

REVIEW

CALORIE RESTRICTION AND PERIODIC FASTING FROM RODENT TO HUMAN: LOST IN TRANSLATION?

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ABSTRACT: In recent years, nutrition has attracted attention and interest from the scientific community, as it has emerged as a fundamental player in improving lifespan and healthspan by preventing non-communicable ageing-related diseases. Preclinical studies have shown that caloric restriction and periodic fasting extend the lifespan in animal models, prevent tumorigenesis, delay the onset of age-related diseases, and enhance the efficacy of anticancer therapies. This review provides the current state of knowledge on the benefits of calorie restriction and periodic fasting on tumor development and ageing in a rodent model and summarizes the clinical progress with calorie restriction and periodic fasting in clinical trials. We also discuss the numerous caveats that might arise with the implementation of these dietary interventions in clinical practice.

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Impact statement: Dietary interventions have the potential to impact life expectancy and prevent age-related diseases like tumors and neurodegenerative disorders. However, the applicability of calorie restriction and fasting interventions from mouse models to human populations remains controversial and inconclusive. In this review, we examine the potential obstacles that may arise when attempting to apply dietary interventions from mouse models to human populations.

Key words: *calorie restriction; fasting; ageing; cancer; metabolism.*

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INTRODUCTION

The ageing of the world population and the increase in life expectancy have led to an increase in age-related diseases, including obesity, cardiovascular diseases (CVDs), type 2 diabetes, neurodegenerative diseases, and cancer (1). However, the health span (the period of life free of chronic disease) does not improve at the same rate as life expectancy. New approaches to therapy are being developed (World Health Organization,

2021) with the goal of minimizing the number of years lived with chronic diseases and the associated suffering, ultimately enhancing the overall well-being of the population and easing the growing burden of healthcare costs (2). Although the advent of molecular genetics in model organisms has made it possible to identify and modulate the genes implicated in the ageing process, these genetic strategies are difficult to apply in clinical practice. To date, caloric restriction (CR) has proven to be the most effective

tive, simplest and robust geroprotective intervention (3, 4).

In recent years, new dietary interventions, which involve alternating periods of fasting (complete abstinence from eating but not drinking) and normal diet, have been shown to have the same beneficial effects as CR in lifespan and healthspan extension (5-11).

CR consists of reducing daily calorie intake below energy demand without deprivation of essential micro- and macronutrients over a given period. In mice and rats, calorie intake is reduced by 10-50% compared to ad libitum food intake (12, 13), whereas it is reduced by 10-25% from the baseline level in humans (7, 14-16).

Periodic fasting (PF) involves several strategies that differ in the duration of fasting. The 5:2 diet involves two non-consecutive fasting days per week (5); alternate-day fasting (ADF) consists of fasting every other day (17); time-restricted eating (TRE) limits meal intake to a daily window of 6-8 hours followed by a fast of 14-16 hours (6-8). The fasting mimicking diet (FMD), on the other hand, is a 5-day plant-based low-calorie diet program alternated with a normal diet, repeated 2-3 times a year, which aims to reproduce the effects of fasting without complete food deprivation (9-11).

In this review, we will discuss the mechanisms regulated by CR and fasting, the effects of such dietary interventions on ageing and cancer, and the pitfalls that might be encountered in translating such dietary programs from the preclinical to clinical phase.

SYSTEMIC METABOLIC AND HORMONAL CHANGES INDUCED BY CR AND PF

Similar to CR, acute fasting triggers catabolic and hormonal responses in mice and humans. Drops in glucose levels due to nutritional restriction or abstinence reduce circulating insulin levels and promote glucagon secretion from the pancreas, which stimulates glucose release from the liver via glycogenolysis and lipolysis in adipocytes via lipase activation (18). Non-esterified fatty acids, released into the circulation upon triglyceride breakdown, undergo beta oxidation mainly in the liver mitochondria and peroxisomes, and are converted into acetyl-CoA, which is used to fuel the mitochondrial Krebs cycle and supply the cell's energy needs.

If the fasting period is prolonged, acetyl-CoA is

converted in the liver to ketone bodies (KB; hydroxybutyrate, acetoacetate, and acetone), which become the main energy source for extrahepatic organs, such as the brain and heart (19). Conversely, KB can be catabolized to acetyl-CoA, which helps sustain the Krebs cycle and energy production (20, 21).

Simultaneously, acetyl-CoA and glucogenic amino acids are metabolized into glycolytic/gluconeogenic or Krebs cycle intermediates that support glucagon-stimulated glucose synthesis (gluconeogenesis) via the cyclic AMP (cAMP)/protein kinase A (PKA) signaling pathway in the liver.

