

## Review Article

# Diet and Aging

**Samo Ribarič**

*Institute of Pathophysiology, Medical Faculty, University of Ljubljana, Zaloška 4, 1000 Ljubljana, Slovenia*

Correspondence should be addressed to Samo Ribarič, samo.ribaric@mf.uni-lj.si

Received 1 June 2012; Revised 12 July 2012; Accepted 16 July 2012

Academic Editor: Paula Ludovico

Copyright © 2012 Samo Ribarič. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Nutrition has important long-term consequences for health that are not only limited to the individual but can be passed on to the next generation. It can contribute to the development and progression of chronic diseases thus effecting life span. Caloric restriction (CR) can extend the average and maximum life span and delay the onset of age-associated changes in many organisms. CR elicits coordinated and adaptive stress responses at the cellular and whole-organism level by modulating epigenetic mechanisms (e.g., DNA methylation, posttranslational histone modifications), signaling pathways that regulate cell growth and aging (e.g., TOR, AMPK, p53, and FOXO), and cell-to-cell signaling molecules (e.g., adiponectin). The overall effect of these adaptive stress responses is an increased resistance to subsequent stress, thus delaying age-related changes and promoting longevity. In human, CR could delay many diseases associated with aging including cancer, diabetes, atherosclerosis, cardiovascular disease, and neurodegenerative diseases. As an alternative to CR, several CR mimetics have been tested on animals and humans. At present, the most promising alternatives to the use of CR in humans seem to be exercise, alone or in combination with reduced calorie intake, and the use of plant-derived polyphenol resveratrol as a food supplement.

## 1. Introduction

Nutrition has important long-term consequences for health. It is one of the lifestyle factors that contribute to the development and progression of chronic diseases including cardiovascular diseases, diabetes, and cancer [1]. The prevention or management of chronic diseases is a global priority since they account for more than half of the deaths worldwide [2]. The effects of nutrition on health are not limited to the individual but can be passed on to the next generation. This observation has been confirmed by epidemiological studies and animal experiments. Epidemiologic observations linked smaller size or low weight at birth or during infancy to increased rates of coronary heart rate disease, type 2 diabetes mellitus, or adiposity in adult life [3–7]. In an animal model, for example, prenatal undernutrition reduced the offspring's life span [8] or lead to inadequate development of nephrons that increased the development of chronic kidney disease in later life [9].

## 2. Epigenetic Modifications by Dietary Factors

The effects of nutrition on the body are also mediated by epigenetic mechanisms [1]. The three known, closely interacting

mechanisms are DNA methylation, histone modification, and noncoding microRNAs (miRNAs) as reviewed by McKay and Mathers [1]. Nutritional factors may induce epigenetic changes via three pathways: (a) a direct influence on gene expression, (b) activation of nuclear receptors by ligands, and (c) modification of membrane receptor signaling cascades [10]. Therefore, epigenetic mechanisms provide the organism with a robust, and time-responsive system for adapting gene expression that is (a) tissue-type specific, (b) appropriate for the developmental state of the organism, and (c) responsive to signals from the external and internal environment [1].

*2.1. DNA Methylation by Diet.* DNA methylation is tissue specific and is regulated by the enzyme DNA methyltransferase (DNMT) that modifies a cytosine base at the CpG dinucleotide residue with a methyl group to form 5-methylcytosine [11]. Examples of processes that are controlled by DNA methylation are X chromosome inactivation, imprinting, and silencing of germline-specific genes, carcinogenesis, and long-term memory formation [12]. Traditionally, DNA methylation was associated with suppression of gene expression. Thus, DNA methylation either physically impedes the binding of transcriptional proteins to the gene,

or the methylated DNA binds to proteins known as methyl-CpG-binding domain proteins that recruit additional proteins to the locus—such as histone deacetylases—that modify histones into compact, inactive chromatin as reviewed in [13, 14]. However, in some patients with cancer, both global DNA-hypomethylation and localized DNA-hypermethylation are present [15, 16].

Dietary constituents that are known to modulate DNA methylation are, for example, folate, vitamin B12, selenium, green tea polyphenols (e.g., epigallocatechin-3-gallate (EGCG), epicatechin, gallocatechin), and bioflavonoids (quercetin, fisetin and myricetin). Folate and vitamin B12 promote global DNA-methylation, whereas selenium, green tea polyphenols, and bioflavonoids reduce global DNA-methylation as reviewed in Davis et al. [17]. However, the local effect of these constituents on DNA methylation can differ from their global one. For example, long-term selenium consumption increases exon-specific DNA methylation of the p53 gene in rat liver and colon mucosa [18].

**2.2. Histone Modification by Diet.** Eukaryotic cell nuclei contain alkaline proteins (due to highly positively charged N-terminus with many lysine and arginine residues) called histones, that package and order the DNA into structural units called nucleosomes. Histones act as spools around which DNA winds and play a role in gene regulation, since genes that are active are less bound to histones; inactive genes are highly associated with histones [19]. The histones N-terminus (i.e., the histone tail) or the side chains at the globular histone core are the sites of epigenetic modifications [20].

Posttranslational modification of histones is significantly more diverse than DNA methylation. Some of the best understood histone modifications are methylation, acetylation, phosphorylation, ribosylation, ubiquitination, sumoylation, or biotinylation [20]. Examples of enzymes involved in posttranslational modification of histones are histone acetyltransferases (HATs), methyltransferases (HMTs), deacetylases (HDACs), and demethylases (HDMs). The effects of diet on histone posttranslational modification were recently reviewed by Link et al. [21]. For example, polyphenols from garlic or cinnamon inhibit HDAC; green tea polyphenols and copper inhibit HAT; EGCG inhibits HMT.

Histone methylation can modulate DNA methylation patterns, and DNA methylation might serve as a template for some histone modifications after DNA replication [20, 22]. It has been suggested that these interactions could be accomplished via direct interactions between histone and DNA methyltransferases [20, 22]. Such DNA-histone interactions could also be initiated or modulated by diet.

**2.3. miRNA Modification by Diet.** miRNAs are a family of approximately 22-nucleotides long noncoding RNAs in eukaryotic cells. miRNAs are posttranscriptional regulators and bind to complementary sequences on target messenger RNA transcripts (mRNAs) leading to posttranscriptional gene silencing due to mRNA translational repression or increased RNA degradation. However, miRNAs can also cause histone modification and DNA methylation of

promoter sites thus regulating the expression of target genes by an alternative pathway. [23, 24]. The human genome encodes over 1000 miRNAs, which target more than 50% of mammalian genes in many human cell types [25–30]. Thus, miRNAs influence the expression of many transcription factors, receptors and transporters [31]. Recent evidence from experiments in human and in animal models suggests that nutrition (e.g., the consumption of fat, protein, alcohol or vitamin E) effects the expression of many miRNA [32].

Polyphenols (e.g., anthocyanin, curcumin and quercetin,) at nutritional doses modulate the expression of liver miRNA in mice *in vivo* [33]. Dietary modulation of miRNA expression could contribute to the cancer protective effects of genistein, curcumin, retinoic acid, or fish oil. Genistein (an isoflavone) inhibits uveal melanoma cell growth in a time and dose-related manner by inhibiting the of miRNA-27a expression [34]. Curcumin treatment upregulates miRNA-22 and downregulated miRNA-199a in a pancreatic cancer cell line [35] and also upregulates the expression of miRNA-15a and miRNA-16 in breast cancer cells [36]. Patients with acute promyelocytic leukemia that were successfully treated with chemotherapy and all-trans-retinoic acid had a downregulation of miRNA-181b and an upregulation of several miRNAs [37]. miRNA-10a downregulation, induced by treatment with retinoic acid, prevented pancreatic cancer metastasis in xenotransplantation experiments in zebrafish embryos [38]. Fish oil reduced the number of differentially expressed miRNAs in experimental animals and may be useful in prevention of colon carcinoma [39]. Indol-3-carbinol downregulated the expression of several miRNAs (i.e., miRNAs -21, -31, -130a, -146b and -377) in mice with induced mouse lung tumors [40]. Inadequate nutrition can also modulate miRNA expression. For example, dietary deficiency of folate was associated with significant overexpression of miRNA -222 in human with low folate intake [41]. Also, rats on a folate-methionine-choline deficient diet developed hepatocellular carcinoma with concurrent overexpression of miRNAs -17 to -92, -21, -23, -130, and -190 [42].

**2.4. TOR Signaling Pathway and Nutrition.** TOR (target of Rapamycin) is a protein kinase that functions as a central controller of cell growth and aging as reviewed elsewhere [43, 44]. Inactivation of the TOR signaling pathway promotes autophagy and prolongs life span [45]. Its function was first characterized in yeast but was also identified in other eukaryotes including mammals (hence mammalian TOR or mTOR). *In vivo*, mTOR exists in two multiprotein complexes, the mTORC1 and mTORC2. The mTORC1 functions as a nutrient-energy-redox sensor and modulates protein synthesis. Therefore, the upstream factors that stimulate the activity of this complex are insulin and other growth factors, amino acids (e.g., leucine), and stress (temperature change, caffeine, oxidative stress). Caffeine, hypoxia, and DNA damage inhibit mTORC1 activity. The upstream regulators of TORC1 activity are the AGC family of kinases (e.g., PKA; PKG and PKC) that are activated by phosphorylation [46]. In mammals, mTORC1 targets are S6 K1 and the eukaryotic initiation factor (4E-BP1) [47–52]. mTORC1-mediated phosphorylation of S6 K1 promotes protein synthesis and

4E-BP1 phosphorylation promotes localization of ribosomes to the cap structure of mRNAs. The phosphorylating activity of mTORC1 is regulated through its association with the RAPTOR (regulatory-associated protein of mTOR) protein [53, 54]. High nutrient or ATP levels activate mTORC1 by phosphorylating and thus inhibiting the TSC1-TSC2 complex as reviewed by Loewith and Hall [43]. This complex is a GTPase activating protein that modifies a second GTPase RHEB into a GTP bound state. RHEB, in the GTP bound state, directly binds and activates mTORC1 thus allowing mTORC1 to phosphorylate downstream targets [55]. Low cellular energy (high AMP levels) or low nutrient levels, in association with the tumor suppressor kinase LBK1, activate AMPK and an activated AMPK phosphorylates both TSC2 and RAPTOR thus inhibiting mTORC1 activity by two pathways [56]. In yeast, TORC1 promotes protein synthesis, ribosome biogenesis, regulates the relationship between cell cycle and cell size, promotes cell growth by inhibiting stress responses, stimulates autophagy, and regulates the signaling of mitochondrial dysfunction to the nucleus via the negative regulator of RTG1-dependent transcription [43, 44]. At the organ and whole body levels, the TORC1/S6 K1 signaling pathway regulates glucose homeostasis, insulin sensitivity, adipocyte metabolism, body mass and energy balance, tissue and organ size, learning, memory formation, and aging [57]. For example, the S6 K1 modulates the differentiation of mesenchymal stem cells into adipocytes. Overstimulation of the mTORC1/S6 K1 signaling pathway by excessive quantities of leucine in infant milk formulas could be the cause of increased adipogenesis and early childhood obesity [58].

The best understood functions of mTORC2 are the control of cell cycle-dependent polarization of actin cytoskeleton, endocytosis, and sphingolipid biosynthesis [43, 59, 60]. The upstream regulators of mTORC2 are insulin and IGF1 [44, 61]. The ribosome maturation factor Nip7 is required for mTORC2 kinase activity in yeast and mammalian cells [44, 61] and the substrates of mTORC2 are the AGC family of kinases including AKT, SGK1, and PKC [44, 62]. For example, mTORC2 promotes cell survival via AKT [63, 64] and also regulates hepatic glucose and lipid metabolism via insulin induced AKT signaling [62].

Although the signaling pathways of TORC1 and TORC2 are to some extent distinct they have a cooperative function to coordinate growth, mitosis, and cell size control. For example, TORC2 activates TORC1 via the AKT signaling pathway. TORC1 activation stimulates anabolic cellular pathways and TORC1 inhibition stimulates catabolic cellular pathways [65]. As a general rule, the sensitivity of the TORC1 and TORC2 signaling pathways could be not only cell-tissue specific but also TORC isoform dependent. For example, the activity of mTORC2 depends on the type of mammalian stress-activated protein kinase interacting protein (mSin1) isoform that constitutes this multiprotein-complex [66].

### 3. Nutrition and Aging

The possibility that mammalian life span could be significantly extended by diet modification was demonstrated in a rodent study published by McCay and coworkers. In 1935

[67]. Rats, as opposed to primates, have the ability to grow their entire life. One of the objects of this study was to determine the effect of retarding growth on the total length of life of rats of both sexes. Growth was retarded by limiting the intake of diet to the quantity necessary for maintaining the rats at fixed levels of body weights at the time of weaning or 2 weeks after weaning. At the same time, care was taken to provide adequate levels of all other diet constituents. Animals of both sexes, subject to diet restriction, had a prolonged total length of life. However, the effect of diet restriction on life span was more pronounced in male than in female rats [67]. In summary, this seminal experiment suggests that life span can be extended by diet restriction without malnutrition as opposed to diet restriction with malnutrition that can have an opposite effect as discussed elsewhere [1].

The standard protocol for studying the positive effects of a limited food intake is the use of caloric restriction, or calorie restriction (CR) diet that does not lead to malnutrition (due to lack of vitamins, minerals or essential biomolecules). CR means limiting calorie intake by 10–30% compared to the base line unrestricted intake for the studied life form and has been shown to improve health at all ages and also to slow the aging process in many eukaryotes [68]. The relevance of CR life span prolonging effects for primates was explored in a 20-year longitudinal adult-onset CR study in rhesus monkeys. The animals' baseline intake of calories was reduced progressively by 10% per month to a final 30% reduction that was maintained for the duration of the experiment. The effect of CR, compared to control, was evaluated by comparing the delay in mortality and the onset of some age-associated conditions most prevalent in humans (e.g., diabetes, cancer, cardiovascular disease, and brain atrophy). The conclusions of the study were that CR lowered the incidence of aging-related deaths (50% in control fed animals versus 20% in CR-fed animals) and also lowered the incidence of diabetes, cancer, cardiovascular disease, and brain atrophy [68].

