mean BMIs of 19.6 after 6 years of self-imposed restriction. They displayed excellent values for a range of cardiovascular risk factors. However, these risk factors are not substantially better than those people who achieve less extreme leanness by other means such as a vegan diet or long-term endurance exercise [10]. On the negative side, DR but not exercise, was associated with reduced lower extremity strength and aerobic capacity, as well as reduced bone mineral density [11,12]. On balance, it still is not clear what level of DR might be generally beneficial to humans under what conditions, even if more people were capable of doing it.

Drosophila research first showed that short-term food restriction, rather than life-long restriction, could alter mortality rate in as little as 1–3 days [13]. Also, studies by

toxicologists and others had shown that DR protected animals against a variety of stressors [14]. These observations led researchers to investigate whether short-term fasting might protect against the stress of surgically induced ischemia injury and the side-effects of cancer chemotherapy using mouse models, which indeed it did [15–17]. These discoveries were followed up by investigations into the molecular basis of these benefits which led to investigation on what aspect of the fasting diet was important. Was it specific macronutrients or fasting itself?

The evidence that calorie, rather than some specific macronutrient, restriction was responsible for the DR effect had always been somewhat equivocal. Even early rodent studies found that restricting protein while keeping calories constant had salubrious effects [18]. Indeed, restricting even a single amino acid had been shown to extend rodent life [19]. Yet protein restriction for extended health received little attention until researchers showed repeatedly that protein, not calorie, restriction was the driving force for extending *Drosophila* lifespan [20–22]. This led to a revived interest in protein and even specific amino acid restriction in mammals. Further research on recovery from surgical ischemia/reperfusion injury paradigm described earlier showed that complete food-free, short-term fasting was not required for the observed protective effect. It could also be achieved by depleting dietary protein or even by depleting a single amino acid [23]. Recently a more sophisticated approach to investigating macronutrient composition in diets has emerged. ‘Nutritional geometry’ manipulates dietary macronutrients in varying combinations rather than individually and is revealing fascinating patterns in nutritional physiology [24–26]. With respect to DR, this work found that reducing calorie intake by food dilution did not extend life in mice as standard DR did, raising the possibility that the neurobiology of satiety may be involved in the DR effect somehow. Other fly and rodent restriction research now began to raise cautionary flags about any single dietary studies focused on single genotypes or ages of experimental animals by showing that genetic background, including sex, could have a major impact on the health impact of either general food restriction or the restriction of specific macronutrients [27–31]. Currently, it seems unlikely that any one dietary formulation will be universally healthful. It will depend on all the variables mentioned above. Human studies combined with extensive ‘omics’ analysis should open the way to more precise and predictable dietary interventions into health.

Other forms of restriction

Another revision to the traditional DR regime came from the observation that because restricted rodents were hungry they ate their food quickly, usually in less than an hour, so that the standard research paradigm could be re-interpreted as a 23 hour daily fast. Modern research on the physiological and molecular biology of fasting shows that fasting can quickly and profoundly alter cellular fuel use as well as gene expression in organ-specific ways [32]. For instance, fasting rodents show a significant elevation in plasma ketones, indicative of a switch from carbohydrate to fatty acid metabolism, within 4–8 hours. Humans display a similar pattern after 8–12 hours of fasting [33]. Also, for years, some rodent studies had restricted food intake by feeding their animals *ad lib* on an every other day schedule. This research approach had similar health and survival effects to daily restriction, yet when food consumption was eventually measured, it sometimes turned out

