Why do the well-fed appear to die young?

A new evolutionary hypothesis for the effect of dietary restriction on lifespan

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Dietary restriction (DR) famously extends lifespan and reduces fecundity across a diverse range of species. A prominent hypothesis suggests that these life-history responses evolved as a survival-enhancing strategy whereby resources are redirected from reproduction to somatic maintenance, enabling organisms to weather periods of resource scarcity. We argue that this hypothesis is inconsistent with recent evidence and at odds with the ecology of natural populations. We consider a wealth of molecular, medical, and evolutionary research, and conclude that the lifespan extension effect of DR is likely to be a laboratory artifact: in contrast with captivity, most animals living in natural environments may fail to achieve lifespan extension under DR. What, then, is the evolutionary significance of the suite of responses that extend lifespan in the laboratory? We suggest that these responses represent a highly conserved nutrient recycling mechanism that enables organisms to maximize immediate reproductive output under conditions of resource scarcity.

Keywords:
aging; autophagy; caloric restriction; dietary restriction; lifespan extension; reproduction; somatic maintenance

Introduction

Dietary restriction (DR), defined as a reduction in nutrients without severe malnutrition, has been widely reported to extend lifespan and reduce fecundity in eukaryotes ranging from yeast to primates [1–4]. DR is the best known and most reproducible environmental intervention demonstrated in laboratory animals to extend lifespan, reduce aging rate, and protect against disease and degeneration [5, 6], and as such, it is the topic of considerable investigation across medical, molecular, evolutionary, and ecological disciplines. But whether this effect is really as universal as believed (e.g. [7]) – as well as the specifics of how DR extends lifespan, and the perplexing evolutionary question of *why* it happens at all – continues to elude researchers.

One major evolutionary hypothesis has dominated the literature for decades: DR's highly conserved effects on the life history represent an evolved strategy to increase survival under nutrient scarcity [8, 9] (and reviewed in [10]). According to this hypothesis, selection has favored a re-allocation of nutrients from reproduction to somatic maintenance and repair, hence increasing chances of surviving the famine (Fig. 1) (e.g. [11, 12] and reviewed in [9, 13–15]). Once nutrients become plentiful ("full feeding"), the organism may then be able to reverse the nutrient re-allocation, and resume reproduction. We will refer to this as the "adaptive resource re-allocation hypothesis".

Nearly a century of laboratory research has yielded an impressive amount of experimental data on DR, and a rapidly increasing understanding of the molecular and physiological bases of its effects. Yet, we are still a long way from understanding the ecological and evolutionary implications of DR, and what applications, if any, it may have for human health. In this paper, we critically re-examine the adaptive resource re-allocation hypothesis. We challenge its two main assertions: (i) the mechanistic explanation that a re-allocation of resources underlies lifespan extension and (ii) the

DOI 10.1002/bies.201300165

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Abbreviations:
BAT, brown adipose tissue; **DR,** dietary restriction; **IGF-1,** insulin-like growth factor-1; **IIS,** insulin/IGF-1 signaling; **TOR,** target of rapamycin.

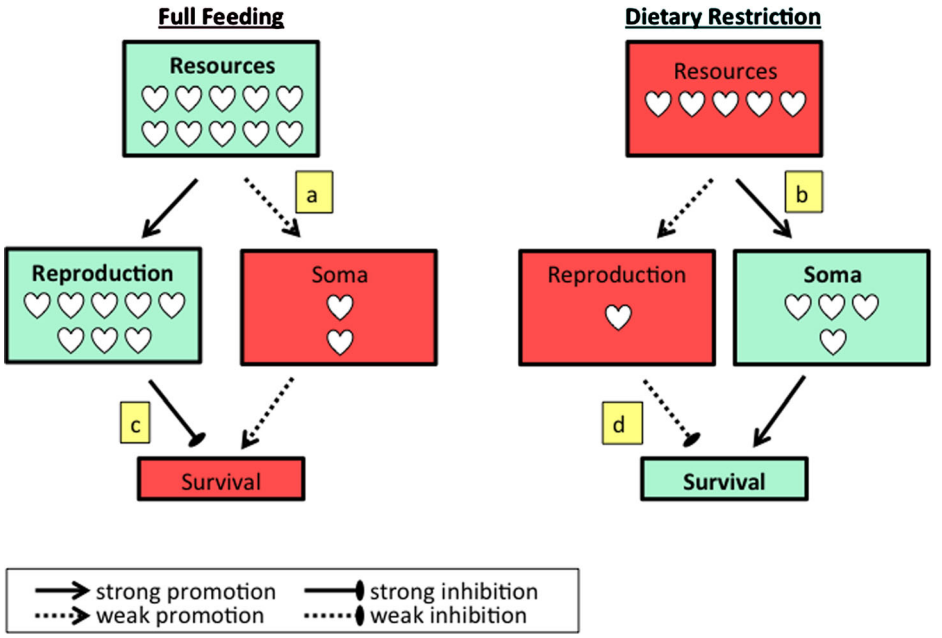


Figure 1. The adaptive resource re-allocation hypothesis. Differential investment of resources is generally thought to underlie the life-history responses to dietary restriction. (a) The adaptive resource re-allocation hypothesis states that under full feeding, most resources are invested in reproduction and few allocated to somatic maintenance or protection, with the result that fully fed organisms neglect survival in order to maximize fecundity. (b) However, under DR, a scarcity of nutrients is thought to trigger an adaptive re-allocation of resources from reproduction to the soma, enhancing survival. (c) Under full feeding, a high reproductive rate entails high somatic costs (i.e. "costs of reproduction", see text), which can further reduce survival. (d) Since reproduction is reduced under DR, costs of reproduction are reduced.

evolutionary hypothesis that the lifespan extension response to DR evolved under selection favoring survival over reproduction in times of resource scarcity. We then suggest a new framework, whereby DR's effects are interpreted as part of a suite of facultative physiological responses that enables organisms to maximize immediate reproductive output in times of famine as well as plenty.

