nature aging

Review article

https://doi.org/10.1038/s43587-026-01091-5

Dietary restriction in aging and longevity

Received: 12 August 2025

Accepted: 9 February 2026

Published online: 06 March 2026

Check for updates

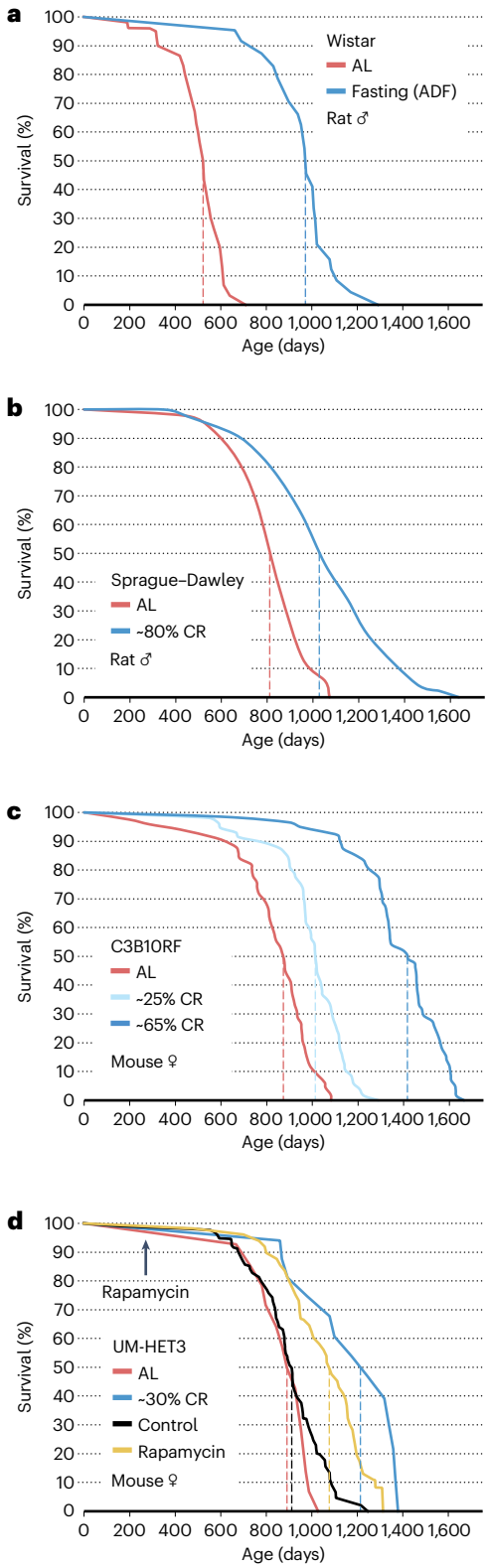
Tomas Schmauck-Medina[1], Sofie Lautrup[1], Andrea Di Francesco[2], Sarah J. Mitchell[3], Christoffer Clemmensen[4], Linda Partridge[5], George S. Roth[6], Rozalyn M. Anderson[7,8], Julie A. Mattison[9], Rafael de Cabo[9], John R. Speakman[10,11], Arlan Richardson[12,13], Donald K. Ingram[14], Richard Weindruch[15], Mark P. Mattson[16] ✉ & Evandro F. Fang[1,17] ✉

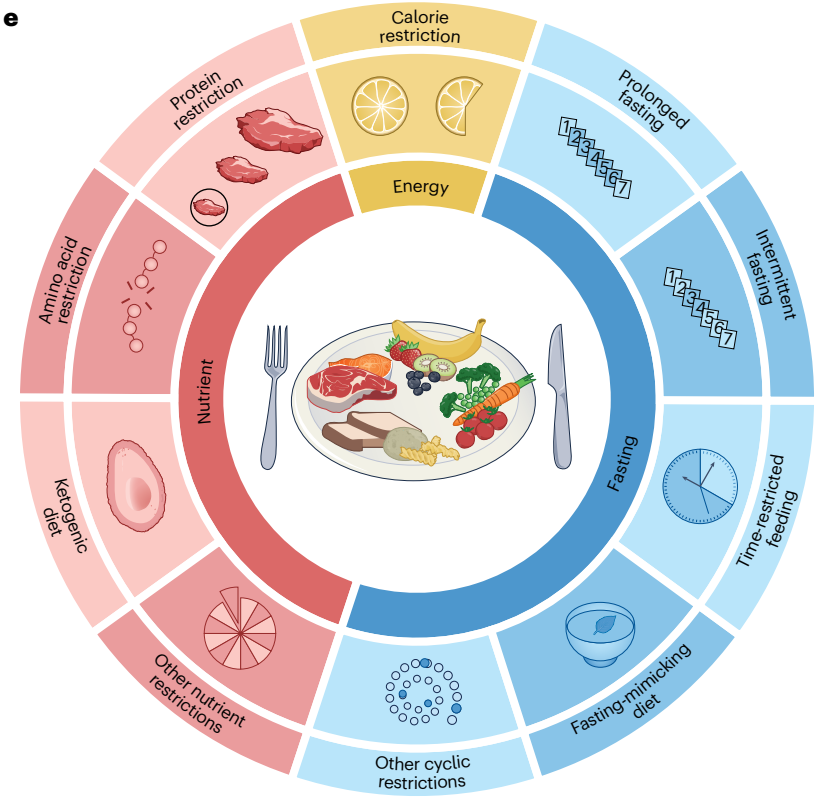
Different types of dietary restriction (DR) have been practiced by humans for religious and medical purposes for millennia, but only during the past three decades has the scientific study of DR at cellular and molecular levels proliferated. Here we review the evidence testing a variety of DR paradigms in the context of aging, focusing on mammalian findings. We discuss potential DR mimetics that modulate autophagy, FGF21, AMPK, mTORC1, NAD^+ metabolism, SIRTs, GLP-1R and other pathways as well as organismal and cellular adaptations to DR, including the roles of fasting, hunger, changes in body temperature and fat loss. We also consider the potential negative effects of DR such as increased vulnerability to infections and impaired wound healing. Further, we discuss preclinical evidence evaluating the potential of DR to improve healthspan and treat, prevent or delay age-related diseases including cancer, cardiovascular diseases and neurodegeneration. Finally, we consider the future opportunities for translation, and the challenges inherent to this complex research field.

The scientific study of DR began in the early 20th century, but progress was slow until the 1980s when several major studies unequivocally demonstrated that caloric restriction (CR) extends lifespan in mice and rats even when initiated well after maturation[1–4]. These findings caused a paradigm shift in the biology of aging and established CR as the field's gold-standard intervention. Today, DR remains the most effective nongenetic intervention for extending maximum lifespan (Fig. 1). In rats, feeding animals on alternate days has been shown to double maximum lifespan[5] (Fig. 1a), and reduction of caloric intake by about 80% (80% CR) produced the longest-lived (1,638 days) laboratory rat[6] (Fig. 1b). Similarly, independent studies have reported the longest mouse lifespans on record under CR: approximately 30% CR resulted in the longest-lived male mouse (1,742 days)[7], while 65% CR produced the longest-lived female mouse (1,660 days[8]; Fig. 1c). These lifespans exceed those reported for dwarf mice on 30% CR[9]. Moreover, indirect comparisons in outbred mice suggest approximately 30% CR

[1]Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital, Lørenskog, Norway. [2]Calico Life Sciences, South San Francisco, CA, USA. [3]Ludwig Princeton Branch, Ludwig Institute for Cancer Research, Princeton University, Princeton, NJ, USA. [4]Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [5]Institute of Healthy Ageing, Department of Genetics, Evolution and Environment, University College London, London, UK. [6]GeroScience and Prolongevity Technologies, Pylesville, MD, USA. [7]Department of Medicine, SMPH, University of Wisconsin, Madison, WI, USA. [8]GRECC, William S Middleton Memorial Veterans Hospital, Madison, WI, USA. [9]Translational Gerontology Branch, National Institute on Aging, National Institutes of Health, Baltimore, MD, USA. [10]Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China. [11]School of Biological Sciences, University of Aberdeen, Aberdeen, UK. [12]Oklahoma City VA Medical Center, Oklahoma City, OK, USA. [13]Department of Biochemistry & Physiology, Oklahoma University Health Science Center, Oklahoma City, OK, USA. [14]Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA, USA. [15]University of Wisconsin, Madison, WI, USA. [16]Department of Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA. [17]The Norwegian Centre on Healthy Ageing (NO-Age) and the Norwegian National Anti-Alzheimer's Disease (NO-AD) Networks, Oslo, Norway. ✉e-mail: mmattso2@jhmi.edu; e.f.fang@medisin.uio.no

https://doi.org/10.1038/s43587-026-01091-5





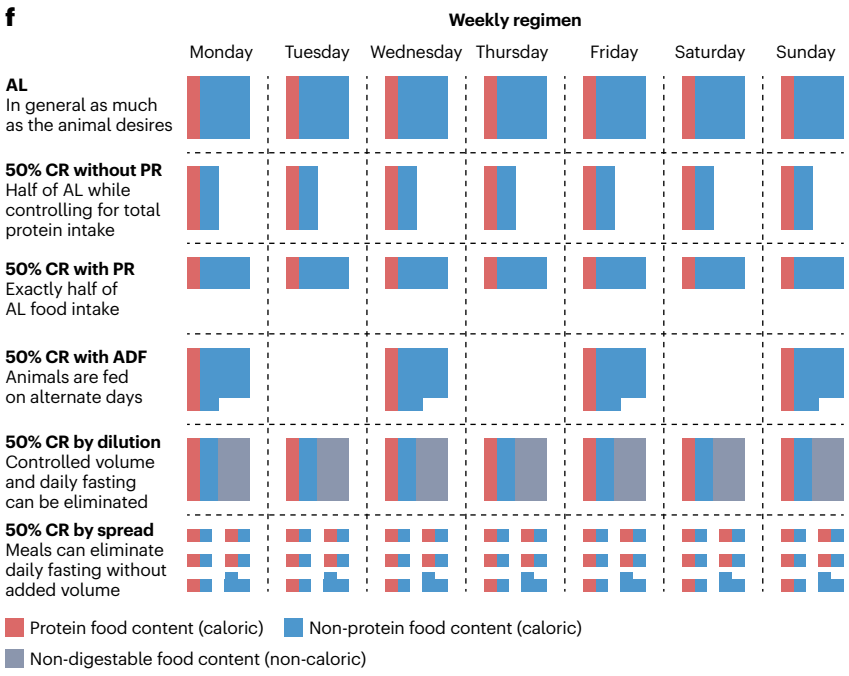


Fig. 1 | DR in aging. a–**d**, Survival curves depict male rats fed from weaning to death an ad libitum (AL) or an alternate day feeding regimen (83% increase mean lifespan)[5] (**a**), male rats fed an ad libitum or an approximately 80% CR diet (the longest-lived rat lived 1,638 days)[6] (**b**), female mice fed from weaning to death an ad libitum, approximately 25% or approximately 65% CR diet (the longest-lived mouse lived 1,660 days)[8] (**c**) and two independent cohorts merged for comparison: female mice fed from 4–5 weeks of age an ad libitum or approximately 30% CR diet[10], and female mice on a control diet or a diet containing 14 ppm rapamycin from day 270 (ref. 11) (**d**). **e**, The illustration depicts the umbrella of DR, with three major forms containing some of the most common dietary regimens practiced. **f**, The illustration depicts the challenge in comparing dietary interventions labeled as 50% CR, which may differ markedly in implementation, for example, daily restriction versus feeding on alternate days, potentially leading to different physiological outcomes and lifespan extensions. Survival curves were reconstructed using published data (Supplementary Methods).

https://doi.org/10.1038/s43587-026-01091-5

BOX 1

Terminology

The many forms of DR can be broadly classified into three types: energy restriction (such as CR), nutrient restriction (including protein and amino acid restriction, ketogenic diet) and different types of fasting regimens. Unfortunately, these forms have been described inconsistently or by ambiguous terminology. For example, '50% CR' may refer to regimens that differ in protein content, feeding frequency, or presence or absence of fasting periods (Fig. 1f). It is now clear that different protocols—with or without CR—can counteract aging. Emerging evidence indicates that omitting fasting windows can attenuate the lifespan benefits of daily CR, and intermittent fasting (IF) without CR can ameliorate metabolic and age-related diseases[139]. Lumping together all the studies under the term DR, therefore, risks oversimplification and may obscure mechanistic insights.

Ad libitum	Typically refers to continuous, unrestricted access to food. In most studies, this serves as the control condition, although mild restrictions may occasionally be applied.
DR	Used as a general descriptor when discussing the field broadly, discussing studies with different methods, or when the type of restriction is unclear or diverse.
CR	Describes a quantitative reduction in caloric intake relative to ad libitum. For example, '40% CR' indicates animals received 60% of the calories consumed by their ad libitum counterparts. Unless otherwise noted, we do not distinguish between protein-controlled and uncontrolled in this context. For a comprehensive classification of CR studies by protein control, see Speakman et al.[310].
Caloric dilution	Involves reducing the caloric density of food while maintaining total volume, often used in invertebrate studies. Dilution is the common approach for DR in invertebrates, and similar approaches in rodents have important implications, which are discussed later.
IF	Describes days within a week where there may be absence of food, for example, '5/2' refers to 5 days of ad libitum eating and 2 days of fasting per week.
ADF	A specific IF variant in which animals are fasted every other day and ad libitum on the feeding days.
TRF	Refers to the deliberate creation of a daily time at which food can be eaten. In general, the ad libitum access is given on those hours. For example, 16:8 refers to a daily 16-h absence of food and a window of 8 h for ad libitum feeding.
Nutrient restriction	This term encompasses restriction of one or more specific nutrients. Examples discussed here include protein restriction or restriction of specific amino acids such as tryptophan or methionine.

extends lifespan more than rapamycin[10,11] (Fig. 1d). Here, we discuss DR by identifying gaps and controversies, disentangling DR into distinct phenomena, and defining key terms for clarity (Fig. 1e,f and Box 1).

Universality of DR on lifespan extension

The geroprotective effects of DR show conservation across the animal kingdom—dogs, cows, fish and spiders all exhibit substantial lifespan extension on DR[12–15]. Even in clonal and non-clonal plants, lifespan is extended through light restriction[16,17]. Furthermore, effects of DR on the different hallmarks of aging are intensely studied, and some of the key mechanisms are summarized here (Fig. 2). Nevertheless, whether DR is truly universal or whether certain species or strains do not benefit remains debated.

Rodents

Strong evidence shows DR extends rodent lifespan across strains, including multiple mouse and rat strains under 40% CR[18] and genetically heterogeneous mice under approximately 30% CR[10]. The efficacy of DR in rodents has been reviewed elsewhere[19]; here, we consider the strength of conflicting evidence.

Genetic models with deficiencies in the growth hormone axis have provided insight on the mechanisms of longevity. Several studies have examined whether DR and growth hormone affect aging via similar mechanisms. Ames dwarf mice display DR-like phenotypes, including reduced size, temperature and fertility, and live about 50% longer[20,21]. Therefore, it was hypothesized that these mice were naturally calorically restricted, and that additional DR might be redundant or harmful[22]. However, Ames mice on 30% CR lived even longer. Interestingly, the slope of the survival curves suggested that while the Ames phenotype primarily delays the onset of age-related mortality, CR appeared to reduce its rate, suggesting that the two interventions influence distinct aspects of aging[9]. Long-lived mice deficient in growth hormone-releasing hormone also exhibit even greater longevity on 40% CR[23]. However, the long-lived growth hormone receptor knockout (Laron dwarf) mouse showed no survival benefit from alternate day feeding (ADF)[24], and under 30% CR only females exhibited a small (about 9%) increase in maximum lifespan[25].

Conflicting evidence for the effects of DR on lifespan have come from additional genetic backgrounds: the ILSXISS strains are a group of 40+ recombinant inbred mouse lines developed to study tolerance for alcohol[26]. In 2010, Liao et al.[27] reported that 40% CR did not extend lifespan of several of these mouse strains and in some even shortened lifespan. However, the study was limited by small cohort sizes (typically $n = 10$ per group) and by group housing, which may also have introduced a 'survival-of-the-fittest' dynamic[28] (compromising food restriction in dominant mice, and inducing extreme underfeeding in subordinate mice). At that time, single housing was standard in DR studies[29]. However, excluding early deaths in this study had little effect on the lifespan shortening across strains[30]. Further, among eight strains that were single housed, the majority still exhibited lifespan shortening from CR. Moreover, prior work by the same group showed that housing mice singly or in groups of four had no effect on median lifespan under ad libitum or 40% CR[29]. A second study using only female ILSXISS mice again reported that CR failed to extend lifespan in many strains and also shortened it in others[31]. Surprisingly, strain-specific responses diverged substantially between the two studies (Supplementary Table 1). Comparative analysis reveals no correlation between strain responses across the two studies, complicating interpretion[32]. These discrepancies probably reflect insufficient sample sizes and suggest a need for a different statistical approach[33]. Recently, a replication study with greater statistical power on both sexes of four strains originally reported by Liao et al. to exhibit lifespan-shortening effects under CR observed lifespan-shortening effects in only male mice from one strain; while in four of the eight groups, CR increased lifespan[34]. Additionally, one of the strains was tested on multiple levels of CR (10%, 20% and 40%), finding that, in female mice, lifespan increased with greater restriction. By contrast, in male mice the mean and median lifespan did not change.