that the animals were gorging sufficiently on the days they ate that they consumed on average as many calories as the control animals eating every day. These observations focused attention on whether the timing of eating or restricting may be as important as the amount eaten or its macronutrient composition [34]. Consequently, a variety of timed fasting or restriction regimes have emerged. These diets include intermittent fasting, typically eating every other day [33], which has been shown to confer health benefits such as improved insulin sensitivity and resistance to renal ischemia-reperfusion injury in mice [35] and improved cardiometabolic risk markers and reduced fat mass in humans [36]. Also, time-restricted eating, typically eating *ad lib* but restricting daily food consumption to a time window, typically 4–6 hours has been found to decrease 24 hour glucose levels and glycemic excursions. This approach is based on chronobiology which finds that more than 10% of expressed genes have circadian expression patterns, combined with the knowledge that in our evolutionary past before artificial sources of light existed, meals would necessarily be eaten during daylight hours [37]. Numerous studies have found that circadian misalignment of feeding behavior has been shown to have deleterious effects such as increased blood pressure, serum glucose and insulin levels and decreased sleep efficiency [38] and circadian alignment has shown beneficial effects, such as improved glucose control, decreased appetite and fat, and inflammatory markers in humans [39,40]. Another incarnation of intermittent restriction is the ‘fast-mimicking,’ that is, low protein, low calorie, diet. In this approach, the low protein/calorie diet is eaten for 5 consecutive days per month with normal food consumption the other days. This diet has shown a variety of beneficial effects in mice such as increased longevity, reduced visceral fat and cancer incidence, and improved immune system markers, and in short-term human studies decreased risk factors for diabetes, cardiovascular disease, and cancer [41]. A somewhat different version of this is the 5:2 diet in which a normal diet is eaten five days per week with a severely restricted number of calories eaten on two consecutive days. That diet has also shown short-term health benefits, including lowering total and visceral adiposity and may have improved liver function in human studies [42]. The tremendous advantage of these new, restriction-based, dietary regimes are they can easily be modeled laboratory species but more importantly, unlike chronic calorie restriction, people actually have a chance of adopting them if the evidence on their health benefits warrants [40]. However, once again research in flies showed the complexity that could be found within these research paradigms. Specifically, the age and duration of intermittent fasting have both been found to affect its health impact [43,44] and presumably so will sex and genotype.

Conclusion

The amount, composition, and timing of nutrient intake are all turning out to be important in the preservation of human health. Now that more translational dietary and nutritional regimes have been developed, along with ‘omic’ tools to assess their impact, we expect rapid expansion in our knowledge of optimized, personalized diets in the coming years.

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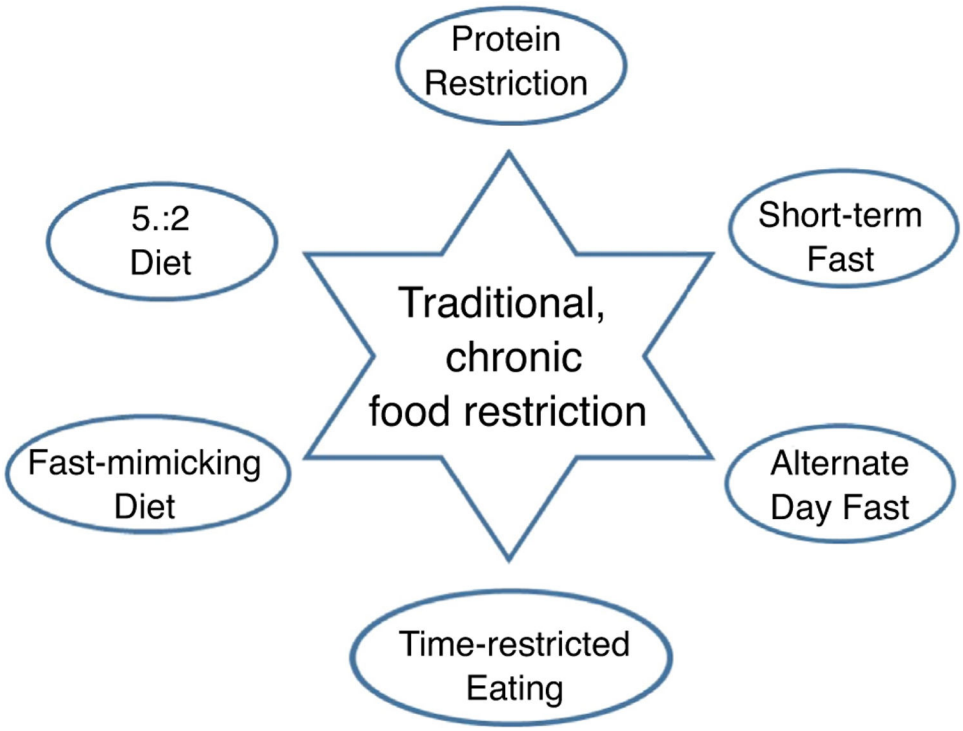

Figure 1.

Some currently experimental variations on traditional daily food restriction as experimentally investigated by early food restriction researchers. All of these have shown some health benefits in short-term human or life-long rodent studies. Protein restriction is self-explanatory, short-term fast refers to 2–3 day water only fasts, alternate day fast is self-explanatory, time-restricted eating describes confining one's eating to a restricted number of hours (usually 4–6) during the day [45], the fast-mimicking diet is consumption of a 'fast mimicking,' that is, low protein, low calorie, diet for 5 consecutive days every month [46], and the 5:2 diet is an intermittent restriction diet in which practitioners severely restrict food intake for two consecutive days each week and eat normally the other five days [42].