Nutrient depletion leads to an increase in ghrelin (hunger hormone) (22), branched-chain amino acids, adiponectin and FGF21 (fibroblast growth factor 21). The latter are involved in both the regulation of glucose levels and energy homeostasis (23). In contrast, the circulatory levels of various factors, such as insulin, IGF-1, glucose, gluconeogenic amino acids, triglycerides, and leptin (satiety hormone), decrease significantly over time.

These metabolic changes are accompanied by a decrease in inflammatory markers and oxidative stress markers, including C-reactive protein (CRP), a liver-derived acute-phase biomarker associated with the inflammatory response (18, 24, 25).

Furthermore, the thyroid gland function and the secretion of the thyroid hormone triiodothyronine (T3) decreases during fasting and caloric restriction and lead a reduction in the body's energy expenditure and a slowing of the metabolic rate in human (17, 26-28). In rodents, low thyroid function leads to a decrease in body temperature, which could contribute to metabolic changes related to CR and fasting (29).

CR and fasting affect intestinal microbiota populations, which depend on the diet's macronutrient composition and metabolome content (30). Clinical and preclinical studies have highlighted that CR and fasting enrich the microbiota with probiotic favorable strains (e.g., *Lactobacillus* and *Bifidobacterium*), while reducing pro-inflammatory strains (e.g., *Desulfovibrionaceae* and *Streptococcaceae*) (31-33). However, returning to a normal diet restores the original microbiota composition and reverses the effects of CR and fasting (34).

The microbiota is a key factor in determining the positive outcome of CR and fasting, as germ-free mice do not benefit to the same extent from CR and fasting-mediated effects as conventional mice (e.g., body weight and fat loss) (34, 35).

CELLULAR AND MOLECULAR MECHANISM UNDERLYING CR AND FASTING

Nutrient availability shapes the cellular metabolome, epigenome, transcriptome, and proteome by modulating several interconnected nutrient-sensing pathways and influencing various cellular processes.

Low glucose and insulin/IGF1 levels and amino acid restriction inhibit mechanistic target of rapamycin (mTOR). mTOR is a threonine-serine kinase that acts as the catalytic domain of two protein complexes: mTORC1 and mTORC2. Its inhibition leads to the arrest of protein synthesis, lipogenesis, ribosome and nucleotide biogenesis, and activation of autophagy.

mTORC1 senses and integrates different environmental stimuli, and coordinates cell growth and division based on environmental nutrient availability. mTORC2 is an effector of PI3K signaling, is sensitive only to growth factor stimulation, and is involved in cytoskeleton organization and cellular insulin sen-

sitivity. Calorie restriction and fasting exert their beneficial effects through inhibition of mTORC1 and activation of mTORC2 (36-41) (**Figure 1**).

Under low-energy conditions, increasing the AMP/ATP ratio activates AMP-activated protein kinase (AMPK), a cellular energy sensor serine/threonine kinase involved in fatty acid oxidation, glucose uptake, and lipogenesis inhibition in various cell types (42).

AMPK activation increases NAD⁺ levels by stimulating the expression and activity of nicotinamide phosphoribosyltransferase (NAMPT), a rate-limiting enzyme in NAD⁺ synthesis, and indirectly by enhancing fatty acid oxidation and mitochondrial respiration (43, 44). Elevated NAD⁺ levels increase sirtuin deacetylase activity, resulting in epigenetic remodeling, metabolic reprogramming, and transcriptional changes. The sirtuin family includes seven members (SIRT1-SIRT7) that differ in their subcellular localization and function (45, 46). Sirtuins also possess ADP ribosylation (SIRT4 and SIRT6) (47, 48), demalonylation and desuccinylation (SIRT5) (49, 50), and lysine decrotonylation (SIRT1 and SIRT2) activities, which could mediate the beneficial effects of CR and fasting (45).

AMPK and SIRT1 enhance mitochondrial respiration and mitochondrial fatty acid (FA) transport and utilization via peroxisome proliferator-activated receptor- γ coactivator 1 α (PGC-1 α), a master transcriptional regulator of mitochondrial biogenesis (51) (**Figure 1**).

To maintain homeostasis during fasting, cells initiate autophagy, which is a process that results in the degradation of damaged organelles and dysfunctional macromolecules via lysosomal pathways. This degradation provides energy and key metabolites for macromolecule synthesis to sustain nutrient-depleted cells (52-55).

Fasting and CR promote autophagy via AMPK-mediated control of mTOR and SIRT1 and through the deacetylation of nuclear and cytoplasmic proteins due to the low availability of acetyl-CoA (56).

The effects of CR and fasting might also be mediated by the nutrient-sensitive GCN2 (general control non-derepressible 2) signaling pathway, which is involved in immune system homeostasis (57) and in the coordination of integrated stress responses and the inflammasome (58).