The extended lifespan response to dietary restriction may be a laboratory artifact

Most wild animals are unlikely to benefit from postponing reproduction

A key question in assessing the plausibility of the adaptive resource re-allocation hypothesis is what kills animals in the wild and in the laboratory. Lifespan extension under DR appears to be achieved in the lab, at least in part, through the reduced incidence of cancer and other old-age pathologies [16, 17], and thus a reduced rate of intrinsic aging. For example, extended lifespan in mouse studies is usually (but not always) correlated with reduced tumor incidence [18]. Reduced rates of cancer may drive lifespan extension in other DR laboratory animals, including species that are not typically considered cancer victims. For example wild-type laboratory flies have been shown to develop cancer late in life in the testes and gut, two areas with mitotic activity throughout life [19]. Yet, organisms in the wild are typically subject to much higher rates of extrinsic (background) mortality (e.g. from predation or infection) than laboratory populations [20, 21], and (with the exception of some long-lived species such as large vertebrates) few individuals in natural populations live long enough to develop cancer and other old-age pathologies [22, 23]. As a result, we argue that a strategy of increasing investment to somatic maintenance would do little to increase survival in the wild for the great majority of taxa in which a lifespan extension effect of DR has been demonstrated (as represented in Fig. 2). The adaptive resource re-allocation hypothesis (Fig. 1) thus appears to be at odds with the ecology of many of the species in which the life-history effects of DR have been demonstrated, such as yeast, nematode worms, flies, and rodents.

Although longitudinal field data on small-bodied, short-lived animals are scarce, the available evidence suggests that high background mortality rates would impose a large cost on any delay in reproduction [20, 24–27]. Likewise, most short-lived organisms are unlikely to have evolved strategies for surviving predictable (e.g. seasonal) periods of resource shortage as adults, often investing heavily instead in a single, brief reproductive period. In species where adults do persist over seasons unsuitable for reproduction, they tend to do so in a fully quiescent state (e.g. diapause). More typically, short-lived organisms weather periods unsuitable for reproduction as eggs or juveniles [28], and may prolong the juvenile phase when resources are scarce, as observed in the "dauer" response of free-living nematode larvae [2]. The situation may be quite different in

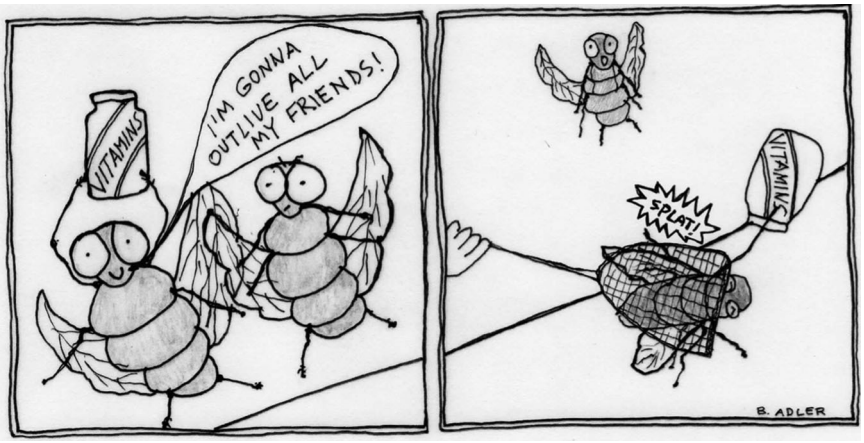


Figure 2. Investing in somatic maintenance is unlikely to pay off when the risk of extrinsic mortality is high.

long-lived species. During sporadic or cyclical (e.g. seasonal) periods of nutrient scarcity, organisms subject to relatively low rates of extrinsic mortality, such as large-bodied vertebrates (including humans), could potentially benefit from increasing their survival prospects at the cost of immediate reproduction (as discussed in [29])(although, as we show in the next section, it is not clear whether DR would actually enhance survival). More typical species – including the ancestral eukaryotes in which the highly conserved suite of physiological responses to DR evolved – would be unlikely to benefit from the hypothesized strategy of shutting down the reproductive system to "wait out" unpredictable periods of resource shortage during the reproductive season because their chances of surviving to reap the benefits would be minimal. Importantly, this suggests that the adaptive resource re-allocation hypothesis cannot account for the evolution of responses to DR.

Dietary restriction may reduce, not prolong, survival in the wild

The adaptive resource re-allocation hypothesis faces an even bigger problem: DR reduces capacity to respond to environmental challenges, and may therefore actually reduce survival prospects for animals in the wild. One situation in which DR would likely entail a lifespan cost is exposure to pathogens and parasites. While numerous studies have reported a reduced age-related decline in immune function under DR, most studies have not measured actual susceptibility to intact, fully functional pathogens [10, 30]. But simply maintaining the immune system in a sterile environment entails relatively low energetic costs as compared to mounting an immune response against a real infection. Indeed, when subjected to intact pathogens, DR animals tend to show a reduced immune response, and are more susceptible to bacterial infection, viruses, and gut macroparasites [10, 30]. For example one study found that aged DR mice inoculated with influenza virus were unable to regain body mass post-infection, and died sooner than fully fed mice [31]. Impairment of the immune response under DR is in line with the fact that the drug rapamycin, which mimics key molecular effects of DR, is used in humans as an immune inhibitor [32] (but see [33]). A negative effect on immune function and recovery after infection is also consistent with findings of a recent meta-analysis on the lifespan extension effect of DR [4], which showed that this effect is weaker in non-model organisms (an interesting recent example of which is provided by [7]). As the authors of the meta-analysis [4] point out, model species are generally cultured in more sterile conditions than non-model species, and DR's life-extending effects on the latter may be reduced by their increased vulnerability to infection.

DR also appears to reduce capacity to avoid and respond to injury. Studies on rodents [34, 35] suggest that DR impairs wound healing – an important survival function for animals in natural environments that must recover quickly from injury in order to evade predators and locate and compete for food. DR has also been shown to result in bone thinning and osteoporosis in humans [34], and reduced bone mineral content in monkeys [7], suggesting that vertebrates facing DR in the wild may be more vulnerable to fractures. Likewise, DR reduces muscle mass in rats, and although it attenuates muscle mass loss with age, the initial decrease has been suggested to severely compromise stamina and strength [34]. DR may thus hinder an animal's ability to escape from or fight off predators or competitors. Such costs may be less important if DR animals reduce activity rates and therefore predator/competitor encounter rates, but the available evidence is equivocal [10]. Indeed, some studies suggest that DR actually increases activity levels, perhaps as a strategy to locate food [36].