These studies, along with findings from wild-caught mice (Box 2), suggest that certain genetic backgrounds may not benefit from DR. However, caution is warranted. For example, 20% and 40% CR and IF (1 and 2 days fasting per week) were shown to extend lifespan in proportion to the degree of restriction in genetically diverse outbred female mice[35]. Crucially, among mice surviving past 6 months, genetic background explained 23.6% of lifespan variation, compared with 7.4% explained by diet, indicating that genetics is a stronger determinant of lifespan than dietary intervention. Interestingly, a recent human population study estimates the heritability of intrinsic lifespan at about 50% after accounting for confounders[36]. Because diverse outbred mice mimic the genetic heterogeneity of natural populations,

https://doi.org/10.1038/s43587-026-01091-5

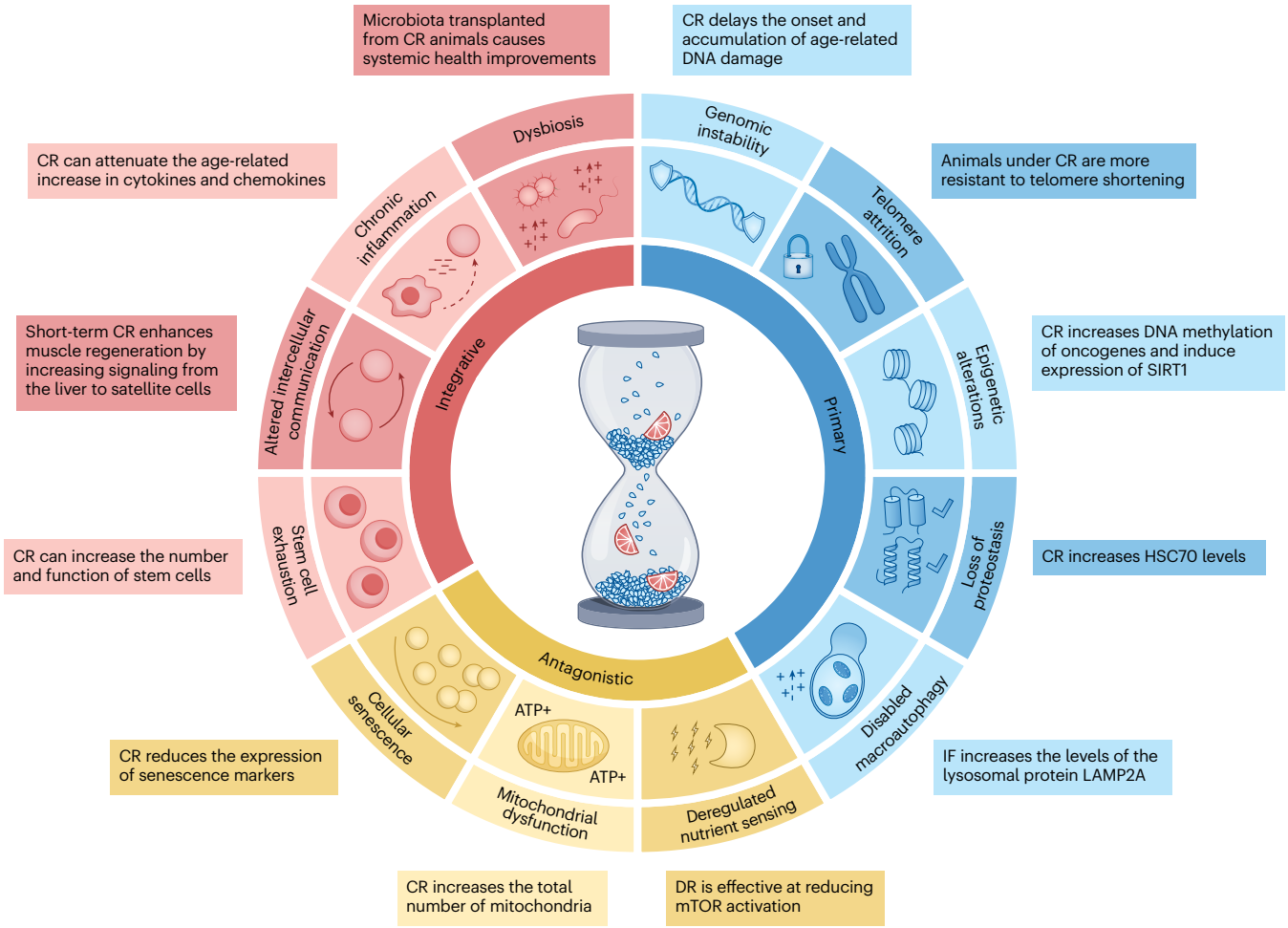


Fig. 2 | DR effects on the hallmarks of aging. The illustration depicts some of the effects of DR on the different hallmarks of aging, including genomic instability[296,297], telomere attrition[298], epigenetic alterations[299–301], loss of proteostasis[302], disabled macroautophagy[303], deregulated nutrient sensing[88], mitochondrial dysfunction[304], cellular senescence[305], stem cell exhaustion[304], altered intercellular communication[306], chronic inflammation[307] and dysbiosis[308]. For a larger and more detailed review covering multiple species (including humans), see de França et al.[309].

these findings may be more generalizable to humans than results from inbred strains, highlighting individual genetic context as a key factor for clinical translation.

Invertebrates

DR also extends lifespan in invertebrate models, including yeast, nematodes and flies[37]. Although these organisms are not the focus of this Review, one study that published conflicting data warrants discussion. Specifically, in medflies, increasing caloric density extended lifespan up to a plateau, while reducing intake eventually shortened lifespan[38]. There are three explanations for this phenomenon: (1) a critical nutrient is being restricted; (2) FOOD dilution may blunt the effects of hunger or fasting; or (3) a different dietary strategy may be needed. Intermittently switching the medflies' diet between a sugar-only diet and a full diet (yeast and sugar) was later shown to increase lifespan[39], suggesting the effects from DR are highly affected by the nature of the protocol[40]. A technical analysis of nutritional geometry performed in a closely related fly species also reconciled the discrepancy between these two studies[41].

Rhesus monkey (*Macaca mulatta*)

Early evidence from a small cohort of rhesus monkeys on a DR protocol for over 9 years, in which the restricted animals ate 40% less without affecting calories per kilogram of body weight (Table 1), reported a reduced risk of death in the DR group[42,43]. However, the small sample size, late introduction of the DR monkeys at varying ages, diet composition, absence of detailed data on body weight and a nonconcurrent control group limited the conclusions[44].

Separately, an early National Institute on Aging (NIA) report on the effects of 30% CR in both the rhesus and the squirrel monkeys[45] (Table 1) indicated a reduced rectal temperature in the CR group[46] and earlier mortality of control monkeys (23% of the control monkeys had died versus 13% in the CR group[47]). Later, the NIA study observed improvements primarily in healthspan, with no significant increase in survival[48]. A separate study at the University of Wisconsin (UW), however, reported benefits from CR on both healthspan and lifespan[49]. The two studies differed in design and in the effects of DR on body weight, with a greater reduction in body weight observed in the UW study, in which lifespan was extended. Indeed, the UW monkeys received a single meal per day and a small snack after food was removed. Furthermore, access to food for all UW monkeys was limited to a daily window of 8 h. At NIA, animals were fed twice daily, and food was not removed at the end of the day. At UW, the diet was semi-purified to ensure consistency throughout the study, while the NIA diet was naturally sourced and, therefore, subject to seasonal variation, lower in fat and higher in protein and fiber compared to the UW diet. Finally, the degree of restriction at UW was determined by each animal's baseline intake, whereas NIA determined restriction by size estimates. In 2017, both groups discussed these differences and concluded that the studies "support the concept that lower food intake in adult or advanced age

https://doi.org/10.1038/s43587-026-01091-5

BOX 2

Wild-caught mice and CR

In the early 2000s, Steven Austad captured wild mice at the University of Idaho and gave their grandchildren a 40% CR diet. While the mice experienced increases in the stress hormone corticosterone, decreases of testosterone and a lower burden of tumors, the CR group had a nonsignificant lower mean lifespan, even though the maximum lifespan was increased[311]. This finding posed the question of whether CR is simply fixing a 'problem', such as their susceptibility to cancer, in laboratory mice. However, there are other possible explanations:

(1) The animals were not raised in a specific pathogen-free SPF barrier colony, opening the doors for infectious diseases. Although the autopsy found that only one animal per group died of infection, the finding does not consider the problem of chronic infections, which may not acutely kill the animal but rather decrease its lifespan potential.
(2) Another consideration is food intake. Perhaps laboratory mice are more gluttonous than wild mice. However, Austad also found that wild mice eat more in nature than in the laboratory, possibly due to different energy requirements. In laboratory conditions, wild and laboratory mice ate similar amounts. However, wild-derived mice (no more than four generations in the laboratory) do eat less than laboratory mice[84]. This suggests, and the authors hypothesize, that a 40% DR may, therefore, be too severe in this genetic background.
(3) Inbred depression in laboratory mice could have created biological dysfunction that is solved by the mechanisms induced by DR. However, as discussed previously, DR might be more powerful in outbred mice[19].

The consistent observation that genetics has a dominant role and that individual responses to CR are highly variable suggests that to understand aging and DR requires moving beyond a one-size-fits-all approach. The conflicting results are a reminder of the need for rigorous, well-powered studies across diverse genetic backgrounds. The evidence supporting lifespan extension by DR across the tree of life is so extensive that it is unlikely to be a coincidence.

is associated with improved survival in nonhuman primates"[50]. A subsequent mouse study directly compared the UW and NIA monkey diets and found that diet composition did not affect the health or lifespan of ad libitum mice, nor did it alter the benefits of DR[51]. Today the UW cohort has no surviving animals, and the maximum observed age was 38.5 years in a restricted female[50]. On the other hand, one survivor remains at the NIA cohort: a control-fed female primate currently aged 40 years. The maximum observed age so far is 44.2 years in a restricted male rhesus, which, to the best of our knowledge, is the longest-lived rhesus monkey ever reported[52].

Squirrel monkeys (*Saimiri sciureus* and *Saimiri boliviensis*)

The NIA also explored 30% CR in the squirrel monkey (Table 1). However, all but one of the monkeys were wild caught, and although the vendor provided age estimates, reassessment based on multiple morphological analyses after the intervention had begun led to readjustment of the groups, causing group imbalances in body mass[53]. While only marginal effects of CR were observed in the adult cohort after 5 years[53], the actual reduction in food intake achieved was 20–25%[53]. In addition, an endemic microfilaria infection further affected the study. Currently, no lifespan data have been published for this cohort. However, in 2002, it was reported that 54% of the controls and 33% of the CR animals had died[47]. The longest-lived and final survivor of the study died in 2011—a control monkey with an estimated age of 30 years.

Gray mouse lemur (*Microcebus murinus*)

Gray mouse lemurs are small primates with a long lifespan relative to their size[54]. The RESTRIKAL program performed a DR study in this species (Table 1). An early analysis reported that about 36% of the CR animals had reached 13 years of age while all control animals had died; this surpassed the previously recorded maximum lifespan for the colony[55]. The final survival data showed a 50% increase in median lifespan[56]. Interestingly, although the CR animals exhibited accelerated loss of gray matter in the brain, no impairment in cognitive performance was detected[55].

Humans

Translating findings from rodents or even nonhuman primates to humans is complex. Carlson and Hoelzel recognized in 1946 that one day of fasting in humans is not equivalent to one day in rats[57]. Small animals have a higher surface area-to-volume ratio, leading to faster heat loss and greater energy expenditure to maintain body temperature. A mouse can lose about 20% of their body weight in a 2-day fast, while humans lose <2% after 4 days[58]. In addition, interpreting human data is complicated by the impracticalities of implementing chronic CR in humans.

In 2002, the NIA began recruitment for a human trial studying the long-term effects of CR: the Comprehensive Assessment of the Long-term Effects of Reducing Intake of Energy (CALERIE). In phase 1, three pilot studies were performed at different centers to address methodological challenges including implementation, adherence, tolerability and safety. After screening, 99 participants were randomized to CR or control, with an average annual dropout rate of 9.2% across centers[59]. The pilots (discussed elsewhere[59,60]) tested 20%, 25% and 30% CR mostly in adults with overweight for 6 or 12 months.

Based on the pilot findings, phase 2 involving a multicenter, 2-year trial began screening in 2007 and randomized 220 adults with normal weight or slight overweight[61]. Of these, 143 initiated a 25% CR and 75 were assigned as controls. In the first 12 months, the CR group lost on average 8.3 ± 0.3 kg (11.5% ± 0.4%) and subsequently stabilized[62]. After 2 years on CR, 78% of total tissue-volume loss was attributable to adipose tissue and only 17.2% to reductions in skeletal muscle lean mass[63]; liver volume decreased, whereas brain, kidney and spleen volumes did not significantly differ. Significant reductions were observed in cardiometabolic risk factors, including triglycerides, low-density lipoprotein cholesterol, systolic and diastolic blood pressure, insulin sensitivity and C-reactive protein[64].

Most recently, effects on multiple methylation clocks have suggested that, while mortality risk did not change (GrimAge), participants' pace of aging was reduced (DunedinPACE)[65]. Unfortunately, the 25% CR target was not achieved: the CR group averaged a restriction of 19.5% ± 0.8% in the first 6 months, and only 9.1% ± 0.7% for the remaining 18 months[62]; indeed, the lack of reduction in body temperature may suggest that the restriction was too mild. Overall, these studies highlight the challenges of translating the DR severity applied to rodents (30–80% CR) to humans. Finally, as noted elsewhere, the benefits may reflect the effect from weight loss rather than from long-term CR[60].

Optimal restriction

Early studies attributed the benefits of DR in adulthood to the avoidance of obesity, distinct from the drastic increases in lifespan observed when DR was implemented in development, attributed to delayed growth[66]. This theory was later abandoned as optimized adult DR protocols produced striking lifespan extension[1,3].

https://doi.org/10.1038/s43587-026-01091-5

Table 1 | A summary of the nonhuman primate studies on DR and survival

Study (by species)	DR	Animals	Age at DR	C[a] (*n*)	DR (*n*)
Macaca mulatta Rhesus monkey					
University of Maryland study[43]	Maintaining the weight of the animals at a normal lean adult body weight of 10–11 kg	8 males	12–19 years	109	8
NIA study[293]	30% CR	1987			
		12 juvenile males	<2 years	6	6
		12 adult males	3–5 years	6	6
		6 old males	>20 years	3	3
		Added in 1988			
		8 juvenile males	1–4 years	4	4
		8 adult males	5–9 years	4	4
		14 old males	>20 years	7	7
		Added in 1992			
		20 juvenile females	1–4 years	10	10
		20 adult females	6–14 years	10	10
		20 old females	>16 years	10	10
UW study[294]	30% CR	1989			
		30 adult males	8–14 years	15	15
		Added in 1994			
		30 adult females	8–14 years	15	15
		16 adult males	8–14 years	8	8
Saimiri sciureus *Saimiri boliviensis* Squirrel monkey					
NIA study[293]	20–25% CR[53]	1987			
		12 juvenile males	1–4 years	7	5
		13 adult males	5–10 years	7	6
		4 old males	>10 years	4	0
		Added in 1988			
		4 old males	>10 years	4	0
Microcebus murinus Gray mouse lemur					
RESTRIKAL study[295]	24% CR[55]	2006–2009			
		34 adult males	~3 years	15	19

[a]The control (C) group consisted of nonconcurrent male (88) and female (21) monkeys that were fed ad libitum.

Age and timing of onset

Traditionally, most DR studies began after weaning, although inconsistent effects were observed. For example, when maternal milk access was limited from week one, no lifespan gain was observed[67]. By contrast, reducing milk access by increasing litter size resulted in significant median (18%) and maximum lifespan extension[68]. However, the age at which DR ceases to be effective at increasing lifespan probably varies not only between species, but also between strains. For example, 19-month-old male B6C3F1 mice gradually transitioned to a 40% CR regimen exhibited a significant approximately 15% and approximately 16% increase in mean and maximum lifespan, respectively[69], whereas male C57BL/6, DBA/2 and B6D2F1 lifespans did not increase (and sometimes decreased) when 40% CR was implemented abruptly at 17 and 24 months of age[70]. In Wistar rats, ADF from 18 months of age potently increased maximum lifespan[2], yet gradual implementation of 33% CR from 18 months of age did not extend the lifespan of male Long Evans rats[71]. Later, the same team reported that although a gradual 32% CR imposed on 18-month-old or 26-month-old male F344 hybrids led to a reduction in body weight, it had no effect on lifespan[72].

Unfortunately, assessing age-specific mortality requires large cohorts. In a study of 800 mice, switching from 40% CR to ad libitum feeding at 24 months sharply increased mortality, while a gradual shift to 40% CR modestly reduced it. Late-life CR failed to trigger major transcriptomic or lipidomic changes in adipose tissue, suggesting a 'nutritional memory' that limits both survival benefits and metabolic remodeling[73]. Alternatively, when cohorts are smaller, model life-table methods still provide informative age-specific mortality patterns for dietary effects[74].

Level of restriction

The optimal degree of energy restriction remains debated, as severe CR may produce diminishing returns or even harm; thus, the optimum may be strain and sex dependent (Box 3). For example, in female C57BL/6J mice, 20% CR increased maximum lifespan by about 40% compared with only about 13% extension under 40% CR[75]. In male F344 rats however, 10% CR can extend mean lifespan as much as 40% CR, although 40% CR still provided greater maximum lifespan[76]. On the other hand, in male Wistar rats, approximately 33% CR (50 kcal per day) produced

https://doi.org/10.1038/s43587-026-01091-5

BOX 3

Sex differences

Harrison et al.[90] showed that genetically obese *ob/ob* female mice under CR outlived ad libitum wild-type controls, despite having more than twice the body fat percentage. This finding was later extended by Harrison and Archer[7], where they demonstrated similar lifespan extension in male *ob/ob* mice, as well as in male B6CBAF1 hybrid mice[7]. However, in the same study, they reported that DR reduced the mean and median lifespan of male C57BL/6J mice. At first glance, this might suggest that certain genotypes or sexes are nonresponders to CR. Yet, as discussed earlier, a more nuanced interpretation is that responses to DR are not binary but rather vary depending on genotype and sex, requiring tailored DR protocols. Supporting this view, Weindruch and Walford[1] showed that male C57BL/6J mice exhibit increased longevity under CR, indicating that the strain and sex are not inherently unresponsive[1]. In 2016, Mitchell et al.[75] showed that, while C57BL/6J female mice exhibited an increase in lifespan under a 20% CR, they did not under 40%. By contrast, males had an increase in lifespan under both 20% and 40% CR. In the same study however, both male and female DBA/2J mice responded well to 20% and 40% CR with regards to lifespan, highlighting not only the differences between strains, but also that these differences can be sex dependent. Furthermore, Green et al.[312] showed that mice under protein restriction exhibit significant differences in health outcomes, gene expression, metabolites and lipidic profile depending on the sex and strain.

the largest lifespan extension, while approximately 66% CR (25 kcal per day) was less effective, and approximately 83% CR (12.5 kcal per day) shortened lifespan compared to ad libitum (75 kcal per day)[77]. Extreme levels of restriction, however, have resulted in extreme cases of longevity in mice and rats. Ross[6] reported a dramatic lifespan extension in Sprague–Dawley rats under approximately 80% CR (16 versus 83 kcal per day), with one rat living 1,638 days[6] (Fig. 1b). In mice, Weindruch et al.[8] showed a progressive lifespan increase with 25%, 55% and 65% CR (about 12, about 7 and about 6 kcal per day, respectively). Remarkably, the 10% longest-lived animals in the 65% CR group averaged around 1,611 days of age, with one mouse living 1,660 days[8] (Fig. 1c).