Upon uncharged tRNAs accumulation or ribosomal stalling (59-61), GCN2 phosphorylates eukaryotic translation initiation factor 2 (eIF2 α) and inhibits mTORC1 and the translation of most mRNAs,

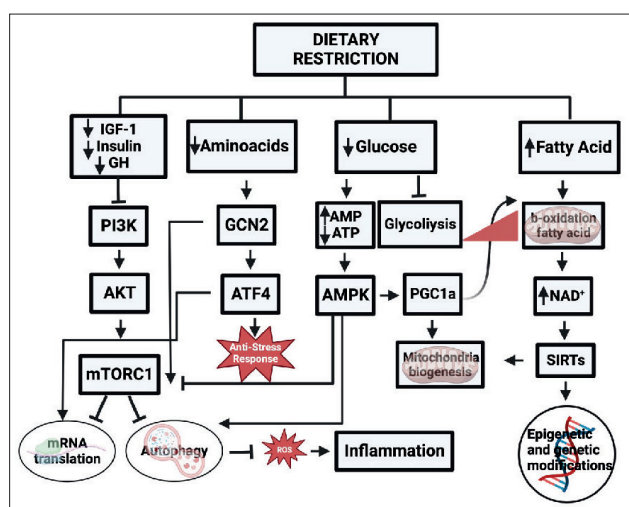


Figure 1. Signaling pathways regulated by CR and PF. Reduced levels of most macronutrients (glucose and amino acids) lead to activation of autophagy, inhibition of mRNA translation, reduced inflammation, and anti-stress response through modulation of the IGF-1-PI3K-AKT-mTORC1 and GCN2-ATF4 signaling pathway. The high AMP/ATP ratio increases AMPK activity which results in mTORC1 inhibition, autophagy activation, and PGC1 α -mediated mitochondria biogenesis. The low glucose level, the increase in fatty acids and their beta oxidation promote the cellular transition from glycolysis to oxidative phosphorylation leading to an increase in the level of NAD⁺, the activation of sirtuins and consequently epigenetic and genetic remodelling. FGF21 = fibroblast growth factor 21, IGF-1 = insulin-like growth factor, mTORC1 = mechanistic target of rapamycin complex 1, NAD⁺ = Nicotinamide adenine dinucleotide, AMP = Adenosine monophosphate, ATP = Adenosine triphosphate, SIRT5 = sirtuins (created with BioRender.com).

except for some selected proteins, such as ATF4 (62, 63) (**Figure 1**).

The transcription factor ATF4 promotes the transcription of genes involved in amino acid import, glutathione biosynthesis, and antioxidative stress response, including the energy balance hormone FGF21 (64). FGF21 is an endocrine hormone that improves metabolic health by increasing insulin sensitivity and energy expenditure by regulating lipid and glucose metabolism.

AMPK activation, low insulin and IGF-1 levels, and PI3K-AKT-mTOR inhibition increase the levels of reactive oxygen species (ROS) and induce a resistance response to oxidative stress by upregulating the expression of genes such as *NRF2*, a master regulator of several cytoprotective and detoxifying genes (20, 65) (**Figure 1**).

KB, produced during fasting, promote not only the switch of cellular metabolism from glycolysis to oxidative phosphorylation but also epigenetic remodeling and transcriptional reprogramming by inhibiting histone deacetylases (66). In addition, KB exerts an anti-inflammatory effect and improves lipid profile (low triglyceride and cholesterol levels combined with high HDL) by binding to G protein-coupled receptors (GPCRs) (67) (**Figure 1**).

CALORIE RESTRICTION, PERIODIC FASTING AND AGEING

Ageing is characterized by the progressive decay of biological functions due to: 1) mitochondrial dysfunction, 2) loss of proteostasis, 3) telomere shortening, 4) cellular senescence, 5) genomic instability, 6) stem cell exhaustion, 7) altered intercellular communication (inflammaging), 8) epigenetic alterations, and 9) deregulated nutrient detection (68, 69).

CR and PF promote longevity in mammals and prevent the onset of ageing hallmarks by regulating nutrient-sensing pathways, such as IGF1-AKT-mTOR, GCN2-ATF4- FGF21, AMPK, and sirtuin pathways (68, 70) (**Figure 2**).

