Intermittent and periodic fasting, longevity and disease

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Abstract

Intermittent and periodic fasting (IF and PF, respectively) are emerging as safe strategies to affect longevity and healthspan by acting on cellular aging and disease risk factors, while causing no or minor side effects. IF lasting from 12 to 48 hours and repeated every 1 to 7 days and PF lasting 2 to 7 days and repeated once per month or less have the potential to prevent and treat disease, but their effect on cellular aging and the molecular mechanisms involved are only beginning to be unraveled. Here, we describe the different fasting methods and their effect on longevity in organisms ranging from yeast to humans, linking them to the major nutrient-sensing signaling pathways and focusing on the benefits of the fasting and the refeeding periods. We also discuss both the therapeutic potential and side effects of IF and PF with a focus on cancer, autoimmunity, neurodegeneration and metabolic and cardiovascular disease.

Dietary restriction (DR) refers to regimens including the reduction of the intake of either calories or of specific components of the diet, such as protein or certain amino acids, and to intermittent and periodic fasting (IF and PF, respectively), which may or may not require an overall reduction in calorie intake. Instead, calorie restriction (CR), which in most cases involves a chronic reduction in calorie intake by 20–40% below the standard, extends lifespan and healthspan in yeast, invertebrates, laboratory rodents and non-human primates[1–7]. In humans, 15% CR reduces markers or risk factors for a range of age-related diseases, including diabetes, cancer and cardiovascular disease, but it also causes side effects, which include a low or very low body mass index (BMI) when applied chronically[1,8]. Moreover, a chronic CR of 20–60% in mice can have positive effects on

aging and immune function[9,10], but can increase susceptibility to certain pathogens, such as the influenza virus and intestinal parasites[11,12].

Protein restriction (PR) independently of calorie intake[13–15] also extends lifespan in mice[16,17] and improves the health of young and middle-age mice and humans, but moderate to severe PR can lead to frailty and/or disease in old mice or individuals over the age of 65 (refs. [16,18]). Severe PR, in which calories from proteins are below 5% of the total, could also have detrimental effects, including weight loss in younger organisms, as shown in mice[18]. Signaling pathways by which CR and PR extend lifespan include those activated by growth hormone, insulin-like growth factor-1 (IGF-1) and insulin, and involve downstream factors, including phosphoinositide 3-kinase (PI3K), mammalian target of rapamycin complex 1 (mTORC1), protein-kinase A (PKA), AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), sirtuins and forkhead transcription factors (FOXOs), that are well established to regulate or affect aging and longevity[19–23]. Thus, both CR and PR can have strong effects on nutrient signaling pathways and aging, but there is a need to identify novel interventions that optimize healthspan while minimizing side effects and the burden imposed by chronic dietary interventions. These studies could allow the identification of specific dietary regimens effective in delaying, and even partially reversing, aging and age-related diseases.

In this Review, we discuss the role of dietary restriction in aging and disease, with a focus on IF and PF regimens (Table 1 and Box 1). The use of generic terms like IF, fasting and even dietary restriction, which include interventions lasting from a few hours to months and represent many dietary compositions and severities of caloric restriction, needs to be limited and replaced by specific terminology referring to a clearly defined intervention so that use can be standardized for the laboratory, the clinic and eventually the public.

These intermittent or periodic dietary interventions can promote cell protection and repair as well as the clearance of damaged cells and intracellular components, in part through the modulation of conserved stress-response or nutrient-sensing pathways. Whereas the dietary-restriction field has mostly focused on the benefits of continuous caloric or macronutrient restriction, here we focus both on the importance of much shorter restriction periods and the refeeding and post-refeeding phases, which is accompanied by a regenerative process that is not observed or is much less active during chronic restrictions[19,21,22,24]. We summarize the metabolic and cellular responses triggered by these feeding regimens, their impact on nutrient signaling pathways and their link to age-related diseases.

Fasting methods and effects

Common fasting methods.

Different fasting regimens (Table 1) can affect metabolism, aging, disease and mortality in simple organisms and mammals. IF includes various eating patterns; water-only fasting or severely restricted (over 50%) calorie intake lasts between 12 and 48 hours, and in most cases is repeated in cycles occurring every day to once per week[25]. There are several types of IF diets commonly adopted in rodents and human clinical studies: complete water-only

fasting that occurs every other day (also called alternate-day fasting (ADF))[26–28]; 70% energy restriction every other day[27–29]; the 5:2 diet, which provides between 500 and 700 calories for 2 days per week[30,31]; and time-restricted feeding (TRF), in which food intake is in most cases restricted to 6–12 hours per day[32,33]. Thus, IF regimens usually encompass a period in which only water is consumed or calorie intake is very low, which is followed by a normal feeding period that in most cases lasts between 12 and 48 hours.

In contrast, PF refers to a prolonged and severely calorie-restricted or water-only fasting period lasting in most cases between 48 hours and 1 week, although several studies have investigated longer fasting periods[25]. Unlike IF, it can occur at specific intervals or on an 'as needed' basis, but it is usually carried out less than once every 2 weeks and in most cases only a limited number of times per year for periods lasting 2 days or longer in mice or 4 days or longer in humans[25,34]. There are two major methods of PF: (1) water-only PF[25] and (2) a fasting-mimicking diet (FMD), which is a plant-based caloric-restricted diet containing low proteins, low sugars and high unsaturated fats that are able to mimic the effects that water-only fasting has on IGF-1, IGF binding protein 1 (IGFBP-1), ketone bodies and glucose[19,34].

Metabolic effects of fasting.

In most mammals, the liver serves as the main reservoir of glucose, which is stored in the form of glycogen. In humans, depending upon their level of physical activity, 12–24 hours of fasting typically results in a decrease in serum glucose and depletion of hepatic glycogen, accompanied by a switch to a metabolic mode in which glucose, fat-derived ketone bodies and free fatty acids are used as energy sources[25]. After hepatic glycogen depletion, lactate, pyruvate, fat-derived glycerol and amino acids account for the gluconeogenesis-dependent generation of approximately 80 g per day of glucose, which is mostly utilized by the brain[25]. Depending on the severity and length of the restriction, fatty acids are mobilized, leading to an increase in circulating ketone bodies and adiponectin and a lowering of circulating leptin[29,35]. In humans, the fed-state blood levels of ketone bodies are at or below the limit of detection and reach levels of 0.2–0.5 mM within 8–12 hours after the onset of fasting, but reach levels between 1 and 2 mM by 48 hours[36,37]. This metabolic switch occurs more rapidly in rodents, as plasma ketone levels are elevated within 4–8 hours after the onset of fasting and reach millimolar levels by 24 hours[38].

Intermittent fasting, metabolism and aging

Intermittent fasting in simple organisms and rodents.

IF affects longevity in multiple organisms. In worms, IF activates mitochondrial-network plasticity as they are switched between fasted and fed states, which may contribute to longevity extension[39]. Moreover, recent studies in mice show that cycles of fasting lasting from 12 to 72 hours followed by a refeeding period have beneficial effects on longevity, markers for health, stress, metabolic response, age-related diseases and tissue regeneration; cycles of fasting lasting 48 hours or longer will be covered in more detail in the 'Effects of periodic fasting and fasting-mimicking diet' section[21,25,40,41]. In flies, chronic IF has failed to extend lifespan, suggesting that flies can be sensitive to a shorter starvation period[42,43].

In mice, however, IF can have both neutral or positive effects on lifespan (see following section) and the beneficial effects of DR on longevity appear to be due, at least in part, to the time-restricted access to food, and therefore to extended daily fasting periods[16]. New studies in flies investigating short-term IF regimens for only a part of the lifespan followed by a switch to ad libitum feeding at later stages indicate stronger effects on the aging process[44]. Again, more marked effects are observed when the flies are switched back to an ad libitum diet after 30 days of IF, in agreement with a series of studies in mice, indicating that the refeeding period is as important as the fasting or restricted stage[19,20,22,23].