### 4. Caloric Restriction Effects in Humans

The fundamental assumption, that caloric restriction can extend the average and maximum life span and delay the onset of age-associated changes, has been proven in many organisms from yeast, worms, and flies to mammals [69–71]. In higher mammals, CR delays many diseases associated with aging including cancer, diabetes, atherosclerosis, cardiovascular disease, and neurodegenerative diseases [68, 72–74]. The incidence of these diseases increases with age and they contribute significantly to mortality. Therefore, CR could increase life span by increasing the body's general state of health and providing a nonspecific, resistance to chronic diseases and metabolic derangements [68, 72–74]. However, the ultimate question, how does CR effect the human body, was studied in a limited number of experiments [73–93]. The study of CR effects on human longevity faces ethical and logistical challenges since the average life span is close to 80 years for the population in developed countries. Therefore, human studies are focused on measuring the CR-related changes that could slow the aging process and

the progression of chronic diseases thus increasing life span. The most convincing evidence that CR could have a positive effect in humans was provided by experiments by Fontana and coworkers, by the Comprehensive Assessment of Long-Term Effects of Reducing Calorie Intake (CALERIE Phase 1), and by data obtained on the members of the Caloric Restriction Society (as discussed below).

Fontana and coworkers evaluated the effect of a 6-year long CR diet on risk factors for atherosclerosis in adult male and female adults (age range 35–82 years) and compared them to age-matched healthy individuals on typical American diets (control group). The total serum cholesterol level and low-density lipoprotein (LDL) cholesterol levels, the ratio of total cholesterol to high-density lipoprotein cholesterol (HDL), triglycerides, fasting glucose, fasting insulin, C-reactive protein (CRP), platelet-derived growth factor AB, and systolic and diastolic blood pressures were all markedly lower in the CR group. The HDL cholesterol was higher after CR. Medical records of individuals in the CR group indicated that, before they began CR, they had serum lipid-lipoprotein and blood pressure levels in the expected range for individuals on typical American diets, and similar to those of the comparison group. The conclusion of the study was that long-term CR can reduce the risk factors for atherosclerosis [74].

The effect of fat loss induced by either (a) a long-term 20% CR or (b) a 20% increased energy expenditure (IEE) by exercise on coronary heart disease (CHD) risk factors was evaluated in a one-year randomized, controlled trial on 48 nonobese male and female subjects. The CR or exercise induced reductions in body fat were quantitatively similar and were accompanied by similar reductions in most of the major CHD risk factors, including plasma LDL-cholesterol, total cholesterol/HDL ratio, and CRP concentrations. The authors concluded that long-term CR or IEE of the same magnitude lead to substantial and similar improvements in the major risk factors for CHD in normal-weight and overweight middle-aged adults [83].

The effects of a 1-year, 20% CR regime or 20% IEE by exercise, on the oxidative damage of DNA and RNA, was evaluated by white blood cell and urine analyses in normal-to-overweight adults. Both interventions significantly reduced oxidative damage to both DNA and RNA in white blood cells compared to baseline. However, urinary levels of DNA and RNA oxidation products did not differ from baseline values following either 1-year intervention program. The conclusion of the study was that either CR or IEE by exercise reduce systemic oxidative stress which is reflected in a decreased DNA or RNA oxidative damage [85].

CALERIE is a research program initiated by the National Institute on aging that involves three research centers. The Phase 1 of CALERIE included three pilot studies to determine whether long-term (6–12 months) effects of 20–25% CR in free-living, nonobese humans could be investigated and to evaluate the adaptive responses to CR. The conclusions of this randomized, controlled, clinical trial were that CR subjects had a lower body weight, a decreased

whole body and visceral fat, a reduced activity energy expenditure, improved fasting insulin levels, improvements in cardiovascular disease markers (LDL, total cholesterol to HDL ratio, and CRP), and no change in bone density compared to controls [76, 77, 83, 86, 92]. The objective of the ongoing CALERIE Phase 2 is to test if 2 years sustained 25% CR of *ad libitum* energy intake, results in beneficial effects that would be similar to those observed in animal studies [91].

Members of the Caloric Restriction Society (CRS) restrict food intake with the expectation that this would delay the disease processes responsible for secondary aging and to slow the primary aging process. Compared to age-matched individuals eating typical American diets, CRS members (average age  $50 \pm 10$  yr) had a lower body mass index, a reduced body fat, significantly lower values for total serum cholesterol, LDL cholesterol, total cholesterol/LDL, and higher HDL cholesterol. Also fasting plasma insulin and glucose values were significantly lower than in the age-matched control group. Left ventricular diastolic function in CRS members was similar to that of about 16 years younger individuals. Chronic inflammation was reduced by CR and this was reflected in significantly lower levels of plasma CRP and tumor necrosis factor alpha (TNF $\alpha$ ) [74, 78, 84].

Aging is associated with a progressive reduction in heart-rate-variability (HRV)—a measure of declining autonomic function—and also a worse health outcome. The effect of a 30% CR on heart autonomic function was assessed by 24-hour monitoring of HRV in adults on self-imposed CR for 3 to 15 years and compared with an age-matched control eating a Western diet. The CR group had a significantly lower heart rate and significantly higher values for HRV. Also, HRV in the CR individuals was comparable to published norms for healthy individuals 20 years younger. The authors suggest that CR reset the balance between the sympathetic/parasympathetic modulation of heart frequency in favor of the parasympathetic drive thus increasing the circadian variability of heart rate [93].

## 5. Cellular Mechanisms of Caloric Restriction

Most age-related changes in gene expression are less than two folds and are tissue specific [94]. Yet despite tissue-specific differences in the effect of age on gene transcription, the rate of aging across tissues appears to be coordinated, suggesting a role for systemic factors in coordinating the aging process at a whole body level [95]. The most common age-related changes include increased expression of genes involved in inflammation and immune responses, and reduced expression of genes involved in mitochondrial (MTH) energy metabolism and CR prevents the majority of these age-associated changes in gene expression [96, 97]. CR is suggested to counteract the age-associated changes by modulating the mTOR signaling pathway, IGF1/insulin signaling, adiponectin expression, DNA methylation, and histone acetylation and deacetylation.

**5.1. Adiponectin Secretion in Caloric Restriction.** A consistent change during CR is a reduction in body fat (i.e., a reduction

in white adipose tissue). White adipose tissue is not only a storage site for lipids but has an important role in blood glucose homeostasis, immune, and inflammatory responses that are mediated by adipocyte-derived, cell-to-cell, signaling molecules adipokines (e.g., adiponectin) [98, 99]. Therefore, adipose tissue could be an important factor for aging and CR-related metabolic changes.

The secretion of adiponectin is increased by reduced caloric intake (e.g., CR) and reduced by both insulin and IGF1 that decrease its synthesis. Cross-sectional studies demonstrate a consistent inverse relationship between plasma insulin and adiponectin concentrations. An increase in adipocyte size will also reduce the secretion of adiponectin [100]. Adiponectin promotes fatty acid oxidation in adipose tissue and reduces lipid accumulation in other peripheral tissues [101]; CR is associated with increased levels of adiponectin [102]. In humans, this hormone suppresses metabolic derangements that may lead to *type 2 diabetes, obesity, atherosclerosis, or metabolic syndrome* [103–105]. Adiponectin regulates mitochondrial energy production via AMPK. AMPK has many functions, it up-regulates cellular uptake of glucose,  $\beta$ -oxidation of fatty acids, expression of glucose transporter 4 (GLUT4), and mitochondrial energy production. The enzyme has an “energy-sensing capability” and responds to fluctuations in the intracellular AMP/ATP ratio. For example, adiponectin treatment of human myotubes leads to an AMPK-dependent increase in MTH biogenesis and reduces reactive oxygen species (ROS) production [106]. AMPK regulates MTH energy production by activating peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1- $\alpha$ ) directly, or through the endothelial nitric oxide synthase (eNOS) and NAD-dependent-deacetylase sirtuin1 (i.e., SIRT1 or silent mating type information regulation 2 homolog 1) signaling pathway. Increased AMPK activity during CR has also a cardioprotective effect, which is abolished in transgenic adiponectin antisense mice or in mice treated with an AMPK inhibitor [102]. Increased AMPK activity also stimulates eNOS activity thus reducing the chances of cerebral ischemic injury [107]. Additional cardioprotective effects that are mediated by increased secretion of adiponectin during CR are (a) inhibition of TNF $\alpha$  release and (b) inhibition of synthesis of adhesion molecules in endothelial cells. The latter suppresses the attachment of monocytes to the endothelial cells and delays the progression of atherosclerosis. Adiponectin modulated inflammatory responses are due to inhibiting the secretion of TNF $\alpha$  (a cytokine involved in systemic inflammation) from monocyte/macrophages and foam cells [108–110]; this may explain the reduced plasma concentration of inflammatory protein CRP in humans subjected to CR.

**5.2. Insulin/IGF1 Signaling in Caloric Restriction.** Insulin resistance is a well-known age-related metabolic change in mammals that can be reversed by CR [94]. CR has been reported to reduce IGF1 blood levels in mice but not in humans [111, 112]. Insulin and IGF1 inhibit FOXOs (an O subclass of the forkhead family of transcription factors) by

a signaling pathway that includes insulin receptor substrate proteins (IRS), 3-phosphoinositide-dependent protein kinase-1 (PDK1), and phosphatidylinositol 3-kinase (PTDINS-3 K), thus translocating FOXOs from the nucleus. FOXOs regulate the rate of aging in response to dietary cues and the dysregulation of this pathway in mammals is associated with obesity and insulin resistance [113]. In a cell-type-specific manner, mammalian FOXO factors control various cellular functions including apoptosis, cell cycle, differentiation, and the expression of genes involved in DNA repair and oxidative stress resistance. These functions are assumed to be the basis for FOXO's ability to control life span [114]. The actions of insulin/IGF1 signaling pathway on FOXO1a are mimicked by black tea polyphenols [113] and polymorphisms in the FOXO3a gene were associated with longevity in humans [115]. CR stimulates SIRT1-mediated deacetylation of the FOXO3a, preventing nuclear FOXO3a activity and inhibiting Rho-associated protein kinase-1 expression thus activating nonamyloidogenic  $\alpha$ -secretase processing of APP and lowering A $\beta$  generation. This reduced A $\beta$  generation is associated with the prevention of Alzheimer's disease type amyloid neuropathology and spatial memory deterioration in a mouse model [114]. The positive effect of CR on the insulin/IGF1 signaling pathway was also associated with a reduction in ROS production in MTH [116].

**5.3. mTOR Signaling Pathway in Caloric Restriction.** The regulation of life span by the mTOR signaling pathway is not completely understood. However, recent experimental work implies that it plays an important role in the cell's aging process [44]. Inhibition of mTOR with rapamycin expands maximal and median life span in mice. This effect was observed even when the treatment was initiated late in life, corresponding roughly to an age of 60 years in humans [44, 117]. The above mentioned, rapamycin-mediated life extension was not associated with change in disease patterns or causes of death suggesting that rapamycin increases life span by slowing-down age-related tissue and organ degeneration [44, 117]. mTORC1 inhibition could prevent tissue degeneration and extend life span by improving stem cell function. For example, reducing mTORC1 signaling with rapamycin restores hematopoietic stem cells self-renewal and hematopoietic function, improves immunity, and increases life span in mice [118]. S6 K1 and 4E-BP1 were suggested as effectors of the mTORC1 signaling pathway that regulates the aging process. As reviewed in Kapahi et al., reduced S6 K1 activity increases life span in various species including in mice [119] and overexpression of 4E-BP1 extends life span under rich nutrient conditions by enhancing mitochondrial activity in flies [120].

mTORC1 could also influence life span through mechanisms that are not associated with modulation of protein synthesis; for example, stimulation of autophagy, as a consequence of mTORC1 inhibition, could promote longevity by stimulating degradation of aberrant proteins and damaged organelles that are accumulating over time and impairing cellular homeostasis [44]. An example how dysregulation of mTORC1 activity can affect life span is seen in the liver

of old mice with impaired fasting-induced ketogenesis and increased mTORC1 activity [121]. This impaired ketogenesis limits the supply of available energy substrates to the peripheral tissues thus reducing the organism's chances of survival during food deprivation.

The age-related decline in MTH function is counteracted by CR that increases the transcription of nuclear-encoded genes involved in the electron transport system [69]. The effects of CR on MTH could also be mediated by the mTOR signaling pathway since mTOR is necessary for the maintenance of mitochondrial oxidative function [122]. Two, S6 K1 and 4E-BP1 independent, mTOR/MTH signaling pathways have been suggested: the TORC1-YY1-PGC-1 $\alpha$  complex [122] demonstrated in a mouse model or the TORC1-regulated complex of BCL-XL and VDAC1 located at the mitochondrial outer membrane in a T-cell leukemic cell model [123].

**5.4. DNA Methylation in Caloric Restriction.** The aging process is associated with a progressively reduced cell homeostasis and altered gene expression [124]. Aging causes a significant change in the distribution of 5-methylcytosine (the product of DNA methylation) across the genome and a decrease in global genome DNA methylation [124–130]. However, the promoter regions of some specific genes tend to switch from unmethylated to methylated status, leading to gene silencing (e.g., promoters of tumor or aging-related genes, such as *RUNX3* and *TIG1* [129, 131]). In summary, the aging process is associated with globally decreased but locally increased DNA methylation [132]. CR is assumed to delay the aging process by reversing aging-related DNA methylation changes thus increasing genomic stability [133, 134]. For example, CR increased the methylation level of proto-oncogene *RAS* in a rat model when compared to *ad libitum* fed animals [135]. A hypermethylated gene promoter is often recognized by transcriptional repressor complexes, thus leading to silencing the expression of these oncogenes, which contributes to the cancer prevention effects of CR [132]. In an *in vitro* human cell model of CR, the E2F-1 binding site in the promoter of the *p16<sup>INK4a</sup>* gene (a tumor suppressor and aging-associated gene) was hypermethylated. This DNA hypermethylation blocked access of E2F-1 (an active transcription factor of *p16<sup>INK4a</sup>*) to the *p16<sup>INK4a</sup>* promoter, resulting in *p16<sup>INK4a</sup>* downregulation, thus contributing to the CR induced life span extension [136].

Obesity is an important metabolic disorder in humans that is closely associated with recognized causes of accelerated aging and increased mortality such as diabetes, hypertension or cancer [137]. Therefore, the antiageing effects of CR should have an impact on the progression of obesity and are used in clinical weight control interventions [138]. The practice of CR by obese humans revealed that hypocaloric diets cause DNA methylation changes in specific loci *ATP10A*, *WT1*, and *TNF- $\alpha$* , which could be used as early indicators of a response to CR [139–141]. Further CR studies in humans are necessary to characterize the pool of DNA methylation-controlled candidate genes that could be closely correlated with metabolic pathways [132].