Genetic mouse models bearing the growth hormone receptor (Ghr) deletion (Ames mice) or Pit1 inactivating mutation (Snell dwarf mice) (71) have a lifespan longer than that of their siblings (36, 37, 41) and show reduced mTORC1 function, elevated mTORC2 activity (71, 72), low circulating levels of IGF1, elevated insulin sensitivity, low AKT activity, and increased chaperone-mediated autophagy

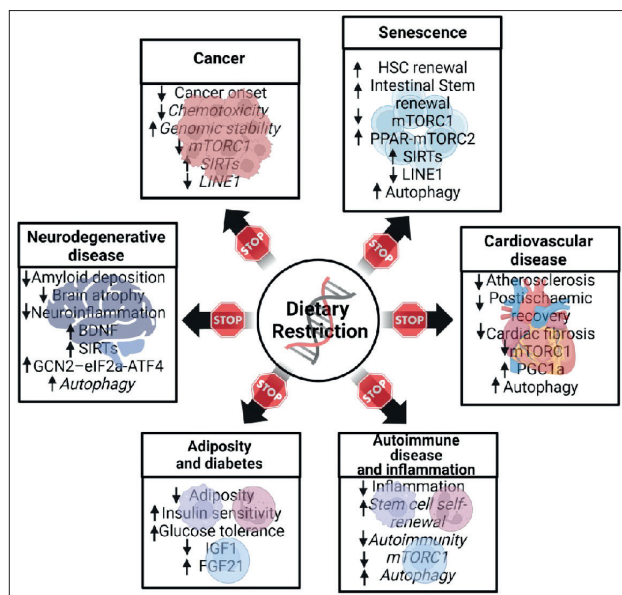


Figure 2. The beneficial effects of calorie restriction and prolonged fasting on age-related disorders and diseases. CR and PF increase health and lifespan in rodents, non-human primates, and humans by preventing obesity and diabetes, cardiovascular diseases, cancer, senescence, autoimmune and inflammatory conditions (created with BioRender.com).

compared to control mice (55). In these mouse models, mTORC1 inhibition and PPAR signaling pathway activation promote hematopoietic and intestinal stem cell renewal and regeneration, and reverse age-associated immune senescence and myelosuppression (**Figure 2**).

The GCN2-eIF2-ATF4 pathway could improve cellular health and preserve cellular and tissue homeostasis by modulating protein quality control mechanisms that remove abnormal proteins. This mechanism ensures cellular functional integrity by preventing progressive deterioration of physiological function with ageing (73).

FGF21 is a potent factor that mediates the beneficial effects of CR and PF on ageing and longevity in rodents. FGF21 treatment or transgenic overexpression extends the lifespan of mice and improves glucose tolerance and insulin sensitivity independently of calorie intake and mTORC1 activity (74-76).

Elevated levels of SIRT6 increase longevity in normal mice, extend the lifespan of progeroid mice (77, 78), and improve the regenerative capacity of hematopoietic and intestinal stem cells (79). Epigenetic remodeling induced by SIRT6 deacetylase activity enhances the anti-stress response, improves DNA double-strand repair through PARP activation (47, 80, 81), and represses LINE1 element transposition. These remodeling activities reduce

oxidative damage, increase DNA stability, prevent telomere shortening, inhibit cellular senescence, and decrease inflammation (82) (**Figure 2**).

Activation of PGC1 α , mediated by SIRT1 and AMPK, increases mitochondrial biogenesis and respiration, reduces oxidative stress, and improves cellular function and health. Furthermore, SIRT1-dependent upregulation of brain-derived neurotrophic factor (BDNF) ameliorates cognitive function in mice (83).

CR- and PF-mediated modulation of nutrient-sensing signaling pathways leads to the activation of autophagy.

Autophagy suppresses inflammatory cytokine secretion by inhibiting ROS-mediated activation of the NLRP3 inflammasome. The p62-mediated autophagosomal degradation of Keap-1 releases the transcription factor NRF2 from its inhibitor and leads to the transcription of antioxidant genes, such as catalase and superoxide dismutase (SOD), which block ROS activity and enhance the cellular anti-stress response (84, 85). Autophagy prevents immune system exhaustion and senescence by attenuating inflammation and oxidative stress (**Figure 2**).

CR and PF protect against myocardial infarction and prevent the onset and progression of neurodegenerative diseases such as Alzheimer disease and Parkinson disease in mouse models via AMPK-mTOR-mediated activation of autophagy (86-88).

CR and PF elicit different ageing outcomes, even though they regulate several common pathways and mechanisms. While CR increases both the mean and maximum lifespan in rodents by up to 50%, PF only improves the median lifespan by 11% without affecting the maximum lifespan (9).

Although CR has proven to be a promising intervention to attenuate age-related decline in rodents, studies on non-human primates have produced controversial and debatable results. In a study conducted by the University of Wisconsin, 30% calorie restriction extended lifespan and healthspan in rhesus monkeys (89, 90), while a similar study at the National Institute on Aging (NIA) demonstrated that CR improves metabolic parameters without extending lifespan (91). However, it must be taken into consideration that in the NIA study, the average lifespan of monkeys was also very high (31.8 years); thus, this aspect may not have allowed the benefits of CR to be detected (92).