A number of studies have investigated the effect of IF on the health and lifespan of rodents. In one of the earliest studies, Goodrick et al. reported an increase of up to 80% in the average lifespan of rats maintained on a regimen of ADF, started at 5 weeks of age[45]. Later studies show much smaller effects of ADF, but several of them confirm an extension of median, as well as maximal, lifespan[46,47]. In addition, longevity in male C57BL/6J mice subjected to ADF is associated with a delayed onset of lethal neoplastic disorders[48] that typically limit natural lifespan in many mouse strains[49,50], but this was achieved without a delay of the aging process[48], in agreement with earlier studies[48]. Thus, the magnitude of the effects of ADF on longevity in rodents depends upon the species (mouse versus rat), strain and age at regimen initiation, and can range from a small negative effect to as much as an 80% lifespan extension[47,51]. Similarly, it has emerged from a genetic screening that CR can exert positive or even detrimental effects on longevity, depending on genetic variations in different mouse backgrounds[52]. For example, in two different mouse genetic backgrounds (A/J and C57BL/6J), IF did not extend mean lifespan, and even reduced lifespan, when initiated at 10 months[47]. When initiated at 1.5 months, IF either increased longevity or had no effect[47]. However, in rodents, IF enhances cognitive performance[53], which may be caused in part by its stimulatory effect on synaptic plasticity[54], improves insulin sensitivity and reduces blood pressure and heart rate[55,56]. Moreover, 2-month-old male C57BL/6J mice fed a time-restricted high-fat diet (16-hour daily fasting period), show protection against obesity, hyperinsulinemia, hepatic steatosis and inflammation with improved motor coordination, despite a caloric intake equivalent to that of the group with ad libitum access to a high-fat diet[57]. At the molecular level, TRF in mice affects the energy signaling mediated by c-AMP responsive element binding protein (CREB) and AMPK, the progrowth mTOR pathways and the expression of circadian clock genes[57].

A deep understanding of the type and length of fasting and mechanisms that can maximize longevity effects, as well as of the detrimental effects that may be counterbalancing the positive ones, is required. Fasting may be consistently protective in young and middle-aged laboratory rodents that are affected by cellular damage and aging, which lead to insulin resistance, inflammation, genomic instability and so on, but may have at least some detrimental effects in old or very old animals after they begin to lose weight or become frail[25].

Human studies on intermittent fasting, aging and disease risk factors.

A rapidly increasing number of studies have investigated the effects of different IF regimens on humans[41]. A recent study showed that 8-hour TRF without intentional calorie limitation

produces mild caloric restriction and weight loss in obese adults[58]. A number of trials testing IF in humans show positive effects on metabolic markers but, together with epidemiological studies, also point to potential side effects, particularly after long-term use. Varady and colleagues showed that ADF in overweight or obese adults with insulin resistance produces a greater reduction in insulin levels and insulin resistance than CR does, despite achieving a similar decrease in body weight[59]. On the other hand, Madeo and colleagues found that either short-term (4 weeks) or long-term (6 months) ADF carried out in healthy middle-aged humans has beneficial effects on metabolic and cardiovascular markers alongside reduced levels of soluble intracellular adhesion molecule-1 (sICAM-1), an age-associated inflammatory marker, low-density lipoprotein (LDL) and the metabolic regulator triiodothyronine[60]. Panda and colleagues, who had shown the beneficial effects of TRF for preventing and treating obesity and metabolic disorders in mice[32–34], showed similar cardiometabolic benefits in people with metabolic syndrome who consumed food for only 10 hours daily[61,62]. TRF prevents excessive body-weight gain, improves sleep, attenuates age- and diet-induced deterioration in cardiac performance and improves blood pressure and accumulation of atherogenic lipids[62]. Cienfuegos and colleagues also reported that 4- and 6-hour TRF reduces body weight, insulin resistance and oxidative stress compared with results from non-time-restricted controls, supporting further studies on TRF as a promising intervention for weight loss and cardiometabolic protection[63].

The health effects of IF are accompanied by weight loss, but how much reduced adiposity affects disease risk factors remains poorly understood. In one trial, 16 healthy participants (aged between 23 and 53 years with a BMI between 20 and 30) assigned to a regimen of ADF for 22 days lost 2.5% of their initial weight and 4% of their fat mass, with a 57% decrease in fasting insulin levels[64]. In two other trials, overweight women (approximately 100 women in each trial) were assigned to either a 5:2 IF regimen or a 25% reduction in daily caloric intake. The women in the two groups lost the same amount of weight during the 6-month period, but those in the group assigned to the 5:2 IF had a greater increase in insulin sensitivity and a larger reduction in waist circumference[30,65].

However, there are also a number of studies indicating that frequent fasting cycles may not only be difficult to carry out for long periods, but also increase side effects and even mortality. For example, the risk of gallstone disease nearly doubles between women who fast for 8 hours per day and those who fast for over 14 hours per day[66]. Furthermore, skipping breakfast, which is perhaps the most common method adopted to reach a daily 14- to 18-hour daily fasting period, is associated with an increased risk of mortality from cardiovascular and all-cause mortality in the US population[67]. Clearly, epidemiological data are not easy to interpret and the association between long daily fasting periods and increased incidence of disease or mortality could be explained by factors other than the fasting itself. However, until further studies, including randomized clinical studies and additional epidemiological studies, are conducted, the use of this type of daily fasting intervention should be limited to short-term periods and applied to only people with disease for which regular IF has been demonstrated to be effective. It is also important to gain an understanding of the effect of long daily fasting periods in which dinner is skipped instead of breakfast. In contrast, daily fasting/TRF periods of approximately 12 hours appear to be associated with benefits without known negative effects.

Effects of periodic fasting on aging

Periodic fasting and fasting-mimicking diet.

In contrast to the short and very frequent fasting periods of IF, PF or FMD last in most cases between 2 and 7 days (2–3 days in mice and 4–7 days in humans) and are followed by a high-nourishment refeeding period of at least 1 week[25,68,69]. Another major difference from IF is that PF can be periodic and does not have to be carried out at a specific interval, but can be applied for one or several cycles either as a preventive measure or to treat a specific disease or condition. PF was traditionally carried out in specialized clinics with water-only or very-low-calorie methods, but outside of a clinic, such a regimen can be difficult to maintain and unsafe because it can cause side effects, including malnourishment, rapid weight loss, reduced blood pressure and hypoglycemia[70,71], as well as the exacerbation of existing micronutrient deficiencies. These safety concerns and the scarcity of preclinical and clinical data may explain why historically the potential benefits of PF have emerged multiple times within the medical community, but have eventually disappeared and have not been integrated into standard-of-care practices. Thus, FMDs were developed to promote the effects of fasting while standardizing dietary composition, providing nourishment and minimizing the burden and side effects associated with water-only fasting[19]. Notably, these steps are necessary for PF and possibly IF to move toward approval from the US Food & Drug Administration and standard-of-care applications.

The FMD composition, which includes low protein, low sugar and high unsaturated fat, achieves a reduction in IGF-1 and glucose, and an increase in ketone bodies, and IGFBP-1, similar to that caused by water-only fasting in mice[34]. Various versions of the rodent FMD have been utilized, but in most cases, they provide between 10% and 50% of the normal caloric intake for periods ranging from 2 to 5 days, with the most severe restrictions lasting from 2 to 3 days[7]. A longer regimen with less caloric restriction is also used, which consists of 5 days with a caloric intake ranging from 50% on the first day to 30% for the rest of the days[19,72]. Mice undergoing FMD cycles lose about 15–20% of their body weight, which is recovered upon refeeding. In fact, the severe caloric restriction is compensated by overeating during the refeeding period, resulting in the same or similar caloric intake in the FMD and control groups[19].