Weight and fat loss

Since the 1980s, it has been debated whether the benefits of DR reflect reduced adiposity and whether ad libitum laboratory feeding is effectively overfeeding, making CR a return to 'normal'[78–80]. However, Weindruch et al.[8] showed that 65% CR still produced a striking extension of maximum lifespan when compared to controls on 25% CR. Furthermore, Weindruch and Walford argued that because about 50% of wild mice die within 6 months, severe CR-induced delayed maturation and reduced fertility would probably reduce fitness in the wild, making it unlikely to reflect a natural physiological state[81,82]. Austad later found that while laboratory mice may eat more than their wild counterparts, they are not 'grossly overfed'[83]. Furthermore, wild mice in nature were found to often consume as much (or even more) energy than ad libitum-fed laboratory mice[84].

Evidence against the fat loss hypothesis, however, has been largely overlooked[85]. Recent articles still argue for CR and other longevity interventions as obesity treatments despite accumulating evidence[86]. In rats, lifespan extension by 40% CR does not appear to be influenced by body fat levels. In fact, the longest-lived rats in the CR group are those with the highest fat mass and maximum body fat percentage[87]. Mice with the longest survival under CR retain the greatest fat mass between their first and second years[75]. Similarly, diverse outbred mice maintaining the most weight and fat live longest, while weight loss at any age correlates with shorter lifespan[35]. Finally, nutritional geometry has shown that diets associated with the greatest longevity are also associated with higher adiposity (and reduced lean mass)[88]. One might argue that leaner animals were harmed by DR; however, more severe restriction (with greater weight loss) actually produces even greater lifespan extension without increased early deaths (Fig. 1b). Furthermore, in genetically obese (leptin-deficient; *ob/ob*) mice, DR extends lifespan beyond that of wild-type mice[89], even when having more than double the body fat[90].

Given the considerable evidence against the fat loss hypothesis, a more plausible hypothesis may involve changes in adipose tissue function. Beyond storing energy, adipose tissue functions as an endocrine organ that releases factors mediating cross-talk between metabolic regulation and inflammation[91,92].

Metabolic rate and body temperature

In 1908, Max Rubner observed that short-lived animals tend to have higher metabolic rates, while long-lived animals have slower ones[93]. Years later, the rate-of-living theory was formalized: live fast, die young[94]. These concepts led to suggestions that CR decreases the rate of aging by decreasing metabolic rate[95]. However, to comply with the laws of thermodynamics, an animal under CR must ultimately expend fewer calories[32], which can be achieved not only by reducing metabolic activity but also by reducing body size. Indeed, although CR rats initially consume fewer calories per gram of body weight, over their lifespan they actually consume more than ad libitum-fed rats when adjusted to body weight[96]. Curiously, as early as 1943, McCay had also observed that restricted rats had the same or even higher "heat production per unit of weight than normal rats"[97]. Further studies showed that although CR initially reduces metabolic rate, this effect is transient[98–100]. For most of their lives, CR rats exhibited metabolic rates comparable to or higher than ad libitum animals. Interestingly, McCarter et al.[101] showed that animals under 40% CR were voluntarily running more than ad libitum animals.

By the end of the 20th century, consensus was forming that at least in mammals, the increases in lifespan observed with CR might not be directly related to slowed growth or reduced metabolism[98,102,103], although conflicting evidence existed[104]. Instead, it was beginning to be appreciated that normalization to body size is confounded by the heterogeneous nature of organ size changes. While some organs scale down proportionally with body weight, others (most notably the brain) are relatively unaffected, an important observation as mass-specific metabolic rates differ across organs[105]. In an extensive analysis of different severities of CR in C57BL/6 mice, the overall basal metabolic rate was shown to fall under CR, but when organ size is taken into account, these differences disappear[106]. These investigators concluded that the apparent metabolic suppression under CR is simply an artifact of insufficient modeling, not a reflection of actual tissue-level hypometabolism. Accounting for these confounders has recently been reviewed[107].

Body temperature (T_b) and lifespan have been robustly linked[108,109]. In 1965, Liu and Walford[110,111] reported that reduced temperature extended lifespan in fish. This finding has since been extended to invertebrates, such as worms and flies[112,113]. These animals, however, are poikilotherms: their core T_b fluctuates with the environment. Meanwhile, mammals are homeotherms, meaning they must thermoregulate their T_b to a constant baseline despite changes in the environment (about 37 °C in humans). Walford's initial interest in CR arose while trying to reduce T_b in homeotherms[114]. In 1978, Walford's group showed that CR lowers T_b in mammals: mice on 40% CR showed an approximately 2.5-°C reduction in T_b relative to ad libitum controls[67]. This observation was further corroborated in male rats, where restricted animals had lower T_b at 19 months of age, even when 40% CR was initiated at 14 weeks of age[115].

https://doi.org/10.1038/s43587-026-01091-5

BOX 4

Thermotolerance

Data on how DR affects tolerance to extreme temperatures is limited. However, rare facility incidents provide some insights. In one case, a power failure raised ambient temperatures above 33 °C, affecting over 100 rats (22-month-old) on ad libitum or 40% CR diets. Twenty-four hours later, only 16% of the ad libitum group survived, compared to 75% of the CR group[313]. In another study, aged rats (>20-month-old) exposed to 41 °C showed 50% mortality in the ad libitum group, while all rats under 33% CR survived[314].

By contrast, when ambient temperature falls, CR may increase vulnerability. In 1939, McCay observed that restricted rats were less able than ad libitum-fed rats to withstand cold temperature during a heating-system failure[315]. This may be a reflection of reduced adipose insulation and the absence of huddling behavior in singly housed animals. Rats under DR exposed to cold stress exhibit a more rapid decline in T_b and a longer recovery time, irrespective of whether DR lasted 5 days or 16 months[316]. Such findings highlight an important disadvantage of DR in natural environments. However, ADF appears to increase cold resistance in old mice[317]. Beyond differences in species and cold severity, a plausible explanation is that the rat study produced greater weight loss, reducing insulation and energy reserves that normally buffer heat loss[317].

In mice, 40% CR lowers subcutaneous T_b in a strain-dependent manner, with reductions ranging from 1.5 °C to 5.0 °C relative to baseline[116]. In male C57BL/6 mice, T_b declines linearly with CR severity: −0.5 °C (10% CR), −0.7 °C (20% CR), −1.6 °C (30% CR) and −3.3 °C (40% CR) relative to ad libitum controls (36.5 °C), measured 11 weeks after CR initiation using intraperitoneal telemetry[117]. The kinetics scale similarly: the time to T_b nadir increased from about 19 days at 10% CR to about 35 days at 40% CR[117]. Some studies have not detected differences in T_b; however, this could reflect the methodologies used to measure temperature[46].

In rhesus monkeys, the gradual implementation of CR is accompanied by a fall in subcutaneous T_b, reaching significance at approximately 30% CR. Under anesthesia, monkeys on 30% CR show an approximately 0.5-°C lower rectal temperature than controls; this difference emerged by year 2 of CR and persisted even 6 years after initiation of CR[46]. In humans, however, the CALERIE trial did not detect a significant T_b difference between CR and ad libitum groups[62]. By contrast, long-term practitioners in the Calorie Restriction Society (about 6 years on CR) showed a small but significant lower T_b (about 0.2 °C lower) compared with both sedentary and exercise-trained individuals consuming a Western diet[118].

Unfortunately, T_b and metabolic rate are closely coupled, making distinguishing their roles complex. However, forced convection has been used for this purpose and showed that increasing T_b reduces lifespan, even when metabolic rate is reduced[119]. Additionally, transgenic mice overexpressing the uncoupling protein 2 (UCP2) in neurons located in the hypothalamus (effectively tricking the mouse thermostat into responding as if body temperature was increased) exhibit chronic lowering of the T_b and a significant increase in lifespan[120].

It is tempting to conclude that CR may increase lifespan primarily by lowering T_b, but, as Walford and Spindler highlighted[121], DR is also robust in animals that do not actively regulate T_b such as fish. They hypothesized that temperature responses to food scarcity would be mechanistically different from temperature changes driven by ambient temperature (Box 4) such as hypothermia[121]. Determining whether, and to what extent, reductions in T_b causally contribute to the effects of CR on lifespan remains an open question.

Hunger and fasting

A major barrier to translating DR to humans is the sensation of hunger. Although humans often report that hunger subsides over time when starting a diet, mice under 20% CR exhibit persistent hunger even after 100 days (equivalent to 11 human years)[122], suggesting that dieting humans may remain suboptimally restricted. Several neuropeptides involved in the hunger signaling pathway are triggered by CR. For example, CR reduces the levels of precursor polypeptide pro-opiomelanocortin (POMC) and the cocaine- and amphetamine-regulated transcript (CART), while increasing the levels of agouti-related peptide (AgRP) and neuropeptide Y (NPY)[123]. Supporting a role for hunger in the downstream effects of DR, damaging the region that produces these neuropeptides, the arcuate nucleus of the hypothalamus (ARC), or knocking out NPY reduces CR-induced protection against tumorigenesis[124]. Furthermore, the lifespan extension seen with 30% CR is abolished in NPY-null mice[125].

Restricted animals are so hungry that rats on 40% CR can consume most of their daily food within 3 h[126], leading to a prolonged fasting period until the next day's meal[127]. Yet, early studies showed that spreading 6 meals over a 12-h period made no difference to the lifespan extension seen with CR[128], a finding later supported using a more restrictive protocol with 2 meals spaced 8 h apart[129].

To eliminate the effects of fasting and hunger, investigators have used caloric dilution by incorporating indigestible cellulose (noncaloric) to lower food energy density[88,130]. Under these conditions, mice consume more food volume but are unable to fully compensate for the lower energy density, resulting in an overall approximately 30% reduction in caloric intake. Surprisingly, however, caloric dilution reduces lifespan, despite animals consuming substantially fewer calories and experiencing weight loss[130]. This raises questions about the potential toxicity of the dilutant, the requirement of fasting for lifespan extension and the physiological relevance of the total food volume. By contrast, it was later demonstrated that 40% CR extends lifespan even when meals are distributed evenly over 24 h[131]. Adding a daily 12-h fasting window further increased lifespan, but extending the fast to 22 h did not grant additional survival benefits. These observations possibly explain why earlier studies saw no effect from reducing fasting windows, as these still involved at least 12 h of fasting. Ultimately, caloric dilution and CR seem to produce distinct physiological effects and are thus not interchangeable strategies[132]. Hunger pathways may be responsible for the benefits observed when calories are not reduced by DR. For example, some metabolic and neuroprotective effects of IF can be dissociated from overall energy intake[133], and IF extended lifespan with little to no reduction in net caloric intake[35]. Further, time-restricted feeding (TRF) protects mice against diet-induced obesity even without a reduction in calorie intake[134].

A working model has proposed that imposing cycles of a metabolic challenge and recovery (eating, resting, sleeping) may bolster health beyond what can be achieved by continuous low-level CR[117]. The underlying cellular molecular mechanisms are being elucidated[135]. For example, the ketone β-hydroxybutyrate acts as a hormone and signaling molecule that mediates at least some important geroprotective and disease-modifying effects of IF[136–138]. During fasting, stress response signaling and resource conservation pathways are activated while cell growth and plasticity pathways are suppressed. During and for many hours after the feeding period, cell growth and plasticity pathways are activated[139].

Circadian regulation

Early work reported that meal timing (whether during light or dark periods) had no effect on DR-induced lifespan extension[128,140], suggesting the effect of DR on the circadian rhythm may be independent from its effects on longevity[141]. However, recent work has revealed a more complex relationship between circadian regulation and DR. Feeding a 40% CR diet at night, aligned with the rodents' natural activity,

increased lifespan extension[131]. Curiously, other DR protocols, such as the ketogenic diet, have been shown to influence circadian rhythms of the liver and intestine[142]. This area requires further investigation and may offer insights into the mechanisms of longevity and DR[143], especially considering the relationships between autophagy, metabolism and circadian rhythms[144].

Protein and amino acid restriction

Early DR research began to investigate whether specific nutrients drove DR benefits[82]. More recently, a large-scale study involving over 20 different diets and 858 mice showed that the most important factor for lifespan extension was the ratio of protein to carbohydrates[88]. However, the caloric content of these diets was modified through dilution, and as a result CR did not increase lifespan. Data suggest that CR with or without protein restriction results in identical lifespan extension, yet protein restriction may have benefits that are different and less pronounced than those from CR[32]. For example, protein restriction does not lower T_b in the same manner as CR under equivalent levels of restriction[117].

Individual amino acids may contribute more particularly to the effects of DR. Indeed, tryptophan restriction alone extends lifespan in rats[145], and methionine restriction also extends lifespan in F344 rats and outbred mice[146,147]. However, the reported lack of differences in lifespan between F344 rats under 40% CR with or without protein or methionine restriction[148] has been used to support claims that caloric intake rather than specific nutrient restriction is responsible for lifespan extension on CR; hence, the widespread adoption of the term 'calorie restriction', as opposed to 'food restriction' or DR[105]. The lack of additive lifespan extensions from protein or methionine restriction in animals already under CR suggests a shared pathway through which both energy and amino acid restriction act. More recently, restricting branched-chain amino acids has shown benefits for metabolic health and lifespan in mice[149–151]. Interestingly, supplementation (instead of restriction) of the amino acid glycine extends lifespan[152]. Tailoring essential amino acid intake timing and dose to match the organism's needs may enhance metabolic efficiency and reduce the negative effects of high protein load[74,153].

Potential DR mimetics

Introduced in 1998, the concept of CR mimetics proposed more potentially translatable interventions that could replicate CR benefits without reducing food intake[154].

Glycolytic inhibitors

The first candidate DR mimetic was 2-deoxy-D-glucose (2-DG). Lane et al.[154] proposed that 2-DG would trigger signaling pathways regulating DR by inhibiting the glycolytic pathway[154]. Results from a short-term study replicated some physiological effects of CR in rats, including reduced T_b and fasting insulin[154–156]. While 2-DG's narrow therapeutic window has limited its practical use, mannoheptulose, a rare seven-carbon sugar that inhibits hexokinase at the first step of glycolysis, has also been shown to lower glucose and insulin levels without affecting food intake in rodents[157,158].

Rapamycin

The nutrient-sensing kinase mTOR regulates growth through two related but functionally distinct complexes[159]. In 2008, CR was reported to suppress mTOR signaling across multiple mouse strains and tissues[160]. Although later studies have shown mixed results and suggest a more complex relationship[161–163], evidence from invertebrates and mTOR's role in nutrient sensing support its involvement in DR-mediated lifespan extension and point to rapamycin as a potential DR mimetic[164–166]. This led the NIA Intervention Testing Program (ITP) to test rapamycin in outbred mice, revealing a striking extension of lifespan when administered late in life[165,166]. However, some observations suggest that DR and rapamycin may work in distinct ways: (1) 40% CR significantly reduced the levels of insulin-like growth factor 1 and leptin, while rapamycin did not[167]; (2) fasting glucose is increased by rapamycin, while CR reduces it; and (3) CR decreased FGF21 in male and female mice, but rapamycin increased it in male mice only. This latter finding stands in contrast to fasting and protein restriction, which elevate FGF21 in both mice and humans[168–170]. Furthermore, CR can ameliorate an mTORC1-driven sarcopenic phenotype without inhibiting mTOR, and that combining CR with rapamycin results in additive and distinct effects in aging muscle[163]. Notably, rapamycin enhances survival during acute infections, whereas DR seems to impair it[171]. Finally, a recent meta-analysis suggests that rapamycin may confer similar lifespan extension to DR in a range of vertebrates[172].

Spermidine

Spermidine is a naturally occurring polyamine that promotes longevity in mice[173]. Levels have been shown to increase in response to various forms of DR across different species, and it has been suggested as essential for the induction of autophagy during fasting[174]. In C57BL/6 mice, spermidine supplementation was found to increase median lifespan by approximately 10% and to delay cardiac aging, whether lifelong administration or administered from 18 months of age[175]. Although the full mechanisms remain unclear, inhibition of the acetyltransferase EP300 may be a key contributor[176].

Sirtuin activators

The roles of sirtuins and their activators in aging biology remain controversial, including in rodent lifespan. Early work reported that in C57BL/6 mice fed a high-calorie diet with or without the purported sirtuin activator, resveratrol, starting at one year of age, by 114 weeks, 58% of control mice had died, compared to 42% of resveratrol-treated mice[177]. The full survival data later showed that resveratrol restored the shortened lifespan of high-calorie-fed mice to that of mice on a standard diet. However, no survival benefit was seen when resveratrol was given to mice on a standard diet[178]. Similarly, when resveratrol treatment was initiated at one year of age in genetically heterogeneous mice, the ITP found no lifespan extension[11]. Based on the hypothesis that, like DR, the effects of resveratrol might be stronger if initiated earlier, the ITP conducted a follow-up study beginning treatment at 4 months of age, but again, observed no effect on survival[179]. However, other studies in male C57BL/6 mice using alternative SIRT1 activators on standard diets have reported modest improvements in survival. For example, SRT2104 increased mean lifespan by 9.7% and maximum lifespan by 4.9%[180]. Similarly, SRT1720 extended mean lifespan in C57BL/6 mice by 8.8% but had no significant effect on median or maximum lifespan[181]. Disentangling the role of SIRT1 in DR-induced lifespan extension is difficult. Homozygous SIRT1 knockout mice are short-lived and do not benefit from 40% CR[182,183], meanwhile heterozygous SIRT1 knockout mice on 40% CR show the same increase in median lifespan as wild-type animals[184]. A possible interpretation is that total SIRT1 loss may shorten lifespan via a distinct pathway that CR cannot rescue, and as a result, CR's effects on survival are masked through early death, whereas partial SIRT1 loss sufficiently preserves lifespan to allow survival benefits of CR to be realized.