CR AND PF IN CANCER PREVENTION AND TREATMENT

Tumor cells contain genetic and epigenetic alterations that reprogram cellular metabolism towards enhanced glycolysis (Warburg effect) to meet the high energy demand to support their uncontrolled cellular proliferation. The high glycolytic and proliferative activity of tumor cells leads to acidification via lactate production and nutrient depletion in the tumor microenvironment.

Tumor cells release growth factors, cytokines, and chemokines and recruit immune cells and stromal cells to hijack their metabolism and transcriptional activity to constrain their antitumor capacity (93).

CR and PF prevent the onset of tumors by slowing tumor progression and improve the efficacy of anticancer therapy by remodeling the tumor micro-environment (TME) and promoting anti-tumor immune responses even in non-immunogenic tumors (93-103).

During CR and PF, glucose levels are reduced, thereby limiting the primary energy source of the tumor. Fasting leads to inhibition of the IGF1-PI3K-mTOR pathway, which is often altered in tumor cells. The metabolic switch from glycolysis to oxidative phosphorylation compromises the tumor growth, reduces tumor plasticity and causes increased vulnerability towards chemotherapies and molecularly targeted therapies (94, 96, 99, 104-110).

These metabolic and hormonal changes also affect the composition of the immune infiltrate and priming and activity of the immune system.

Low levels of IGF1 and inhibition of the stress-responsive enzyme heme oxygenase 1 (HO1) reduce immunosuppressive T cells and MDSCs in the TME. Fasting conditions increase fatty acid beta-oxidation, oxidative phosphorylation, short-chain fatty acid (SCFA) release, and reduced methionine levels in the TME. These changes in metabolism and metabolites reshape the epigenetic landscape of T cells leading to the activation of signaling pathways involved in stemness and secondary immune response (93, 96, 111-116).

In mouse models, CR and PF not only improve the antitumor response but also exert a protective effect against the adverse effects of chemotherapy and immunotherapy. CR and PF reverse chemotherapy-induced immunosuppression by promoting hematopoietic stem cell (HSC) self-renewal, while preventing immune-related cardiotoxicity of

checkpoint inhibitors by reducing T-cell infiltration into the heart and levels of inflammatory markers, such as the NLRP3 inflammasome and leukotrienes (9, 97, 98, 100, 117, 118).

To date, clinical studies evaluating the effects of CR and PF on cancer patients are few, controversial, and have been conducted on small cohorts of patients.

However, the reduced cancer rates of Okinawan people, who eat a low-calorie, antioxidant-rich diet of mostly vegetables, fruit, fish, and seafood (119, 120), might suggest that CR could have the same antineoplastic potential found in mouse models. Furthermore, recent clinical studies conducted on a limited number of cancer patients have shown that PF is safe and well tolerated, modulates the immune system and improves the quality of life by reducing the adverse effects of chemotherapy (98, 100-102, 121-123).

However, to confirm the benefits of CR and PF on tumor prevention and antitumor response in humans, new double-blind clinical trials in larger cohorts of patients with cancer are urgently needed.

TRANSLATABILITY OF CR AND PF EFFECTS FROM RODENTS TO HUMAN: LIMITS AND CONSIDERATIONS

Caloric restriction and periodic fasting have multiple effects on the health and lifespan of rodents; however, are these benefits transferable from mice to humans?

Although mice and humans share a high degree of genomic, anatomical, and physiological similarities, they differ in physiology, morphometric parameters, and evolutionary biology. Humans are approximately 3,000 times the size of mice, live 30 times longer than mice, and have a basal metabolic rate per gram of body weight (mass-specific metabolic rate) seven times lower than that of mice (**Figure 3**). A high mass-specific metabolic rate in mice implies high production of free radicals, an increase in oxidative damage, and enhancement of cellular senescence. This difference in metabolic rate could translate into conflicting effects on ageing and tumorigenesis in these two species (124-126) (**Figure 3**).

Mice and humans age at different rates, implying that the ageing mechanism is different between the two species. The decline in physiological functions associated with ageing depends on metabolic stability, that is, the cellular ability to maintain metabolic homeostasis in response to external stresses (127, 128).

Humans have higher metabolic stability and maximum potential lifespan than mice, which are susceptible to significant metabolic alterations in response to external stress (129).