Rodent studies on prolonged fasting or fasting-mimicking diet and longevity.

Sixteen-month-old female C57BL/6 mice placed on a periodic 4-day FMD twice per month, alternating with a normal diet, display an 11% increase in their median lifespan, in addition to significant weight and visceral-fat loss, without loss of muscle mass. Moreover, FMD cycles reduce tumor incidence by 45% and delay tumor development, with most being detected after 26 months of age. FMD cycles that are started at middle age reduce skin inflammation including dermatitis by 50% and improve motor coordination along with long- and short-term memory[19]. Notably, the FMD cycles also promote changes leading to an immune-system profile in 20.5-month-old mice more similar to that of younger mice (4 months old), in agreement with the effect of PF on hematopoietic stem cell (HSC)-dependent regeneration of immune cells[21]. In addition, FMD cycles selectively reduce visceral fat without an overall reduction in per-month calorie intake, indicating a

potential acceleration in metabolism during the refeeding period. In summary, similarly to the well-established effects of CR[73], FMD cycles delay the onset and reduce the incidence of age-related diseases[19], but achieve this with minimal or no long-term reduction in calorie intake and with positive effects on immunity and a targeted reduction in visceral fat. Thus, PF/FMD but potentially also certain DR, including IF, may achieve many beneficial effects by mechanisms that are independent of reduced calorie intake. In fact, chronic protein restriction, without calorie restriction, is well established to extend longevity and healthspan[16,74]. Notably, IF and PF, similarly to chronic CR, delay disease incidence but also reduce the lifelong portion of animals that develop any type of disease, and particularly cancer[19,25,34].

One limitation of studies with PF and IF is that they have been conducted on only a few mouse strains, and mostly on C57BL6 mice[19,57,75,76], so we do not yet know whether the health benefits and lifespan increase is not dependent on rodent strain. On the other hand, several of these studies were conducted in both female[19] and male mice, and some of them began when the mice were young[75] and others when the mice were already middle aged (16 months)[19], indicating that the health- and lifespan benefits of IF or PF can be achieved when started at middle age. A recent study has shown that there is memory of CR done during early mouse life, and that age-dependent mortality can depend on the nutrition earlier in life[77]. This is another effect of CR, IF and PF that is poorly understood and could help achieve the goal of maximizing healthspan and longevity with minimal burden.

Human studies on prolonged fasting/fasting-mimicking diet and longevity.

A study assessed longer periods of fasting in large cohorts that included non-obese participants[78,79]. In a 1-year observational study, 1,422 non-obese participants aged between 18 and 99 participated in a fasting program consisting of fasting periods of between 4 and 21 days in which they fasted with a daily caloric intake of 200–250 kcal accompanied by a moderate-intensity lifestyle program. Significant reductions in weight, abdominal circumference and blood pressure were observed, along with a reduction in blood glucose levels and increase in ketone bodies, proving the fasting-related metabolic switch[79]. However, these effects are detected during the fasting period. Also, a single period of extended fasting with concomitant weight reduction leads to significant, rapid improvement of fatty-liver index in people with or without type 2 diabetes (age   18 and BMI   19 kg per m^2) by the end of the fasting cycle, but the long-term effect of this regimen on fatty liver after return to the normal diet is not known[78]. The major limitation of these very severe and prolonged fasting periods is that they must be carried out in a specialized clinic to avoid adverse events. Furthermore, we do not know the long-term effects of the long periods (weeks) of water-only or very-low-calorie fasting on health. In fact, long periods of CR have been associated with a reduction in metabolic rates[80], which could actually promote, rather than reduce, fat accumulation after the end of the fasting or CR period, which would result in the regain of weight as established in studies involving severe restrictions or fasting lasting 10 days and longer. In addition, multiple cycles of weight loss (ranging between 7% and 10%) and regain (yo-yo diets) are associated with increased mortality[81], indicating that long and severe fasting periods could have short-term benefits followed by long-term beneficial as well as detrimental effects.

The periodic use of FMD and refeeding cycles was studied to identify interventions to maximize effects against aging and age-related diseases, while minimizing side effects and the burden of frequent restrictions. In a randomized clinical trial with 100 relatively healthy volunteers, FMD cycles lasting 5 days, carried out once per month for 3 months, reduce multiple risk factors for age-related diseases, including diabetes, cancer and cardiovascular disease[72]. These effects include reduced body weight and trunk fat, lowered blood pressure and decreased IGF-1, along with decreased BMI, glucose, triglycerides, cholesterol and C-reactive protein 5 to 7 days after returning to a normal diet in people that displayed elevated levels of these markers at baseline[72]. Notably, the beneficial effects of the FMD on several of these markers/risk factors were maintained for months after subjects returned to a normal diet[72]. These studies underline the potential of PF, FMDs and other types of fasting for extension of not only healthspan but also 'youthspan' (the range of time in which an organism remains youthful, healthy and fully functional)[82]. Thus, PF and FMD cycles that alternate with normal refeeding periods appear to be safe in both rodents and humans and to have beneficial effects on disease or disease risk factors when started at middle-old age (16 months in rodents). Although FMDs appear to be safe, their use should be limited to three times per year in healthy people with normal levels of disease risk factors, until additional and long-term clinical studies demonstrating the safety of more frequent use are carried out. In contrast, the more severe and longer forms of fasting should only be done in a specialized clinic in the presence of medical personnel. How much of the benefit of PF is due to effects on cellular aging and dysfunction versus the effect of weight loss remains unclear and is addressed in the following sections.

Because there is a concern that all types of fasting methods may in the long term cause side effects, including those listed earlier, when done too frequently, the beneficial effects of IF and PF must be weighed against their potential side effects, particularly in healthy or relatively healthy individuals. Both basic and clinical research should focus more on interventions that maximize efficacy against aging and age-related diseases while minimizing side effects and the burden of the intervention, which is usually inversely correlated with long-term compliance.

Periodic fasting, intermittent fasting and nutrient signaling pathways

Reduced activity of the nutrient-sensing pathways that regulate aging in yeast (Fig. 1), worms and flies can also extend longevity in rodents (Fig. 2). Thus, inhibition of the mTOR pathway either pharmacologically with rapamycin[83] or genetically by deletion of the ribosomal S6 kinase 1 (S6K1) extends longevity in mice[84]. In addition, S6K1-mutant mice show a delayed onset of age-related phenotypes, such as bone-matrix loss, immune and motor dysfunction and insulin resistance[84].

The GH–IGF-1 axis, acting upstream of mTOR–PI3K–AKT-1 and PKA signaling, has been intensively studied in mammals because of its effects on the incidence of age-related diseases and lifespan. Indeed, mice lacking the GH receptor binding protein (GHR-BP) display a 40% longer average lifespan than that of controls, with reduced tumor incidence[85].

A number of additional studies have shown similar extended lifespans for mice with defects leading to both GH and IGF-1 deficiencies[86], which are also associated with downregulation of TOR signaling in multiple cell types. Whereas the mTORC1 inhibitor rapamycin increases longevity in wild-type mice[83,87,88], in mice with knocked out growth hormone receptor that have constitutively suppressed mTORC1 and upregulated mTORC2 signaling, it leads to a drastic reduction in mTORC2 in liver, muscle and subcutaneous fat, which in turn causes an elevation of the inflammation marker interleukin-6, reduced numbers of functional immune cells and a shortened lifespan[89]. These results together, with a series of previous studies, indicate that mTORC2 is not as clearly linked to aging as mTORC1 and that its inhibition may even be deleterious[90–92].