NAD^+ precursors

Nicotinamide adenine dinucleotide (NAD^+) is an essential metabolite for life that has been observed to decline with age and is linked to multiple hallmarks of aging[185]. Interest in its therapeutic potential increased after studies showed improved healthspan and lifespan in models of premature aging, with several clinical trials now completed or ongoing (reviewed elsewhere[186,187]). Unfortunately, these effects appear less pronounced in wild-type animals. In old C57BL/6 mice (22–24 months old), the NAD^+ precursor nicotinamide riboside increased mean lifespan by about 5% upon 6 weeks of treatment (400 mg per kg body weight)[188]. By contrast, the ITP found no effect on lifespan in UM-HET3 mice on

nicotinamide riboside (1,000 mg per kg body weight) continuously from 8 months of age[189]. In other studies, the NAD^+ precursors nicotinamide mononucleotide (100 or 300 mg per kg body weight; 5–17 months of age) and nicotinamide (500 or 1,000 mg per kg body weight; from 1 year of age) improved the health but not survival of C57BL/6 mice[190,191].

Acarbose, metformin and lithocholic acid

Three other drugs investigated for their potential to counteract aging are the diabetes medications acarbose and metformin, and the bile acid lithocholic acid. Acarbose is an α-glucosidase inhibitor that has been shown by the ITP to increase both median and maximum lifespan[192,193]. Metformin is an AMPK activator, but ITP studies have failed to show effects on lifespan when administered alone[193]. Lithocholic acid increased lifespan in nematodes and flies (but not in mice) in an AMPK-dependent manner[194].

GLP-1R agonists

Long-acting glucagon-like peptide-1 receptor (GLP-1R) agonists such as semaglutide have transformed the treatment landscape for diabetes and obesity. This class of drugs promotes weight loss and reduces cardiovascular mortality even in nondiabetic patients[195–197]. GLP-1R agonists were initially recognized for their ability to enhance glucose-stimulated insulin secretion and thereby facilitate glycemic control[198]. Their metabolic benefits also involve suppression of glucagon secretion and delayed gastric emptying[198]. Beyond glycemia, the effects of GLP-1R agonists on appetite and body weight are predominantly mediated by GLP-1R-expressing neurons in the hypothalamus and brainstem[199]. Within the hypothalamus, these agents directly activate POMC neurons[200] while indirectly suppressing AgRP neuron activity[201].

Consequently, GLP-1R agonist-induced weight loss differs fundamentally from that induced by CR, which engages orexigenic circuits, including AgRP neurons, to promote hunger and reduce satiety. By contrast, GLP-1R agonists robustly suppress appetite and prolong satiety[202]. CR also elicits a characteristic 'metabolic adaptation', a disproportionate decline in energy expenditure that contributes to weight regain. Interestingly, when CR is followed by GLP-1R agonist treatment, this adaptive response is counteracted, that is, semaglutide restores the CR-induced decrease in energy expenditure[203]. Whether this effect translates to humans remains uncertain[204]. Although GLP-1R agonists can be categorized as DR inducers rather than DR mimetics, their ability to bypass the nutrient-sensing and hunger-promoting responses typically associated with CR raises new questions, such as whether GLP-1R agonists can extend lifespan to a similar extent as CR on matched caloric intake. These agents may elucidate the importance of hunger in the extension of lifespan on DR. Finally, the emergence of potent, clinically validated oral GLP-1R agonists[205] may enable testing whether sustained GLP-1R activation can extend maximal lifespan in laboratory rodents, including in paradigms compatible with long-term drug administration. A practical limitation, however, is that current small-molecule GLP-1R agonists show weak activity at the rodent receptor. Instead, humanized GLP-1R models are required to help to clarify the effects on health and longevity[206].

The field of obesity pharmacotherapy is advancing at remarkable speed, with next-generation, multi-agonist drugs now targeting receptors beyond GLP-1R, including the glucose-dependent insulinotropic polypeptide receptor (GIPR), the glucagon receptor (GCGR) and the amylin receptor[199]. As our understanding of the molecular pathways that govern healthspan deepens, future work in drug development should aim to bridge insights from weight loss trials with discoveries in aging biology.

Negative effects from DR

Recent work in diverse outbred mice highlights that improving physical fitness and extending lifespan are not necessarily synonymous: despite having the largest lifespan-extending effect, 40% CR led to loss of lean mass and immune changes that may increase susceptibility to infections[35]. While potential side effects of DR including effects on bone health, brain atrophy and reproductive function have been reviewed elsewhere[207], here we examine the effects of DR on immune function and tissue regeneration.

Immunity and infections

Immune function declines during aging, rendering older individuals vulnerable to morbidity and mortality upon infection[208,209]. Early observations showed that CR had a profound effect on the immune system, leading to the hypothesis that CR might slow the development of the immune system[210]. While numerous immune-related benefits have been documented, several concerns remain[207,211,212]. For example, nonhuman primates under 30% CR elicit a lower antibody response to the influenza vaccine[213]. In diverse outbred mice, 40% CR induces changes to the immune cell repertoire, including reductions in circulating B cells, mature natural killer cells and eosinophils[35]. However, the clinical relevance of these immune alterations remains unclear without in vivo infections. A meta-analysis of infection experiments tentatively concluded that CR makes mice less likely to survive infections[171], but this analysis was limited by the small number of studies and large heterogeneity.

Sepsis

In C57BL/6 mice, exposure to a 40% CR diet from 6 weeks of age resulted in significantly shorter survival compared to mice fed ad libitum upon polymicrobial sepsis induction at 6 months by cecal ligation and puncture[214]. Similarly, upon a drastic reduction of food for 7 days (146 g per kg versus only 35.5 g per kg of body weight), restricted mice exhibited a dramatic reduction in survival[215]. By contrast, another study induced polymicrobial sepsis by injecting 12-month-old C57BL/6 mice intraperitoneally with cecal contents. Remarkably, mice on 40% CR initiated just 3 weeks before infection showed improved survival relative to ad libitum controls[216]. Furthermore, ADF initiated 8 days before cecal ligation and puncture improved survival in male C57BL/6 mice[217].

Bacterial infection

In female A/J mice on various levels of protein restriction, with or without 50% CR for 3 weeks, before *Salmonella* infection, mild but not severe CR appeared to be protective, and survival worsened with increasing severity of protein restriction[218]. By contrast, in 6-month-old male Balb/c mice (40% CR from 2 months of age) infected with *Salmonella*, no significant difference in survival compared to ad libitum controls was observed[219].

Parasitic infection

In the 1960s–1970s, some reports suggested malnourished children had fewer malaria symptoms, with refeeding sometimes linked to symptom resurgence[220]. In *Plasmodium berghei*-infected mice, exposure to different forms of DR reduced mortality (10–53%) compared to ad libitum animals[221]. Similarly, in young female C57BL/6 mice (8–10 weeks old) on graded CR (10–50%, 7 days before infection), most ad libitum mice died within 12 days, but none of the 40% CR mice died or showed symptoms[222]. Notably, initiating CR on the day of infection—unlike 2 days after infection—still conferred protection.

Viral infection

Aged C57BL/6 mice (>23 months old) on 40% CR from 3 months of age experienced further weight loss upon influenza infection, and showed significantly shorter survival compared to ad libitum animals[223]. These findings were replicated in 6-month-old mice, and further, ad libitum refeeding for 14 days before infection dramatically improved survival in the CR group[224,225]. Similarly, 40% CR reduced survival after West Nile virus infection in both young (4-month-old) and old (>18-month-old) mice of the same strain[226].

The relationship between DR and immunity is complex and dependent on context and environmental conditions but also on the specific metrics used to assess immune function. Caution is warranted as our understanding is clouded by the reliance of most modern studies on specific pathogen-free conditions, which may not accurately reflect the immunological challenges encountered in natural environments[227]. Given that CR produced similar outcomes across two different viral infections, these findings strongly suggest that long-term CR may increase vulnerability to viral pathogens. While Walford hypothesized that CR delays immune system development[210], this would not fully explain the weakened immune response, as short periods of refeeding seem to reverse the effect, and older animals remain affected. Interestingly, CR may offer some protection against parasitic infections or sepsis, but mechanistic understanding is missing.

Tissue repair and regeneration

An intuitively expected downside of reduced energy intake is impaired tissue repair. In mammals, this can manifest as slowed hair growth and delayed wound healing.

Wound healing

In male C57BL/6J mice and in B6CBAF1 mice, tail wounds heal more slowly under approximately 33% CR than with ad libitum feeding[7]. There is conflicting evidence linking CR to collagen synthesis upon wounding in rats: in F344 rats, acute (7–14 days) and chronic (4 months) 40% CR reduced collagen crosslinking, and chronic CR decreased collagen accumulation[228], yet no changes in collagen or protein synthesis under acute CR in female Sprague–Dawley rats were reported[229]. Finally, only complete absence of protein (100% protein restriction) significantly impaired wound contraction and re-epithelialization in a study using graded protein restriction[230].

Conversely, other data suggest no delay in wound healing in aged CR mice (30–33 months) versus ad libitum controls and even reported restoration of age-related decline in healing upon refeeding before injury, suggesting that CR could preserve regenerative capacity but requires adequate nutrition to be realized[231]. The absence of differences between ad libitum and CR mice may reflect a general decline in regenerative capacity in both groups; however, other work in a different strain reported differences between CR and ad libitum mice at 22 months[7]. In 7-month-old F344 rats, the slower healing observed under 40% CR was rescued by refeeding 4 weeks before a skin biopsy punch[232]. However, contrasting observations were made in male Wistar rats on 40% CR (from 3 months of age) and in rhesus monkeys (ages 9–13 and 22–32) under 30% CR[233]: while a marked decline in wound closure speed was observed with aging in both species, no statistical differences were found between ad libitum and CR groups.

Hair growth

In male C57BL/6J mice, approximately 33% CR has been shown to slow hair regrowth[7]. However, this appears to be a strain-dependent effect; in B6CBAF1 mice, CR actually accelerated regrowth compared to ad libitum controls (69% versus 53%), a trend that persisted in older age (67% versus 36%)[7]. Similarly, Swiss mice on a 40% CR diet initiated at 8 weeks of age exhibited faster hair regrowth[234]. In these mice, the regrown coat appeared thicker and longer, suggesting a thermoprotective adaptation to cold stress often associated with CR. By contrast, various forms of TRF (including 21/3, 19/5 and 16/8) as well as ADF, significantly impaired hair regrowth in C57BL/6 mice[235]. Interestingly, when the fasting window was further reduced to 12/12 TRF, hair growth rates were nearly identical to those of ad libitum-fed animals, despite all groups consuming equivalent daily caloric intake. In parallel, the authors conducted a human trial and found that both CR (1,200–1,500 kcal per day) and TRF (18/6) slowed hair growth compared to control participants[235]. Further studies assessing different degrees of restriction at various ages, both with and without refeeding, should help to clarify the role of DR in hair growth.

Diseases and injury

There is considerable interest in determining whether DR interventions can be used to prevent and treat diseases. Animal studies support the potential of DR for cancer, neurodegeneration and cardiovascular disease, and recent clinical trials suggest that some forms of DR translate well to human conditions[236–238]. We urge caution when interpreting human studies, as it remains unclear to what extent the observed benefits reflect the treatment of overweight/obesity rather than the mechanisms elicited by the severe restrictions used in laboratory animals.

Cancer

Cancer is the major cause of death in rodents such as C57BL6 mice[239], and it was observed in very early work that tumors did not grow as quickly in underfed mice[240]. This was later supported by evidence that underfeeding delays and reduces the occurrence of induced and spontaneous tumors in mice[241,242]. Further, compared to those fed ad libitum, female rats maintained on lifelong IF developed fewer mammary tumors[57], later replicated and extended to other types of cancer[1,8]. Curiously, others have attributed DR effects on lifespan to cancer delay. In an analysis of 200 phenotypes in old male C57BL/6J mice on ADF, limited effects on readouts of aging were observed[243], leading the authors to argue that the increase in longevity may be attributable to delaying the onset of neoplasia. This interpretation is less straightforward when extended to species where DR extends lifespan and in which cancer does not dominate mortality, and a critical point for translation to humans where demographic analysis indicates that eliminating cancer specifically would increase overall life expectancy by only about 3 years, suggesting that aging reflects a broader systemic decline that cannot be resolved by targeting any single pathology alone[244,245]. Nonetheless, cancer suppression is a conserved feature of DR: the NIA and UW showed that CR also delays the development of spontaneous tumors in rhesus monkeys[48,49]. More recent work has reported that DR can suppress the growth of a range of cancers (breast, pancreatic, hepatic, melanoma) in mouse models in which the cancer cells were implanted subcutaneously or where cancers were induced by exposure to a carcinogenic chemical[246–250]. One study in which Balb/c mice exposed to a range of dietary regimens were implanted with a highly metastatic breast cancer reported that diet cycling (4 days of a low-calorie diet, followed by 10 days ad libitum) slowed primary tumor growth, regardless of diet composition[251]. However, daily 20% CR slowed tumor growth more, and unlike diet cycling, significantly reduced lung metastases. CR also showed a dose-dependent effect (10–40%), with greater restriction producing larger reductions in tumor mass and growth rate. Recent exploration of the anticancer effects of DR highlights two main mechanisms: metabolic compromise of cancer cells and stimulation of immune surveillance[252–254]. Hursting et al.[255] found that 40% CR suppresses tumor development in cancer-prone p53-deficient mice, suggesting an antitumor mechanism upstream of p53. Finally, clinical trials in patients with cancer suggest that DR can complement chemotherapy and radiation[256].

Cognition and neurological disorders

Early evidence showed that approximately 40% CR from youth preserved learning, memory and motor function in female mice at 31–35 months, indicating that DR could mitigate age-related cognitive decline[257]. In a randomized 8-week trial, either a healthy diet or the same diet combined with IF (5:2) improved cognition and lowered BrainAGE in adults ≥55 years with insulin resistance[258]. Further, in a randomized controlled trial of obese postmenopausal women in which participants followed an 8-week very-low-calorie diet (about 800 kcal per day) followed by a phase of weight maintenance, increases in regional gray matter density and cognitive measures were observed. However, the increase in gray matter density waned after the maintenance phase, and specific cognitive aspects such as improvements in recognition

https://doi.org/10.1038/s43587-026-01091-5

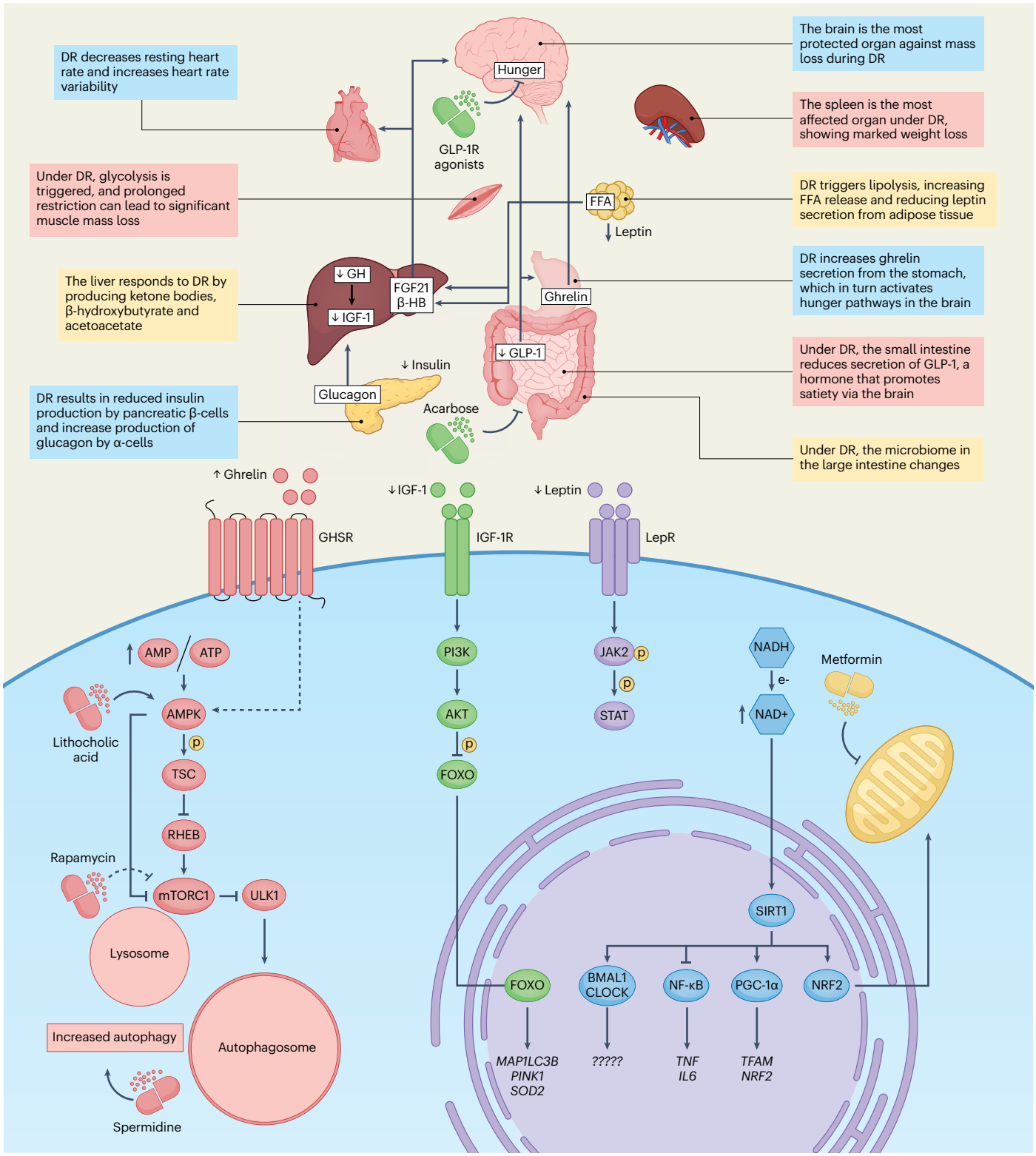


Fig. 3 | Physiological and cellular effects of DR and DR mimetics. The illustration summarizes some of the effects that DR and DR mimetics seem to have on the organism. The effects may vary or overlap between different DRs: for example, under CR, FGF21 is decreased[167]; meanwhile, fasting and protein restriction elevate FGF21 in both mice and humans[168–170]. These differences highlight the need for comprehensive disentanglement of diet-specific effects to further understand overlap and mechanistic differences. In addition, the role of some of these changes in promoting survival remains unclear. FFA, free fatty acid.

memory disappeared[259]. In a CALERIE pilot, 6 months of CR in participants with overweight had no measurable effect in cognitive performance (for example, memory and attention)[260]. The generalizability of these studies involving participants with overweight or obesity may be limited, however.