### 5.5. Posttranslational Modification of Histones in Caloric Restriction

**5.5.1. Histone Acetylation/Deacetylation.** Histone modifications are associated with gene activation or gene repression. The combination of modifications within histone tails directly changes nucleosomes configuration switching chromatin to either a compacted (tight-close) or a relaxed configuration (loose-open) [142]. Therefore, histone modifications determine the (tight-close: loose-open) ratio of chromatin and thus the degree of gene activity within a certain DNA region. For example, a deacetylated histone lysine residue has the positive charge, which attracts the negatively charged DNA strands producing a compact chromatin state that is associated with transcriptional repression. Alternatively, histone acetylation removes the positive charge and results in an open chromatin structure, which promotes gene transcription [132]. HDAC activity is increased during CR, therefore, global deacetylation may be a protective mechanism against nutrition stress and may influence the aging processes [136]. For example, enhanced activity of HDAC1 on the promoter regions of the *p16<sup>INK4a</sup>* and human telomerase reverse transcriptase (*hTERT*) genes, the former a tumor suppressor in many human cancers and the latter a key regulator of telomerase activity modified by aging regulation, leads to beneficial expression changes of these two genes and contributes to longevity under CR conditions [136, 143, 144].

Several HDAC families have been identified, including class III NAD<sup>+</sup>-dependent HDACs like Sirtuin1. Sirtuin1 (SIRT1 in mammals), and its orthologs in other species (e.g., Sirtuin2 in yeast) are important for aging regulation and CR-related lifespan extension [145–149]. The enzymatic activity of SIRT1 depends on NAD<sup>+</sup>/NADH ratio, a key indicator for oxygen consumption, thus suggesting that this protein is responsive to the metabolic state of cells. The role of SIRT1 in mediating CR and lifespan extension is supported by several animal models, human subjects, and *in vitro* CR cellular systems [136, 145, 146, 148–154]. CR induces SIRT1 expression in several tissues of mice or rats [146]. SIRT1 is assumed to mediate CR-induced metabolic alterations and subsequent aging retardation by (a) increasing stress resistance by negative regulation of p53 and FOXO [155–159] and (b) by initiating a series of endocrine responses, including inhibition of adipogenesis and insulin secretion in pancreatic  $\beta$  cells by regulation of key metabolism-associated genes such as peroxisome proliferator-activated receptor G coactivator 1 $\alpha$  (PGC-1 $\alpha$ ) [160, 161]. Although SIRT1 is classified as an HDAC, it also deacetylates nonhistone substrates [146, 152] including key transcription factors (e.g., FOXO), regulatory proteins (e.g., p53, *p16<sup>INK4a</sup>*), and DNA repair proteins (e.g., Ku70) that are involved in lifespan extension by CR. For example, downregulation of p53 by SIRT1 deacetylation may affect lifespan by inhibiting cellular apoptosis and replicative senescence processes [155–157, 162–164]. FOXO protein can be directly deacetylated by SIRT1 at lysine residues and its expression is reduced, thereby repressing FOXO-mediated apoptosis [158, 159]. The DNA repair protein, Ku70, can become deacetylated by SIRT1, allowing it to inactivate

the proapoptotic factor BAX, thus inhibiting apoptosis [165, 166].  $p16^{INK4\alpha}$  is a cyclin-dependent kinase inhibitor, an important tumor suppressor protein and a potential aging biomarker since it is significantly accumulated during the aging processes [167–171]. CR-activated SIRT1 can directly bind to the  $p16^{INK4\alpha}$  promoter and decrease its expression through a deacetylation effect, which contributes to delaying the aging process and to life span extension in human cells *in vitro* [153]. As stated previously, SIRT1 also regulates the expression of genes that are involved in metabolic pathways. PGC-1 $\alpha$  is a key regulator of gluconeogenesis and fatty acid oxidation [160, 161] and is upregulated during CR by SIRT1-mediated deacetylation, which increases its ability to coactivate HNF4 $\alpha$ , a transcription factor that promotes the expression of gluconeogenic genes and represses genes involved in glycolysis [147, 152]. In summary, SIRT1 plays an important role in the cross-talk between epigenetic and genetic pathways [132].

**5.5.2. Histone Methylation.** In contrast to histone acetylation, associated with open chromatin status and subsequent gene activation, differentially methylated forms of histones show unique association patterns with specific proteins that recognize these markers and thus lead to gene silencing or activation [132]. Histone lysine residues can be mono-, di-, or tri-methylated, leading to either activation or repression depending upon the particular lysine residue that is modified [172, 173]. For example, CR elicited histone methylation modifications such as di- or tri-methylated histone H3 at lysine residue 3 or 4 regulate expression of key aging-related genes,  $p16^{INK4\alpha}$  and *hTERT*, thereby contributing to CR-induced lifespan extension of human cells [136, 153].

**5.6. miRNA Expression in Caloric Restriction.** miRNA expression patterns change with age; some miRNAs are downregulated and some are upregulated. Expression profile analysis of 800 miRNAs in human peripheral blood mononuclear cells revealed that the majority of miRNAs decreased in quantity including miRNAs involved in cancer development [174]. Since human tumors are often associated with a general downregulation of miRNAs, the reported age-related global decrease in miRNA could increase the frequency of cellular transformation and tumor genesis thus reducing life span. The decrease of these latter miRNAs with advanced age was also associated with an increased expression of target proteins phosphatidylinositol 3-kinase, stem cell factor receptor (c-KIT) and histone H2A [174]. Animal studies also support the role of miRNAs in aging. For example compared to wild-type controls, *C. elegans* mutants with deletion of miRNA-239 have a significantly prolonged lifespan and *C. elegans* mutants with deletions of miRNA-71, miRNA-238, and miRNA-246 have a significantly reduced lifespan [175]. The longevity of Ames dwarf mouse—attributed to their increased insulin sensitivity, increased stress resistance and reduced tumor frequency as a result of reduced IGF-1 activity—was associated with liver miRNA-27a suppression of regulatory proteins ornithine decarboxylase and spermidine synthase that occurred sooner in postnatal life than in wild-type mice [176].

CR changes miRNA expression profile. In mouse breast tissue, of animals restricted to 70% of normal diet for 6 months, CR increased the expression of miR-203 that targets caveolin-1 and p63 important factors affecting growth and invasive potential of cancer cells [177]. The authors concluded that CR could reduce the incidence, progression and metastasis of breast cancer thus contributing to an increased life span [177]. The brain of CR mice—after an 8-month reduction of calories to 60% of normal *ad libitum* intake—shows a decreased expression of miRNA-181a, miRNA-30e, and miRNA-34a with a concomitant increase in BCL2 expression and a concomitant decrease in BAX expression with reduced activities of Caspases 9 and -3. Decreased activities of Caspases 9 and 3 are associated with a reduced rate of apoptosis [178]. BAX and Caspase 3 activity is increased in Alzheimer's and Parkinson's disease; therefore, the progress of these common neurodegenerative diseases could be delayed by CR thus prolonging life span [179–183].

## 6. Mimetics of Caloric Restriction

Since long-term CR is necessary to produce beneficial effects on health and longevity observed in experimental conditions, alternatives have been investigated that could produce the positive effects of CR without food restriction. An ideal calorie restriction mimetic (CRM) should (a) elicit metabolic, hormonal and physiological effects similar to CR, (b) not require a significant reduction in long-term food intake, (c) activate stress response pathways similar to CR and (d) extend life span and reduce or delay the onset of age-related diseases [184]. To speedup the search for a candidate CRM the National Institute on Aging established the Interventions Testing Program as a multi-institutional program to test substances predicted to “extend lifespan and delay disease and dysfunction” [185–189].

**6.1. Caloric Restriction Combined with Exercise.** Male rats are the favorite animal model to study whether exercise in combination with CR (i.e., CE) potentiates the health-promoting benefits caused by CR alone, because these animals do not increase their caloric intake to compensate for their exercise-induced caloric expenditure [180]. Some studies concluded that CE does not have health-promoting benefits beyond those elicited by CR [111, 190–192]; there was no significant change in oxidative stress levels or pro-inflammatory protein levels in exercised animals fed an 80% CR diet [191, 192] and no effect on the animal's maximal life span [190]. On the other hand, CE reduced CRP levels to a greater extent than CR by itself [193] and reduced the chances of developing both myocardial necrosis and myocardial ischemia [194, 195].

Several human CE studies investigated the effect of a 25% total caloric reduction with 12.5% coming from exercise induced expenditure and another 12.5% coming from CR. The majority of them found no significant difference between CR and CE in respect to fasting insulin levels, DNA damage, muscle mitochondrial gene expression, triglyceride levels, and liver lipid content [76, 196–198]. The exceptions are two studies that reported a further reduction in both

diastolic blood pressure and LDL cholesterol when CR with exercise was compared to CR alone [198, 199]. Also, CE has been shown to increase bone mineral density at the femoral neck and reduce sTNFR1, an inflammatory biomarker, in overweight postmenopausal women [200]. The main advantage of combining CR with exercise over CR alone is that it may be easier for an individual to comply with a CE regimen where the total energy (i.e., caloric reduction) is divided between exercise-induced expenditure and calorie restriction [201].

**6.2. Dietary Restriction.** Dietary restriction (DR) refers to the modification of the quantity ratio between protein, fat, and carbohydrates with or without reducing the total intake of calories. Neither carbohydrate restriction nor lipid restriction are effective alternatives to CR and both failed to decrease reactive oxygen species production or oxidative DNA damage [202–208]. In an animal model, protein DR seems to be an alternative to CR. Protein DR was reported to increase the maximum lifespan in rodents by 20% [206]. The life-extending benefits of protein DR were attributed to a methionine restriction in diet [209–215]. For example, a 40% methionine restriction has been reported to decrease both mitochondrial reactive oxygen species generation and oxidative damage in mitochondrial DNA [216, 217]. Evidence that supports the link between methionine restriction and increased life span includes (a) inverse relationship between methionine content and maximum life span in mammals [218], (b) methionine supplementation increases LDL cholesterol oxidation [219] and (c) increased methionine intake increases plasma homocysteine concentrations, thus increasing the risk of cardiovascular disease and mortality [219]. Also, it has been demonstrated that a 40% restriction of all dietary amino acids except methionine failed to reduce both mitochondrial reactive oxygen species generation and oxidative damage in mitochondrial DNA [220]. In summary, animal experiments suggest that about half of the life extension effect of CR can be attributed to methionine restriction [206]. Therefore, further work in humans is justifiable since methionine DR is feasible and tolerable [221].

**6.3. Alternate Day Fasting.** Alternate day fasting (ADF) alternates 24-hour periods of *ad libitum* intake with partial or complete restriction of calorie intake. Therefore, ADF does not necessarily reduce overall caloric consumption or bodyweight, since subjects may compensate for the reduced caloric intake during fasting periods by overeating in the *ad libitum* intake period [222, 223]. ADF extended lifespan in animal trials [223–225]. Some authors attributed the increased life span during ADF to the concomitant increase in brain-derived neurotrophic factor [215]. ADF also attenuated or prevented the development of age-related disease processes, including cardiovascular disease, kidney disease, cancers, and diabetes [222, 223, 225–230]. Human trials have established the feasibility of ADF in humans [231]. The preliminary results of ADF-human trials [231–233] cannot be compared to CR-human trials since the ADF-trial periods were relatively short (from a few days to 20 weeks)

compared to the CR-trial periods (6 months to 6 years) [74, 83, 85]. However, even during such brief trial periods, some potentially beneficial effects were noted: a decrease in fasting insulin with no difference in fasting glucose [231] and an improved bronchial responsiveness to medication [233]. It has been reported that peripheral blood mononuclear cells of normal weight middle-age male and female subjects on a 2-month long ADF responded with a reduced capability to produce cytokines upon stimulation [234]. To date, there are no reports in regard to changes in biomarkers specific to blood lipids and oxidative stress in ADF subjects.

**6.4. Resveratrol.** Resveratrol (RSV), a plant-derived polyphenol in the skins of red grapes, is the most studied caloric restriction mimetic. RSV is reported to activate Sir2 (SIRT1 homolog) [235], thus mimicking the benefits of CR—without restricting calorie intake—such as increasing lifespan in yeast, worms, flies, and fish [235–238]. Recently, the assumption that activation of Sir2 by direct binding with RSV is responsible for extended lifespan has been challenged in experiments in multiple organisms [239–248]. For example, RSV is known to produce a wide array of effects in mammalian cells, including activation of AMP-activated protein kinase (AMPK) that is involved in some of the same pathways as SIRT1 and directly phosphorylates PGC-1 $\alpha$ . [249, 250]. SIRT1 can activate the kinase upstream of AMPK, but this pathway does not appear to be necessary for AMPK stimulation by RSV [251]. Recently, it was reported that SIRT1 is essential for moderate doses of resveratrol to stimulate AMPK and improve mitochondrial function *in vitro* and *in vivo* [252].

Although the mechanism of RSV-mediated CR-like effects are not fully understood, it appears that RSV treatment produces a transcriptional response similar to CR [253], and in the presence of a high-fat diet, both health and longevity benefits have been reported with RSV use in a mouse model [249]. The beneficial effects of RSV use in obese mice were increased insulin sensitivity, improved motor coordination, and decreased incidence of cataracts [253, 254]. There was no significant life span increase in adult mice when RSV was added to a normal diet [254, 255]. This finding implies that RSV is not a true CRM [256]. A one-year treatment with RSV increased resting metabolic rate and total daily energy expenditure in nonhuman primate with any adverse health effects, implying that long-term use of RSV is effective and safe [257, 258]. CR, in the same animal model and experimental protocol, reduced total daily energy expenditure but did not change resting metabolic rate [258].