In mice, one day of fasting causes profound changes in circulating levels of metabolites (glucose, amino acids, lipids, ketone bodies), hormones (thyroid, insulin, adiponectin), and inflammatory factors (C-reactive proteins, cytokines, and chemokines), and consequently, lowers body temperature and transiently lowers metabolism. One-day fasting also implies strong inhibition of endocrine hormone

MOUSE			HUMAN	
	20-35 grams	Body Weight	70 kg	
	8 W/kg	Metabolic Rate	1.25 W/kg	
	1747.3 kcal/kg/day	Energy Expenditure Liver	224.5 kcal/kg/day	
	207.7 kcal/kg/day	Energy Expenditure Whole Body	24.6 kcal/kg/day	
	500-700	Heart Rate	60-80	
	255 per minute	Respiratory rate	12-18 per minute	
	19-21 days	Gestation	280 days	
	50 days	Sexual Maturity	11.5 years	
	26-30 months	Life span	80 years	
	2-3 days	Starvation	30 days	

Figure 3. Physiological properties for mouse (low metabolic stability) and human (strong metabolic stability) (created with BioRender.com).

signaling pathways (130-134) and nutrient-sensing systems (135).

In some mouse strains, the glucose and thyroid hormone T3 levels are drastically reduced after 24 h of fasting, causing pathological and life-threatening conditions such as hypoglycemia, hypothyroidism, and hypothermia (132-134). In fact, 48 h of fasting can be lethal for some mouse strains because of their genetic and epigenetic features (2). In humans, 24-hour fasting has negligible to mild effects on glucose and T3 levels and must be extended for several days to achieve metabolic outcomes similar to those in mice (136, 137). The release of KB rises within a few hours in mice until it reaches millimolar concentrations after 24 h, while in humans, KB production increases after 24 hours (0.2-0.5 mM) and reaches millimolar concentrations after 48 h (134, 135). Therefore, the timescales of the physiological processes in rodents and humans are extremely different. Laboratory mice have an average lifespan of 2 years, whereas humans live on average up to 80 years. Thus, 24 h of fasting in mice corresponds to at least 5 days of fasting in humans (2) (**Figure 3**).

These fasting-induced metabolic changes lead to weight loss of at least 20% in mice over a short period of time. Caloric restriction of 20-30% reduces the body weight of mice by up to 20% within a few weeks, while fasting causes a 20% drop in body weight in 2-3 days. In humans, CR or PF cause weight loss of up to 10% continuously and gradually over a few months (9).

Therefore, the adaptation of cellular metabolism to food reduction or deprivation follows completely different dynamics in mice and humans. Human cellular metabolism is much more resistant to stress than murine cellular metabolism, and the human metabolic network and increased energy reserves confer greater metabolic stability during fasting, while minimizing systemic changes. Thus, it is possible that CR and PF may have only minimal effects on the metabolic stability of humans, but could have significant effects on the metabolic stability of mice, leading to an extension of lifespan (138).

The differences in metabolic rates between the two species could influence the pathogenesis of diseases, such as cancer susceptibility. If tumor genesis had been similar in mice and humans, we would expect to have a higher tumor incidence in human than in mice, given that humans live 30 times longer than mice. As the lifetime tumor incidence is similar between humans and mice, cancer development and progression follow different patterns in the two

species (139, 140). In mice, genetic tumor alterations occur within a time window of 6 to 18 months and increase exponentially with age, whereas in humans, the multistep process of carcinogenesis takes several years to occur, begins at 40 years of age, and ceases at age 80 (141, 142). Approximately 30% of laboratory mice develop cancer during their lifetime, whereas in humans, this percentage is reached only after the age of 70 years (143).

CLINICAL TRIAL OUTCOMES OF THE CR AND PF EFFECTS ON HUMAN HEALTH

Given the large metabolic and physiological differences between the two species, it is uncertain whether such dietary interventions can have the same benefits as those found in rodents. So far, clinical studies aimed at evaluating the benefits of these dietary interventions in humans remain scarce and controversial. The main limitations of these human clinical studies are: 1) low adherence to diet; 2) the short duration of the study; 3) the limited number of participants; 4) enrolled participants are mostly overweight or obese (9, 144-146).

The compliance of obese and non-obese individuals to CR and PR interventions is low and represents the main challenge to overcome (15, 147). In the CALERIE-2 study, conducted on normal-weight healthy people to test the effect of CR on health and markers of longevity, adherence to 18% CR decreased significantly after 20 weeks, and the drop in body weight was significant in people who adhered to such dietary interventions for a long period (148). In the CALERIE clinical trial, participants who adhered to 18% CR for 6 months, followed by 10% CR for the remaining 18 months, demonstrated an average 8% reduction in body weight. Weight loss is far from the 20% observed in mice, and as such, the overall health impacts are less pronounced.