Likewise, there is evidence that cold tolerance is compromised under DR. In mammals and birds, maintaining a relatively constant body temperature is a basic physiological function. When exposed to cold temperatures, the desirable body temperature is achieved through an energy-costly process activated in brown adipose tissue (BAT) known as non-shivering thermogenesis [37]. DR may appear to increase cold tolerance because DR animals save energy by tolerating a lower body temperature, associated with increased BAT activation, and because DR reduces age-related decline in BAT function [37]. However, as in the case of immunity, simply maintaining a functional level may be much less energy-costly than mounting a response to a challenging cold-exposure. Indeed, DR mammals exposed to cold have been shown to progressively lose body weight [38] or to have a reduced rate of BAT hypertrophy and resultant higher mortality [39, 40]. Humans practicing DR report increased cold sensitivity, attributed to the lower threshold to initiate thermogenesis, reduced fat stores and reduced shivering thermogenesis due to muscle mass loss, making DR humans more

vulnerable to hypothermia and thus at higher risk for stroke, myocardial fibrillation, and death [34]. Indeed, a study on rats from the 1930s – one of the earliest reports of the DR effect – noted that half the animals in the DR group died as a result of laboratory heating system failures, while all of the fully fed animals survived the temperature drops [41]. Thus, small-bodied, homoiothermic DR animals may experience increased mortality under natural conditions, where cold-exposure is a constant threat [9, 42].

Laboratory conditions may exaggerate the survival costs of full feeding

While most studies have found that any extension in lifespan is accompanied by a reduction in fecundity, this trade-off is not always observed [2, 14]. The apparent absence of such a trade-off suggests that the standard "full-feeding" diets on which laboratory animals are maintained are not optimized for their physiological needs, and thus impose survival costs with no corresponding fecundity benefit [43, 44]. Lifespan "extension" on certain DR regimens may therefore simply reflect reduced intake of a poorly balanced diet. For example, Lee et al. [45] report that, at some protein:carbohydrate ratios, increased total nutrient intake reduces both fecundity and longevity in *Drosophila melanogaster*. Such an effect is suggested by the results of Grandison et al. [13], who found that adding only the amino acid methionine to the standard *Drosophila* DR diet resulted in increased fecundity without reduced longevity. When additional essential amino acids were added to this DR + methionine diet, however, fecundity increased above the levels of the standard "fully fed" animals, while longevity was reduced. These results show that the standard full-feeding laboratory diet reduces longevity as much as it does per unit gain in fecundity simply because it is nutritionally unbalanced, and is therefore an inappropriate "control" diet against which to compare DR animals. Unbalanced laboratory diets could increase both immediate (age-independent) and age-dependent mortality [4]. This suggests that, relative to captivity, the mortality costs associated with full feeding may be lower in natural populations, where animals have greater opportunity to optimize their diet by selecting among a variety of foods.

The poor nutrient balance of laboratory diets raises an important caveat in interpreting DR studies: because there is no universally agreed upon DR regimen for any species, DR effects vary, and results are difficult to generalize and compare across studies and species [44]. Different DR regimens may also elicit lifespan extension in different ways, as has been shown for *C. elegans*, in which different genetic pathways are activated depending on which DR regimen is used [46]. One example of methodology confounding interpretation of results comes from a study by Ja et al. [47], showing that a commonly used DR regimen in *Drosophila* resulted in dehydration of fully fed flies, and that providing additional water abolished the lifespan extension effect. The same study also confirmed results from Lee et al. [45] that protein:carbohydrate ratio, not total nutrient intake, is the main determinant of diet-dependent lifespan in *Drosophila*. This illustrates the value of a "nutritional geometry" approach to DR research [48], and suggests that results from older studies should be interpreted with caution.

Moreover, some authors have suggested that strong selection for rapid growth and large litter/clutch sizes in laboratory cultures may favor "gluttony", which might amplify the mortality costs of ad libitum feeding in lab-adapted animals [18, 49]. This might explain Harper et al.'s [18] finding that the grand-offspring of wild mice did not exhibit a longevity response to DR. This interpretation is also consistent with the considerable variation in the strength and even sign of the DR effect on longevity among lab-adapted mouse strains [50], and the finding that DR effects are generally weaker in non-model animals, which are likely to be less lab-adapted [4].

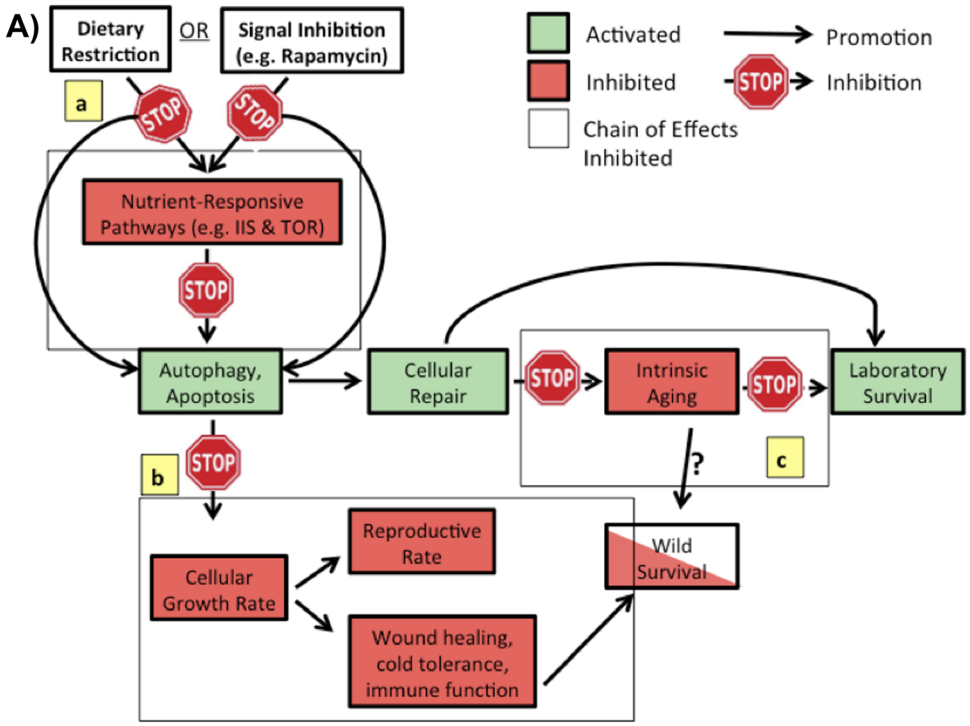
Finally, the longevity-reducing effects of full feeding may be exaggerated in the lab because of the relatively sedentary lifestyles of captive animals. He et al. [51] found that exercise induces autophagy after feeding in mice, and therefore acts as a potent inhibitor of diet-induced cellular damage, as discussed below. Animals in the wild are likely to get far more exercise than their captive counterparts, and may therefore avoid some of the costs of full feeding.