Suggesting therapeutic relevance for Alzheimer's disease, several studies across species and strains have reported effects of DR on amyloid-β (Aβ) load. Using a transgenic Alzheimer's disease mouse model (Tg2576), 30% CR initiated at 3 months was shown to lower $Aβ_{1–40}$ and $Aβ_{1–42}$ levels versus controls in both hippocampus and neocortex[261].

Similarly, 40% CR initiated at 14–15 weeks (for 6 weeks) in the J20 mouse and initiated at 9 weeks (for 14 weeks) in the PS/APP mouse reduced the development of Aβ plaque by 40–55% in the cortex, and only for J20 in the hippocampus[262]. CR of 40% was shown to ameliorate Aβ volume in the APPSwe/PSEN1dE9 mouse even when initiated at 13–14 months of age[263]. However, one study in the APPswe/PSEN1dE9 (line 85) mouse reports that ADF started at 5 months of age (for 5 months) reduced $A\beta_{1-42}$ plaques in the cerebral cortex[264]. In the 3xTg mouse, the Mattson group showed that both ADF and 40% CR started at 5 months protected the mice from cognitive deficits in learning and memory[265]. However, while 40% CR reduced the development of $A\beta_{1-40}$, $A\beta_{1-42}$ and phospho-tau, ADF had no effect on these measures. Both 30% CR and 30% caloric dilution started at 6 months of age (for 9 months) significantly reduced Aβ plaque density, but only CR reduced phospho-tau[266]. Meanwhile, a study in the 5xFAD (B6SJL) mouse reported that ADF started at 2 months of age (for 4 months) did not affect Aβ levels in the cortex or hippocampus and led to increased inflammation in the cortex, reduction in synaptic plasticity and neuronal injury[267]. By contrast, in the same model, ADF started at 3 months of age (for 5.5–6 months) reduced Aβ in both cortex and hippocampus and attenuated glial overactivation and cognitive decline[268]. Finally, squirrel monkeys under 30% CR exhibit reduced levels of $A\beta_{1-40}$ and $A\beta_{1-42}$ in the temporal cortex[269].

Beyond Alzheimer's disease, DR has shown potential benefit in models of neurological conditions including frontotemporal dementia, multiple sclerosis and traumatic brain injury[270–273]. For example, DR was shown to reduce the vulnerability of the brain to acute insults and age-related neurodegenerative disorders. In the first three studies, rats or mice were maintained for several months on either ADF (eating about 30–40% less) or ad libitum feeding, and subjected to acute brain insults in models of relevance to epilepsy (exposure to the excitotoxin kainic acid), Huntington's disease (exposure to the mitochondrial toxin 3-nitropropionic acid), Parkinson's disease (exposure to the dopaminergic neurotoxin MPTP) or stroke (middle cerebral artery occlusion and reperfusion). All the animals in the ADF group exhibited less damage to neurons and improved functional outcomes associated with the particular neurons affected by the insults[274–276]. Reports of clinical trials of DR in patients with neurological disorders are sparse. DR has been reported to have beneficial effects on well-being and disease biomarkers in patients with multiple sclerosis[277,278]. There are numerous clinical trials of DR, particularly IF, in patients with a range of neurological disorders currently in progress, including trials for patients with epilepsy, early Alzheimer's disease, Parkinson's disease and stroke.

Cardiovascular disease

During World War II, food restrictions in Oslo, Norway reduced average daily caloric intake by about 18%: from 3,470 kcal (1936–37) to 2,850 kcal (1942–45). Alongside weight loss, deaths from circulatory diseases fell by about 21% in 1943–1945 compared with 1938–1940, then rose again after the war[279]. Today it is well established that DR can reduce risk factors of cardiovascular disease, including hypercholesterolemia, abdominal obesity and insulin resistance. Adults (35–82 years old) following a CR diet for 3–15 years show favorable atherosclerosis risk profiles: levels of low-density lipoprotein and total cholesterol in the lowest 10% for their age group, triglycerides in the lowest 5% among 20 year olds and systolic/diastolic blood pressure similar to that of 10 year olds[280]. In normotensive men with overweight, CR also reduces blood pressure[281]. In people with obesity, 20 days of a very-low-calorie diet improved left ventricular function and reduced systolic blood pressure[282]. However, rats fed ADF (eating about 30% less) exhibit a decrease in resting heart rate and blood pressure, which return to baseline within 2 weeks of resuming ad libitum feeding[283]. Furthermore, reduced heart rate variability is a sign of heart failure in humans, yet both 40% CR and ADF have been shown to increase it in rats[284]. In rat ischemic injury, 55% CR improved cardiac recovery after global ischemia–reperfusion, whereas exercise alone did not[285]. Rats maintained on ADF for 3 months before coronary artery ligation experienced less cardiomyocyte apoptosis and improved survival[286]. A later study showed that ADF even if initiated 2 weeks after ligation was successful at improving cardiac function and survival[287]. Very recently, a clinical trial in people after a heart attack showed that TRF (16/8) improved heart function and reduced diastolic blood pressure[288]. Further clinical trials of DR in patients who suffer from other chronic or acute cardiological conditions are warranted.

Concluding comments and future perspectives

To date, to our knowledge, no published survival curve has shown a laboratory mouse that has outlived the longest-lived male mouse (1,742 days) from Harrison and Archer on approximately 30% CR[7], or the longest-lived female mouse from Weindruch et al. (1,660 days), in which 65% CR increased maximum lifespan by 51%[8] (Fig. 1b). Notably, this extreme CR regimen also followed an ADF schedule, with food provided on Monday and Wednesday and a double portion on Friday. Likewise, no individual rat has exceeded the lifespan of the longest-lived rat (1,638 days) reported by Ross[6], where 80% CR extended maximum lifespan by about 83%[6] (Fig. 1c). Furthermore, a male rhesus monkey from the NIA cohort fed a CR diet that lived for 44.2 years represents the longest lifespan ever recorded for this species[52]. Thus, despite the expanding universe of longevity interventions, DR remains the force defining the ceiling of maximum lifespan.

A major complexity of DR research is that it is not a single intervention or mechanism but a family of related regimens and processes that are often conflated by experimental design. Studies that disentangle the physiological effects from each specific scenario, for example, energy restriction versus periods of complete food absence, thus hold particular value. Similarly, studies should isolate the effects on health attributable to (1) obesity, (2) the dynamic state of active weight loss, (3) a maintenance state at a healthy body weight and (4) chronic maintenance at an unusually low body weight. Clarifying these components will not only define the nature of DR, but also potentially shed light on the mechanisms of aging.

The challenge is further complicated by the fact that most of our genetic understanding comes from invertebrate models, where different protocols produce different outcomes[37]. Furthermore, differences in strain, severity of restriction and distinct regimens may operate via distinct combinations of pathways, perhaps sharing some but not all mechanistic features that are causal to lifespan extension. Nevertheless, we compiled a molecular model that shares features from multiple forms of DR and that may be applicable to mammals (Fig. 3). Some hints suggest the involvement of cellular pathways related to mTORC1, autophagy, FOXOs, NAD^+ and sirtuins.

There is an urgent need to systematically catalog and interrogate these shared and distinct molecular signatures of different DRs, including whether they exert causal effects on healthspan and lifespan. Currently, it is still unclear what type and level of restriction would be optimal for human translation. Approaches like the geometric framework may further elucidate dietary responses in animals and help to disentangle the varied number of interventions under the umbrella of DR[289]. Moreover, given the complex role of hunger, simply inhibiting the pathway that drives calorie intake may not be sufficient, or even appropriate. Finally, whether early-life diets will have effects in later life[73] and the real-world implications of DR remain matters of investigation. Given the global 'silver tsunami' and the accompanying healthcare pressures, and growing public engagement in health, feasible strategies to implement CR in humans (while preserving its benefits) and clinically validated DR mimetics are urgent priorities with major potential health and socioeconomic impact[290–292].

References

1. Weindruch, R. & Walford, R. L. Dietary restriction in mice beginning at 1 year of age: effect on life-span and spontaneous cancer incidence. *Science* **215**, 1415–1418 (1982).

2. Goodrick, C. L., Ingram, D. K., Reynolds, M. A., Freeman, J. R. & Cider, N. L. Differential effects of intermittent feeding and voluntary exercise on body weight and lifespan in adult rats. *J. Gerontol.* **38**, 36–45 (1983).
3. Yu, B. P., Masoro, E. J. & McMahan, C. A. Nutritional influences on aging of Fischer 344 rats: I. Physical, metabolic, and longevity characteristics. *J. Gerontol.* **40**, 657–670 (1985).
4. Beauchene, R. E., Bales, C. W., Bragg, C. S., Hawkins, S. T. & Mason, R. L. Effect of age of initiation of feed restriction on growth, body composition, and longevity of rats. *J. Gerontol.* **41**, 13–19 (1986).
5. Goodrick, C. L., Ingram, D. K., Reynolds, M. A., Freeman, J. R. & Cider, N. L. Effects of intermittent feeding upon growth and life span in rats. *Gerontology* **28**, 233–241 (1982).
6. Ross, M. H. Length of life and nutrition in the rat. *J. Nutr.* **75**, 197–210 (1961).
7. Harrison, D. E. & Archer, J. R. Genetic differences in effects of food restriction on aging in mice. *J. Nutr.* **117**, 376–382 (1987).
8. Weindruch, R., Walford, R. L., Fligiel, S. & Guthrie, D. The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. *J. Nutr.* **116**, 641–654 (1986).
9. Bartke, A. et al. Extending the lifespan of long-lived mice. *Nature* **414**, 412–412 (2001).
10. Flurkey, K., Astle, C. M. & Harrison, D. E. Life extension by diet restriction and *N*-acetyl-L-cysteine in genetically heterogeneous mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **65**, 1275–1284 (2010).
11. Miller, R. A. et al. Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **66**, 191–201 (2011).
12. Kealy, R. D. et al. Effects of diet restriction on life span and age-related changes in dogs. *J. Am. Vet. Med. Assoc.* **220**, 1315–1320 (2002).
13. Pinney, D. O., Stephens, D. F. & Pope, L. S. Lifetime effects of winter supplemental feed level and age at first parturition on range beef cows. *J. Anim. Sci.* **34**, 1067–1074 (1972).
14. Comfort, A. The effect of age on growth-resumption in fish (*Lebistes*) checked by food restriction. *Gerontologia* **4**, 177–186 (1960).
15. Austad, S. N. Life extension by dietary restriction in the bowl and doily spider, *Frontinella pyramitela*. *Exp. Gerontol.* **24**, 83–92 (1989).
16. Chmilar, S. L. et al. Caloric restriction extends lifespan in a clonal plant. *Ecol. Lett.* **27**, e14444 (2024).
17. Minina, E. A. et al. Autophagy mediates caloric restriction-induced lifespan extension in *Arabidopsis*. *Aging Cell* **12**, 327–329 (2013).
18. Turturro, A. et al. Growth curves and survival characteristics of the animals used in the biomarkers of aging program. *J. Gerontol. A Biol. Sci. Med. Sci.* **54**, B492–B501 (1999).
19. Swindell, W. R. Dietary restriction in rats and mice: a meta-analysis and review of the evidence for genotype-dependent effects on lifespan. *Ageing Res. Rev.* **11**, 254–270 (2012).
20. Brown-Borg, H. M., Borg, K. E., Meliska, C. J. & Bartke, A. Dwarf mice and the ageing process. *Nature* **384**, 33–33 (1996).
21. Bartke, A. & Brown-Borg, H. Life extension in the dwarf mouse. *Curr. Top. Dev. Biol.* **63**, 189–225 (2004).
22. Mattison, J. A. et al. Studies of aging in ames dwarf mice: effects of caloric restriction. *J. Am. Aging Assoc.* **23**, 9–16 (2000).
23. Sun, L. Y. et al. Growth hormone-releasing hormone disruption extends lifespan and regulates response to caloric restriction in mice. *eLife* **2**, e01098 (2013).
24. Arum, O., Bonkowski, M. S., Rocha, J. S. & Bartke, A. The growth hormone receptor gene-disrupted mouse fails to respond to an intermittent fasting diet. *Aging Cell* **8**, 756–760 (2009).
25. Bonkowski, M. S., Rocha, J. S., Masternak, M. M., Regaiey, K. A. A. & Bartke, A. Targeted disruption of growth hormone receptor interferes with the beneficial actions of calorie restriction. *Proc. Natl Acad. Sci. USA* **103**, 7901–7905 (2006).
26. Smolen, A., Smolen, T. N. & van de Kamp, J. L. Sensitivity of inbred and selectively bred mice to ethanol. *Alcohol* **4**, 57–62 (1987).
27. Liao, C., Rikke, B. A., Johnson, T. E., Diaz, V. & Nelson, J. F. Genetic variation in the murine lifespan response to dietary restriction: from life extension to life shortening. *Aging Cell* **9**, 92–95 (2010).
28. Mattson, M. P. Genes and behavior interact to determine mortality in mice when food is scarce and competition fierce. *Aging Cell* **9**, 448–449 (2010).
29. Ikeno, Y. et al. Housing density does not influence the longevity effect of calorie restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **60**, 1510–1517 (2005).
30. Liao, C., Rikke, B. A., Johnson, T. E., Diaz, V. & Nelson, J. F. No evidence that competition for food underlies lifespan shortening by dietary restriction in multiply housed mice: response to commentary. *Aging Cell* **9**, 450–452 (2010).
31. Rikke, B. A., Liao, C. -Y., McQueen, M. B., Nelson, J. F. & Johnson, T. E. Genetic dissection of dietary restriction in mice supports the metabolic efficiency model of life extension. *Exp. Gerontol.* **45**, 691–701 (2010).
32. Speakman, J. R. & Mitchell, S. E. Caloric restriction. *Mol. Aspects Med.* **32**, 159–221 (2011).
33. Senior, A. M. Estimating genetic variance in life-span response to diet: insights from statistical simulation. *J. Gerontol. A Biol. Sci. Med. Sci.* **78**, 392–396 (2022).
34. Unnikrishnan, A. et al. Reevaluation of the effect of dietary restriction on different recombinant inbred lines of male and female mice. *Aging Cell* **20**, e13500 (2021).
35. Francesco, A. D. et al. Dietary restriction impacts health and lifespan of genetically diverse mice. *Nature* https://doi.org/10.1038/s41586-024-08026-3 (2024).
36. Shenhar, B. et al. Heritability of intrinsic human life span is about 50% when confounding factors are addressed. *Science* **391**, 504–510 (2026).
37. Kapahi, P., Kaeberlein, M. & Hansen, M. Dietary restriction and lifespan: lessons from invertebrate models. *Ageing Res. Rev.* **39**, 3–14 (2017).
38. Carey, J. R. et al. Life history response of Mediterranean fruit flies to dietary restriction. *Aging Cell* **1**, 140–148 (2002).
39. Carey, J. R. et al. Food pulses increase longevity and induce cyclical egg production in Mediterranean fruit flies. *Funct. Ecol.* **16**, 313–325 (2002).
40. Carey, J. R. et al. Stochastic dietary restriction using a Markov-chain feeding protocol elicits complex, life history response in medflies. *Aging Cell* **4**, 31–39 (2005).
41. Fanson, B. G., Weldon, C. W., Pérez-Staples, D., Simpson, S. J. & Taylor, P. W. Nutrients, not caloric restriction, extend lifespan in Queensland fruit flies (*Bactrocera tryoni*). *Aging Cell* **8**, 514–523 (2009).
42. Hansen, B. C., Ortmeyer, H. K. & Bodkin, N. L. Prevention of obesity in middle-aged monkeys: food intake during body weight clamp. *Obes. Res.* **3**, 199s–204s (1995).
43. Bodkin, N. L., Alexander, T. M., Ortmeyer, H. K., Johnson, E. & Hansen, B. C. Mortality and morbidity in laboratory-maintained rhesus monkeys and effects of long-term dietary restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **58**, B212–B219 (2003).
44. Lane, M. A., Mattison, J. A., Roth, G. S., Brant, L. J. & Ingram, D. K. Effects of long-term diet restriction on aging and longevity in primates remain uncertain. *J. Gerontol. A Biol. Sci. Med. Sci.* **59**, B405–B407 (2004).
45. Ingram, D. K. et al. Dietary restriction and aging: the initiation of a primate study. *J. Gerontol.* **45**, B148–B163 (1990).
46. Lane, M. A. et al. Calorie restriction lowers body temperature in rhesus monkeys, consistent with a postulated anti-aging mechanism in rodents. *Proc. Natl Acad. Sci. USA* **93**, 4159–4164 (1996).