CALERIE clinical trials showed that participants who adhered to 11% CR over 2 years demonstrated a reduction in only some age-related cardiovascular and metabolic disease risk factors and improved only some longevity markers; therefore, it did not have the same impact on humans as rodents. Furthermore, this study found that CR reduced lean mass in young and older adults, muscle strength in older groups, and bone mass in some areas (149). In the Minnesota Starvation Experiment, which was designed to assess the physiological effects of se-

vere and prolonged dietary restriction, participants following a 40% CR for 6 months lost 25% of their weight and developed severe socio-behavioral changes (e.g., depression and chronic fatigue) (150). In an experiment conducted in Biosphere 2, eight participants subjected to a 20% CR for 2 years lost approximately 15% of their body weight and improved their metabolic profile. However, many participants experienced severe adverse events and psychological changes such as hunger, tiredness, mental confusion, licking of every dish rather obsessively, elaborate eating rituals, and depression (151).

Contrary to what has been observed in mice (152), prolonged CR or PF significantly reduced circulating levels of the neuroprotective factor BDNF in humans (153, 154), confirming that the mechanisms of adaptation to fasting in rodents and humans are vastly different.

It is necessary to assess whether the CR or PF metabolic and physiological benefits are extended and maintained after the end of the dietary intervention or reversed, or even worsened, when the participant returns to a normal diet.

At the 6-month follow-up of the Biosphere2 study, the participants' weight recovery was exclusively due to an increase in body fat reserves. Their lean mass did not change, while reduced energy expenditure due to low spontaneous physical activity could subsequently promote weight gain and obesity (151). The same phenomenon also occurred in famine victims and emaciated prisoners of World War II (155, 156), in patients with anorexia nervosa (157), cancer (158), and in subjects during the refeeding period of the Minnesota experiment (150, 159).

Therefore, such dietary interventions cannot be pursued for a long period of time in humans and are not recommended for lean people, minors, very elderly people, pregnant or breastfeeding women, anorexic people, those with low bone density, and patients affected by specific diseases, as they could cause psychological stress, depression, and a harmful impact on mental and physical health (160).

For instance, advanced-stage cancer patients already have high catabolism, are at risk of cachexia, and would not be able to tolerate such stringent regimens. Furthermore, in such patients, the lack of appetite and reduced absorption of nutrients should already activate the mechanisms regulated by CR and PF involved in enhancing the effectiveness of chemotherapies, targeted molecular therapies, and immunotherapies. In any case, a nutrient

supplement is necessary for patients with cancer to avoid worsening their physical conditions. Correct nutrition during therapy and at the end of treatment is an aspect to be considered, which has only been receiving the right and necessary attention in recent years (161).

Furthermore, nutritional restriction or deprivation impairs immune function by suppressing the mTOR signaling pathway and makes mice more susceptible to viral infections (162, 163). Although the reduction in circulating leukocytes during fasting is much more marked in mice than in humans (9, 96, 100), such dietary interventions may not be suitable for immunosuppressed cancer patients, as they are more exposed to infections and experience serious complications that could lead to death (36, 164-166).

However, small clinical studies have shown that fasting for a few days is feasible and safe in patients with HER2 negative breast cancer, gynecological cancers, advanced stage cancers undergoing chemotherapy, suggesting that fasting can reduce toxicity and modulate the immune system (94, 98, 100-102, 122). Although these data are encouraging, it is not possible to draw conclusions on the effectiveness of these interventions because these studies were conducted on a limited number of cancer patients with various forms of cancer. The main objective of these studies was to evaluate the feasibility of the intervention and did not consider tumor stratification and different treatment strategies. Future studies are needed to determine whether immune system activation in cancer patients during fasting is indicative of an enhanced antitumor response or a stress condition associated with increased CRP. In fact, fasting-induced modulation of the immune system occurs even in healthy individuals and fades when oncologic patients return to their normal diet. Therefore, the activation of the immune system in oncologic patients may not be associated with a specific antitumor response, but could be a stress-induced effect.

Adopting an appropriate lifestyle, based on a balanced diet combined with physical exercise, plays a fundamental role in decreasing the onset and development of the most common tumors in Western countries. It has been shown that following a diet based on the Mediterranean model helps prevent the risk of tumors. In general, a diet rich in vegetables (fruit and vegetables), whole grains, and legumes and low in animal fats and meat constitutes a protective factor against the onset of tumors.