Because carbohydrates and proteins play a central role in growth, it is not surprising that the protein and sugar restriction associated with PF, IF and CR causes conserved changes in growth factors and nutrient signaling. It is well established that higher protein levels increase IGF-1 levels[93] and that several amino acids are sufficient to promote an increase in the levels of this growth factor in the serum and also in TOR–S6K signaling[94,95]. For example, methionine regulates the growth hormone (GH)–IGF-1 axis, whereas amino acids including leucine and arginine can activate TOR–S6K signaling[74,95,96]. Moreover, the balance of macronutrients, not just their levels, and particularly of protein and carbohydrate can affect lifespan. In the mouse liver, when protein is replaced with carbohydrate, compensatory mechanisms are inhibited and protein uptake is suppressed, leading to mTOR inhibition[16]. Furthermore, the quality of macronutrient, for instance animal- versus plant-derived proteins, can influence aging and disease. In fact, high protein intake in adult life (up to age 65) is associated with increased risk of overall mortality and cancer-related death, however this association is attenuated or eliminated when the higher intake of proteins is from plant-derived sources[18]. It has also emerged that amino-acid quality can have an important effect on aging, and in fact an imbalance of branched-chain amino acids compared with other amino acids (high BCAA:non-BCAA ratio) leads to reduced longevity through a mechanism independent of mTOR activation and caused by hyperphagia[97].

Sugars can also activate various pathways including Ras or PKA signaling either through insulin action or by an insulin-independent mechanism of insulin and resulting in reduced antioxidant protection and cellular stress sensitivity[98]. Thus, it is not surprising that GH- or GHR-deficient mice and those undergoing IF or PF, all of which reduce common proaging pathways, share an extended longevity and a major reduction in disease incidence[99]. For example, CR (from 10% to 50%) reduces IGF-1 and insulin, as well as Tor–S6K signaling, in rodent models. In agreement with mouse studies, in humans CR reduces IGF-1 levels only when protein intake is also restricted, underlining the need to focus both on calorie intake and dietary composition and their effects on nutrient signaling pathways[1,100]. IF, including ADF and TRF (8-week fast, 16 hours per day (16:8)), also decrease IGF-1, blood glucose and insulin levels while increasing insulin sensitivity and adiponectin levels[101].

PF and FMDs also affect the levels TOR–S6K, IGF-1, insulin and glucose in mice, but these effects are reversed when animals return to the normal diet[19,25,34]. Thus, it is likely that a long-lasting reduction of factors including glucose, insulin and IGF-1 caused by CR is not necessary for at least part of the lifespan and healthspan effects[1]. However, in humans, FMD

cycles can have long-lasting effects on IGF-1, insulin and glucose, raising the possibility that at least some of the effects of dietary restriction and FMDs on longevity may involve long-term effects on the levels of these factors[72].

Another mechanism that may explain the role of the temporary reduction of these factors on the longevity extension caused by PF is the activation of HSC-based and other regenerative processes discussed later in this Review. Notably, these effects depend on the activation of regenerative processes that begin during fasting periods but are completed after mice return to a normal diet[19–21]. IF lasting between 12 and 24 hours can also have effects on IGF-1, IGFBP-1, glucose and ketone bodies, but most of the changes are smaller than those obtained by the longer PF or the chronic CR. For example, in mice, 4 weeks of every-other-day IF did not elevate ketone bodies but instead caused a reduction in β-hydroxybutyrate levels and β-hydroxybutyrate dehydrogenase activity in the mouse liver but not in the cerebral cortex, where levels remained unchanged or enhanced, supporting the importance of further investigating the mechanisms of ketone-body production, release and delivery[102]. However, circulating IGF-1, insulin and glucose were decreased after resistance-trained men (aged 29–33 and weighing 85–92 kg) were placed on a 16:8 fast, indicating that IF can have a positive effect on these factors[101]. Notably, because this IF period also caused loss of fat mass, it is difficult to establish whether the effects of IF on insulin and glucose and possibly IGF-1 are mediated by effects on adipose tissue. Importantly, these positive effects in men undergoing the 16:8 TRF were also accompanied by negative ones, including an over 20% decrease in total testosterone[101]. These results point to the importance of continuing to study both the short-term and long-term molecular changes caused by IF, PF and CR and to connect them to the positive as well as negative effects on metabolic markers, but also diseases and other conditions.

Fasting–refeeding and regeneration

Fasting and refeeding regimens are powerful promoters of stem-cell self-renewal mechanisms and activators of tissue regeneration, in part through inhibition and reactivation of the IGF-1, PKA and mTOR pathways[19–21,103] (Fig. 3). The PF and FMD regimens can promote a rejuvenation process in tissues, organs and cells through the activation of cell death and autophagy followed by the activation of stem or progenitor cells[19–21]. Notably, the refeeding period appears to be responsible for a major component of the regeneration process leading to the replacement of senescent or damaged cells with new cells arising from tissue-specific stem cells[19–22]. Not surprisingly, the effects of short-term fasting and FMD on stem-cell function and regeneration depend on the content of the diet, its effects on different pathways and the timing and duration of the regimen. For example, lifelong CR does not prevent the age-related functional decline of HSCs in mice[24], whereas short-term fasting periods, as well as FMD regimens followed by refeeding periods, do promote regenerative and rejuvenating effects in the hematopoietic and immune systems[19]. This is a fundamental distinction between cycles of fasting and refeeding compared with chronic dietary interventions, which may have smaller regenerative effects compared to fasting/refeeding by preventing or limiting the regenerative phase which requires high levels of macro- and micronutrients and possibly higher calories to support macromolecular synthesis, cellular division and growth and tissue and organ expansion[18].

Stem-cell-based regeneration is stimulated by IF and PF through nutrient-sensing pathways[19,21,103]. The positive effects of fasting have been described in multiple stem-cell types, including muscle stem cells, HSCs, intestinal stem cells (ISCs) and neuronal stem cells (NSCs)[19,21,103–105]. The common mechanisms through which fasting affects stem-cell function involve modulation of the amino-acid- and glucose-sensing pathways, including IGF-1–TOR–PKA, also implicated in longevity regulation. However, the fasting–refeeding cycles also affect inflammation and can cause long-lasting epigenetic changes or changes in the stem-cell niche, which could contribute to the biological age of cells and organs[40].

Whether changes in stem-cell function in response to nutrient availability are mediated by epigenetic changes is still unclear, but a few studies have indicated this possibility. For instance, the regulation of NSC proliferation in response to decreased glucose availability is governed by the nutrient sensors CREB and SIRT1, and the effects of CR are accompanied by an increase in histone H3 acetylated at Lys 9 (H3K9Ac)[106]. Changes in dietary intake are accompanied by massive changes in chromatin states in several species[107,108]. On the basis of these studies and on previous results of PF and FMD on tissue regeneration[19–22,72], we speculate that FMD and/or refeeding periods may cause long-lasting epigenetic changes, although further studies are required to test this hypothesis and to understand its possible role in stem-cell maintenance during aging.

During aging, a skewing in the lineage of differentiation of HSCs occurs with relatively more myeloid cells being produced than lymphoid cells. This skewing contributes to an age-related impairment of adaptive immunity[109]. PF reduces IGF-1–PKA signaling and promotes HSC self-renewal and long-term repopulation capacity upon serial transplantations and lineage-balanced regeneration of the immune system[21]. Also, four periodic cycles of PF or FMD started at 16 months of age rejuvenate the hematopoietic system, leading to an increased number of white blood cells 1 week after refeeding, and to a partial reversion of the age-dependent lineage skewing[19]. Moreover, FMD cycles can partially revert the age-dependent decline of mesenchymal stem and progenitor cells, thus indicating that they promote proliferation of new stem and progenitor cells in various tissues[19].