47. Lane, M. A., Mattison, J., Ingram, D. K. & Roth, G. S. Caloric restriction and aging in primates: relevance to humans and possible CR mimetics. *Microsc. Res. Tech.* **59**, 335–338 (2002).
48. Mattison, J. A. et al. Impact of caloric restriction on health and survival in rhesus monkeys from the NIA study. *Nature* **489**, 318–321 (2012).
49. Colman, R. J. et al. Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science* **325**, 201–204 (2009).
50. Mattison, J. A. et al. Caloric restriction improves health and survival of rhesus monkeys. *Nat. Commun.* **8**, 14063 (2017).
51. Mitchell, S. J. et al. Daily fasting improves health and survival in male mice independent of diet composition and calories. *Cell Metab* **29**, 221–228 (2019).
52. Stayer-Wilburn, O. et al. Dysregulation of astrocytic Aquaporin-1 in the brains of oldest-old rhesus macaques: the NIA caloric restriction study. *GeroScience* **47**, 781–793 (2025).
53. Weindruch, R. et al. Measures of body size and growth in rhesus and squirrel monkeys subjected to long-term dietary restriction. *Am. J. Primatol.* **35**, 207–228 (1995).
54. Stuart, J. A. & Page, M. M. Plasma IGF-1 is negatively correlated with body mass in a comparison of 36 mammalian species. *Mech. Ageing Dev.* **131**, 591–598 (2010).
55. Pifferi, F. et al. Caloric restriction increases lifespan but affects brain integrity in grey mouse lemur primates. *Commun. Biol.* **1**, 30 (2018).
56. Pifferi, F. et al. Promoting healthspan and lifespan with caloric restriction in primates. *Commun. Biol.* **2**, 107 (2019).
57. Carlson, A. J. & Hoelzel, F. Apparent prolongation of the life span of rats by intermittent fasting one figure. *J. Nutr.* **31**, 363–375 (1946).
58. Pietrocola, F. et al. Metabolic effects of fasting on human and mouse blood in vivo. *Autophagy* **13**, 567–578 (2017).
59. The CALERIE Research Group. *Final Protocol for the Phase 2 Study*, version 1.5 (The CALERIE Research Group, 2009).
60. Most, J., Tosti, V., Redman, L. M. & Fontana, L. Calorie restriction in humans: an update. *Ageing Res. Rev.* **39**, 36–45 (2017).
61. Stewart, T. M. et al. Comprehensive assessment of long-term effects of reducing intake of energy phase 2 (CALERIE Phase 2) screening and recruitment: methods and results. *Contemp. Clin. Trials* **34**, 10–20 (2013).
62. Ravussin, E. et al. A 2-year randomized controlled trial of human caloric restriction: feasibility and effects on predictors of health span and longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **70**, 1097–1104 (2015).
63. Shen, W. et al. Effect of 2-year caloric restriction on organ and tissue size in nonobese 21- to 50-year-old adults in a randomized clinical trial: the CALERIE study. *Am. J. Clin. Nutr.* **114**, 1295–1303 (2021).
64. Kraus, W. E. et al. 2 years of calorie restriction and cardiometabolic risk (CALERIE): exploratory outcomes of a multicentre, phase 2, randomised controlled trial. *Lancet Diabetes Endocrinol.* **7**, 673–683 (2019).
65. Waziry, R. et al. Effect of long-term caloric restriction on DNA methylation measures of biological aging in healthy adults from the CALERIE trial. *Nat Aging* **3**, 248–257 (2023).
66. McCay, C. M. in *Cowdry's Problems of Ageing* (ed. Lansing, A. I.) 139–202 (The Williams & Wilkins Company, 1952).
67. Weindruch, R. H., Kristie, J. A., Cheney, K. E. & Walford, R. L. Influence of controlled dietary restriction on immunologic function and aging. *Fed. Proc.* **38**, 2007–2016 (1979).
68. Sun, L., Akha, A. A. S., Miller, R. A. & Harper, J. M. Life-span extension in mice by preweaning food restriction and by methionine restriction in middle age. *J. Gerontol. A Biol. Sci. Med. Sci.* **64A**, 711–722 (2009).
69. Dhahbi, J. M., Kim, H. -J., Mote, P. L., Beaver, R. J. & Spindler, S. R. Temporal linkage between the phenotypic and genomic responses to caloric restriction. *Proc. Natl Acad. Sci. USA* **101**, 5524–5529 (2004).
70. Forster, M. J., Morris, P. & Sohal, R. S. Genotype and age influence the effect of caloric intake on mortality in mice. *FASEB J.* **17**, 690–692 (2003).
71. Lipman, R. D., Smith, D. E., Bronson, R. T. & Blumberg, J. Is late-life caloric restriction beneficial? *Aging Clin. Exp. Res.* **7**, 136–139 (1995).
72. Lipman, R. D., Smith, D. E., Blumberg, J. B. & Bronson, R. T. Effects of caloric restriction or augmentation in adult rats: longevity and lesion biomarkers of aging. *Aging Clin. Exp. Res.* **10**, 463–470 (1998).
73. Hahn, O. et al. A nutritional memory effect counteracts the benefits of dietary restriction in old mice. *Nat. Metab.* **1**, 1059–1073 (2019).
74. Senior, A. M. et al. Dietary macronutrient content, age-specific mortality and lifespan. *Proc. Biol. Sci.* **286**, 20190393 (2019).
75. Mitchell, S. J. et al. Effects of sex, strain, and energy intake on hallmarks of aging in mice. *Cell Metab.* **23**, 1093–1112 (2016).
76. Richardson, A., Austad, S. N., Ikeno, Y., Unnikrishnan, A. & McCarter, R. J. Significant life extension by ten percent dietary restriction. *Ann. N. Y. Acad. Sci.* **1363**, 11–17 (2016).
77. Everitt, A. V., Porter, B. D. & Wyndham, J. R. Effects of caloric intake and dietary composition on the development of proteinuria, age-associated renal disease and longevity in the male rat. *Gerontology* **28**, 168–175 (1982).
78. Cherkin, A. Letter to editor. *Age* **2**, 51–51 (1979).
79. Cutler, R. G. in *Testing the Theories of Aging* (eds. Adelman, R. C. et al.) 25–114 (CRC Press, 1982).
80. Hayflick, L. *How and Why We Age?* (Random House, 1994).
81. Berry, R. J., Jakobson, M. E. & Triggs, G. S. Survival in wild-living mice. *Mammal Rev.* **3**, 46–57 (1973).
82. Weindruch, R. & Walford, R. L. *The Retardation of Aging and Disease by Dietary Restriction* (C. C. Thomas, 1988).
83. Austad, S. N. Does caloric restriction in the laboratory simply prevent overfeeding and return house mice to their natural level of food intake? *Sci. Aging Knowledge Environ.* **2001**, pe3 (2001).
84. Austad, S. N. & Kristan, D. M. Are mice calorically restricted in nature?. *Aging Cell* **2**, 201–207 (2003).
85. Masoro, E. J. in *Calorie Restriction, Aging and Longevity* (eds. Everitt, A. V. et al.) 3–14 (Springer, 2010).
86. Wolf, A. M. Rodent diet aids and the fallacy of caloric restriction. *Mech. Ageing Dev.* **200**, 111584 (2021).
87. Bertrand, H. A., Lynd, F. T., Masoro, E. J. & Yu, B. P. Changes in adipose mass and cellularity through the adult life of rats fed ad libitum or a life-prolonging restricted diet. *J. Gerontol.* **35**, 827–835 (1980).
88. Solon-Biet, S. M. et al. The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab.* **19**, 418–430 (2014).
89. Lane, P. W. & Dickie, M. M. The effect of restricted food intake on the life span of genetically obese mice. *J. Nutr.* **64**, 549–554 (1958).
90. Harrison, D. E., Archer, J. R. & Astle, C. M. Effects of food restriction on aging: separation of food intake and adiposity. *Proc. Natl Acad. Sci. USA* **81**, 1835–1838 (1984).
91. Ahima, R. S. Adipose tissue as an endocrine organ. *Obesity* **14**, 242S–249S (2006).
92. Funcke, J. -B. & Scherer, P. E. Beyond adiponectin and leptin: adipose tissue-derived mediators of inter-organ communication. *J. Lipid Res.* **60**, 1648–1697 (2019).
93. Rubner, M. *Das Problem der Lebensdauer und Seine Beziehungen zu Wachstum und Ernährung* (Druck und Verlag von R. Oldenbourg, 1908).

94. Pearl, R. The Rate of Living (Univ. London Press, 1928).
95. Sacher, G. A. in *Handbook of The Biology of Aging* (eds. Finch, C. E. et al.) 582–638 (Litton Educational Publishing, 1977).
96. Masoro, E. J., Yu, B. P. & Bertrand, H. A. Action of food restriction in delaying the aging process. *Proc. Natl Acad. Sci. USA* **79**, 4239–4241 (1982).
97. Will, L. C. & McCay, C. M. Ageing, basal metabolism, and retarded growth. *Arch. Biochem.* **481**, 485 (1943).
98. McCarter, R., Masoro, E. J. & Yu, B. P. Does food restriction retard aging by reducing the metabolic rate?. *Am. J. Physiol. Endocrinol. Metab.* **248**, E488–E490 (1985).
99. McCarter, R. J. & McGee, J. R. Transient reduction of metabolic rate by food restriction. *Am. J. Physiol. Endocrinol. Metab.* **257**, E175–E179 (1989).
100. McCarter, R. J. & Palmer, J. Energy metabolism and aging: a lifelong study of Fischer 344 rats. *Am. J. Physiol. Endocrinol. Metab.* **263**, E448–E452 (1992).
101. McCarter, R. J. M. et al. Physical activity as a factor in the action of dietary restriction on aging: effects in Fischer 344 rats. *Aging Clin. Exp. Res.* **9**, 73–79 (1997).
102. Finch, C. E. *Longevity, Senescence, and The Genome* (The University of Chicago Press, 1990).
103. Guarente, L. & Picard, F. Calorie restriction—the SIR2 connection. *Cell* **120**, 473–482 (2005).
104. Sohal, R. S., Ferguson, M., Sohal, B. H. & Forster, M. J. Life span extension in mice by food restriction depends on an energy imbalance. *J. Nutr.* **139**, 533–539 (2009).
105. Masoro, E. J. Overview of calorie restriction and aging. In *Calorie Restriction Society Conference III*, vol. 2 (DVD set) (Calorie Restriction Society, 2004).
106. Mitchell, S. E. et al. The effects of graded levels of calorie restriction: VIII. impact of short term calorie and protein restriction on basal metabolic rate in the C57BL/6 mouse. *Oncotarget* **8**, 17453–17474 (2017).
107. Banks, A. S. et al. A consensus guide to preclinical indirect calorimetry experiments. *Nat. Metab.* **7**, 1765–1780 (2025).
108. Keil, G., Cummings, E. & de Magalhães, J. P. Being cool: how body temperature influences ageing and longevity. *Biogerontology* **16**, 383–397 (2015).
109. Conti, B. & Cabo, R. de. Promoting health and survival through lowered body temperature. *Nat. Aging* https://doi.org/10.1038/s43587-025-00850-0 (2025).
110. Walford, R. L. & Liu, R. K. Husbandry, life span, and growth rate of the annual fish, *Cynolebias adloffi*. *Exp. Gerontol.* **1**, 161–168 (1965).
111. Liu, R. K. & Walford, R. L. Increased growth and life-span with lowered ambient temperature in the annual fish, *Cynolebias adloffi*. *Nature* **212**, 1277–1278 (1966).
112. Klass, M. R. Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. *Mech. Ageing Dev.* **6**, 413–429 (1977).
113. Miquel, J., Lundgren, P. R., Bensch, K. G. & Atlan, H. Effects of temperature on the life span, vitality and fine structure of *Drosophila melanogaster*. *Mech. Ageing Dev.* **5**, 347–370 (1976).
114. Rikke, B. A. & Johnson, T. E. Lower body temperature as a potential mechanism of life extension in homeotherms. *Exp. Gerontol.* **39**, 927–930 (2004).
115. Duffy, P. H. et al. Effect of chronic caloric restriction on physiological variables related to energy metabolism in the male Fischer 344 rat. *Mech. Ageing Dev.* **48**, 117–133 (1989).
116. Rikke, B. A. et al. Strain variation in the response of body temperature to dietary restriction. *Mech. Ageing Dev.* **124**, 663–678 (2003).
117. Mitchell, S. E. et al. The effects of graded levels of calorie restriction: III. Impact of short term calorie and protein restriction on mean daily body temperature and torpor use in the C57BL/6 mouse. *Oncotarget* **6**, 18314–18337 (2015).
118. Soare, A., Cangemi, R., Omodei, D., Holloszy, J. O. & Fontana, L. Long-term calorie restriction, but not endurance exercise, lowers core body temperature in humans. *Aging* **3**, 374–379 (2011).
119. Zhao, Z. et al. Body temperature is a more important modulator of lifespan than metabolic rate in two small mammals. *Nat. Metab.* **4**, 320–326 (2022).
120. Conti, B. et al. Transgenic mice with a reduced core body temperature have an increased life span. *Science* **314**, 825–828 (2006).
121. Walford, R. L. & Spindler, S. R. The response to calorie restriction in mammals shows features also common to hibernation: a cross-adaptation hypothesis. *J. Gerontol. A Biol. Sci. Med. Sci.* **52A**, B179–B183 (1997).
122. Hambly, C., Mercer, J. G. & Speakman, J. R. Hunger Does not diminish over time in mice under protracted caloric restriction. *Rejuvenation Res.* **10**, 533–542 (2007).
123. Derous, D. et al. The effects of graded levels of calorie restriction: VI. Impact of short-term graded calorie restriction on transcriptomic responses of the hypothalamic hunger and circadian signaling pathways. *Aging* **8**, 642–661 (2016).
124. Minor, R. K. et al. The arcuate nucleus and neuropeptide Y contribute to the antitumorigenic effect of calorie restriction. *Aging Cell* **10**, 483–492 (2011).
125. Chiba, T. et al. A key role for neuropeptide Y in lifespan extension and cancer suppression via dietary restriction. *Sci. Rep.* **4**, 4517 (2014).
126. Duffy, P. H., Feuers, R., Nakamura, K. D., Leakey, J. & Hart, R. W. Effect of chronic caloric restriction on the synchronization of various physiological measures in old female Fischer 344 rats. *Chronobiol. Int.* **7**, 113–124 (1990).
127. Acosta-Rodríguez, V. A., de Groot, M. H. M., Rijo-Ferreira, F., Green, C. B. & Takahashi, J. S. Mice under caloric restriction self-impose a temporal restriction of food intake as revealed by an automated feeder system. *Cell Metab.* **26**, 267–277 (2017).
128. Nelson, W. & Halberg, F. Meal-timing, circadian rhythms and life span of mice. *J. Nutr.* **116**, 2244–2253 (1986).
129. Masoro, E. J., Shimokawa, I., Higami, Y., McMahan, C. A. & Yu, B. P. Temporal pattern of food intake not a factor in the retardation of aging processes by dietary restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **50A**, B48–B53 (1995).
130. Pak, H. H. et al. Fasting drives the metabolic, molecular and geroprotective effects of a calorie-restricted diet in mice. *Nat Metab.* **3**, 1327–1341 (2021).
131. Acosta-Rodríguez, V. et al. Circadian alignment of early onset caloric restriction promotes longevity in male C57BL/6J mice. *Science* **376**, 1192–1202 (2022).
132. Liu, X. et al. Calorie restriction and calorie dilution have different impacts on body fat, metabolism, behavior, and hypothalamic gene expression. *Cell Rep.* **39**, 110835 (2022).
133. Anson, R. M. et al. Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. *Proc. Natl Acad. Sci. USA* **100**, 6216–6220 (2003).
134. Hatori, M. et al. Time-restricted feeding without reducing caloric intake prevents metabolic diseases in mice fed a high-fat diet. *Cell Metab.* **15**, 848–860 (2012).
135. Calabrese, E. J. & Mattson, M. P. The catabolic - anabolic cycling hormesis model of health and resilience. *Ageing Res. Rev.* **102**, 102588 (2024).
136. Marosi, K. et al. 3-Hydroxybutyrate regulates energy metabolism and induces BDNF expression in cerebral cortical neurons. *J. Neurochem.* **139**, 769–781 (2016).
137. Youm, Y. -H. et al. The ketone metabolite β-hydroxybutyrate blocks NLRP3 inflammasome–mediated inflammatory disease. *Nat. Med.* **21**, 263–269 (2015).