Resource re-allocation is unlikely to underlie the life-history responses to DR

Signals, not nutrients per se, control the lifespan response

In contrast to the hypothesis that a reallocation of nutrients from reproduction to somatic functions promotes survival under DR, empirical evidence strongly suggests that signaling, even in the absence of nutrient manipulation, is sufficient to trigger the responses of lifespan and aging to nutrient availability. Recent research shows that the suite of life-history responses to nutrient availability is largely controlled by the activity of highly conserved nutrient-responsive pathways (reviewed in [1–3, 6]). Two of the best-described pathways that have been implicated in regulating the life-history responses to nutrient availability are insulin/IGF-1 signaling (IIS) and target of rapamycin (TOR). Both the IIS and TOR pathways are activated by plentiful nutrients, and when switched on, they result in organism-wide changes in gene expression as well as in cellular growth and regulatory activity [2, 52]. Some of the most important and relevant downstream effects of the combined activity of the IIS and TOR pathways include up-regulation of cellular growth and proliferation rates (directly increasing reproductive rates) as well as down-regulation (inhibition) of cellular recycling, repair, and error-prevention mechanisms, most notably autophagy and apoptosis [5] (Fig. 3Ba,b).

Deactivation of nutrient-responsive pathways or of some of their downstream effects, through mutations affecting receptors, or infusion with signal inhibitors such as the TOR-inhibiting drug rapamycin, has been found to mimic the life-history responses to DR in multiple species: extending lifespan, reducing the incidence, and delaying the onset of cancers and age-related



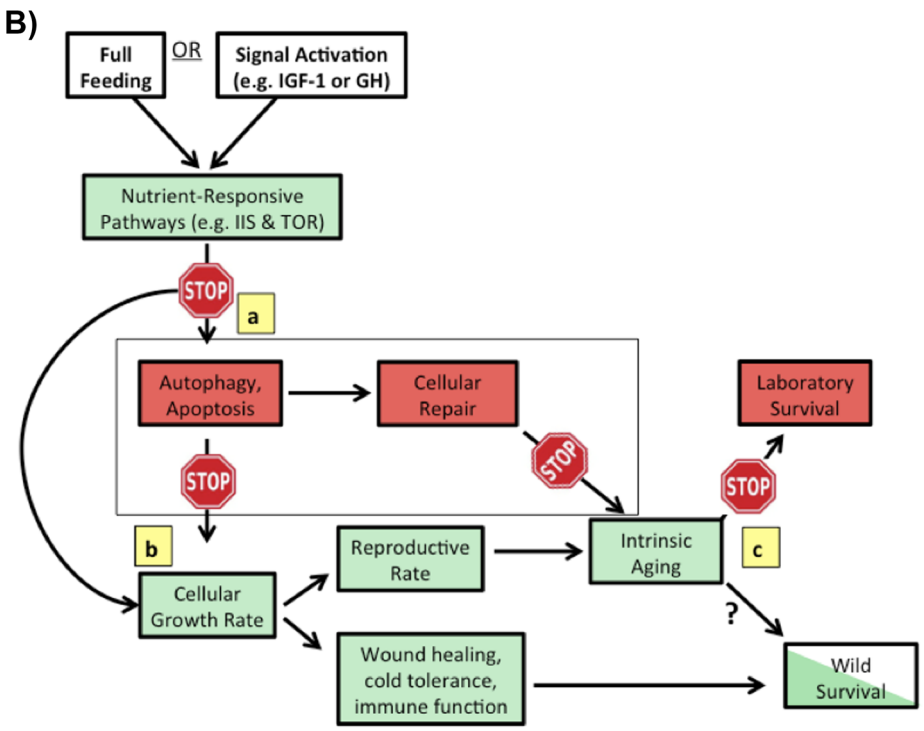


Figure 3. Nutrient-responsive pathways control multiple physiological and life-history responses. **A: Dietary restriction.** (a) Under DR or nutrient-signal inhibition (e.g. administration of rapamycin), nutrient-responsive pathways are inhibited and thus do not obstruct autophagy and apoptosis. (b) Autophagy and apoptosis inhibit cell growth rate, precluding high rates of reproduction and somatic responses likely to be important for wild survival. (c) Autophagy and apoptosis enhance cellular repair, reducing intrinsic aging by lowering rates of cancer and old-age pathologies, which bolsters survival in the lab. It is unclear how wild survival would be affected. **B: Full feeding.** (a) Full feeding or infusion with signal agonists, such as Insulin-like growth factor-1 (IGF-1) or growth hormone (GH), activate nutrient-responsive pathways, which inhibit autophagy and apoptosis, precluding cellular repair and leading to a high rate of intrinsic aging. (b) The inhibition of autophagy and apoptosis allows cell growth and proliferation to operate at a high level, enabling a high rate of reproduction (costs of which may contribute to intrinsic aging), and also enhancing somatic responses that are likely to bolster wild survival. (c) High intrinsic aging rate reduces survival in the lab, but effects in the wild are unknown.