Quiescent satellite cells express the transcription factor paired box 7 (Pax-7), and when activated, coexpress Pax-7 and MyoD, the myoblast determination protein, which promotes transcription of muscle-specific target genes and plays a role in muscle differentiation. When they proliferate, they downregulate Pax-7 and differentiate[110]. Interestingly, mice undergoing FMD cycles show increases in Pax-7 and MyoD in muscle tissue, with associated reversal of markers of impaired autophagy[19]. Of note, the boost in protein expression of the regenerative markers was detected during the refeeding period, while no major changes were measured at the end of the fasting period. These results indicate that different systems may activate quiescent stem or progenitor cells at different stages during the fasting and refeeding periods. Notably, in old mice, short-term administration of spermidine, a known CR mimetic[111], reverses the age-associated defect of autophagy in muscle cells, normally characterized by loss of proteostasis, increased mitochondrial dysfunction and oxidative stress. The molecular mechanism of spermidine action involves the increase of protein deacetylation and autophagy activation through induction of arginine

and NO (nitric oxide) metabolism, as well as downregulation of proinflammatory cytokines in muscle stem cells (satellite cells)[112].

In the mouse pancreas, PF and FMD promote a decrease in the numbers of differentiated or committed pancreatic cells, followed by the induction of transitional α-to-β or β-to-α cells that coexpress both α (that is, glucagon) and β (that is, PDX-1 or insulin) cell markers, and finally a major increase in the proliferation and number of insulin-generating β cells[20]. The metabolic reprogramming caused by the FMD also affects lineage determination in pancreatic islets with increased pluripotency and β cell reprogramming markers, especially one day after refeeding[20]. Results in human pancreatic islets from healthy donors and people with type 1 diabetes indicate that FMD induces the expression of SOX-2, NGN3 and insulin in part by reducing IGF-1 and inhibiting both mTOR and PKA signaling[20]. Recent work confirms the effect of FMD on pancreatic regeneration and diabetes in a genetic mouse model of type 2 diabetes[20,113] and also confirms that a FMD promotes reduced blood glucose, increased insulin sensitivity, β cell proliferation and NGN3 expression[113]. The protein content of this specific FMD formulation was higher (17% versus 6%), whereas fat was lower (14% versus 65%), than the content of the Cheng et al. formulation[20], but the diet was administered for nearly twice as long and was much more calorically restricted (7 days at 30% of daily calorie intake). Thus, in the latter study, changes in the key signaling pathways, similar to those caused by the shorter FMD, were likely to be achieved by a longer and more severely restricted period with a less-fasting-mimicking macronutrient ratio. Thus, the beneficial regenerative effects achieved are affected by dietary composition, severity of the calorie restriction and length of the PF or FMD period.

In another study, one day of fasting was sufficient to increase ISCs and progenitor activity both in young and old mice by inducing a fatty-acid oxidation program[103]. Interestingly, a recent study carried out in a mouse model of intestinal bowel disease shows that FMD cycles stimulate protective gut microbiota, reduce intestinal inflammation, increase stem-cell number and reverse intestinal pathology[23]. Notably, water-only fasting increases regenerative and reduces inflammatory markers without reversing pathology[23], supporting the possibility that specific nutrients contained in the FMD, and possibly prebiotic ingredients contained in plant-based foods, can have a crucial impact on microbiota and consequently on the course of the disease. A recent study highlights that dietary macronutrients, in particular carbohydrate and protein, can also be major drivers of microbial response and can reshape microbiome by dictating nitrogen availability to bacteria[114].

Previous studies also indicate that long-term dietary restriction can increase the regenerative capacity of ISCs via an extrinsic mechanism involving reduction of mTORC1 in the niche surrounding Paneth cells, suggesting that the refeeding period is not necessary for ISC activation[104], although it may enhance or maximize regeneration as indicated in other studies[19–22,72]. Taken together, these studies suggest that the mechanisms of regeneration may vary depending on the timing, duration, composition and severity of both the fasting and refeeding diet. However, mechanisms involving IGF-1, TOR and PKA are likely to represent common denominators in the protective and regenerative effects of IF, PF and CR. Notably, both the inhibition of these signaling proteins and enzymes during the fasting and

their activation during the refeeding are likely to be important for the regenerative process. In fact, their role in regeneration during the refeeding period is poorly understood and should be investigated further in multiple tissues.

Intermittent fasting, periodic fasting and aging-related diseases

Advancing age is the major risk factor for most major diseases, including cancer, diabetes and neurodegenerative[115], cardiovascular and immunological diseases[116–118]. Because IF, PF and FMD cycles have been shown to slow down and partially reverse cellular aging in rodent models, a number of studies have investigated their potential application to the prevention and treatment of age-related diseases (Table 2).

Neurodegeneration.

Fasting has been shown to protect neurons and ameliorate cognitive impairment in animal models[119]. A triple transgenic mouse model of Alzheimer's disease (AD), which expresses familial AD mutations in the β-amyloid precursor protein (APP), presenilin 1 and Tau, fed either a 40% CR or ADF dietary regimen for 1 year starting at 5 months of age, develop reduced cognitive impairment compared with that in ad libitum-fed control mice; interestingly, 40% CR, but not ADF, reduces the levels of β amyloid (Aβ) and Tau accumulation in the brains of the AD mice[120]. The intermittent restriction of essential amino acids also protects mice from pathology and cognitive decline in triple transgenic AD mice, suggesting that the protein-restriction component of the fasting plays a key role in its protective effects[121]. The latter study did not detect reduced levels of Aβ after restriction of essential amino acids, although it did observe reduced accumulation of phosphorylated Tau in the hippocampus[121]. This suggests that fasting and protein restriction can protect the nervous system even in the presence of high levels of Aβ, although their effect on the fibrillar versus soluble forms of Aβ remain poorly understood. The mechanism by which fasting protects neurons from degeneration has been linked to increased expression of neurotrophic factors important for neuronal cell growth and stress resistance, including BDNF and FGF2 (refs. [122],[123]). Studies also suggest important roles for the ketone body β-hydroxybutyrate and the mitochondrial sirtuin SIRT3 in the neuroprotective mechanism of IF. Ketone bodies may be protective against GABAergic interneurons degeneration through a mechanism dependent on SIRT3, which was shown to reduce anxiety-like behavior and to improve hippocampus-dependent memory in mouse models of AD[124],[125].

A common genetic mouse model for Parkinson's disease (PD) that overexpresses human α-synuclein exhibits progressive accumulation in neurons and shows motor dysfunction and premature death[126]. When these mice were treated with ADF, the autonomic nervous system deficit was reversed, while it was exacerbated even more in mice on a high-fat diet[127]. Bai et al. in a recent study showed that, in response to rapamycin treatment and the consequent inhibition of mTOR, these PD model mice displayed reduced oxidative stress and synaptic damage and an overall improvement of motor function[128]. Hence, ADF cycles have the potential to improve the overall pathology progression. Although it has not been tested with AD mouse models yet, PF or FMD has been shown to increase neural stem cells and increase cognitive performance in normal, old mice[19]. Major issues remaining to

be addressed when considering human trials are the burden of fasting every other day and the side effects described earlier, particularly in people with PD or AD, who in most cases will be over age 70. The identification of the specific dietary compositions responsible for neuroprotective and regenerative effects and the description of the mechanisms involved should eventually allow the design and development of fasting-like interventions that are able to protect against neurodegeneration with minimal side effects.