138. Shimazu, T. et al. Suppression of oxidative stress by β-hydroxybutyrate, an endogenous histone deacetylase inhibitor. *Science* **339**, 211–214 (2013).
139. Mattson, M. P. The cyclic metabolic switching theory of intermittent fasting. *Nat. Metab.* **7**, 665–678 (2025).
140. Nelson, W. & Halberg, F. Schedule-shifts, circadian rhythms and lifespan of freely-feeding and meal-fed mice. *Physiol. Behav.* **38**, 781–788 (1986).
141. Nelson, W. Food restriction, circadian disorder and longevity of rats and mice. *J. Nutr.* **118**, 284–289 (1988).
142. Tognini, P. et al. Distinct circadian signatures in liver and gut clocks revealed by ketogenic diet. *Cell Metab.* **26**, 523–538 (2017).
143. Longo, V. D. & Panda, S. Fasting, circadian rhythms, and time-restricted feeding in healthy lifespan. *Cell Metab.* **23**, 1048–1059 (2016).
144. Zhang, S.-Q. et al. NR1D1 inhibition enhances autophagy and mitophagy in Alzheimer's disease models. *Aging Dis.* https://doi.org/10.14336/ad.2024.1654 (2025).
145. Segall, P. E. & Timiras, P. S. Patho-physiologic findings after chronic tryptophan deficiency in rats: a model for delayed growth and aging. *Mech. Ageing Dev.* **5**, 109–124 (1976).
146. Orentreich, N., Matias, J. R., DeFelice, A. & Zimmerman, J. A. Low methionine ingestion by rats extends life span. *J. Nutr.* **123**, 269–274 (1993).
147. Miller, R. A. et al. Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Aging Cell* **4**, 119–125 (2005).
148. Masoro, E. J. et al. Dietary modulation of the progression of nephropathy in aging rats: an evaluation of the importance of protein. *Am. J. Clin. Nutr.* **49**, 1217–1227 (1989).
149. Green, C. L., Lamming, D. W. & Fontana, L. Molecular mechanisms of dietary restriction promoting health and longevity. *Nat. Rev. Mol. Cell Biol.* **23**, 56–73 (2022).
150. Green, C. L. et al. Dietary restriction of isoleucine increases healthspan and lifespan of genetically heterogeneous mice. *Cell Metab.* **35**, 1976–1995 (2023).
151. Yeh, C. -Y. et al. Late-life protein or isoleucine restriction impacts physiological and molecular signatures of aging. *Nat. Aging* **4**, 1760–1771 (2024).
152. Miller, R. A. et al. Glycine supplementation extends lifespan of male and female mice. *Aging Cell* **18**, e12953 (2019).
153. Piper, M. D. W. et al. Matching dietary amino acid balance to the in silico-translated exome optimizes growth and reproduction without cost to lifespan. *Cell Metab.* **25**, 610–621 (2017).
154. Lane, M. A., Ingram, D. K. & Roth, G. S. 2-deoxy-D-glucose feeding in rats mimics physiologic effects of calorie restriction. *J. Anti Aging Med.* **1**, 327–337 (1998).
155. Lane, M. A., Ingram, D. K. & Roth, G. S. The serious search for an anti-aging pill. *Sci. Am.* **287**, 36–41 (2002).
156. Ingram, D. K. & Roth, G. S. Calorie restriction mimetics: can you have your cake and eat it, too? *Ageing Res. Rev.* **20**, 46–62 (2015).
157. Pistell, P. J. et al. An avocado extract enriched in mannoheptulose prevents the negative effects of a high-fat diet in mice. *Nutrients* **14**, 155 (2021).
158. Ingram, D. K. et al. Characterization and mechanisms of action of avocado extract enriched in mannoheptulose as a candidate calorie restriction mimetic. *J. Agric. Food Chem.* **69**, 7367–7376 (2021).
159. Simcox, J. & Lamming, D. W. The central moTOR of metabolism. *Dev. Cell* **57**, 691–706 (2022).
160. Moore, T. et al. Dietary energy balance modulates signaling through the Akt/mammalian target of rapamycin pathways in multiple epithelial tissues. *Cancer Prev. Res.* **1**, 65–76 (2008).
161. Sharma, N., Castorena, C. M. & Cartee, G. D. Tissue-specific responses of IGF-1/insulin and mTOR signaling in calorie restricted rats. *PLoS ONE* **7**, e38835 (2012).
162. Tulsian, R., Velingkaar, N. & Kondratov, R. Caloric restriction effects on liver mTOR signaling are time-of-day dependent. *Aging* **10**, 1640–1648 (2018).
163. Ham, D. J. et al. Distinct and additive effects of calorie restriction and rapamycin in aging skeletal muscle. *Nat. Commun.* **13**, 2025 (2022).
164. Unnikrishnan, A., Kurup, K., Salmon, A. B. & Richardson, A. Is rapamycin a dietary restriction mimetic?. *J. Gerontol. A Biol. Sci. Med. Sci.* **75**, glz060 (2019).
165. Sharp, Z. D. & Strong, R. The role of mTOR signaling in controlling mammalian life span: what a fungicide teaches us about longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **65**, 580–589 (2010).
166. Harrison, D. E. et al. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* **460**, 392–395 (2009).
167. Miller, R. A. et al. Rapamycin-mediated lifespan increase in mice is dose and sex dependent and metabolically distinct from dietary restriction. *Aging Cell* **13**, 468–477 (2014).
168. Inagaki, T. et al. Endocrine regulation of the fasting response by PPARα-mediated induction of fibroblast growth factor 21. *Cell Metab.* **5**, 415–425 (2007).
169. Fazeli, P. K. et al. FGF21 and the late adaptive response to starvation in humans. *J. Clin. Invest.* **125**, 4601–4611 (2015).
170. Laeger, T. et al. FGF21 is an endocrine signal of protein restriction. *J. Clin. Invest.* **124**, 3913–3922 (2014).
171. Phillips, E. J. & Simons, M. J. P. Rapamycin not dietary restriction improves resilience against pathogens: a meta-analysis. *GeroScience* **45**, 1263–1270 (2023).
172. Ivimey-Cook, E. R., Sultanova, Z. & Maklakov, A. A. Rapamycin, not metformin, mirrors dietary restriction-driven lifespan extension in vertebrates: a meta-analysis. *Aging Cell* https://doi.org/10.1111/acel.70131 (2025).
173. Soda, K., Dobashi, Y., Kano, Y., Tsujinaka, S. & Konishi, F. Polyamine-rich food decreases age-associated pathology and mortality in aged mice. *Exp. Gerontol.* **44**, 727–732 (2009).
174. Hofer, S. J. et al. Spermidine is essential for fasting-mediated autophagy and longevity. *Nat. Cell Biol.* **26**, 1571–1584 (2024).
175. Eisenberg, T. et al. Cardioprotection and lifespan extension by the natural polyamine spermidine. *Nat. Med.* **22**, 1428–1438 (2016).
176. Pietrocola, F. et al. Spermidine induces autophagy by inhibiting the acetyltransferase EP300. *Cell Death Differ.* **22**, 509–516 (2015).
177. Baur, J. A. et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* **444**, 337–342 (2006).
178. Pearson, K. J. et al. Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. *Cell Metab.* **8**, 157–168 (2008).
179. Strong, R. et al. Evaluation of resveratrol, green tea extract, curcumin, oxaloacetic acid, and medium-chain triglyceride oil on life span of genetically heterogeneous mice. *J. Gerontol. A Biol. Sci. Med. Sci.* **68**, 6–16 (2013).
180. Mercken, E. M. et al. SRT2104 extends survival of male mice on a standard diet and preserves bone and muscle mass. *Aging Cell* **13**, 787–796 (2014).
181. Mitchell, S. J. et al. The SIRT1 activator SRT1720 extends lifespan and improves health of mice fed a standard diet. *Cell Rep.* **6**, 836–843 (2014).
182. Boily, G. et al. SirT1 regulates energy metabolism and response to caloric restriction in mice. *PLoS ONE* **3**, e1759 (2008).
183. Li, Y., Xu, W., McBurney, M. W. & Longo, V. D. SirT1 inhibition reduces IGF-I/IRS-2/Ras/ERK1/2 signaling and protects neurons. *Cell Metab.* **8**, 38–48 (2008).

184. Mercken, E. M. et al. SIRT1 but not its increased expression is essential for lifespan extension in caloric-restricted mice. *Aging Cell* **13**, 193–196 (2014).
185. Fang, E. F. et al. NAD+ in aging: molecular mechanisms and translational implications. *Trends Mol. Med.* **23**, 899–916 (2017).
186. Lautrup, S., Sinclair, D. A., Mattson, M. P. & Fang, E. F. NAD^+ in brain aging and neurodegenerative disorders. *Cell Metab.* **30**, 630–655 (2019).
187. Zhang, J. et al. Emerging strategies, applications and challenges of targeting NAD^+ in the clinic. *Nat. Aging* **5**, 1704–1731 (2025).
188. Zhang, H. et al. NAD+ repletion improves mitochondrial and stem cell function and enhances life span in mice. *Science* **352**, 1436–1443 (2016).
189. Harrison, D. E. et al. 17-α-estradiol late in life extends lifespan in aging UM-HET3 male mice; nicotinamide riboside and three other drugs do not affect lifespan in either sex. *Aging Cell* **20**, e13328 (2021).
190. Mills, K. F. et al. Long-term administration of nicotinamide mononucleotide mitigates age-associated physiological decline in mice. *Cell Metab.* **24**, 795–806 (2016).
191. Mitchell, S. J. et al. Nicotinamide improves aspects of healthspan, but not lifespan, in mice. *Cell Metab.* **27**, 667–676 (2018).
192. Harrison, D. E. et al. Acarbose improves health and lifespan in aging HET3 mice. *Aging Cell* **18**, e12898 (2019).
193. Strong, R. et al. Longer lifespan in male mice treated with a weakly estrogenic agonist, an antioxidant, an α-glucosidase inhibitor or a Nrf2-inducer. *Aging Cell* **15**, 872–884 (2016).
194. Qu, Q. et al. Lithocholic acid phenocopies anti-ageing effects of calorie restriction. *Nature* https://doi.org/10.1038/s41586-024-08329-5 (2024).
195. Wilding, J. P. H. et al. Once-weekly semaglutide in adults with overweight or obesity. *N. Engl. J. Med.* **384**, 989–1002 (2021).
196. Garvey, W. T. et al. Two-year effects of semaglutide in adults with overweight or obesity: the STEP 5 trial. *Nat. Med.* **28**, 2083–2091 (2022).
197. Lincoff, A. M. et al. Semaglutide and cardiovascular outcomes in obesity without diabetes. *N. Engl. J. Med.* **389**, 2221–2232 (2023).
198. Holst, J. J. GLP-1 physiology in obesity and development of incretin-based drugs for chronic weight management. *Nat. Metab.* **6**, 1866–1885 (2024).
199. Johansen, V. B. I. et al. Brain control of energy homeostasis: implications for anti-obesity pharmacotherapy. *Cell* **188**, 4178–4212 (2025).
200. Secher, A. et al. The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *J. Clin. Invest.* **124**, 4473–4488 (2014).
201. McMorrow, H. E. et al. Incretin receptor agonism rapidly inhibits AgRP neurons to suppress food intake in mice. *J. Clin. Invest.* https://doi.org/10.1172/jci186652 (2025).
202. Friedrichsen, M., Breitschaft, A., Tadayon, S., Wizert, A. & Skovgaard, D. The effect of semaglutide 2.4 mg once weekly on energy intake, appetite, control of eating, and gastric emptying in adults with obesity. *Diabetes Obes. Metab.* **23**, 754–762 (2021).
203. Petersen, J., Merrild, C., Lund, J., Holm, S. & Clemmensen, C. Lead-in calorie restriction enhances the weight-lowering efficacy of incretin hormone-based pharmacotherapies in mice. *Mol. Metab.* **89**, 102027 (2024).
204. Ravussin, E. et al. Tirzepatide did not impact metabolic adaptation in people with obesity, but increased fat oxidation. *Cell Metab.* **37**, 1060–1074 (2025).
205. Wharton, S. et al. Orforglipron, an oral small-molecule GLP-1 receptor agonist for obesity treatment. *N. Engl. J. Med.* https://doi.org/10.1056/nejmoa2511774 (2025).
206. Sonne, N. et al. 2182-LB: development and characterization of a humanized GLP-1 receptor mouse model for the evaluation of small-molecule GLP1R agonists. *Diabetes* **74**, 2182-LB (2025).
207. Wang, A. & Speakman, J. R. Potential downsides of calorie restriction. *Nat. Rev. Endocrinol.* https://doi.org/10.1038/s41574-025-01111-1 (2025).
208. Aw, D., Silva, A. B. & Palmer, D. B. Immunosenescence: emerging challenges for an ageing population. *Immunology* **120**, 435–446 (2007).
209. Pawelec, G. Age and immunity: what is "immunosenescence"? *Exp. Gerontol.* **105**, 4–9 (2018).
210. Gerbase-DeLima, M., Liu, R. K., Cheney, K. E., Mickey, R. & Walford, R. L. Immune function and survival in a long-lived mouse strain subjected to undernutrition. *Gerontology* **21**, 184–202 (1975).
211. Nikolich-Žugich, J. & Messaoudi, I. Mice and flies and monkeys too: caloric restriction rejuvenates the aging immune system of non-human primates. *Exp. Gerontol.* **40**, 884–893 (2005).
212. Ingram, D. K. & de Cabo, R. Calorie restriction in rodents: caveats to consider. *Ageing Res. Rev.* **39**, 15–28 (2017).
213. Roecker, E. B., Kemnitz, J. W., Ershler, W. B. & Weindruch, R. Reduced immune responses in Rhesus monkeys subjected to dietary restriction. *J. Gerontol. A Biol. Sci. Med. Sci.* **51A**, B276–B279 (1996).
214. Sun, D., Muthukumar, A. R., Lawrence, R. A. & Fernandes, G. Effects of calorie restriction on polymicrobial peritonitis induced by cecum ligation and puncture in young C57BL/6 mice. *Clin. Diagn. Lab. Immunol.* **8**, 1003–1011 (2001).
215. Kang, W., Saito, H., Fukatsu, K., Hidemura, A. & Matsuda, T. Diet restriction impairs extracellular signal-regulated kinase activation of peritoneal exudative cells after *N*-formyl-methionyl-leucyl-phenylalanine stimulation in a murine peritonitis model. *JPEN J. Parenter. Enteral Nutr.* **26**, 259–264 (2002).
216. Starr, M. E., Steele, A. M., Cohen, D. A. & Saito, H. Short-term dietary restriction rescues mice from lethal abdominal sepsis and endotoxemia and reduces the inflammatory/coagulant potential of adipose tissue. *Crit. Care Med.* **44**, e509–e519 (2016).
217. Hasegawa, A. et al. Alternate day calorie restriction improves systemic inflammation in a mouse model of sepsis induced by cecal ligation and puncture. *J. Surg. Res.* **174**, 136–141 (2012).
218. Peck, M. D., Babcock, G. F. & Alexander, J. W. The role of protein and calorie restriction in outcome from salmonella infection in mice. *JPEN J. Parenter. Enteral Nutr.* **16**, 561–565 (1992).
219. Trujillo-Ferrara, J. et al. Caloric restriction increases free radicals and inducible nitric oxide synthase expression in mice infected with *Salmonella typhimurium*. *Biosci. Rep.* **31**, 273–282 (2011).
220. Murray, M., Murray, A., Murray, N. & Murray, M. Diet and cerebral malaria: the effect of famine and refeeding. *Am. J. Clin. Nutr.* **31**, 57–61 (1978).
221. Hunt, N. H., Manduci, N. & Thumwood, C. M. Amelioration of murine cerebral malaria by dietary restriction. *Parasitology* **107**, 471–476 (1993).
222. Mejia, P. et al. Dietary restriction protects against experimental cerebral malaria via leptin modulation and T-cell mTORC1 suppression. *Nat. Commun.* **6**, 6050 (2015).
223. Gardner, E. M. Caloric restriction decreases survival of aged mice in response to primary influenza infection. *J. Gerontol. A Biol. Sci. Med. Sci.* **60**, 688–694 (2005).
224. Ritz, B. W., Aktan, I., Nogusa, S. & Gardner, E. M. Energy restriction impairs natural killer cell function and increases the severity of influenza infection in young adult male C57BL/6 mice. *J. Nutr.* **138**, 2269–2275 (2008).
225. Clinthorne, J. F., Adams, D. J., Fenton, J. I., Ritz, B. W. & Gardner, E. M. Short-term re-feeding of previously energy-restricted C57BL/6 male mice restores body weight and body fat and attenuates the decline in natural killer cell function after primary influenza infection. *J. Nutr.* **140**, 1495–1501 (2010).