There have been only a few studies on RSV effects in humans, however the results are encouraging. The use of 0.1 mM RSV in cultures of human mesenchymal stem cells promotes cell regeneration by inhibiting cellular senescence; at higher concentrations (5 mM or more) RSV inhibits cell regeneration by increasing senescence rate, cell doubling time, and S-phase cell cycle arrest [259]. In human peritoneal mesothelial cells RSV delays replicative senescence by mobilization of antioxidative and DNA repair mechanisms



as measured by increased expression of proliferating cell nuclear antigen, augmented fraction of cells in the S phase of the cell cycle, increased number of divisions, diminished expression and activity of senescence-associated  $\beta$ -galactosidase, upregulated biogenesis of mitochondria, increased activity of superoxide dismutase and reduced DNA damage [260]. RSV and other polyphenols have low bioavailability in humans. However, RSV and its metabolites do accumulate within human cells *in vivo* in a tissue-specific and dose-dependent manner [261]. A six-week supplementation regime with RSV suppressed the binding of nuclear factor kappa B (NF- $\kappa$ B), decreased ROS generation, and decreased the levels of TNF $\alpha$  and interleukin-6 (IL-6) in mononuclear cells. The plasma levels of TNF $\alpha$  and CRP were significantly reduced as well. There were no significant changes in fasting plasma concentrations of cholesterol (total, LDL and HDL), triglycerides, or leptin in RSV-treated group compared to the control group of healthy individuals receiving placebo [262]. A high-fat, high-carbohydrate diet induces inflammation and oxidative stress [263]. Healthy humans on a high-fat, high-carbohydrate meal, taking a single-dose supplement of RSV and other grape polyphenols, had a significantly increased messenger RNA (mRNA) expression of the *NAD(P)H dehydrogenase [quinone] 1* and *glutathione S-transferase-p1* genes—implying a strong anti-oxidant effect. The single-dose RSV supplement also attenuated the meal-induced increase of plasma endotoxin and lipoprotein binding protein concentrations and attenuated the expression of p47<sup>phox</sup>, TLR-4, CD14, SOCS-3, IL-1 $\beta$ , and KEAP-1 [264]. Therefore, RSV reduces the oxidative and inflammatory responses of a high-fat, high-carbohydrate meal, and it may reduce the risk of atherosclerosis and diabetes [261]. Preliminary results suggest that RSV also improves the glucose tolerance and insulin sensitivity [265]. The improved insulin sensitivity was attributed to decreased oxidative stress [265].

The causal association between red wine and grape juice consumption and the reduction of risk factors for cardiovascular disease (reduced blood flow, increased oxidative stress and inflammation) is well known [266–269]. RSV upregulates eNOS, thus promoting nitric oxide mediated vasodilatation and increased blood flow [270–272]. Also, RSV attenuates hemostasis-related activation of human platelets [273]. Increased arterial blood flow, after a single bolus of RSV, was measured in the brain and arm [274, 275]. However, increased brain blood flow after RSV treatment was not associated with an enhanced cognitive function [274].

Improved insulin resistance, arterial blood flow, and decreased oxidative stress and inflammation are associated with short-term use of RSV but there are no human data on the long-term health benefits [261]. In summary, further research is needed to clarify the biochemical pathways of RSV mediated effects and to establish its long-term effects in humans [276].

**6.5. Rapamycin.** Rapamycin (RAP) is an antibiotic and inhibitor of TOR (target of rapamycin) signaling in cells, with known immunosuppressive and antiproliferative effects

[277]. TOR is a mediator of nutrient signaling in cells and is proposed to play a role in aging and the CR response (see Section 6.3). When RAP was administered to mice at about 20 months of age there was a significant, about 10% increase in mean life span extension in male and female mice. Since there were no significant changes in the organ pathology in the RAP feed mice, compared to control, the authors suggested that the longevity benefits of RAP could be at least partially mediated by biochemical pathways independent of the CR response [117]. The existence of multiple, RAP activated life-extension biochemical pathways were also suggested in flies. RAP feed adult *Drosophila* had an increased life span. The suggested mechanism for this RAP increased longevity was by the TORC1 branch of the TOR pathway, with alterations to autophagy and translation. However, RAP could increase life span of weak insulin/IGF1 signaling pathway mutants and of flies with life span maximized by CR, suggesting additional mechanisms for life span extension [278]. Lifelong administration of rapamycin, administered intermittently 2 weeks per month, extended lifespan in normal inbred female mice. Significantly, rapamycin inhibited age-related weight gain, decreased aging rate, increased life span and delayed spontaneous cancer [279]. Adult mice treated with rapamycin, starting at 2 months of age, perform significantly better on a task measuring spatial learning and memory compared to age-matched mice on the control diet. However, rapamycin did not improve cognition in adult mice with pre-existing, age-dependent learning and memory deficits. The rapamycin-mediated improvement in learning and memory was associated with a decrease in IL-1 $\beta$  levels and an increase in NMDA signaling. [280]. Since rapamycin is used as an immunosuppressive, its relevance for longevity in humans has yet to be established [117].

## 7. Diet and the Aging Population

An important demographic tendency in the developed world is a progressive increase in the percentage of the population over 65 years of age and a simultaneous decline in the percentage of the working age population. The health implications of these trends are a shift from acute to chronic and age related illnesses (e.g., Alzheimer's disease, osteoporosis, cardiovascular diseases, and cancer), increasing health costs and an increasing economic burden to the society and to the individual [281–283]. Therefore, any dietary intervention that has the potential of delaying the progression of chronic and age-related illnesses could have a significant impact not only on the individual's quality of life but also on the society's ability to deal with the health and economic implications of an aging population. There is a body of data suggesting that CR significantly reduces the rate of age-related changes in humans [73–93]. However, there is no data that CR promotes longevity in humans. Studies of people with exceptional longevity suggest that a family history of longevity and of a low prevalence of age-related diseases enables a significantly prolonged life span even when the subjects were obese, smoked or did not exercise regularly. Therefore, exceptional longevity in humans could be more dependent on genetics than lifestyle [284–286].

## 8. Conclusions

Caloric restriction or calorie restriction mimetics elicit coordinated adaptive stress responses at the cellular and whole-organism level by modulating the signaling pathways of adiponectin, insulin/IGF1, AMPK, mTOR, FOXO, p53, and sirtuins [287]. Sirtuins could play an important role in the cross-talk between epigenetic and genetic pathways [132]. The activation of these adaptive stress responses may prevent the initiation of apoptosis by the intrinsic pathway [288]. Furthermore, it may stimulate autophagy to provide substrates for energy production and for the anabolic processes involved in cellular regeneration and synthesis of antioxidants and heat-shock proteins [287]. A large body of experimental evidence proves that the overall effect of these adaptive stress responses is an increased resistance to subsequent stress, thus delaying age related changes and promoting longevity. Therefore, CR, alone or in combination with caloric restriction mimetics, could improve the quality of life of the aging population.

## Abbreviations

4E-BP1:	Eukaryotic translation initiation factor 4E binding protein 1	DR:	Dietary restriction
ADF:	Alternate day fasting	E2F-1:	Transcription factor E2F1 protein
AGC:	Acronym of the protein kinase A, G, and C families	EGCG:	Epigallocatechin-3-gallate
AKT:	Serine-threonine-specific protein kinase also known as protein kinase B (PKB)	eNOS:	Endothelial nitric oxide synthase
AMP:	Adenosine monophosphate	FOXO:	O subclass of the forkhead family of transcription factors; known FOXO family members are FOXO1, FOXO3, FOXO4 and FOXO6
AMPK:	5' adenosine monophosphate-activated protein kinase	GLUT4:	Glucose transporter 4
ATP:	Adenosine-5'-triphosphate	GTP:	Guanosine-5'-triphosphate
ATP10A:	Probable phospholipid-transporting ATPase VA also known as ATPase class V type 10A or aminophospholipid translocase VA gene	GTPase:	Enzyme that hydrolyses GTP
A $\beta$ :	Amyloid beta	HAT(s):	Histone acetyltransferase(s)
B12 vitamin:	Cobalamin	HDAC(s):	Histone deacetylase(s)
BAX:	Bcl-2 associated X protein	HDAC(s):	Histone deacetylase(s)
BCL-XL:	B-cell lymphoma-extra large, a transmembrane mitochondrial protein	HDL:	High-density lipoprotein
CALERIE:	Comprehensive Assessment of Long-Term Effects of Reducing Calorie Intake	HDM(s):	Histone demethylase(s)
CD14:	Cluster of differentiation 14 protein also known as CD14 protein	hmdC:	5-hydroxymethyl-2'-deoxycytidine
CE:	Exercise in combination with CR	HNF4 $\alpha$ :	Hepatocyte nuclear factor 4 $\alpha$ also known as nuclear receptor subfamily 2, group A, member 1
CHD:	Coronary heart disease	HMT(s):	Histone methyltransferase(s)
CpG dinucleotide:	Cytosine-phosphate-guanine dinucleotide	HNF4 $\alpha$ :	Hepatocyte nuclear factor 4 $\alpha$
CR:	Caloric restriction or calorie restriction diet	HRV:	Heart-rate-variability
CRM:	Calorie restriction mimetic	<i>hTERT</i> :	Gene encoding human telomerase reverse transcriptase a catalytic subunit of the enzymetelomerase
CRP:	C-reactive protein	IEE:	Increased energy expenditure
CRS:	Caloric Restriction Society	IGF1:	Insulin-like growth factor 1 also known as somatomedin C
DNA:	Deoxyribonucleic acid	IL-1 $\beta$ :	Human interleukin 1 $\beta$
DNMT:	DNA methyltransferase	c-KIT:	Proto-oncogene c-Kit also known as mast/stem cell growth factor receptor, also known as tyrosine-protein kinase Kit or CD117
		IRS:	Insulin receptor substrate
		KEAP-1:	Kelch-like ECH-associated protein 1
		Ku70:	Protein encoded in humans by the gene <i>XRCC6</i>
		LBK1:	Tumor suppressor kinase enzyme that activates AMPK
		LDL:	Low-density lipoprotein
		miRNA(s):	microRNA(s)
		mRNA:	Messenger RNA
		mSin1:	Mammalian stress-activated protein kinase-interacting protein
		MTH:	Mitochondrion, mitochondrial
		mTOR:	Mammalian target of rapamycin
		mTORC1:	Mammalian target of rapamycin complex 1
		mTORC2:	Mammalian target of rapamycin complex 2
		NAD <sup>+</sup> :	Nicotinamide adenine dinucleotide
		NADH:	NADH dehydrogenase
		NF- $\kappa$ B:	nuclear factor kappa B
		NIP7:	60S ribosome subunit biogenesis protein NIP7 homolog
		NMDA:	N-Methyl-D-aspartic acid or N-Methyl-D-aspartate

$p16^{INK4a}$ :	Gene encoding the tumor suppressor protein cyclin-dependent kinase inhibitor 2A or CDKN2A or multiple tumor suppressor 1 (MTS-1)
PDPK1:	3-phosphoinositide-dependent protein kinase-1
PGC1- $\alpha$ :	Peroxisome proliferator-activated receptor G co-activator 1 $\alpha$
p53:	Tumor suppressor protein p53 also known as tumor protein 53
p47phox:	Subunit of NADPH oxidase, that has to be phosphorylated for the activation of NADPH oxidase
PKA:	Protein kinase A
PKC:	Protein kinase C
PKG:	Protein kinase G, or cGMP-dependent protein kinase
PtdIns-3K:	Phosphatidylinositol 3-kinase
RAP:	Rapamycin
RAPTOR:	Regulatory-associated protein of mTOR
RHEB:	RAS homolog enriched in brain protein, binds GTP
RNA:	Ribonucleic acid
ROS:	Reactive oxygen species
RSV:	Resveratrol
RAS:	Protein superfamily of small GTPases
RTG1:	Retrograde regulation protein 1
RUNX3:	Gene encoding runt-related transcription factor 3
S6 K1:	Ribosomal protein S6 kinase $\beta$ -1
SGK1:	Serum-and glucocorticoid-regulated kinase; a serine/threonine protein kinase
SIRT1:	NAD-dependent-deacetylase sirtuin1 also known as silent mating type information regulation 2 homolog 1
SOCS-3:	Suppressor of cytokine signaling 3
sTNRF1:	Soluble tumor necrosis factor receptor 1
TLR-4:	Toll-like receptor 4
TNF $\alpha$ :	Tumor necrosis factor $\alpha$
TOR:	Target of rapamycin
TSC1:	Tuberous sclerosis protein 1 also known as hamartin
TSC2:	Tuberous sclerosis protein 2 also known as tuberin
VDAC1:	Voltage-dependent anion-selective channel protein 1
TIG1:	Tazarotene-induced gene-1
WT1:	Gene encoding Wilms tumor protein
YY1:	Transcriptional repressor protein YY1.

## References

- [1] J. A. McKay and J. C. Mathers, "Diet induced epigenetic changes and their implications for health," *Acta Physiologica*, vol. 202, no. 2, pp. 103–118, 2011.
- [2] "Diet, nutrition and the prevention of chronic diseases," *World Health Organization Technical Report Series*, vol. 916, no. 1–8, pp. 1–149, 2003.
- [3] D. J. P. Barker and C. Osmond, "Infant mortality, childhood nutrition, and ischaemic heart disease in England and Wales," *The Lancet*, vol. 1, no. 8489, pp. 1077–1081, 1986.
- [4] O. A. Kensara, S. A. Wootton, D. I. Phillips, M. Patel, A. A. Jackson, and M. Elia, "Fetal programming of body composition: relation between birth weight and body composition measured with dual-energy X-ray absorptiometry and anthropometric methods in older Englishmen," *The American Journal of Clinical Nutrition*, vol. 82, no. 5, pp. 980–987, 2005.
- [5] C. Osmond, D. J. P. Barker, P. D. Winter, C. H. D. Fall, and S. J. Simmonds, "Early growth and death from cardiovascular disease in women," *British Medical Journal*, vol. 307, no. 6918, pp. 1519–1524, 1993.
- [6] C. N. Hales and D. J. P. Barker, "Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis," *Diabetologia*, vol. 35, no. 7, pp. 595–601, 1992.
- [7] C. Cooper, C. Fall, P. Egger, R. Hobbs, R. Eastell, and D. Barker, "Growth in infancy and bone mass in later life," *Annals of the Rheumatic Diseases*, vol. 56, no. 1, pp. 17–21, 1997.
- [8] S. E. Ozanne and C. N. Hales, "Lifespan: catch-up growth and obesity in male mice," *Nature*, vol. 427, no. 6973, pp. 411–412, 2004.
- [9] V. M. Vehaskari, "Prenatal programming of kidney disease," *Current Opinion in Pediatrics*, vol. 22, no. 2, pp. 176–182, 2010.
- [10] A. Gabory, L. Attig, and C. Junien, "Sexual dimorphism in environmental epigenetic programming," *Molecular and Cellular Endocrinology*, vol. 304, no. 1–2, pp. 8–18, 2009.
- [11] A. Bird, "DNA methylation patterns and epigenetic memory," *Genes & Development*, vol. 16, no. 1, pp. 6–21, 2002.
- [12] H. Wu, J. Tao, and Y. E. Sun, "Regulation and function of mammalian DNA methylation patterns: a genomic perspective," *Briefings in Functional Genomics*, vol. 11, no. 3, pp. 240–250, 2012.
- [13] X. Zou, W. Ma, I. A. Solov'yov, C. Chipot, and K. Schulten, "Recognition of methylated DNA through methyl-CpG binding domain proteins," *Nucleic Acids Research*, vol. 40, no. 6, pp. 2747–2758, 2012.
- [14] K. S. Crider, T. P. Yang, R. J. Berry, and L. B. Bailey, "Folate and DNA methylation: a review of molecular mechanisms and the evidence for folate's role," *Advances in Nutrition*, vol. 3, no. 1, pp. 21–38, 2012.
- [15] T. A. Rauch, X. Zhong, X. Wu et al., "High-resolution mapping of DNA hypermethylation and hypomethylation in lung cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 1, pp. 252–257, 2008.
- [16] M. Ehrlich, "DNA hypomethylation in cancer cells," *Epigenomics*, vol. 1, pp. 239–259, 2009.
- [17] C. D. Davis, E. O. Uthus, and J. W. Finley, "Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon," *The Journal of Nutrition*, vol. 130, no. 12, pp. 2903–2909, 2000.
- [18] H. Zeng, L. Yan, W. H. Cheng, and E. O. Uthus, "Dietary selenomethionine increases exon-specific DNA methylation of the p53 gene in rat liver and colon mucosa," *The Journal of Nutrition*, vol. 141, no. 8, pp. 1464–1468, 2011.
- [19] E. M. E. Van Straten, V. W. Bloks, N. C. A. Huijckman et al., "The liver X-receptor gene promoter is hypermethylated in a mouse model of prenatal protein restriction," *American Journal of Physiology*, vol. 298, no. 2, pp. R275–R282, 2010.