pathologies, and reducing fecundity, even when the animal is fully fed [2, 5, 14] (Fig. 3A). Intriguingly, the lifespan extension effect of DR in *Drosophila* is partially reversed in flies exposed to odorants from live yeast, demonstrating that the lifespan response to nutrients is controlled in part by signaling rather than ingestion [53]. Conversely, infusing DR animals with hormones such as growth hormone (GH) or insulin-like growth factor-1 (IGF-1), which activate the downstream targets of the IIS pathway, has been shown to reverse the beneficial effects of DR on lifespan and aging [5] (Fig. 3B) – fecundity is not, of course, increased in hormone-infused DR animals, since actual nutrients are necessary for reproduction. If lifespan extension (or shortening) can be achieved independently of nutrients, this suggests that the resource re-allocation explanation for the extension of lifespan under DR may be misguided. A study in *D. melanogaster*, which used stable isotopes to track nutrient investment in reproductive and somatic tissues, bolsters this conclusion by showing that fully fed flies invest more resources than their long-lived DR counterparts into reproductive *and* somatic tissues, suggesting that the longer lifespans of the DR flies cannot be explained by increased somatic investment [15]. Moreover, reduced reproductive rate in DR animals is not necessarily indicative of resource re-allocation. Compared to fully fed animals, DR animals may reproduce less as a straightforward consequence of resource limitation.

Costs of reproduction matter, but cannot be the whole story

Given that fully fed animals typically reproduce more than DR animals, costs of reproduction could contribute to the difference in lifespan between fully fed and DR animals (as considered by, e.g. [14, 54]) (see Figs. 1 and 3). In this context, costs of reproduction refer to direct somatic wear-and-tear associated with production of gametes or nourishment of young [54], and do not include costs of mating, such as exposure to toxic substances in semen [55, 56], which may not be reduced in DR animals if nutritional state is unrelated to mating rate. While costs of reproduction clearly have the potential to decrease lifespan (Fig. 1c and Fig. 3Bb), there is strong evidence, at least in flies, that reproduction itself is unnecessary for the observed reduction in lifespan under full feeding or through interventions that activate pathways such as IIS and TOR independently of nutrients. Mair et al. [57] prevented female *D. melanogaster* from reproducing through removal of the ovaries or a mutation that blocks vitellogenesis (yolk formation), yet these sterile flies still experienced lifespan reductions under full feeding in comparison to DR. Moreover, Bjedov et al. [58] found that rapamycin extends lifespan and reduces reproduction in wild-type *D. melanogaster* females, but also extends lifespan in sterile mutants. The same study reported that rapamycin reduces reproduction in a dose-dependent manner, while different doses of the drug had roughly the same effect on lifespan, suggesting that reduced fecundity cannot be the sole cause of the lifespan extension [58]. The lack of a straightforward trade-off between survival and fecundity is also demonstrated by the results of Lee et al. [45]. Thus, while costs of reproduction may contribute to the negative association between nutrient availability and longevity, they are not the sole cause of this association.

An alternative hypothesis for the life-history responses to dietary restriction

Above, we showed that extended lifespan under DR does not appear to result from a re-allocation of resources from reproduction to somatic maintenance, and that such a re-allocation would in any case be unlikely to increase fitness – or even prolong life – for most animals in the wild. The evidence outlined above thus casts doubt on the long-held idea that DR's effects reflect an evolved response geared to increased survival. Why, then, did these responses evolve in ancestral eukaryotes, and why are they so highly conserved across diverse eukaryote lineages? To answer these questions, we suggest that it is necessary to refocus attention from the life-history outcomes observed in the non-natural environment of the laboratory, to the underlying physiology that appears to trigger these outcomes.

At the heart of all organismal responses to nutrient availability are the highly conserved nutrient-responsive pathways, including IIS and TOR, which have been linked to lifespan extension in diverse species such as yeast, flies, nematode worms, and mice [2]. These pathways initiate a cascade of physiological responses, a number of which have been suggested to act separately or in concert to modulate the lifespan response to nutrient availability. Below we examine one of the key responses – the modulation of cellular recycling and repair mechanisms, including autophagy and apoptosis – and consider why this response may have evolved in natural populations. Although the physiological response to DR is highly complex and multi-faceted, we focus on these particular processes because they are exceptionally well studied, and provide a foundation for an alternative evolutionary hypothesis of DR. In the following sections, we construct this new hypothesis as a series of propositions.

Autophagy and apoptosis are inhibited in fully fed animals to maximize growth

Autophagy and apoptosis are two of the best described cellular recycling and repair mechanisms that respond plastically to nutrient availability, but many others are likely to play a role as well. Autophagy is an intra-cellular process whereby portions of the cell are sequestered, broken down, and recycled [59], promoting protection and survival of the cell [60] and aiding in the prevention of neurodegeneration and cancer [61]. Apoptosis (i.e. programmed cell death) is a systemic process required for normal organismal functioning that also removes cells that are cancerous or damaged by disease [62]. Apoptotic cells are dismantled from within and then recycled [63]. When an animal is fully fed, its nutrient-responsive pathways are activated, and these pathways in turn inhibit cellular recycling and repair mechanisms, including autophagy and apoptosis [5]

(Fig. 3Ba). In contrast, when nutrient-responsive pathways are down-regulated under DR, these cellular mechanisms are dis-inhibited (i.e. up-regulated) [59] (Fig. 3Aa). But why has selection favored the up- or down-regulation of these processes in response to nutrient availability?

The likely answer is that these mechanisms entail a trade-off: although they can protect, repair, and recycle aging and damaged cells, resulting in long-term benefits to the organism (detailed below), they also limit the rate of cellular growth and proliferation [64, 65], which in turn limits the immediate reproductive rate [66] (Fig. 3Ab). By increasing its cell growth rate, the animal can increase its conversion rate of nutrients into reproductive products such as eggs and, in mammals, nourishment of young and development of the endometrium [67]. This is also true for milk production, as cell growth and proliferation are necessary for initiating and maintaining lactation (e.g. [68, 69]). Increasing the rate of cellular growth and proliferation appears to be beneficial not only for increasing reproductive rate but also for an animal's ability to respond to environmental challenges, through functions such as increased cold tolerance, wound healing, and immune function (Fig. 3Bb). We suggest, therefore, that autophagy and apoptosis are inhibited under full feeding as an efficient way of allowing the animal to increase its cell growth rate to take immediate advantage of available nutrients. Although this may result in latent damage, standard evolutionary theory predicts that selection will strongly favor reproduction early in life, even if it entails late-life costs [70]. Moreover, as we argued above, those costs will be negligible for many small, short-lived species with high rates of extrinsic mortality.