226. Goldberg, E. L. et al. Lifespan-extending caloric restriction or mTOR inhibition impair adaptive immunity of old mice by distinct mechanisms. *Aging Cell* **14**, 130–138 (2015).
227. Beura, L. K. et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature* **532**, 512–516 (2016).
228. Reiser, K., McGee, C., Rucker, R. & McDonald, R. Effects of aging and caloric restriction on extracellular matrix biosynthesis in a model of injury repair in rats. *J. Gerontol. A Biol. Sci. Med. Sci.* **50A**, B40–B47 (1995).
229. Emery, P. W. & Sanderson, P. Effect of dietary restriction on protein synthesis and wound healing after surgery in the rat. *Clin. Sci.* **89**, 383–388 (1995).
230. Otranto, M., Souza-Netto, I., guila, M. B. & Monte-Alto-Costa, A. Male and female rats with severe protein restriction present delayed wound healing. *Appl. Physiol. Nutr. Metab.* **34**, 1023–1031 (2009).
231. Reed, M. J. et al. Enhanced cell proliferation and biosynthesis mediate improved wound repair in refed, caloric-restricted mice. *Mech. Ageing Dev.* **89**, 21–43 (1996).
232. Hunt, N. D. et al. Effect of calorie restriction and refeeding on skin wound healing in the rat. *Age* **34**, 1453–1458 (2012).
233. Roth, G. S. et al. Effect of age and caloric restriction on cutaneous wound closure in rats and monkeys. *J. Gerontol. A Biol. Sci. Med. Sci.* **52A**, B98–B102 (1997).
234. Forni, M. F. et al. Caloric restriction promotes structural and metabolic changes in the skin. *Cell Rep.* **20**, 2678–2692 (2017).
235. Chen, H. et al. Intermittent fasting triggers interorgan communication to suppress hair follicle regeneration. *Cell* **188**, 157–174 (2025).
236. Harvie, M. N. et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. *Int. J. Obes.* **35**, 714–727 (2011).
237. Liu, D. et al. Calorie restriction with or without time-restricted eating in weight loss. *New Engl. J. Med.* **386**, 1495–1504 (2022).
238. Dote-Montero, M. et al. Effects of early, late and self-selected time-restricted eating on visceral adipose tissue and cardio-metabolic health in participants with overweight or obesity: a randomized controlled trial. *Nat. Med.* **31**, 524–533 (2025).
239. Blackwell, B. -N., Bucci, T. J., Hart, R. W. & Turturro, A. Longevity, body weight, and neoplasia in ad libitum-fed and diet-restricted C57BL6 mice fed NIH-31 open formula diet. *Toxicol. Pathol.* **23**, 570–582 (1995).
240. Moreschi, C. Beziehungen zwischen Ernährung und Tumorwachstum. *Zeitschrift für Immunitaetsforschung* **2**, 651–675 (1909).
241. Rous, P. The influence of diet on transplanted and spontaneous mouse tumors. *J. Exp. Med.* **20**, 433–451 (1914).
242. Tannenbaum, A. The initiation and growth of tumors: introduction. I. Effects of underfeeding. *Am. J. Cancer* **38**, 335–350 (1940).
243. Xie, K. et al. Every-other-day feeding extends lifespan but fails to delay many symptoms of aging in mice. *Nat. Commun.* **8**, 155 (2017).
244. Olshansky, S., Carnes, B. & Cassel, C. In search of Methuselah: estimating the upper limits to human longevity. *Science* **250**, 634–640 (1990).
245. Olshansky, S. J. & Carnes, B. A. Inconvenient truths about human longevity. *J. Gerontol. A Biol. Sci. Med. Sci.* **74**, S7–S12 (2019).
246. Konno, A., Hishinuma, K., Hashimoto, Y., Kimura, S. & Nishimura, T. Dietary restriction reduces the incidence of 3-methylcholanthrene-induced tumors in mice: close correlation with its potentiating effect on host T cell functions. *Cancer Immunol. Immunother.* **33**, 293–298 (1991).
247. Kolaja, K. L., Bunting, K. A. & Klaunig, J. E. Inhibition of tumor promotion and hepatocellular growth by dietary restriction in mice. *Carcinogenesis* **17**, 1657–1664 (1996).
248. Nogueira, L. M. et al. Dose-dependent effects of calorie restriction on gene expression, metabolism, and tumor progression are partially mediated by insulin-like growth factor-1. *Cancer Med.* **1**, 275–288 (2012).
249. Harvey, A. E. et al. Calorie restriction decreases murine and human pancreatic tumor cell growth, nuclear factor-κB activation, and inflammation-related gene expression in an insulin-like growth factor-1–dependent manner. *PLoS ONE* **9**, e94151 (2014).
250. Novelle, M. G. et al. Caloric restriction induces heat shock response and inhibits B16F10 cell tumorigenesis both in vitro and in vivo. *Aging* **7**, 233–239 (2015).
251. Pomatto-Watson, L. C. D. et al. Daily caloric restriction limits tumor growth more effectively than caloric cycling regardless of dietary composition. *Nat. Commun.* **12**, 6201 (2021).
252. Seyfried, B. T. N., Kiebish, M., Marsh, J. & Mukherjee, P. Targeting energy metabolism in brain cancer through calorie restriction and the ketogenic diet. *J. Cancer Res. Ther.* **5**, 7–15 (2009).
253. Vernieri, C. et al. Fasting-mimicking diet is safe and reshapes metabolism and antitumor immunity in patients with cancer. *Cancer Discov.* **12**, 90–107 (2022).
254. Udumula, M. P. et al. Intermittent fasting induced ketogenesis inhibits mouse epithelial ovarian cancer by promoting antitumor T cell response. *iScience* **26**, 107839 (2023).
255. Hursting, S. D., Perkins, S. N. & Phang, J. M. Calorie restriction delays spontaneous tumorigenesis in p53-knockout transgenic mice. *Proc. Natl Acad. Sci. USA* **91**, 7036–7040 (1994).
256. Vernieri, C., Ligorio, F., Tripathy, D. & Longo, V. D. Cyclic fasting-mimicking diet in cancer treatment: preclinical and clinical evidence. *Cell Metab.* **36**, 1644–1667 (2024).
257. Ingram, D. K., Weindruch, R., Spangler, E. L., Freeman, J. R. & Walford, R. L. Dietary restriction benefits learning and motor performance of aged mice. *J. Gerontol.* **42**, 78–81 (1987).
258. Kapogiannis, D. et al. Brain responses to intermittent fasting and the healthy living diet in older adults. *Cell Metab.* **36**, 1668–1678 (2024).
259. Prehn, K. et al. Caloric restriction in older adults—differential effects of weight loss and reduced weight on brain structure and function. *Cereb. Cortex* **27**, 1765–1778 (2017).
260. Martin, C. K. et al. Examination of cognitive function during six months of calorie restriction results of a randomized controlled trial. *Rejuvenation Res.* **10**, 179–190 (2007).
261. Wang, J. et al. Caloric restriction attenuates β-amyloid neuropathology in a mouse model of Alzheimer's disease. *FASEB J.* **19**, 1–18 (2005).
262. Patel, N. V. et al. Caloric restriction attenuates Aβ-deposition in Alzheimer transgenic models. *Neurobiol. Aging* **26**, 995–1000 (2005).
263. Mouton, P. R., Chachich, M. E., Quigley, C., Spangler, E. & Ingram, D. K. Caloric restriction attenuates amyloid deposition in middle-aged dtg APP/PS1 mice. *Neurosci. Lett.* **464**, 184–187 (2009).
264. Zhang, J. et al. Intermittent fasting protects against Alzheimer's disease possible through restoring aquaporin-4 polarity. *Front. Mol. Neurosci.* **10**, 395 (2017).
265. Halagappa, V. K. M. et al. Intermittent fasting and caloric restriction ameliorate age-related behavioral deficits in the triple-transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* **26**, 212–220 (2007).
266. Babygirija, R. et al. Fasting is required for many of the benefits of calorie restriction in the 3xTg mouse model of Alzheimer's disease. *Nat. Commun.* **16**, 7147 (2025).
267. Lazic, D. et al. Every-other-day feeding exacerbates inflammation and neuronal deficits in 5XFAD mouse model of Alzheimer's disease. *Neurobiol. Dis.* **136**, 104745 (2020).

268. Pan, R. -Y. et al. Intermittent fasting protects against Alzheimer's disease in mice by altering metabolism through remodeling of the gut microbiota. *Nat. Aging* **2**, 1024–1039 (2022).
269. Qin, W. et al. Calorie restriction attenuates Alzheimer's disease type brain amyloidosis in squirrel monkeys (*Saimiri sciureus*). *J. Alzheimer's Dis.* **10**, 417–422 (2006).
270. Liu, Y. et al. SIRT3 mediates hippocampal synaptic adaptations to intermittent fasting and ameliorates deficits in APP mutant mice. *Nat. Commun.* **10**, 1886 (2019).
271. Rubovitch, V. et al. Dietary energy restriction ameliorates cognitive impairment in a mouse model of traumatic brain injury. *J. Mol. Neurosci.* **67**, 613–621 (2019).
272. Whittaker, D. S. et al. Circadian modulation by time-restricted feeding rescues brain pathology and improves memory in mouse models of Alzheimer's disease. *Cell Metab.* **35**, 1704–1721 (2023).
273. Cogut, V., McNeely, T. L., Bussian, T. J., Graves, S. I. & Baker, D. J. Caloric restriction improves spatial learning deficits in tau mice. *J. Alzheimer's Dis.* **98**, 925–940 (2024).
274. Bruce-Keller, A. J., Umberger, G., McFall, R. & Mattson, M. P. Food restriction reduces brain damage and improves behavioral outcome following excitotoxic and metabolic insults. *Ann. Neurol.* **45**, 8–15 (1999).
275. Duan, W. & Mattson, M. P. Dietary restriction and 2-deoxyglucose administration improve behavioral outcome and reduce degeneration of dopaminergic neurons in models of Parkinson's disease. *J. Neurosci. Res.* **57**, 195–206 (1999).
276. Yu, Z. F. & Mattson, M. P. Dietary restriction and 2-deoxyglucose administration reduce focal ischemic brain damage and improve behavioral outcome: evidence for a preconditioning mechanism. *J. Neurosci. Res.* **57**, 830–839 (1999).
277. Fitzgerald, K. C. et al. Effect of intermittent vs. daily calorie restriction on changes in weight and patient-reported outcomes in people with multiple sclerosis. *Mult. Scler. Relat. Disord.* **23**, 33–39 (2018).
278. Fitzgerald, K. C. et al. Intermittent calorie restriction alters T cell subsets and metabolic markers in people with multiple sclerosis. *eBioMedicine* **82**, 104124 (2022).
279. Strøm, A. & Jensen, R. A. Mortality from circulatory diseases in Norway 1940–1945. *Lancet* **257**, 126–129 (1951).
280. Fontana, L., Meyer, T. E., Klein, S. & Holloszy, J. O. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc. Natl Acad. Sci. USA* **101**, 6659–6663 (2004).
281. Fortmann, S. P., Haskell, W. L., Wood, P. D. & Team, S. W. C. P. Effects of weight loss on clinic and ambulatory blood pressure in normotensive men. *Am. J. Cardiol.* **62**, 89–93 (1988).
282. Caviezel, F. et al. Early improvement of left ventricular function during caloric restriction in obesity. *Int. J. Obes.* **10**, 421–426 (1986).
283. Wan, R., Camandola, S. & Mattson, M. P. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. *FASEB J.* **17**, 1133–1134 (2003).
284. Mager, D. E. et al. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. *FASEB J.* **20**, 631–637 (2006).
285. Broderick, T. L., Driedzic, W. R., Gillis, M., Jacob, J. & Belke, T. Effects of chronic food restriction and exercise training on the recovery of cardiac function following ischemia. *J. Gerontol. A Biol. Sci. Med. Sci.* **56**, B33–B37 (2001).
286. Ahmet, I., Wan, R., Mattson, M. P., Lakatta, E. G. & Talan, M. Cardioprotection by intermittent fasting in rats. *Circulation* **112**, 3115–3121 (2005).
287. Katare, R. G., Kakinuma, Y., Arikawa, M., Yamasaki, F. & Sato, T. Chronic intermittent fasting improves the survival following large myocardial ischemia by activation of BDNF/VEGF/PI3K signaling pathway. *J. Mol. Cell. Cardiol.* **46**, 405–412 (2009).
288. Dutzmann, J. et al. Intermittent fasting after ST-segment–elevation myocardial infarction improves left ventricular function: the randomized controlled INTERFAST-MI trial. *Circ. Heart Fail.* **17**, e010936 (2024).
289. Piper, M. D. W., Partridge, L., Raubenheimer, D. & Simpson, S. J. Dietary restriction and aging: a unifying perspective. *Cell Metab.* **14**, 154–160 (2011).
290. Partridge, L., Fuentealba, M. & Kennedy, B. K. The quest to slow ageing through drug discovery. *Nat. Rev. Drug Discov.* **19**, 513–532 (2020).
291. Fang, E. F. et al. A research agenda for ageing in China in the 21st century (2nd edition): focusing on basic and translational research, long-term care, policy and social networks. *Ageing Res. Rev.* **64**, 101174 (2020).
292. Fang, E. F. et al. Adapting health, economic and social policies to address population aging in China. *Nat. Aging* **5**, 2176–2187 (2025).
293. Lane, M. A. et al. Dietary restriction in nonhuman primates: progress report on the NIA study. *Ann. N. Y. Acad. Sci.* **673**, 36–45 (1992).
294. Ramsey, J. J. et al. Dietary restriction and aging in rhesus monkeys: the University of Wisconsin study. *Exp. Gerontol.* **35**, 1131–1149 (2000).
295. Dal-Pan, A. et al. Caloric restriction or resveratrol supplementation and ageing in a non-human primate: first-year outcome of the RESTRIKAL study in *Microcebus murinus*. *Age* **33**, 15–31 (2011).
296. Kaneko, T., Tahara, S. & Matsuo, M. Retarding effect of dietary restriction on the accumulation of 8-hydroxy-2'-deoxyguanosine in organs of Fischer 344 rats during aging. *Free Radic. Biol. Med.* **23**, 76–81 (1997).
297. Kisby, G. E. et al. Effect of caloric restriction on base-excision repair (BER) in the aging rat brain. *Exp. Gerontol.* **45**, 208–216 (2010).
298. Vera, E., Jesus, B. B. de, Foronda, M., Flores, J. M. & Blasco, M. A. Telomerase reverse transcriptase synergizes with calorie restriction to increase health span and extend mouse longevity. *PLoS ONE* **8**, e53760 (2013).
299. Hass, B. S., Hart, R. W., Lu, M. H. & Lyn-Cook, B. D. Effects of caloric restriction in animals on cellular function, oncogene expression, and DNA methylation in vitro. *Mutat. Res.* **295**, 281–289 (1993).
300. Cohen, H. Y. et al. Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase. *Science* **305**, 390–392 (2004).
301. Hahn, O. et al. Dietary restriction protects from age-associated DNA methylation and induces epigenetic reprogramming of lipid metabolism. *Genome Biol.* **18**, 56 (2017).
302. Bonelli, M. A. et al. Low-level caloric restriction rescues proteasome activity and Hsc70 level in liver of aged rats. *Biogerontology* **9**, 1–10 (2008).
303. Ntsapi, C. M. & Loos, B. Neurons die with heightened but functional macro- and chaperone mediated autophagy upon increased amyloid-ß induced toxicity with region-specific protection in prolonged intermittent fasting. *Exp. Cell. Res.* **408**, 112840 (2021).
304. Cerletti, M., Jang, Y. C., Finley, L. W. S., Haigis, M. C. & Wagers, A. J. Short-term calorie restriction enhances skeletal muscle stem cell function. *Cell Stem Cell* **10**, 515–519 (2012).
305. Fontana, L. et al. The effects of graded caloric restriction: XII. Comparison of mouse to human impact on cellular senescence in the colon. *Aging Cell* **17**, e12746 (2018).
306. Bareja, A. et al. Liver-derived plasminogen mediates muscle stem cell expansion during caloric restriction through the plasminogen receptor Plg-RKT. *Cell Rep.* **43**, 113881 (2024).
307. Deepa, S. S., Unnikrishnan, A., Matyi, S., Hadad, N. & Richardson, A. Necroptosis increases with age and is reduced by dietary restriction. *Aging Cell* **17**, e12770 (2018).

308. Sbierski-Kind, J. et al. Effects of caloric restriction on the gut microbiome are linked with immune senescence. *Microbiome* **10**, 57 (2022).
309. de França, N. A. G., Rolland, Y., Guyonnet, S. & de Barreto, P. S. The role of dietary strategies in the modulation of hallmarks of aging. *Ageing Res. Rev.* **87**, 101908 (2023).
310. Speakman, J. R., Mitchell, S. E. & Mazidi, M. Calories or protein? The effect of dietary restriction on lifespan in rodents is explained by calories alone. *Exp. Gerontol.* **86**, 28–38 (2016).
311. Harper, J. M., Leathers, C. W. & Austad, S. N. Does caloric restriction extend life in wild mice? *Aging Cell* **5**, 441–449 (2006).
312. Green, C. L. et al. Sex and genetic background define the metabolic, physiologic, and molecular response to protein restriction. *Cell Metab.* **34**, 209–226 (2022).
313. Heydari, A. R., Wu, B., Takahashi, R., Strong, R. & Richardson, A. Expression of heat shock protein 70 is altered by age and diet at the level of transcription. *Mol. Cell. Biol.* **13**, 2909–2918 (1993).
314. HALL, D. M. et al. Caloric restriction improves thermotolerance and reduces hyperthermia-induced cellular damage in old rats. *FASEB J.* **14**, 78–86 (2000).
315. McCay, C. M., Maynard, L. A., Sperling, G. & Barnes, L. L. Retarded growth, life span, ultimate body size and age changes in the albino rat after feeding diets restricted in calories four figures. *J. Nutr.* **18**, 1–13 (1939).
316. Campbell, B. A. & Richardson, R. Effect of chronic undernutrition on susceptibility to cold stress in young adult and aged rats. *Mech. Ageing Dev.* **44**, 193–202 (1988).
317. Talan, M. I. & Ingram, D. K. Effect of intermittent feeding on thermoregulatory abilities of young and aged C57BL/6J mice. *Arch. Gerontol. Geriatr.* **4**, 251–259 (1985).

Acknowledgements

We thank A. Molière for assistance and comments on the content of multiple sections; A. Anisimov and A. Ferrante for their comments on early drafts; J. Teeters, B. M. Delaney and A. de Grey for helping with the facilitation of valuable materials related to the CR Society; F. Pifferi for assisting with information related to the RESTRIKAL study; the Medical Library of Akershus University Hospital for their immense help with obtaining several non-digitalized materials. E.F.F. is supported by Cure Alzheimer's Fund (282952; 284930), HELSE SØR-ØST (2020001, 2021021, 2023093), the Research Council of Norway (262175, 334361), Molecule AG/VITADAO (282942), NordForsk Foundation (119986), the National Natural Science Foundation of China (81971327; 82574074), Akershus University Hospital (269901, 261973, 262960), the Civitan Norges Forskningsfond for Alzheimers sykdom (281931), the Czech Republic-Norway KAPPA program (with M. Vyhnálek, TO01000215), the Rosa sløyfe/Norwegian Cancer Society & Norwegian Breast Cancer Society (207819), HORIZON-TMA-MSCA-DN (101073251, with R. Houtkooper), Wellcome Leap's Dynamic Resilience Program (jointly funded by Temasek Trust; 104617) and the Alzheimer's Association (PTC-Gene-25-1439553). C.C. is supported by Novo Nordisk Foundation (NNF22OC0073778, NNF24OC0089508, NNF23SA0084103) and the European Research Council under the European Union's Horizon Europe research and innovation program (grant agreement no. 101170678, PDC-MAIN). A.R. is supported by the Department of Veterans Affairs (1IK6BX005238). R.d.C. is supported by the Intramural Research Program of the NIA, NIH. Figures were designed in Adobe Illustrator. Language refinement was assisted by ChatGPT to improve readability and clarity. Survival curves were extracted from published sources using WebPlotDigitizer.

Author contributions

Original conceptualization: T.S.-M., E.F.F. and M.P.M.; Writing, review and editing: T.S.-M., E.F.F., M.P.M., S.L., A.D.F., S.J.M., C.A., L.P., G.S.R., R.M.A., J.A.M., R.d.C., J.R.S., A.R., D.K.I. and R.W.

Competing interests

E.F.F. is a co-owner of Fang-S Consultation AS (organization no. 931 410 717) and NO-Age AS (organization no. 933 219 127). E.F.F. has a material transfer agreement with LMITO Therapeutics (South Korea), a CRADA arrangement with ChromaDex (USA), a commercialization agreement with Molecule AG/VITADAO, and material transfer agreements with GeneHarbor (Hong Kong) Biotechnologies Limited and Hong Kong Longevity Science Laboratory (Hong Kong). E.F.F. is a consultant to MindRank AI (China), NYO3 (Norway), AgeLab (Vitality Nordic AS, Norway) and Hong Kong Longevity Science Laboratory (Hong Kong). D.K.I. and G.S.R. are founders and Chief Scientific Officers for GeroScience (USA) and Prolongevity Technologies (USA). D.K.I. is Chief Scientific Officer for Functional Longevity Labs (USA). C.C. is a cofounder of Ousia Pharma, a biotech company developing therapeutics for the treatment of obesity. M.P.M. is the author of and receives royalties from *The Intermittent Fasting Revolution*. A.D.F. is employed by Calico Life Sciences. The remaining authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43587-026-01091-5.

Correspondence and requests for materials should be addressed to Mark P. Mattson or Evandro F. Fang.

Peer review information *Nature Aging* thanks Valter Longo and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.