Diseases of the immune system.

Aging is associated with progressive immunosenescence, which is caused in part by the age-dependent impairment of HSC function[129]. This results in a higher ratio of myeloid cells relative to lymphoid cells, accompanied by a decline in common lymphoid progenitors, and ultimately reduced T- and B-cell lymphogenesis as well as stem-cell exhaustion and reduced regenerative capacity[130]. Dysfunctional lymphocytes can consequently give rise to immunosuppression or immunosenescence, but may also contribute to autoimmune disorders such as asthma, systemic lupus erythematosus, multiple sclerosis (MS) and rheumatoid arthritis[131]. Certain dietary restriction regimens have the potential to prevent and/or reverse age-dependent immune dysfunction by killing or altering autoimmune cells and activating HSC-dependent regeneration[21,132,133]. Notably, severe CR (66% CR) and 8 weeks of ADF regimen prevent autoimmune encephalomyelitis (EAE) in mice[134,135].

Although the molecular mechanisms of autoimmune disease suppression are not yet known, these diet regimens have been shown to decrease the amount of circulating T cell and inflammatory cytokines and chemokines[132]. In contrast to the prevention of autoimmunity described above, for the treatment of already-established MS symptoms and pathology, FMD–refeeding cycles were shown to attenuate EAE by modulating immune cells and promoting regeneration of oligodendrocyte precursor cells[132]. The FMD cycle increased apoptosis in autoreactive T cells, which are replaced in part by newly generated naive T cells during the refeeding period[132]. Notably, several studies show that fasting and CR can cause both the death and relocalization of different immune-cell populations[136–138]. In agreement with the rodent study, a clinical trial in humans also reported a reduction in lymphocytes upon FMD intervention and an improvement in quality of life in people with MS[132]. However, larger studies are necessary to determine whether the FMD can reduce multiple sclerosis pathology and progression in people.

Cancer.

Recently, a series of studies in animal models has shown that PF and FMD lasting two or more days can be as effective as chemotherapy at delaying the progression of a wide range of cancers but, more importantly, can protect normal cells from the toxic effects of chemotherapy while sensitizing cancer cells to the treatment[68,69,139,140]. PF and FMD cycles appear to increase the killing of cancer cells by causing system-wide changes that affect the ability of malignant cells to survive or adapt, which includes a reduction in IGF-1, insulin, glucose, leptin and cytokines, but likely also changes in hundreds of enzymes or pathways[72,141]. Notably, fasting and FMD are most effective against cancer cells when combined with chemotherapy, radiotherapy, kinase inhibitors, metabolic drugs or hormone therapy[142–146]. Another important mode of action of cycles of PF or FMDs and refeeding

is the activation of the immunosurveillance to promote T-cell-dependent killing of cancer cells[143,147]. These effects of the fasting/FMD on immunity-dependent attack of cancer cells was confirmed by a recent study from Collins et al. showing that mice that underwent severe 50% CR for few weeks accumulated memory T cells in the bone marrow, and that caused enhanced protection against infections and tumors[136]. The broad effect of fasting and FMD in decreasing circulating levels of glucose, and IGF-1 and increasing ketone bodies, IGFBP-1 and so on together with the targeted toxicities of standard cancer therapies have the potential to promote major improvements in therapeutic index and cancer- or progression-free survival.

Diabetes and cardiovascular disease.

Both IF and PF or FMDs can be effective in reducing not only weight, abdominal circumference and body fat, but also risk factors for metabolic syndrome, diabetes and cardiovascular disease.

ADF and chronic CR caused similar effects on weight loss, reducing body and abdominal fat and lipids and improving insulin sensitivity in humans[27]. Notably, ADF had stronger effects on high-density lipoprotein (HDL) and LDL than did CR[27]. Another study on people with one or more risk factors for metabolic syndrome compared a 6-month ADF against chronic CR. Both groups experienced a similar decrease in body weight (8–9 kg), as well as improvements in blood pressure, triglycerides and HDL (Table 2).

In another study, 8 weeks of a 10-hour time-restricted eating decreased body weight (4 kg), waist circumference (4 cm), systolic BP (13 mmHg) and glucose in people with metabolic syndrome. No significant changes were found in LDL cholesterol, HDL and insulin or insulin resistance[62] (Table 2).

For the PF interventions, three monthly cycles of a FMD were effective at reducing risk factors for diabetes and CVD in higher risk people. Independently of initial weight, people underwent a reduction in BMI, although individuals with BMI > 30 displayed a greater decrease (Table 2). Significant improvements in total cholesterol, LDL, fasting glucose, C-reactive protein and triglycerides were also observed in people with high levels of these risk factors at baseline[72] (Table 2). In summary, both IF and PF or FMDs can cause a wide range of improvements in cardiometabolic risk factors, which are likely to lead to a reduction in diabetes and CVDs. Additional and larger studies will be important to determine which of these interventions can become standard of care in disease prevention and treatment.

Conclusion and future perspectives

IF and PF or FMD activate ancient programs that promote entry into alternative metabolic modes focused on conserving energy and on protecting the organism while enduring extended periods of food deprivation to optimize survival and reproduction once food becomes available. In fact, it is the refeeding period that has more recently emerged as an equally important process involved in the regeneration, and possibly rejuvenation, of systems, including organs, cells and organelles. In humans, the alternation of fasting and refeeding periods is accompanied by positive effects on risk factors for aging, diabetes,

autoimmunity, cardiovascular disease, neurodegeneration and cancer. But not all fasting interventions are equal, and some are associated with smaller beneficial effects as well as side effects, including, in some cases, reduced longevity.

The IF and PF field should expand the investigation of the effects of dietary macronutrient composition, ratio and quality on healthspan. In fact, lifespan and healthspan extension can be reached by a specific macronutrient balance, independently of caloric intake. It is also important to identify the mediators of the effects of IF and PF not only on different types of mammalian cells and organs and on disease, but also on the microorganisms of the digestive system, with a focus on the molecular mechanisms mediating key effects on cellular protection, aging and regeneration.

It will not only be important to separate the positive effects of IF and PF from the adverse effects, but also to match the type and length of IF and PF with goals including healthspan extension, the prevention and treatment of specific diseases and weight management.

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Box 1 |

Glossary

Autophagy. The digestion of cellular components by enzymes of the same cell to allow for their degradation and recycling.

Calorie restriction (CR). A dietary regimen with a 20–40% reduction of the normal daily calorie intake without malnutrition.

Chromatin regulators. Factors that allow dynamic chromatin remodeling by accessing condensed genomic DNA regions to control gene expression.

Dietary restriction (DR). A dietary regiment which accounts restriction of one or more macronutrients. Calorie intake can be normal or restricted.

Epigenetic drift. Epigenetic changes acquired with age that modify the DNA-methylation landscape of a cell.

Fasting-mimicking diet (FMD). A diet designed to achieve fasting-like effects on the serum levels of IGF-1, IGFBP-1, glucose and ketone bodies, while providing both macro- and micronutrients to minimize the burden and adverse effects of water-only fasting.

Forkhead transcription factor FOXO. A subgroup of the forkhead transcription factor family regulated by the insulin–PI3K–AKT pathway and involved in activation of stress response and survival genes.

Healthspan. The period of an organism's life during which it is generally healthy and free from serious or chronic illness.

Insulin–IGF-1 signaling. A conserved nutrient-sensing pathway among different organisms that coordinates growth, differentiation, metabolism and longevity in response to changing environmental conditions and nutrient availability.

Intermittent fasting (IF). An eating pattern in which short periods of fasting (16–48 hours) and eating periods (8–120 hours) are alternated.