- [20] A. J. Bannister and T. Kouzarides, "Regulation of chromatin by histone modifications," *Cell Research*, vol. 21, no. 3, pp. 381–395, 2011.
- [21] A. Link, F. Balaguer, and A. Goel, "Cancer chemoprevention by dietary polyphenols: promising role for epigenetics," *Biochemical Pharmacology*, vol. 80, no. 12, pp. 1771–1792, 2010.
- [22] X. Cheng and R. M. Blumenthal, "Coordinated chromatin control: structural and functional linkage of DNA and histone methylation," *Biochemistry*, vol. 49, no. 14, pp. 2999–3008, 2010.
- [23] Y. Tan, B. Zhang, T. Wu et al., "Transcriptional inhibition of Hoxd4 expression by miRNA-10a in human breast cancer cells," *BMC Molecular Biology*, vol. 10, article 12, 2009.
- [24] P. G. Hawkins and K. V. Morris, "RNA and transcriptional modulation of gene expression," *Cell Cycle*, vol. 7, no. 5, pp. 602–607, 2008.
- [25] D. P. Bartel, "MicroRNAs: target recognition and regulatory functions," *Cell*, vol. 136, no. 2, pp. 215–233, 2009.
- [26] B. Kusenda, M. Mraz, J. Mayer, and S. Pospisilova, "MicroRNA biogenesis, functionality and cancer relevance," *Biomedical papers of the Medical Faculty of the University Palacký, Olomouc, Czechoslovakia*, vol. 150, no. 2, pp. 205–215, 2006.
- [27] I. Bentwich, A. Avniel, Y. Karov et al., "Identification of hundreds of conserved and nonconserved human microRNAs," *Nature Genetics*, vol. 37, no. 7, pp. 766–770, 2005.
- [28] B. P. Lewis, C. B. Burge, and D. P. Bartel, "Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets," *Cell*, vol. 120, no. 1, pp. 15–20, 2005.
- [29] R. C. Friedman, K. K. H. Farh, C. B. Burge, and D. P. Bartel, "Most mammalian mRNAs are conserved targets of microRNAs," *Genome Research*, vol. 19, no. 1, pp. 92–105, 2009.
- [30] L. P. Lim, N. C. Lau, E. G. Weinstein et al., "The microRNAs of *Caenorhabditis elegans*," *Genes & Development*, vol. 17, no. 8, pp. 991–1008, 2003.
- [31] A. Esquela-Kerscher and F. J. Slack, "Oncomirs—microRNAs with a role in cancer," *Nature Reviews Cancer*, vol. 6, no. 4, pp. 259–269, 2006.
- [32] J. C. Mathers, G. Strathdee, and C. L. Relton, "Induction of epigenetic alterations by dietary and other environmental factors," *Advances in Genetics*, vol. 71, pp. 4–39, 2010.
- [33] D. Milenkovic, C. Deval, E. Gouranton et al., "Modulation of miRNA expression by dietary polyphenols in apoE deficient mice: a new mechanism of the action of polyphenols," *PLoS One*, vol. 7, no. 1, Article ID e29837, 2012.
- [34] Q. Sun, R. Cong, H. Yan et al., "Genistein inhibits growth of human uveal melanoma cells and affects microRNA-27a and target gene expression," *Oncology Reports*, vol. 22, no. 3, pp. 563–567, 2009.
- [35] M. Sun, Z. Estrov, Y. Ji, K. R. Coombes, D. H. Harris, and R. Kurzrock, "Curcumin (diferuloylmethane) alters the expression profiles of microRNAs in human pancreatic cancer cells," *Molecular Cancer Therapeutics*, vol. 7, no. 3, pp. 464–473, 2008.
- [36] J. Yang, Y. Cao, J. Sun, and Y. Zhang, "Curcumin reduces the expression of Bcl-2 by upregulating miR-15a and miR-16 in MCF-7 cells," *Medical Oncology*, vol. 27, no. 4, pp. 1114–1118, 2010.
- [37] S. Careccia, S. Mainardi, A. Pelosi et al., "A restricted signature of miRNAs distinguishes APL blasts from normal promyelocytes," *Oncogene*, vol. 28, no. 45, pp. 4034–4040, 2009.
- [38] F. U. Weiss, I. J. Marques, J. M. Woltering et al., "Retinoic acid receptor antagonists inhibit miR-10a expression and block metastatic behavior of pancreatic cancer," *Gastroenterology*, vol. 137, no. 6, pp. 2136–2145, 2009.
- [39] L. A. Davidson, N. Wang, M. S. Shah, J. R. Lupton, I. Ivanov, and R. S. Chapkin, "n-3 Polyunsaturated fatty acids modulate carcinogen-directed non-coding microRNA signatures in rat colon," *Carcinogenesis*, vol. 30, no. 12, pp. 2077–2084, 2009.
- [40] T. Melkamu, X. Zhang, J. Tan, Y. Zeng, and F. Kassie, "Alteration of microRNA expression in vinyl carbamate-induced mouse lung tumors and modulation by the chemopreventive agent indole-3-carbinol," *Carcinogenesis*, vol. 31, no. 2, Article ID bgp208, pp. 252–258, 2010.
- [41] C. J. Marsit, K. Eddy, and K. T. Kelsey, "MicroRNA responses to cellular stress," *Cancer Research*, vol. 66, no. 22, pp. 10843–10848, 2006.
- [42] H. Kutay, S. Bai, J. Datta et al., "Downregulation of miR-122 in the rodent and human hepatocellular carcinomas," *Journal of Cellular Biochemistry*, vol. 99, no. 3, pp. 671–678, 2006.
- [43] R. Loewith and M. N. Hall, "Target of rapamycin (TOR) in nutrient signaling and growth control," *Genetics*, vol. 189, no. 4, pp. 1177–1201, 2011.
- [44] M. Laplante and D. M. Sabatini, "mTOR signaling in growth control and disease," *Cell*, vol. 149, no. 2, pp. 274–293, 2012.
- [45] W. L. Yen and D. J. Klionsky, "How to live long and prosper: autophagy, mitochondria, and aging," *Physiology*, vol. 23, no. 5, pp. 248–262, 2008.
- [46] L. R. Pearce, D. Komander, and D. R. Alessi, "The nuts and bolts of AGC protein kinases," *Nature Reviews*, vol. 11, no. 1, pp. 9–22, 2010.
- [47] Y. Sancak, C. C. Thoreen, T. R. Peterson et al., "PRAS40 is an insulin-regulated inhibitor of the mTORC1 protein kinase," *Molecular Cell*, vol. 25, no. 6, pp. 903–915, 2007.
- [48] C. A. Easley IV, A. Ben-Yehudah, C. J. Redinger et al., "MTOR-mediated activation of p70 S6K induces differentiation of pluripotent human embryonic stem cells," *Cellular Reprogramming*, vol. 12, no. 3, pp. 263–273, 2010.
- [49] L. A. Julien, A. Carriere, J. Moreau, and P. P. Roux, "mTORC1-activated S6K1 phosphorylates rictor on threonine 1135 and regulates mTORC2 signaling," *Molecular and Cellular Biology*, vol. 30, no. 4, pp. 908–921, 2010.
- [50] A. Y. Choo, S. O. Yoon, S. G. Kim, P. P. Roux, and J. Blenis, "Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 45, pp. 17414–17419, 2008.
- [51] D. Zhang, R. Contu, M. V. G. Latronico et al., "mTORC1 regulates cardiac function and myocyte survival through 4E-BP1 inhibition in mice," *The Journal of Clinical Investigation*, vol. 120, no. 8, pp. 2805–2816, 2010.
- [52] R. J. O. Dowling, I. Topisirovic, T. Alain et al., "mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs," *Science*, vol. 328, no. 5982, pp. 1172–1176, 2010.
- [53] K. G. Foster, H. A. Acosta-Jaquez, Y. Romeo et al., "Regulation of mTOR complex 1 (mTORC1) by raptor Ser863 and multisite phosphorylation," *The Journal of Biological Chemistry*, vol. 285, no. 1, pp. 80–94, 2010.
- [54] D. Kwak, S. Choi, H. Jeong et al., "Osmotic stress regulates mammalian target of rapamycin (mTOR) complex 1 via c-Jun N-terminal Kinase (JNK)-mediated Raptor protein phosphorylation," *The Journal of Biological Chemistry*, vol. 287, no. 22, pp. 18398–18407, 2012.
- [55] T. Sato, A. Nakashima, L. Guo, and F. Tamanoi, "Specific activation of mTORC1 by Rheb G-protein in vitro involves

- enhanced recruitment of its substrate protein," *The Journal of Biological Chemistry*, vol. 284, no. 19, pp. 12783–12791, 2009.
- [56] D. M. Gwinn, D. B. Shackelford, D. F. Egan et al., "AMPK phosphorylation of raptor mediates a metabolic checkpoint," *Molecular Cell*, vol. 30, no. 2, pp. 214–226, 2008.
- [57] B. Magnuson, B. Ekim, and D. C. Fingar, "Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks," *The Biochemical Journal*, vol. 441, no. 1, pp. 1–21, 2012.
- [58] B. C. Melnik, "Excessive Leucine-mTORC1-signalling of cow milk-based infant formula: the missing link to understand early childhood obesity," *Journal of Obesity*, vol. 2012, Article ID 197653, 2012.
- [59] A. K. A. DeHart, J. D. Schnell, D. A. Allen, J. Y. Tsai, and L. Hicke, "Receptor internalization in yeast requires the Tor2-Rho1 signaling pathway," *Molecular Biology of the Cell*, vol. 14, no. 11, pp. 4676–4684, 2003.
- [60] T. Powers, S. Aronova, and B. Niles, "TORC2 and sphingolipid biosynthesis and signaling. lessons from budding yeast," *The Enzymes*, vol. 27, pp. 177–197, 2010.
- [61] V. Zinzalla, D. Stracka, W. Oppliger, and M. N. Hall, "Activation of mTORC2 by association with the ribosome," *Cell*, vol. 144, no. 5, pp. 757–768, 2011.
- [62] A. Hagiwara, M. Cornu, N. Cybulski et al., "Hepatic mTORC2 activates glycolysis and lipogenesis through Akt, glucokinase, and SREBP1c," *Cell Metabolism*, vol. 15, no. 5, pp. 725–738, 2012.
- [63] N. Cybulski and M. N. Hall, "TOR complex 2: a signaling pathway of its own," *Trends in Biochemical Sciences*, vol. 34, no. 12, pp. 620–627, 2009.
- [64] C. A. Sparks and D. A. Guertin, "Targeting mTOR: prospects for mTOR complex 2 inhibitors in cancer therapy," *Oncogene*, vol. 29, no. 26, pp. 3733–3744, 2010.
- [65] N. Ikai, N. Nakazawa, T. Hayashi, and M. Yanagida, "The reverse, but coordinated, roles of Tor2 (TORC1) and Tor1 (TORC2) kinases for growth, cell cycle and separase-mediated mitosis in *Schizosaccharomyces pombe*," *Open Biology*, vol. 1, 2011.
- [66] M. A. Frias, C. C. Thoreen, J. D. Jaffe et al., "mSin1 is necessary for Akt/PKB phosphorylation, and its isoforms define three distinct mTORC2s," *Current Biology*, vol. 16, no. 18, pp. 1865–1870, 2006.
- [67] C. M. McCay, M. F. Crowell, and L. A. Maynard, "The effect of retarded growth upon the Length of life span and upon the ultimate body size," *The Journal of Nutrition*, vol. 10, no. 1, pp. 63–79, 1935.
- [68] R. J. Colman, R. M. Anderson, S. C. Johnson et al., "Caloric restriction delays disease onset and mortality in rhesus monkeys," *Science*, vol. 325, no. 5937, pp. 201–204, 2009.
- [69] R. Anderson and R. Weindruch, "Metabolic reprogramming in dietary restriction," *Interdisciplinary Topics in Gerontology*, vol. 35, pp. 18–38, 2007.
- [70] B. K. Kennedy, K. K. Steffen, and M. Kaeberlein, "Ruminations on dietary restriction and aging," *Cellular and Molecular Life Sciences*, vol. 64, no. 11, pp. 1323–1328, 2007.
- [71] M. D. W. Piper and A. Bartke, "Diet and aging," *Cell Metabolism*, vol. 8, no. 2, pp. 99–104, 2008.
- [72] G. S. Roth, D. K. Ingram, and M. A. Lane, "Caloric restriction in primates and relevance to humans," *Annals of the New York Academy of Sciences*, vol. 928, pp. 305–315, 2001.
- [73] R. L. Walford, D. Mock, R. Verdery, and T. MacCallum, "Calorie restriction in biosphere 2: alterations in physiologic, hematologic, hormonal, and biochemical parameters in humans restricted for a 2-year period," *The Journals of Gerontology A*, vol. 57, no. 6, pp. B211–B224, 2002.
- [74] L. Fontana, T. E. Meyer, S. Klein, and J. O. Holloszy, "Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 17, pp. 6659–6663, 2004.
- [75] V. Tsagareli, M. Noakes, and R. J. Norman, "Effect of a very-low-calorie diet on in vitro fertilization outcomes," *Fertility and Sterility*, vol. 86, no. 1, pp. 227–229, 2006.
- [76] L. K. Heilbronn, L. De Jonge, M. I. Frisard et al., "Effect of 6-month calorie restriction on biomarkers of longevity, metabolic adaptation, and oxidative stress in overweight individuals: a randomized controlled trial," *Journal of the American Medical Association*, vol. 295, no. 13, pp. 1539–1548, 2006.
- [77] S. B. Racette, E. P. Weiss, D. T. Villareal et al., "One year of caloric restriction in humans: feasibility and effects on body composition and abdominal adipose tissue," *The Journals of Gerontology A*, vol. 61, no. 9, pp. 943–950, 2006.
- [78] T. E. Meyer, S. J. Kovács, A. A. Ehsani, S. Klein, J. O. Holloszy, and L. Fontana, "Long-term caloric restriction ameliorates the decline in diastolic function in humans," *Journal of the American College of Cardiology*, vol. 47, no. 2, pp. 398–402, 2006.
- [79] L. Fontana, S. Klein, J. O. Holloszy, and B. N. Premachandra, "Effect of long-term calorie restriction with adequate protein and micronutrients on thyroid hormones," *The Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 8, pp. 3232–3235, 2006.
- [80] L. Fontana and S. Klein, "Aging, adiposity, and calorie restriction," *Journal of the American Medical Association*, vol. 297, no. 9, pp. 986–994, 2007.
- [81] J. O. Holloszy and L. Fontana, "Caloric restriction in humans," *Experimental Gerontology*, vol. 42, no. 8, pp. 709–712, 2007.
- [82] B. J. Willcox, D. C. Willcox, H. Todoriki et al., "Caloric restriction, the traditional okinawan diet, and healthy aging: the diet of the world's longest-lived people and its potential impact on morbidity and life span," *Annals of the New York Academy of Sciences*, vol. 1114, pp. 434–455, 2007.
- [83] L. Fontana, D. T. Villareal, E. P. Weiss et al., "Calorie restriction or exercise: effects on coronary heart disease risk factors. A randomized, controlled trial," *American Journal of Physiology*, vol. 293, no. 1, pp. E197–E202, 2007.
- [84] J. O. Holloszy and L. Fontana, "Caloric restriction in humans," *Experimental Gerontology*, vol. 42, no. 8, pp. 709–712, 2007.
- [85] T. Hofer, L. Fontana, S. D. Anton et al., "Long-term effects of caloric restriction or exercise on DNA and RNA oxidation levels in white blood cells and urine in humans," *Rejuvenation Research*, vol. 11, no. 4, pp. 793–799, 2008.
- [86] L. M. Redman, J. Rood, S. D. Anton, C. Champagne, S. R. Smith, and E. Ravussin, "Calorie restriction and bone health in young, overweight individuals," *Archives of Internal Medicine*, vol. 168, no. 17, pp. 1859–1866, 2008.
- [87] C. Cruzen and R. J. Colman, "Effects of caloric restriction on cardiovascular aging in non-human primates and humans," *Clinics in Geriatric Medicine*, vol. 25, no. 4, pp. 733–743, 2009.
- [88] R. Cangemi, A. J. Friedmann, J. O. Holloszy, and L. Fontana, "Long-term effects of calorie restriction on serum sex-hormone concentrations in men," *Aging Cell*, vol. 9, no. 2, pp. 236–242, 2010.