Overweight or obese people certainly tolerate these dietary interventions for longer periods and benefit from both weight loss and improvements in cardiometabolic markers (15, 153, 167). However, it is unclear whether the beneficial effects of these dietary interventions on cardiometabolic health are mediated by weight loss, the modulation of nutrient-sensing pathways, or dietary components. Several studies have shown that weight loss mainly depends on caloric intake. CR and PF cause different effects even if they act on the same signaling pathways and the composition of the diet influences cardiometabolic health and long-term metabolomic reprogramming in mice (147, 168-170). In patients with thyroiditis (inflammation of the thyroid due to an autoimmune pathology), a balanced, carbohydrate-free diet (bread, pasta, fruit, and rice) reduces autoimmune antibodies (antithyroid, anti-mitochondrial, and antiperoxidase antibodies), body weight, body mass index, and fat mass by inhibiting the translocation of carbohydrate-responsive element-binding protein (ChREBP) into the nucleus and consequently the transcription of genes involved in lipogenesis and autoantibody production (171). Recent work has shown that isocaloric CR and PF (25% net calorie restriction), tested on lean subjects for 2 years, reduced body weight equally; however, PF causes greater loss of lean mass than CR, probably due to specific losses affecting skeletal muscle. Furthermore, the positive effects of PF on metabolic regulation or cardiovascular health are essentially due to reduced caloric intake and not to mechanisms regulated by fasting, as PF without energy restriction is less effective in reducing body fat mass and improving metabolic parameters or cardiovascular health (147). In addition, CR provides greater protection against tumor growth and lung metastasis compared to PF in the tumor xenograft mouse model, by enhancing the immune response (170). Collectively, CR and PF could improve metabolic and cardiovascular health and have antineoplastic potential also in humans, however it is still early to draw conclusions in the absence of double-arm randomized clinical trials performed on a statistically significant sample (172).

FOOD DRUG INTERACTION: WARNING AND PRECAUTIONS

The composition of food can be a factor capable of interacting pharmacodynamically with chemother-

apeutics. Food can influence some oral drugs that must be taken between meals, while others have only a mild effect on others. Foods rich in fats or proteins can significantly increase the absorption of the drug at the intestinal level and therefore its bioavailability, whereas foods with a high fiber content can alter the bioavailability of oral chemotherapy drugs owing to the link between the drugs and the fiber. Liquids accelerate passage through the stomach, thereby reducing the time interval between drug administration and the onset of its effects (173, 174). Grapefruit, cranberry, and cranberry juice must not be taken together with chemotherapy because they enhance the toxic effects of chemotherapy by increasing the concentration of the drug in the blood. Garlic sensitizes prostatic tumor cells to chemotherapy because biologically active garlic components interact with chemotherapy and suppress the growth of human prostate cancer cells (175).

Therefore, it is particularly important to respect the prescribed methods of administering oral chemotherapy drugs with respect to meals.

It should not be underestimated that cancer patients often consume complementary medicinal products, such as multivitamin or mineral salt supplements, to replenish nutritional deficiencies due to reduced food intake. Even the most popular nutritional supplements should be consumed after careful evaluation of their effectiveness.

Fish oil and its main components, omega 3 fatty acids, are commonly used as supplements for cancer patients. They can enhance the effects of chemotherapy while simultaneously reducing toxicity to healthy tissues and systemic inflammation. Instead, Vitamin D has poor antitumor activity and potential toxic effects; therefore, indiscriminate supplementation (*i.e.*, without an actual need) of vitamin D, alone or in combination with standard treatments, must be avoided (175).

Many cancer patients consume dietary supplements, vitamins, minerals, and herbal products along with cancer treatments because they perceive them to be anticancer and antitoxic agents. Herbal remedies have clinical implications, with a potential effect on drug metabolism. In fact, herbal products are not subjected to rigorous scientific investigations to evaluate their effectiveness, tolerability, or quality control. Furthermore, they are rarely sold with a contraindication leaflet because they are not required by law; therefore, it is very important to pay attention to their consumption.

St. John's wort, most often used to treat depression, can modify hepatic metabolism and reduce chemotherapy efficacy. Ginseng, used by cancer patients for its alleged anti-neoplastic properties, increases the concentration of chemotherapeutic drugs in circulation and enhances their toxic effects. Green tea, known for its antioxidant and anti-inflammatory properties, exerts an antagonistic action with some chemotherapeutic drugs; therefore, the use of this drink in combination with therapy should be avoided. The consumption of Aloe Vera-based products reduces the absorption and effectiveness of the drugs as they have a laxative effect (175). For these reasons, supplements and other substances with pharmacological effects should be taken under the supervision of a specialist in the sector because they can interact with chemotherapeutic drugs and affect their efficacy and toxicity.

CONCLUSION

In recent years, preclinical studies have shown that CR and PF prevent the development of diseases associated with ageing (cardiovascular, neurodegenerative, and cancer) and prolong life extension by improving metabolic and physiological profiles. Although these results are promising, it is too early to establish whether the broad health benefits of these dietary interventions could be translated from rodents to humans.

To date, few clinical studies have been conducted on small cohorts, in most cases including overweight or obese individuals, for short periods of time. Although these dietary interventions also appear promising in humans, new clinical trials need to be implemented to establish long-term benefits and safety in a large-scale population.

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The article provides a comprehensive review of the latest studies in the field, with accurate citations.

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