Ketone bodies. Intermediates of the lipid metabolism that are produced by the liver from fatty acids during periods of low food intake, such as fasting or calorie restriction.

Protein restriction (PR). A dietary regiment with a reduction of total protein intake or a reduction of specific amino acids.

p16. A tumor suppressor protein with an important role in cell-cycle arrest. Its increase is related to increased senescence and cellular aging.

Periodic fasting (PF). An extreme dietary restriction period characterized by a complete restriction of food (water-only) or a severely calorie-restricted diet (FMD) for a prolonged duration (more than two days), followed by an undertermined ad-libitum refeeding period. PF can be repeated but infrequently.

Pluripotency markers. Markers that identify pluripotent cells, or cells that are able to differentiate into any cell type.

Ribosomal biogenesis. A cellular process that results in the biosynthesis assembly and arrangement of constituent parts of ribosome subunits.

Sirtuins. Sirtuins are both histone deacetylases (HDAC, class III), able to remove the acetyl group from acetylated lysines inhistones) and ADP-ribosyltransferases. They regulate cellular metabolic signaling and protein post-translational modifications.

Stem-cell self-renewal. Stem cells' ability to go through cycles of cell division while maintaining the undifferentiated state.

Starvation. A chronic nutritional restriction that, in contrast to IF and PF, results in detrimental effects, which can include a deficit in fat stores and the atrophy of muscles.

Time-restricted feeding (TRF). A daily eating pattern in which all nutrient intake occurs within a limited number of hours (usually 12 hours).
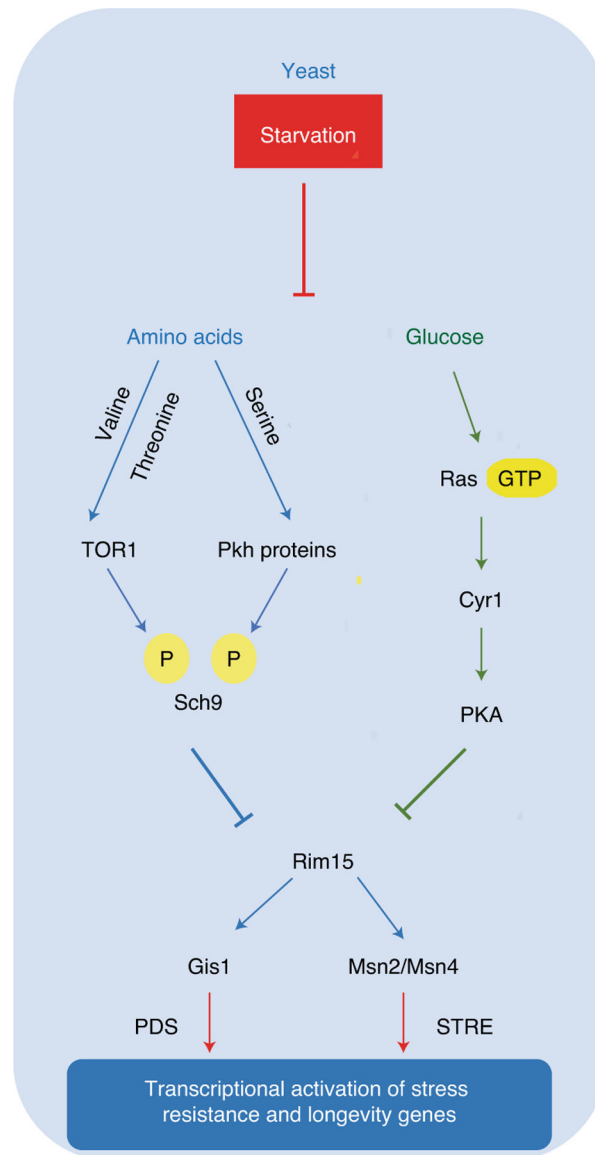
Fig. 1 |. Fasting, nutrient signaling and longevity in yeast.

Starvation conditions in yeast cause a major lifespan extension mediated in large part by the lack of amino acids and sugars. On one hand, amino-acid restriction causes the inactivation of TOR–Pkh–S6k signaling; on the other hand, low glucose levels promote reduced activity of the Ras–adenylate cyclase (Cyr1)–PKA pathway. Both the amino-acid and the sugar pathways converge on and inactivate the serine threonine kinase Rim15. This, in turn, contributes to the activation of stress-resistance transcription factors Gis1, which binds to post diauxic shift (PDS) motif, and Msn2 and Msn4, orthologs of mammalian early growth response protein 1 (EGR1)[1,19], which bind to stress-responsive element (STRE) motif.
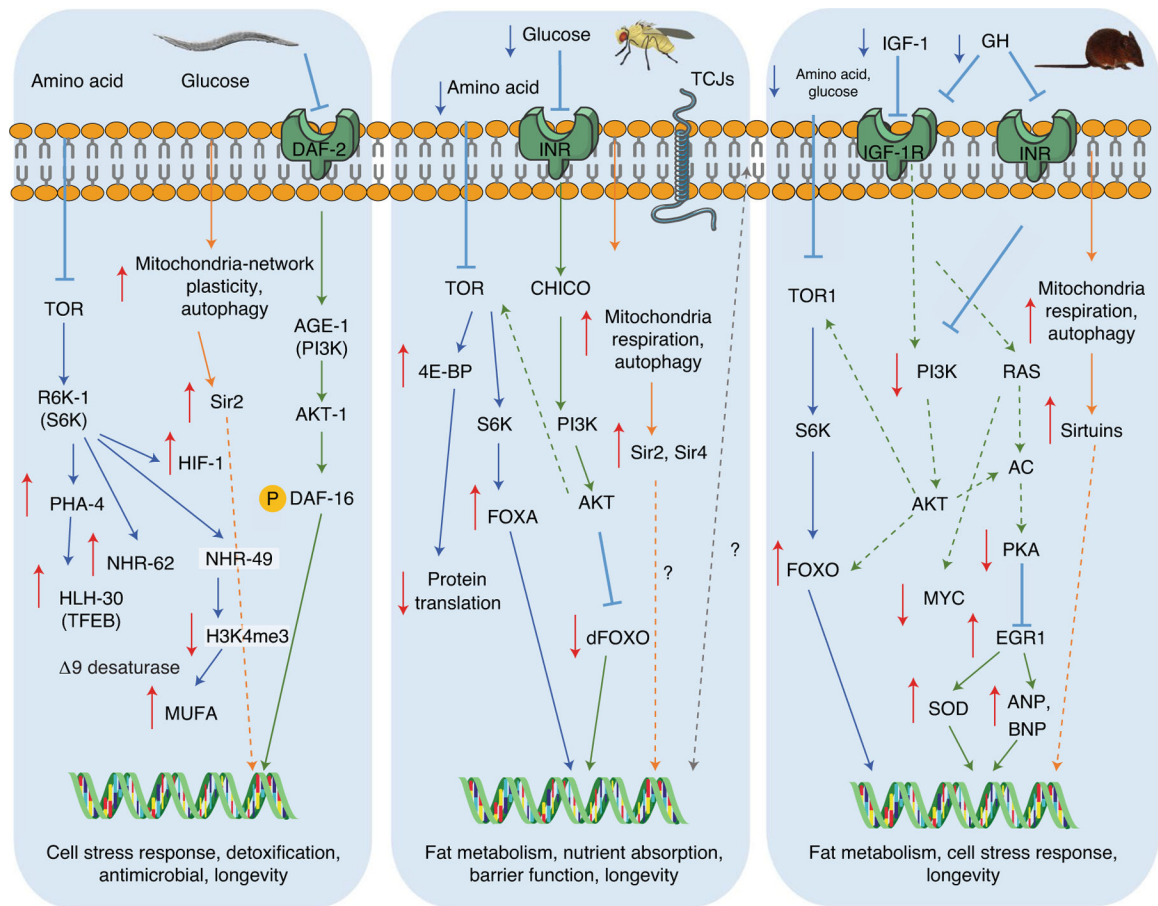
Fig. 2 |. Conserved nutrient-sensing response pathways in worms, flies and mammals.
This model summarizes the conserved nutrient-sensing pathways that regulate longevity
and stress-response mechanisms in different model organisms[1]. Fasting or calorie restriction
reduces the activity of amino acids and glucose signaling pathways through membrane
receptors by reducing circulating ligands such as growth factors like mammalian IGF-1.
The fasting-inhibited TOR–S6K pathway (labeled in blue) promotes the expression of
nuclear transcription factors such as the hypoxia-inducible factor-1 (HIF-1), the FOXA
ortholog PHA-4, the nuclear hormone receptors NHR-62 and NHR-49 and the TFEB
ortholog HLH-30 (worms), the FOXA nuclear factor (flies) and the increase of the FOXO
nuclear factor (mammals). These transcription factors commonly activate antiaging systems
and processes, including autophagy and ribosomal biogenesis, stress response and cellular-
protection genes, including antioxidant SODs. The RAS–AC–PKA pathway (labeled in
green) is also partially conserved between species. Similar to what was observed in
yeast, glucose in certain mammalian cells can signal through the PKA pathway and the
transcription factor EGR1, the mammalian ortholog of Msn2/Msn4 in yeast. In worms,
flies and mice, downregulation of TOR–S6K signaling has conserved proaging effects[1].
The fasting-dependent effects on longevity in different organisms may also involve sirtuin
pathway activation (labeled in orange), the increase in mitochondria respiration and the
activation of autophagy[39,40]. Also, fasting in flies delays the disruption of tricellular