- [89] J. F. Trepanowski and R. J. Bloomer, "The impact of religious fasting on human health," *Nutrition Journal*, vol. 9, no. 1, article 57, 2010.
- [90] A. Soare, R. Cangemi, D. Omodei, J. O. Holloszy, and L. Fontana, "Long-term calorie restriction, but not endurance exercise, lowers core body temperature in humans," *Aging*, vol. 3, no. 4, pp. 374–379, 2011.
- [91] J. Rochon, C. W. Bales, E. Ravussin et al., "Design and conduct of the CALERIE study: comprehensive assessment of the long-term effects of reducing intake of energy," *The Journals of Gerontology A*, vol. 66, no. 1, pp. 97–108, 2011.
- [92] C. K. Martin, S. K. Das, L. Lindblad et al., "Effect of calorie restriction on the free-living physical activity levels of nonobese humans: results of three randomized trials," *Journal of Applied Physiology*, vol. 110, no. 4, pp. 956–963, 2011.
- [93] K. Stein, A. Soare, T. E. Meyer, R. Cangemi, J. O. Holloszy, and L. Fontana, "Caloric restriction may reverse age-related autonomic decline in humans," *Aging Cell*, vol. 11, no. 4, pp. 644–650, 2012.
- [94] R. M. Anderson and R. Weindruch, "Metabolic reprogramming, caloric restriction and aging," *Trends in Endocrinology and Metabolism*, vol. 21, no. 3, pp. 134–141, 2010.
- [95] J. M. Zahn, S. Poosala, A. B. Owen et al., "AGEMAP: a gene expression database for aging in mice," *PLoS Genetics*, vol. 3, no. 11, p. e201, 2007.
- [96] J. P. de Magalhães, J. Curado, and G. M. Church, "Meta-analysis of age-related gene expression profiles identifies common signatures of aging," *Bioinformatics*, vol. 25, no. 7, pp. 875–881, 2009.
- [97] S. K. Park and T. A. Prolla, "Lessons learned from gene expression profile studies of aging and caloric restriction," *Ageing Research Reviews*, vol. 4, no. 1, pp. 55–65, 2005.
- [98] G. S. Hotamisligil, "Inflammation and metabolic disorders," *Nature*, vol. 444, no. 7121, pp. 860–867, 2006.
- [99] F. Lago, C. Dieguez, J. Gómez-Reino, and O. Gualillo, "The emerging role of adipokines as mediators of inflammation and immune responses," *Cytokine & Growth Factor Reviews*, vol. 18, no. 3-4, pp. 313–325, 2007.
- [100] U. Meier and A. M. Gressner, "Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin," *Clinical Chemistry*, vol. 50, no. 9, pp. 1511–1525, 2004.
- [101] M. Zhu, G. D. Lee, L. Ding et al., "Adipogenic signaling in rat white adipose tissue: modulation by aging and calorie restriction," *Experimental Gerontology*, vol. 42, no. 8, pp. 733–744, 2007.
- [102] K. Shinmura, K. Tamaki, K. Saito, Y. Nakano, T. Tobe, and R. Bolli, "Cardioprotective effects of short-term caloric restriction are mediated by adiponectin via activation of AMP-activated protein kinase," *Circulation*, vol. 116, no. 24, pp. 2809–2817, 2007.
- [103] J. J. Díez and P. Iglesias, "The role of the novel adipocyte-derived hormone adiponectin in human disease," *European Journal of Endocrinology*, vol. 148, no. 3, pp. 293–300, 2003.
- [104] O. Ukkola and M. Santaniemi, "Adiponectin: a link between excess adiposity and associated comorbidities?" *Journal of Molecular Medicine*, vol. 80, no. 11, pp. 696–702, 2002.
- [105] O. Renaldi, B. Pramono, H. Sinorita, L. B. Purnomo, R. H. Asdie, and A. H. Asdie, "Hypoadiponectinemia: a risk factor for metabolic syndrome," *Acta medica Indonesiana*, vol. 41, no. 1, pp. 20–24, 2009.
- [106] A. E. Civitaresse, B. Utkropcova, S. Carling et al., "Role of adiponectin in human skeletal muscle bioenergetics," *Cell Metabolism*, vol. 4, no. 1, pp. 75–87, 2006.
- [107] M. Nishimura, Y. Izumiya, A. Higuchi et al., "Adiponectin prevents cerebral ischemic injury through endothelial nitric oxide synthase-dependent mechanisms," *Circulation*, vol. 117, no. 2, pp. 216–223, 2008.
- [108] J. M. Fernández-Real, A. López-Bermejo, R. Casamitjana, and W. Ricart, "Novel interactions of adiponectin with the endocrine system and inflammatory parameters," *The Journal of Clinical Endocrinology & Metabolism*, vol. 88, no. 6, pp. 2714–2718, 2003.
- [109] W. Aldhahi and O. Hamdy, "Adipokines, inflammation, and the endothelium in diabetes," *Current Diabetes Reports*, vol. 3, no. 4, pp. 293–298, 2003.
- [110] N. Ouchi, S. Kihara, T. Funahashi, Y. Matsuzawa, and K. Walsh, "Obesity, adiponectin and vascular inflammatory disease," *Current Opinion in Lipidology*, vol. 14, no. 6, pp. 561–566, 2003.
- [111] D. M. Huffman, D. R. Moellering, W. E. Grizzle, C. R. Stockard, M. S. Johnson, and T. R. Nagy, "Effect of exercise and calorie restriction on biomarkers of aging in mice," *American Journal of Physiology*, vol. 294, no. 5, pp. R1618–R1627, 2008.
- [112] L. Fontana, E. P. Weiss, D. T. Villareal, S. Klein, and J. O. Holloszy, "Long-term effects of calorie or protein restriction on serum IGF-1 and IGFBP-3 concentration in humans," *Aging Cell*, vol. 7, no. 5, pp. 681–687, 2008.
- [113] A. R. Cameron, S. Anton, L. Melville et al., "Black tea polyphenols mimic insulin/insulin-like growth factor-1 signalling to the longevity factor FOXO1a," *Aging Cell*, vol. 7, no. 1, pp. 69–77, 2008.
- [114] W. Qin, W. Zhao, L. Ho et al., "Regulation of forkhead transcription factor FOXO3a contributes to calorie restriction-induced prevention of Alzheimer's disease-type amyloid neuropathology and spatial memory deterioration," *Annals of the New York Academy of Sciences*, vol. 1147, pp. 335–347, 2008.
- [115] F. Flachsbarth, A. Caliebe, R. Kleindorp et al., "Association of FOXO3A variation with human longevity confirmed in German centenarians," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 8, pp. 2700–2705, 2009.
- [116] E. J. Anderson, M. E. Lustig, K. E. Boyle et al., "Mitochondrial H<sub>2</sub>O<sub>2</sub> emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans," *The Journal of Clinical Investigation*, vol. 119, no. 3, pp. 573–581, 2009.
- [117] D. E. Harrison, R. Strong, Z. D. Sharp et al., "Rapamycin fed late in life extends lifespan in genetically heterogeneous mice," *Nature*, vol. 460, no. 7253, pp. 392–395, 2009.
- [118] C. Chen, Y. Liu, Y. Liu, and P. Zheng, "mTOR regulation and therapeutic rejuvenation of aging hematopoietic stem cells," *Science Signaling*, vol. 2, no. 98, p. ra75, 2009.
- [119] P. Kapahi, D. Chen, A. N. Rogers et al., "With TOR, less is more: a key role for the conserved nutrient-sensing TOR pathway in aging," *Cell Metabolism*, vol. 11, no. 6, pp. 453–465, 2010.
- [120] B. M. Zid, A. N. Rogers, S. D. Katewa et al., "4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*," *Cell*, vol. 139, no. 1, pp. 149–160, 2009.
- [121] S. Sengupta, T. R. Peterson, M. Laplante, S. Oh, and D. M. Sabatini, "mTORC1 controls fasting-induced ketogenesis and its modulation by ageing," *Nature*, vol. 468, no. 7327, pp. 1100–1106, 2010.