Autophagy and apoptosis are dis-inhibited under DR to increase efficiency

We suggest that selection would favor the dis-inhibition (i.e. up-regulation) of cellular recycling mechanisms under DR because they allow animals to make more efficient use of limited resources, possibly allowing for some immediate reproduction. Autophagy frees up stored nutrients in cells, a function that has been suggested to allow the organism to operate with lower resource intake [71, 72], and apoptosis recycles whole cells and reduces cell number [63, 73], allowing the organism to function more efficiently and with reduced cell mass to maintain. One interesting example of this form of nutrient recycling is observed in the cockroach *Nauphoeta cinerea*, in which females can initiate apoptosis, even in some oocytes, when nutrients are too scarce to produce a full clutch – in this case, when the females were held under starvation (e.g. [74]). Of course, survival is a prerequisite of reproduction, and a baseline level of nutrients must be provided before reproduction will be possible. DR responses appear to lower this baseline, making immediate reproduction more attainable when nutrients are scarce (Fig. 4).

Processes such as autophagy and apoptosis could be viewed as mechanisms of differential resource allocation because, under DR, stored resources are recycled and put to use for survival or reproduction. However, this form of differential allocation differs fundamentally from the form envisioned under the adaptive resource re-allocation hypothesis because it does not involve sacrificing reproduction for the sake of somatic maintenance. Instead, we suggest that it represents an alternate, "making-the-best-of-a-bad-situation" resource-use mode: the organism makes more efficient use of incoming resources, but with a slower conversion rate that is more-than-sufficient to accommodate the lower resource intake rate under DR. That is, a low rate of cell growth and proliferation will not be as costly to an animal that has few nutrients to convert to new growth anyway. This slower nutrient conversion rate would, however, entail a cost if more nutrients were available, explaining why IIS and TOR inhibition in fully fed animals reduces fecundity [2], and would very likely increase susceptibility to environmental challenges that necessitate a cell growth response.

Cellular recycling mechanisms affect lifespan as a side effect

There is strong empirical evidence that cellular recycling and repair

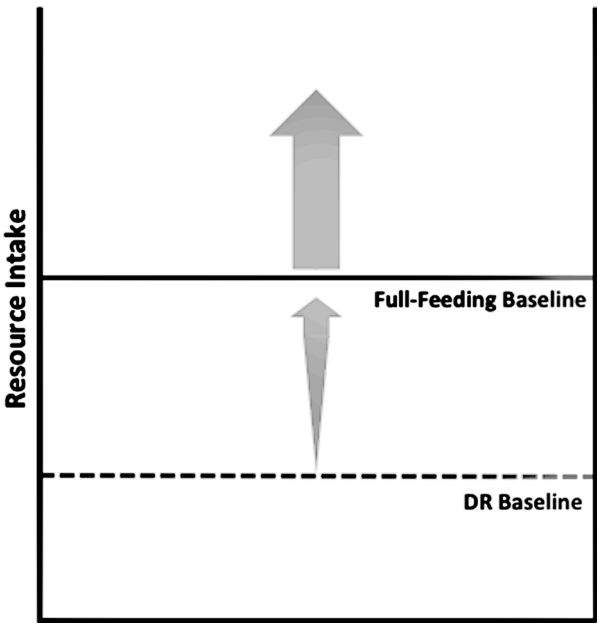


Figure 4. Adaptive responses to DR make reproduction more attainable. Responses to DR lower the baseline level of resource intake needed for basic functions, enabling reproductive investment (gray arrows) at a lower nutrient level. This is achieved in part through the up-regulation of cellular recycling mechanisms, such as autophagy and apoptosis, which reduce nutrient requirements by decreasing the size and number of cells that must be maintained, and which recycle stored nutrients to supplement incoming resources.

mechanisms play a key role in the response of lifespan to nutrient availability in laboratory animals (Fig. 3). The clearest evidence exists for autophagy, which has been shown to be required for DR to extend lifespan, as genetic interventions that block autophagy preclude the lifespan extension effect of DR and TOR inhibition [71, 75], as well as that of IIS inhibition [76]. More than just extending lifespan, up-regulation of autophagy under DR has been shown to mediate numerous anti-aging effects across model species, including reduced loss of muscle function and reduced lipofuscin levels in the heart; blocking autophagy prevents these beneficial effects [59]. The response to DR, mediated by mechanisms such as autophagy, thus appears to comprise a suite of beneficial physiological responses. But the fact that these are all at least partly dependent on autophagy suggests that the common mechanism of reduced cellular damage has numerous phenotypic manifestations, and this suggests a simple link among the various anti-aging phenotypes triggered by DR in the lab. In other words, while DR is often represented as adaptively initiating a complex array of organismal responses to ensure survival until resources return, we suggest a simpler scenario: DR up-regulates cellular repair and recycling, which, in turn, down-regulates cellular growth and proliferation, and slows aging both systemically and within individual organs and tissues.

Although the role of apoptosis in the lifespan response to nutrient availability is less clear, abundant evidence suggests that its dis-inhibition under DR may also promote lifespan extension in the laboratory. It is well established that inhibition of apoptosis is a central cause of tumor development leading to cancer [77], and while the precise effects of DR on apoptosis are unknown, the higher level of apoptosis enabled when nutrient-responsive pathways are down-regulated may contribute to the reduced aging rate [17]. However, as we have noted above, the survival-promoting effects of autophagy and apoptosis are unlikely to be realized in natural populations of short-lived animals (Fig. 3Ac,Bc and Fig. 5).