junctions (TCJs), which is linked to improved intestinal barrier integrity and therefore longevity (labeled in gray)[148].
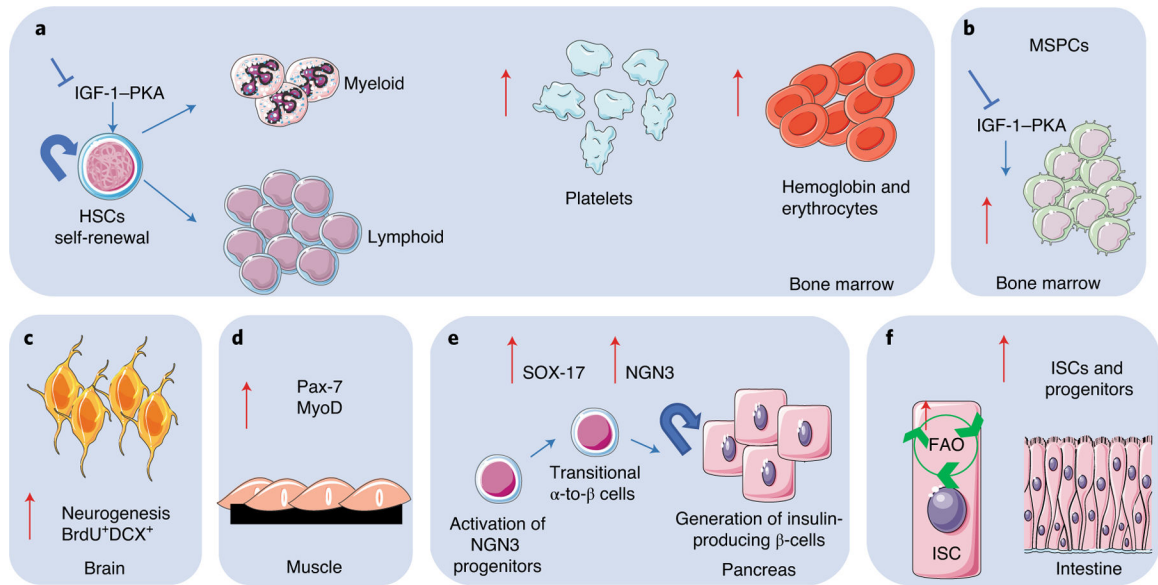
Fig. 3 |. Periodic fasting and tissue regeneration and rejuvenation in mice.
Periodic fasting or FMD can affect tissue regeneration in multiple systems and organs in mice. **a**, In bone marrow, PF or FMD drives self-renewal of HSCs and lineage-balance regeneration of the immune system, leading to a lymphoid-biased phenotype. **b**, PF and FMD increase mesenchymal stem and progenitor cells (MSPCs) in bone marrow. **c**, PF and FMD increase neurogenesis in brain tissue, represented by increase in doublecortin (DCX) levels in newly generated bromodeoxyuridine (BrdU$^+$) neurons. **d**, In muscle tissue, PF or FMD modulates the expression of the pair box protein Pax-7, a stem-cell marker mainly expressed by muscle satellite stem cells, and MyoD, a marker of early muscle differentiation. **e**, In the pancreas, PF and FMD drive increased expression of early developmental markers, including SOX-17, and of the downstream NGN3 transcription factor, leading to the regeneration of insulin-producing β cells. **f**, In intestinal tissue acute one-day-only fasting, PF and FMD increase levels of ISCs and progenitors in part by inducing a fatty-acid oxidation (FAO) program or by modulating gut microbiota.

Table 1 |

Experimental strategies to promote healthspan in different organisms

	Type of fasting regimens	Macronutrients composition	Feeding pattern	Description	Refs.
Intermittent fasting (IF)	ADF	Standard (15% protein, 30% fat, 55% carbohydrate)	24-hour fast (water-only)/24-hour eating period	Complete fasting every other day	12–15
	5:2	Standard (15% protein, 30% fat, 55% carbohydrate)	2-day fast, or very low calorie consumption (500–700 kcal)/5-day eating period	Alternation of 2 days of 500–700 kcal with a 5-day ad libitum eating period. The 2 days of fasting can be consecutive or separated	17,18
	TRF	Standard (15% protein, 30% fat, 55% carbohydrate) Obesogenic (10% protein, 60% fat, 30% carbohydrate)	12- to 18-hour fast/6- to 12-hour eating period	Food intake restricted to 6–12 hours per day	19,20
	Modified ADF	Standard (15% protein, 30% fat, 55% carbohydrate)	24-hour restriction (75% calories)/24-hour eating period	Alternation of 24 hours of a very low calorie consumption with a 24-hour ad libitum eating period	14,16
Periodic fasting (PF)	Prolonged fasting (water-only)	Standard (15% protein, 30% fat, 55% carbohydrate)	3- to 21-day water-only fast/ 7 days or longer-day refeeding period	Up to 21 days of water-only fasting followed an ad libitum eating period of at least 7 days	9,65,66,107
	FMD	FMD (9–10% protein, 50–60% fat, 30–40% carbohydrate)	4- to 7-day FMD/10- to 25-day refeeding period	30–50% of the normal caloric intake for 4–7 consecutive days, followed by a refeeding ad libitum period once per month	7,8,10,78

Table 2 |

Fasting and disease risk factors in humans

Diet intervention	Disease risk factors decreased with fasting	References
ADF	Weight loss, total cholesterol, triglycerides, fat mass, LDL	14,15,60,149
5:2	Weight loss, insulin resistance	17,61
TRF	weight loss, fat mass, leptin, systolic blood pressure	54,110,150
FMD	Weight loss, systolic blood pressure, diastolic blood pressure, IGF-1, glucose, triglycerides, total cholesterol, LDL, C-reactive protein	78