- [122] J. T. Cunningham, J. T. Rodgers, D. H. Arlow, F. Vazquez, V. K. Mootha, and P. Puigserver, "mTOR controls mitochondrial oxidative function through a YY1-PGC-1 $\alpha$  transcriptional complex," *Nature*, vol. 450, no. 7170, pp. 736–740, 2007.
- [123] A. Ramanathan and S. L. Schreiber, "Direct control of mitochondrial function by mTOR," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 52, pp. 22229–22232, 2009.
- [124] J. Knapowski, K. Wieczorowska-Tobis, and J. Witowski, "Pathophysiology of ageing," *Journal of Physiology and Pharmacology*, vol. 53, no. 2, pp. 135–146, 2002.
- [125] J. P. J. Issa, N. Ahuja, M. Toyota, M. P. Bronner, and T. A. Brentnall, "Accelerated age-related CpG island methylation in ulcerative colitis," *Cancer Research*, vol. 61, no. 9, pp. 3573–3577, 2001.
- [126] J. P. J. Issa, Y. L. Ottaviano, P. Celano, S. R. Hamilton, N. E. Davidson, and S. B. Baylin, "Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon," *Nature Genetics*, vol. 7, no. 4, pp. 536–540, 1994.
- [127] J. P. J. Issa, P. M. Vertino, C. D. Boehm, I. F. Newsham, and S. B. Baylin, "Switch from monoallelic to biallelic human IGF2 promoter methylation during aging and carcinogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 21, pp. 11757–11762, 1996.
- [128] R. P. Singhal, L. L. Mays-Hoopers, and G. L. Eichhorn, "DNA methylation in aging of mice," *Mechanisms of Ageing and Development*, vol. 41, no. 3, pp. 199–210, 1987.
- [129] T. Waki, G. Tamura, M. Sato, and T. Motoyama, "Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples," *Oncogene*, vol. 22, no. 26, pp. 4128–4133, 2003.
- [130] V. L. Wilson, R. A. Smith, S. Ma, and R. G. Cutler, "Genomic 5-methyldeoxycytidine decreases with age," *The Journal of Biological Chemistry*, vol. 262, no. 21, pp. 9948–9951, 1987.
- [131] T. Y. Kim, H. J. Lee, K. S. Hwang et al., "Methylation of RUNX3 in various types of human cancers and premalignant stages of gastric carcinoma," *Laboratory Investigation*, vol. 84, no. 4, pp. 479–484, 2004.
- [132] Y. Li, M. Daniel, and T. O. Tollefsbol, "Epigenetic regulation of caloric restriction in aging," *BMC Medicine*, vol. 9, article 98, 2011.
- [133] A. Vaquero and D. Reinberg, "Calorie restriction and the exercise of chromatin," *Genes & Development*, vol. 23, no. 16, pp. 1849–1869, 2009.
- [134] U. Muñoz-Najar and J. M. Sedivy, "Epigenetic control of aging," *Antioxidants & Redox Signaling*, vol. 14, no. 2, pp. 241–259, 2011.
- [135] B. S. Hass, R. W. Hart, M. H. Lu, and B. D. Lyn-Cook, "Effects of caloric restriction in animals on cellular function, oncogene expression, and DNA methylation in vitro," *Mutation Research*, vol. 295, no. 4–6, pp. 281–289, 1993.
- [136] Y. Li, L. Liu, and T. O. Tollefsbol, "Glucose restriction can extend normal cell lifespan and impair precancerous cell growth through epigenetic control of hTERT and p16 expression," *The FASEB Journal*, vol. 24, no. 5, pp. 1442–1453, 2010.
- [137] R. S. Ahima, "Connecting obesity, aging and diabetes," *Nature Medicine*, vol. 15, no. 9, pp. 996–997, 2009.
- [138] T. M. Larsen, S. Dalskov, M. Van Baak et al., "The diet, obesity and genes (diogenes) dietary study in eight European countries—a comprehensive design for long-term intervention," *Obesity Reviews*, vol. 11, no. 1, pp. 76–91, 2010.
- [139] F. I. Milagro, J. Campión, P. Cordero et al., "A dual epigenomic approach for the search of obesity biomarkers: DNA methylation in relation to diet-induced weight loss," *The FASEB Journal*, vol. 25, no. 4, pp. 1378–1389, 2011.
- [140] L. Bouchard, R. Rabasa-Lhoret, M. Faraj et al., "Differential epigenomic and transcriptomic responses in subcutaneous adipose tissue between low and high responders to caloric restriction," *The American Journal of Clinical Nutrition*, vol. 91, no. 2, pp. 309–320, 2010.
- [141] J. Campión, F. I. Milagro, E. Goyenechea, and J. A. Martínez, "TNF- $\alpha$  promoter methylation as a predictive biomarker for weight-loss response," *Obesity*, vol. 17, no. 6, pp. 1293–1297, 2009.
- [142] A. L. Clayton, C. A. Hazzalin, and L. C. Mahadevan, "Enhanced histone acetylation and transcription: a dynamic perspective," *Molecular Cell*, vol. 23, no. 3, pp. 289–296, 2006.
- [143] M. Meyerson, C. M. Counter, E. N. Eaton et al., "hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization," *Cell*, vol. 90, no. 4, pp. 785–795, 1997.
- [144] T. Kanaya, S. Kyo, M. Takakura, H. Ito, M. Namiki, and M. Inoue, "hTERT is a critical determinant of telomerase activity in renal-cell carcinoma," *International Journal of Cancer*, vol. 78, no. 5, pp. 539–543, 1998.
- [145] S. J. Lin, P. A. Defossez, and L. Guarente, "Requirement of NAD and SIR2 for life-span extension by calorie restriction in *Saccharomyces cerevisiae*," *Science*, vol. 289, no. 5487, pp. 2126–2128, 2000.
- [146] L. Guarente and F. Picard, "Calorie restriction—the SIR2 connection," *Cell*, vol. 120, no. 4, pp. 473–482, 2005.
- [147] I. B. Leibiger and P. O. Berggren, "Sirt1: a metabolic master switch that modulates lifespan," *Nature Medicine*, vol. 12, no. 1, pp. 34–36, 2006.
- [148] L. Bordone, D. Cohen, A. Robinson et al., "SIRT1 transgenic mice show phenotypes resembling calorie restriction," *Ageing Cell*, vol. 6, no. 6, pp. 759–767, 2007.
- [149] H. Y. Cohen, C. Miller, K. J. Bitterman et al., "Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase," *Science*, vol. 305, no. 5682, pp. 390–392, 2004.
- [150] Y. Kanfi, V. Peshti, Y. M. Gozlan, M. Rathaus, R. Gil, and H. Y. Cohen, "Regulation of SIRT1 protein levels by nutrient availability," *FEBS Letters*, vol. 582, no. 16, pp. 2417–2423, 2008.
- [151] A. B. Crujeiras, D. Parra, E. Goyenechea, and J. A. Martínez, "Sirtuin gene expression in human mononuclear cells is modulated by caloric restriction," *European Journal of Clinical Investigation*, vol. 38, no. 9, pp. 672–678, 2008.
- [152] L. A. Wakeling, L. J. Ions, and D. Ford, "Could Sirt1-mediated epigenetic effects contribute to the longevity response to dietary restriction and be mimicked by other dietary interventions?" *Age*, vol. 31, no. 4, pp. 327–341, 2009.
- [153] Y. Li and T. O. Tollefsbol, "P16<sup>INK4a</sup> suppression by glucose restriction contributes to human cellular lifespan extension through SIRT1-mediated epigenetic and genetic mechanisms," *PLoS ONE*, vol. 6, no. 2, Article ID e17421, 2011.
- [154] M. C. Haigis and L. P. Guarente, "Mammalian sirtuins—emerging roles in physiology, aging, and calorie restriction," *Genes & Development*, vol. 20, no. 21, pp. 2913–2921, 2006.
- [155] J. Luo, A. Y. Nikolaev, S. I. Imai et al., "Negative control of p53 by Sir2 $\alpha$  promotes cell survival under stress," *Cell*, vol. 107, no. 2, pp. 137–148, 2001.
- [156] E. Langley, M. Pearson, M. Faretta et al., "Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence," *The EMBO Journal*, vol. 21, no. 10, pp. 2383–2396, 2002.

- [157] H. Vaziri, S. K. Dessain, E. N. Eaton et al., “*hSIR2<sup>SIRT1</sup>* functions as an NAD-dependent p53 deacetylase,” *Cell*, vol. 107, no. 2, pp. 149–159, 2001.
- [158] A. Brunet, L. B. Sweeney, J. F. Sturgill et al., “Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase,” *Science*, vol. 303, no. 5666, pp. 2011–2015, 2004.
- [159] M. C. Motta, N. Divecha, M. Lemieux et al., “Mammalian SIRT1 represses forkhead transcription factors,” *Cell*, vol. 116, no. 4, pp. 551–563, 2004.
- [160] M. M. Schilling, J. K. Oeser, J. N. Boustead, B. P. Flemming, and R. M. O’Brien, “Gluconeogenesis: re-evaluating the FOXO1-PGC-1 $\alpha$  connection,” *Nature*, vol. 443, no. 7111, pp. E10–E11, 2006.
- [161] R. B. Vega, J. M. Huss, and D. P. Kelly, “The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor  $\alpha$  in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes,” *Molecular and Cellular Biology*, vol. 20, no. 5, pp. 1868–1876, 2000.
- [162] J. Koubova and L. Guarente, “How does calorie restriction work?” *Genes & Development*, vol. 17, no. 3, pp. 313–321, 2003.
- [163] R. S. Sohal and R. Weindruch, “Oxidative stress, caloric restriction, and aging,” *Science*, vol. 273, no. 5271, pp. 59–63, 1996.
- [164] B. J. Merry, “Molecular mechanisms linking calorie restriction and longevity,” *The International Journal of Biochemistry & Cell Biology*, vol. 34, no. 11, pp. 1340–1354, 2002.
- [165] J. Jeong, K. Juhn, H. Lee et al., “SIRT1 promotes DNA repair activity and deacetylation of Ku70,” *Experimental & Molecular Medicine*, vol. 39, no. 1, pp. 8–13, 2007.
- [166] H. Y. Cohen, S. Lavu, K. J. Bitterman et al., “Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis,” *Molecular Cell*, vol. 13, no. 5, pp. 627–638, 2004.
- [167] H. Wong and K. Riabowol, “Differential CDK-inhibitor gene expression in aging human diploid fibroblasts,” *Experimental Gerontology*, vol. 31, no. 1–2, pp. 311–325, 1996.
- [168] J. Gil and G. Peters, “Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all,” *Nature Reviews*, vol. 7, no. 9, pp. 667–677, 2006.
- [169] J. Krishnamurthy, C. Torrice, M. R. Ramsey et al., “Ink4a/Arf expression is a biomarker of aging,” *The Journal of Clinical Investigation*, vol. 114, no. 9, pp. 1299–1307, 2004.
- [170] D. A. Alcorta, Y. Xiong, D. Phelps, G. Hannon, D. Beach, and J. C. Barrett, “Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 24, pp. 13742–13747, 1996.
- [171] A. Melk, B. M. W. Schmidt, O. Takeuchi, B. Sawitzki, D. C. Rayner, and P. F. Halloran, “Expression of p16<sup>INK4a</sup> and other cell cycle regulator and senescence associated genes in aging human kidney,” *Kidney International*, vol. 65, no. 2, pp. 510–520, 2004.
- [172] W. Fischle, Y. Wang, and C. D. Allis, “Histone and chromatin cross-talk,” *Current Opinion in Cell Biology*, vol. 15, no. 2, pp. 172–183, 2003.
- [173] T. Kouzarides, “Histone methylation in transcriptional control,” *Current Opinion in Genetics & Development*, vol. 12, no. 2, pp. 198–209, 2002.
- [174] N. Noren Hooten, K. Abdelmohsen, M. Gorospe, N. Ejiogu, A. B. Zonderman, and M. K. Evans, “microRNA expression patterns reveal differential expression of target genes with age,” *PloS One*, vol. 5, no. 5, Article ID e10724, 2010.
- [175] A. De Lencastre, Z. Pincus, K. Zhou, M. Kato, S. S. Lee, and F. J. Slack, “MicroRNAs both promote and antagonize longevity in *C. elegans*,” *Current Biology*, vol. 20, no. 24, pp. 2159–2168, 2010.
- [176] D. J. Bates, N. Li, R. Liang et al., “MicroRNA regulation in Ames dwarf mouse liver may contribute to delayed aging,” *Aging Cell*, vol. 9, no. 1, pp. 1–18, 2010.
- [177] U. A. Ørom, M. K. Lim, J. E. Savage et al., “MicroRNA-203 regulates caveolin-1 in breast tissue during caloric restriction,” *Cell Cycle*, vol. 11, no. 7, pp. 1291–1295, 2012.
- [178] A. Khanna, S. Muthusamy, R. Liang, H. Sarojini, and E. Wang, “Gain of survival signaling by down-regulation of three key miRNAs in brain of calorie-restricted mice,” *Aging*, vol. 3, no. 3, pp. 223–236, 2011.
- [179] E. Paradis, H. Douillard, M. Koutroumanis, C. Goodyer, and A. LeBlanc, “Amyloid  $\beta$  peptide of Alzheimer’s disease down-regulates bcl-2 and upregulates bax expression in human neurons,” *Journal of Neuroscience*, vol. 16, no. 23, pp. 7533–7539, 1996.
- [180] C. Perier, J. Bové, D. C. Wu et al., “Two molecular pathways initiate mitochondria-dependent dopaminergic neurodegeneration in experimental Parkinson’s disease,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 19, pp. 8161–8166, 2007.
- [181] N. Louneva, J. W. Cohen, L. Y. Han et al., “Caspase-3 is enriched in postsynaptic densities and increased in Alzheimer’s disease,” *The American Journal of Pathology*, vol. 173, no. 5, pp. 1488–1495, 2008.
- [182] M. Yamada, K. Kida, W. Amutuhaire, F. Ichinose, and M. Kaneki, “Gene disruption of caspase-3 prevents MPTP-induced Parkinson’s disease in mice,” *Biochemical and Biophysical Research Communications*, vol. 402, no. 2, pp. 312–318, 2010.
- [183] W. Kudo, H. P. Lee, M. A. Smith, X. Zhu, S. Matsuyama, and H. G. Lee, “Inhibition of Bax protects neuronal cells from oligomeric  $A\beta$  neurotoxicity,” *Cell Death & Disease*, vol. 3, Article ID e309, 2012.
- [184] D. K. Ingram, M. Zhu, J. Mamczarz et al., “Calorie restriction mimetics: an emerging research field,” *Aging Cell*, vol. 5, no. 2, pp. 97–108, 2006.
- [185] H. R. Warner, D. Ingram, R. A. Miller, N. L. Nadon, and A. G. Richardson, “Program for testing biological interventions to promote healthy aging,” *Mechanisms of Ageing and Development*, vol. 115, no. 3, pp. 199–207, 2000.
- [186] N. L. Nadon, R. Strong, R. A. Miller et al., “Design of aging intervention studies: the NIA interventions testing program,” *Age*, vol. 30, no. 4, pp. 187–199, 2008.
- [187] R. A. Miller, D. E. Harrison, C. M. Astle et al., “An aging interventions testing program: study design and interim report,” *Aging Cell*, vol. 6, no. 4, pp. 565–575, 2007.
- [188] R. Strong, R. A. Miller, C. M. Astle et al., “Nordihydroguaiaretic acid and aspirin increase lifespan of genetically heterogeneous male mice,” *Aging Cell*, vol. 7, no. 5, pp. 641–650, 2008.
- [189] N. L. Nadon, “Exploiting the rodent model for studies on the pharmacology of lifespan extension,” *Aging Cell*, vol. 5, no. 1, pp. 9–15, 2006.
- [190] J. O. Holloszy, “Mortality rate and longevity of food-restricted exercising male rats: a reevaluation,” *Journal of Applied Physiology*, vol. 82, no. 2, pp. 399–403, 1997.
- [191] K. C. Deruisseau, A. N. Kavazis, S. Judge et al., “Moderate caloric restriction increases diaphragmatic antioxidant enzyme mRNA, but not when combined with lifelong