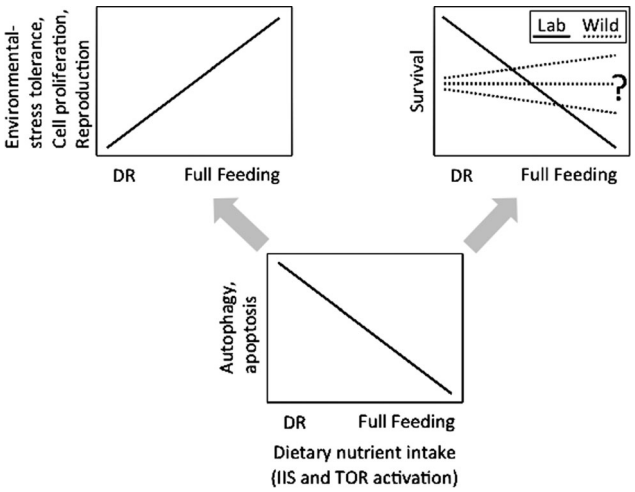


Figure 5. Responses to nutrient availability entail trade-offs. Levels of autophagy and apoptosis are inversely correlated with nutrient availability, with resulting effects in the opposite direction on cell growth rate, reproductive rate, and environmental-stress tolerance (e.g. wound healing, cold tolerance, and immune function). Laboratory survival trades off with these functions. Effects on wild survival are unknown, but may be weak or even opposite to effects in the lab.

Responses to DR are highly conserved because they promote immediate reproduction

The evidence outlined above suggests a plausible evolutionary explanation for the highly conserved responses to DR. Under DR, autophagy and apoptosis are up-regulated (i.e. dis-inhibited) to free up stored nutrients in the cells and make the animal function more efficiently, potentially enabling some immediate reproduction. Because high rates of autophagy and apoptosis reduce the intrinsic aging rate, survival is extended in the laboratory (but probably not in the wild) as a secondary consequence (Fig. 3Ac). We therefore suggest that immediate reproductive output, not survival to reproduce later, has been the key target of selection in the evolution of physiological responses to DR. In animals lacking parental care, these responses may allow males to continue to pursue matings and allow females to produce at least some eggs. Similarly, in animals with extended parental care, these responses may allow for the production of at least some viable offspring, which may be able to compensate for poor early nutrition if conditions improve later on. Moreover, if poor conditions persist, females may recycle their investment, as in the case of small mammals in which infanticide is commonly practiced under nutrient scarcity [78, 79]. A positive effect on immediate reproductive capacity offers a plausible explanation for the evolutionary origin and conservation of physiological responses to DR in eukaryotes.

While offering an alternative to the problematic "adaptive resource reallocation hypothesis", our hypothesis also furnishes an evolutionary basis for a key premise of the recent "hyperfunction" or "bloated soma" theory [6, 80]. This theory suggests that biosynthetic processes that promote cell proliferation and tissue growth in early life – thereby enhancing reproductive capacity – continue non-adaptively into late life, resulting in damaging cellular hypertrophy (cell mass growth) and hyperplasia (organ or tissue growth due to increased cell number). According to the bloated soma theory, such processes continue despite causing somatic damage and ageing because there is little selection to stop them; but because DR down-regulates nutrient-responsive pathways that control growth and reproduction, it reduces the rate of biosynthesis and thus the rate of aging [6]. However, the bloated soma theory fails to explain why selection would favor down-regulation of biosynthesis under DR. Our hypothesis

Table 1. The propositions that make up our alternative hypothesis for the life-history effects of DR observed in the laboratory entail a number of specific predictions. Strong evidence exists in support of some of these predictions, while others need to be tested, and we suggest possible experiments.

Proposition	Prediction(s)	Available evidence/suggested experiments
Lifespan is extended under DR in part because cellular recycling mechanisms (e.g. autophagy, apoptosis) are up-regulated	Blocking some or all of these mechanisms would preclude the lifespan extension response to DR	A few studies have inhibited genes necessary for autophagy in *C. elegans* held under DR, and found that autophagy is required for DR to extend lifespan [71, 75, 76]. Some authors have suggested that apoptosis may also be important in the lifespan extension effect of DR [17], but as far as we are aware, a clear causal link has not yet been established
Selection favors the (otherwise perplexing) inhibition of key cellular recycling mechanisms, such as autophagy and apoptosis, under full feeding because they reduce cell growth rate. This would prevent the animal from taking advantage of available nutrients by reducing: (1) Reproductive rate (2) Ability to respond to somatic challenges	Up-regulating autophagy and apoptosis under full feeding would: (1) Decrease reproductive rate (2) Reduce ability to respond to environmental challenges.	(1) A number of studies have blocked nutrient-responsive pathways while fully feeding the animal, through mutations and signal inhibitors. These studies have thus indirectly increased autophagy and apoptosis in fully fed animals (although pleiotropic effects of blocking these pathways could make results difficult to interpret). Many of these studies have reported reduced reproduction [2, 58] (2) In terms of effects on the ability to cope with environmental hazards, it would be very interesting to use the method of blocking nutrient-responsive pathways in fully fed animals to measure survival, compared to a fully fed control group, when exposed to challenges such as cold temperatures, injuries, and live pathogens
The benign environment of the laboratory masks the survival costs of DR	(1) Making the laboratory environment more realistic, in terms of environmental challenges, would negate or possibly reverse the beneficial effects of DR on lifespan (2) DR in the wild would reduce, rather than prolong, survival (at least for populations with relatively high rates of extrinsic mortality)	(1) DR animals exposed to cold temperatures [38–40], injuries [34, 35], and live pathogens [10, 30] in the laboratory have demonstrated reduced ability to respond to these challenges, and sometimes experienced increased mortality as a result (2) To the best of our knowledge, no study has yet managed to dietary restrict wild animals, due to numerous methodological challenges. Holding wild animals under DR could potentially be achieved by manipulating the animals' ability to consume or process food, or by manipulating food availability in the wild environment. If such manipulation were successful, animals could be tracked and survival recorded. A related, but less direct, tactic is to regularly capture marked wild animals and feed supplementary nutrients to one group, while the non-supplemented group would be the control, representing something closer to DR. Such studies have tended to find supplemental feeding has no effect on survival or that it increases survival; very few have observed that survival decreases in response to supplemental feeding in the wild [18, 29, 81]
DR extends lifespan in the laboratory by reducing intrinsic aging (old-age pathologies and degeneration), but these benefits would be unlikely to translate to the harsher environment of the wild, where animals (particularly small, short-lived species) do not often live long enough to develop old-age pathologies	(1) DR animals in the laboratory will have delayed or reduced incidence of cancer and other old-age pathologies compared to their fully fed counterparts, accounting for their longer lifespans (2) In small, short-lived species, most animals will die in the wild due to extrinsic causes before old-age pathologies can play much of a role	(1) DR is well known in laboratory animals to reduce the occurrence and delay the onset of cancers and numerous other pathologies and deterioration associated with old age [2, 5]. However, these discoveries are generally made by killing the animal prematurely to detect tumors and other signs of pathology, precluding an assessment of mortality factors. More work is needed on causes of death in DR vs. fully fed animals. One useful experiment might be to create numerous replicates of paired animals – each animal in the pair treated identically, except one would be fully fed and the other DR. When the fully fed animal dies, an autopsy could be performed to detect possible causes of death. The paired DR animal would be killed at the same point to examine whether the same pathologies are absent or reduced

(*Continued*)

Table 1. (*Continued*)

Proposition	Prediction(s)	Available evidence/suggested experiments
		(2) Wild animals have been shown to live a significantly shorter time than their laboratory counterparts [20, 21], and predation has been identified as the main cause of mortality in some natural populations [82–85]. However, more studies specifically examining causes of death in wild animals, using methods such as autopsies on carcasses, tracking of individuals to determine predation, and recording environmental conditions at time of death, are needed
Up-regulating cellular recycling mechanisms such as autophagy and apoptosis is beneficial under DR because these mechanisms allow the animal to make more efficient use of scarce resources, potentially allowing for some immediate reproduction that would not otherwise have been possible	Blocking autophagy and/or apoptosis under DR would reduce reproduction compared to DR controls	We are not aware of any studies that have tested this idea, but the methods are available for doing so, at least for autophagy. The methodology used in the studies that have inhibited genes necessary for autophagy, and measured effects in DR animals on lifespan compared to wild-type DR controls [71, 75, 76], could be used to measure effects on reproduction. Such an experiment might be extended using a nutritional geometry approach, in order to reveal a wider range of nutritional effects

complements the bloated soma theory by suggesting a selective advantage for the modulation of cellular growth mechanisms under DR: at least in short-lived animals, organismal responses to varying levels of nutrient abundance function to maximize immediate reproductive output.

Conclusions and outlook

In summary, we have shown that the adaptive resource re-allocation hypothesis is inconsistent with both the physiology of DR's effects, and with the ecology of natural populations. The typical effects of DR – reduced reproduction and increased longevity – do not appear to result from changes in nutrient allocation. Rather, effects on lifespan and aging are mediated by cellular signaling pathways, and reproduction is reduced under DR because there are simply fewer resources available to support it and because cellular growth rate is reduced. Moreover, although DR tends to increase longevity in the protected environment of the lab, it is unlikely to do so in natural populations because DR reduces capacity to respond to a variety of environmental challenges. Because most species of animals (and probably the early eukaryotes in which the physiological responses to DR evolved) are subject to a high extrinsic mortality rate in the wild, they would be unlikely to benefit from a strategy of postponing reproduction in any case. We suggest an alternative, more plausible evolutionary hypothesis: the highly conserved physiological responses to DR, including the up-regulation of autophagy and apoptosis, represent a nutrient-recycling/resource-efficiency mode that may allow for some immediate reproduction in times of famine.

The key propositions of our hypothesis can be tested experimentally (Table 1). Perhaps most importantly, there is a pressing need for better understanding of differences between laboratory and natural environments in selective pressures and the consequences of variation in diet. One of the key goals in seeking to understand why and how DR extends lifespan and whether this may be relevant outside of the laboratory is to discover what usually kills animals in the lab and the wild. If animals such as rodents, insects, and nematodes regularly die of predation, injury, or exposure to parasites or extreme temperatures, and if DR increases susceptibility to these mortality causes, then the potential for DR to extend lifespan in natural populations is low. Conversely, if susceptibility to old-age pathologies has a substantive effect on fitness in natural populations of such organisms, and DR substantially reduces susceptibility to extrinsic sources of mortality such as predation (e.g. by reducing activity rates), then this would lend credence to the idea that increasing investment in survival under nutrient scarcity could be adaptive across diverse eukaryotic taxa. These questions can be addressed through studies on DR's effects in natural populations, or through laboratory experiments that simulate the harsh conditions experienced by wild animals.

Finally, although we have argued that increased survival under DR is unlikely to be realized in natural populations, the fact that we can elicit this response in benign environments creates enormous potential for applications to human health. Nonetheless, we must be aware that some of the factors that limit or negate the potential benefits of DR in natural populations may also apply to humans (see [34] for a review of known costs in humans). Any intervention that reduces the capacity for reproduction, immune response, wound healing, and other functions, may be undesirable. For example rapamycin has often been considered as a human anti-aging agent because of its ability to inhibit activation of the TOR pathway without restricting nutrients [10], but it is essential to know what functions would suffer as a side-effect. An enhanced integration of insights from evolutionary, molecular, and medical research will be crucial in elucidating the functions and underlying mechanisms

of life-history responses to nutrient availability, as well as the potential for DR, or substances that elicit its effects, as promoters of healthy longevity in humans.

Acknowledgments

We thank Mark Brown, Jonny Anomaly, Angela Crean, Edith Aloise King, Michael Garratt, David Sinclair, Andreas Flouris, Marcos Vidal, and Lindsay Wu for their expertise and insightful discussion and comments, Bernice and Morton Hunt for copy-editing the manuscript, and Barbara Adler for drawing the cartoon in Fig. 2. We are also very grateful for the thorough and thoughtful reviewer and editorial comments we received in revising the manuscript. This work was funded through a PhD scholarship to M.A. from the University of New South Wales and the Evolution & Ecology Research Centre, and an Australian Research Council Research Fellowship and Discovery grant to R.B.

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