- exercise," *Antioxidants & Redox Signaling*, vol. 8, no. 3-4, pp. 539–547, 2006.
- [192] A. Y. Seo, T. Hofer, B. Sung, S. Judge, H. Y. Chung, and C. Leeuwenburgh, "Hepatic oxidative stress during aging: effects of 8% long-term calorie restriction and lifelong exercise," *Antioxidants & Redox Signaling*, vol. 8, no. 3-4, pp. 529–538, 2006.
- [193] R. Kalani, S. Judge, C. Carter, M. Pahor, and C. Leeuwenburgh, "Effects of caloric restriction and exercise on age-related, chronic inflammation assessed by C-reactive protein and interleukin-6," *The Journals of Gerontology A*, vol. 61, no. 3, pp. 211–217, 2006.
- [194] P. Abete, G. Testa, G. Galizia et al., "Tandem action of exercise training and food restriction completely preserves ischemic preconditioning in the aging heart," *Experimental Gerontology*, vol. 40, no. 1-2, pp. 43–50, 2005.
- [195] D. L. Crandall, R. P. Feirer, D. R. Griffith, and D. C. Beitz, "Relative role of caloric restriction and exercise training upon susceptibility to isoproterenol-induced myocardial infarction in male rats," *The American Journal of Clinical Nutrition*, vol. 34, no. 5, pp. 841–847, 1981.
- [196] A. E. Civitarese, S. Carling, L. K. Heilbronn et al., "Calorie restriction increases muscle mitochondrial biogenesis in healthy humans," *PLoS Medicine*, vol. 4, no. 3, article e76, 2007.
- [197] D. E. Larson-Meyer, B. R. Newcomer, L. K. Heilbronn et al., "Effect of 6-month calorie restriction and exercise on serum and liver lipids and markers of liver function," *Obesity*, vol. 16, no. 6, pp. 1355–1362, 2008.
- [198] M. Lefevre, L. M. Redman, L. K. Heilbronn et al., "Caloric restriction alone and with exercise improves CVD risk in healthy non-obese individuals," *Atherosclerosis*, vol. 203, no. 1, pp. 206–213, 2009.
- [199] D. E. Larson-Meyer, L. Redman, L. K. Heilbronn, C. K. Martin, and E. Ravussin, "Caloric restriction with or without exercise: the fitness versus fatness debate," *Medicine and Science in Sports and Exercise*, vol. 42, no. 1, pp. 152–159, 2010.
- [200] N. E. Silverman, B. J. Nicklas, and A. S. Ryan, "Addition of aerobic exercise to a weight loss program increases BMD, with an associated reduction in inflammation in overweight postmenopausal women," *Calcified Tissue International*, vol. 84, no. 4, pp. 257–265, 2009.
- [201] J. F. Trepanowski, R. E. Canale, K. E. Marshall, M. M. Kabir, and R. J. Bloomer, "Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings," *Nutrition Journal*, vol. 10, article 107, 2011.
- [202] K. Iwasaki, C. A. Gleiser, E. J. Masoro, C. A. McMahan, E. Seo, and B. P. Yu, "The influence of dietary protein source on longevity and age-related disease processes of Fischer rats," *Journals of Gerontology*, vol. 43, no. 1, pp. B5–B12, 1988.
- [203] I. Shimokawa, Y. Higami, B. P. Yu, E. J. Masoro, and T. Ikeda, "Influence of dietary components on occurrence of and mortality due to neoplasms in male F344 rats," *Aging*, vol. 8, no. 4, pp. 254–262, 1996.
- [204] M. Khorakova, Z. Deil, D. Khausman, and K. Matsek, "Effect of carbohydrate-enriched diet and subsequent food restriction on life prolongation in Fischer 344 male rats," *Fiziologicheskii Zhurnal*, vol. 36, no. 5, pp. 16–21, 1990.
- [205] C. Kubo, B. C. Johnson, A. Gajjar, and R. A. Good, "Crucial dietary factors in maximizing life span and longevity in autoimmune-prone mice," *The Journal of Nutrition*, vol. 117, no. 6, pp. 1129–1135, 1987.
- [206] R. Pamplona and G. Barja, "Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection," *Biochimica et Biophysica Acta*, vol. 1757, no. 5-6, pp. 496–508, 2006.
- [207] A. Sanz, P. Caro, J. G. Sanchez, and G. Barja, "Effect of lipid restriction on mitochondrial free radical production and oxidative DNA damage," *Annals of the New York Academy of Sciences*, vol. 1067, no. 1, pp. 200–209, 2006.
- [208] A. Sanz, J. Gómez, P. Caro, and G. Barja, "Carbohydrate restriction does not change mitochondrial free radical generation and oxidative DNA damage," *Journal of Bioenergetics and Biomembranes*, vol. 38, no. 5-6, pp. 327–333, 2006.
- [209] P. E. Segall and P. S. Timiras, "Pathophysiologic findings after chronic tryptophan deficiency in rats: a model for delayed growth and aging," *Mechanisms of Ageing and Development*, vol. 5, no. 2, pp. 109–124, 1976.
- [210] H. Ooka, P. E. Segall, and P. S. Timiras, "Histology and survival in age-delayed low-tryptophan-fed rats," *Mechanisms of Ageing and Development*, vol. 43, no. 1, pp. 79–98, 1988.
- [211] R. A. Miller, G. Buehner, Y. Chang, J. M. Harper, R. Sigler, and M. Smith-Wheelock, "Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance," *Aging Cell*, vol. 4, no. 3, pp. 119–125, 2005.
- [212] N. Orentreich, J. R. Matias, A. DeFelice, and J. A. Zimmerman, "Low methionine ingestion by rats extends life span," *The Journal of Nutrition*, vol. 123, no. 2, pp. 269–274, 1993.
- [213] J. P. Richie Jr., Y. Leutzinger, S. Parthasarathy, V. Malloy, N. Orentreich, and J. A. Zimmerman, "Methionine restriction increases blood glutathione and longevity in F344 rats," *The FASEB Journal*, vol. 8, no. 15, pp. 1302–1307, 1994.
- [214] J. P. Richie Jr., D. Komninou, Y. Leutzinger et al., "Tissue glutathione and cysteine levels in methionine-restricted rats," *Nutrition*, vol. 20, no. 9, pp. 800–805, 2004.
- [215] J. A. Zimmerman, V. Malloy, R. Krajcik, and N. Orentreich, "Nutritional control of aging," *Experimental Gerontology*, vol. 38, no. 1-2, pp. 47–52, 2003.
- [216] A. Sanz, P. Caro, V. Ayala, M. Portero-Otin, R. Pamplona, and G. Barja, "Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins," *The FASEB Journal*, vol. 20, no. 8, pp. 1064–1073, 2006.
- [217] P. Caro, J. Gómez, M. López-Torres et al., "Forty percent and eighty percent methionine restriction decrease mitochondrial ROS generation and oxidative stress in rat liver," *Biogerontology*, vol. 9, no. 3, pp. 183–196, 2008.
- [218] M. C. Ruiz, V. Ayala, M. Portero-Otin, J. R. Requena, G. Barja, and R. Pamplona, "Protein methionine content and MDA-lysine adducts are inversely related to maximum life span in the heart of mammals," *Mechanisms of Ageing and Development*, vol. 126, no. 10, pp. 1106–1114, 2005.
- [219] N. Hidiroglou, G. S. Gilani, L. Long et al., "The influence of dietary vitamin E, fat, and methionine on blood cholesterol profile, homocysteine levels, and oxidizability of low density lipoprotein in the gerbil," *The Journal of Nutritional Biochemistry*, vol. 15, no. 12, pp. 730–740, 2004.
- [220] P. Caro, J. Gomez, I. Sanchez et al., "Effect of 40% restriction of dietary amino acids (except methionine) on mitochondrial oxidative stress and biogenesis, AIF and SIRT1 in rat liver," *Biogerontology*, vol. 10, no. 5, pp. 579–592, 2009.
- [221] M. F. McCarty, J. Barroso-Aranda, and F. Contreras, "The low-methionine content of vegan diets may make

- methionine restriction feasible as a life extension strategy," *Medical Hypotheses*, vol. 72, no. 2, pp. 125–128, 2009.
- [222] K. A. Varady and M. K. Hellerstein, "Alternate-day fasting and chronic disease prevention: a review of human and animal trials," *The American Journal of Clinical Nutrition*, vol. 86, no. 1, pp. 7–13, 2007.
- [223] R. M. Anson, Z. Guo, R. de Cabo et al., "Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 10, pp. 6216–6220, 2003.
- [224] O. Descamps, J. Riondel, V. Ducros, and A. M. Roussel, "Mitochondrial production of reactive oxygen species and incidence of age-associated lymphoma in OF1 mice: effect of alternate-day fasting," *Mechanisms of Ageing and Development*, vol. 126, no. 11, pp. 1185–1191, 2005.
- [225] W. Duan, Z. Guo, H. Jiang, M. Ware, and M. P. Mattson, "Reversal of behavioral and metabolic abnormalities, and insulin resistance syndrome, by dietary restriction in mice deficient in brain-derived neurotrophic factor," *Endocrinology*, vol. 144, no. 6, pp. 2446–2453, 2003.
- [226] I. Ahmet, R. Wan, M. P. Mattson, E. G. Lakatta, and M. Talan, "Cardioprotection by intermittent fasting in rats," *Circulation*, vol. 112, no. 20, pp. 3115–3121, 2005.
- [227] D. E. Mager, R. Wan, M. Brown et al., "Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats," *The FASEB Journal*, vol. 20, no. 6, pp. 631–637, 2006.
- [228] C. R. Pedersen, I. Hagemann, T. Bock, and K. Buschard, "Intermittent feeding and fasting reduces diabetes incidence in BB rats," *Autoimmunity*, vol. 30, no. 4, pp. 243–250, 1999.
- [229] K. Tikoo, D. N. Tripathi, D. G. Kabra, V. Sharma, and A. B. Gaikwad, "Intermittent fasting prevents the progression of type I diabetic nephropathy in rats and changes the expression of Sir2 and p53," *FEBS Letters*, vol. 581, no. 5, pp. 1071–1078, 2007.
- [230] R. Wan, S. Camandola, and M. P. Mattson, "Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats," *The FASEB Journal*, vol. 17, no. 9, pp. 1133–1134, 2003.
- [231] L. K. Heilbronn, S. R. Smith, C. K. Martin, S. D. Anton, and E. Ravussin, "Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism," *The American Journal of Clinical Nutrition*, vol. 81, no. 1, pp. 69–73, 2005.
- [232] N. Halberg, M. Henriksen, N. Söderhamn et al., "Effect of intermittent fasting and refeeding on insulin action in healthy men," *Journal of Applied Physiology*, vol. 99, no. 6, pp. 2128–2136, 2005.
- [233] J. B. Johnson, W. Summer, R. G. Cutler et al., "Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma," *Free Radical Biology and Medicine*, vol. 42, no. 5, pp. 665–674, 2007.
- [234] V. D. Dixit, H. Yang, K. S. Sayeed et al., "Controlled meal frequency without caloric restriction alters peripheral blood mononuclear cell cytokine production," *Journal of Inflammation*, vol. 8, article 6, 2011.
- [235] K. T. Howitz, K. J. Bitterman, H. Y. Cohen et al., "Small molecule activators of sirtuins extend *Saccharomyces cerevisiae* lifespan," *Nature*, vol. 425, no. 6954, pp. 191–196, 2003.
- [236] J. G. Wood, B. Rogina, S. Lavu et al., "Sirtuin activators mimic caloric restriction and delay ageing in metazoans," *Nature*, vol. 430, no. 7000, pp. 686–689, 2004.
- [237] H. Yang, J. A. Baur, A. Chen, C. Miller, and D. A. Sinclair, "Design and synthesis of compounds that extend yeast replicative lifespan," *Ageing Cell*, vol. 6, no. 1, pp. 35–43, 2007.
- [238] D. R. Valenzano, E. Terzibasi, T. Genade, A. Cattaneo, L. Domenici, and A. Cellerino, "Resveratrol prolongs lifespan and retards the onset of age-related markers in a short-lived vertebrate," *Current Biology*, vol. 16, no. 3, pp. 296–300, 2006.
- [239] M. Kaeberlein, T. McDonagh, B. Heltweg et al., "Substrate-specific activation of sirtuins by resveratrol," *The Journal of Biological Chemistry*, vol. 280, no. 17, pp. 17038–17045, 2005.
- [240] M. Kaeberlein and R. W. Powers III, "Sir2 and calorie restriction in yeast: a skeptical perspective," *Ageing Research Reviews*, vol. 6, no. 2, pp. 128–140, 2007.
- [241] M. Kaeberlein and B. K. Kennedy, "Does resveratrol activate yeast Sir2 in vivo?" *Ageing Cell*, vol. 6, no. 4, pp. 415–416, 2007.
- [242] D. L. Smith Jr., C. Li, M. Matecic, N. Maqani, M. Bryk, and J. S. Smith, "Calorie restriction effects on silencing and recombination at the yeast rDNA," *Ageing Cell*, vol. 8, no. 6, pp. 633–642, 2009.
- [243] T. M. Bass, D. Weinkove, K. Houthoofd, D. Gems, and L. Partridge, "Effects of resveratrol on lifespan in *Drosophila melanogaster* and *Caenorhabditis elegans*," *Mechanisms of Ageing and Development*, vol. 128, no. 10, pp. 546–552, 2007.
- [244] E. L. Greer and A. Brunet, "Different dietary restriction regimens extend lifespan by both independent and overlapping genetic pathways in *C. elegans*," *Ageing Cell*, vol. 8, no. 2, pp. 113–127, 2009.
- [245] T. L. Kaeberlein, E. D. Smith, M. Tsuchiya et al., "Lifespan extension in *Caenorhabditis elegans* by complete removal of food," *Ageing Cell*, vol. 5, no. 6, pp. 487–494, 2006.
- [246] S. Zou, J. R. Carey, P. Liedo et al., "The longevity effect of resveratrol depends on dietary composition and calorie intake in a tephritid fruit fly," *Experimental Gerontology*, vol. 44, no. 6-7, pp. 472–476, 2009.
- [247] M. Riesen and A. Morgan, "Calorie restriction reduces rDNA recombination independently of rDNA silencing," *Ageing Cell*, vol. 8, no. 6, pp. 624–632, 2009.
- [248] M. Pacholec, J. E. Bleasdale, B. Chrnyk et al., "SRT1720, SRT2183, SRT1460, and resveratrol are not direct activators of SIRT1," *The Journal of Biological Chemistry*, vol. 285, no. 11, pp. 8340–8351, 2010.
- [249] J. A. Baur, K. J. Pearson, N. L. Price et al., "Resveratrol improves health and survival of mice on a high-calorie diet," *Nature*, vol. 444, no. 7117, pp. 337–342, 2006.
- [250] M. Zang, S. Xu, K. A. Maitland-Toolan et al., "Polyphenols stimulate AMP-activated protein kinase, lower lipids, and inhibit accelerated atherosclerosis in diabetic LDL receptor-deficient mice," *Diabetes*, vol. 55, no. 8, pp. 2180–2191, 2006.
- [251] B. Dasgupta and J. Milbrandt, "Resveratrol stimulates AMP kinase activity in neurons," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 17, pp. 7217–7222, 2007.
- [252] N. L. Price, A. P. Gomes, A. J. Ling et al., "SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function," *Cell Metabolism*, vol. 15, no. 5, pp. 675–690, 2012.
- [253] J. L. Barger, T. Kayo, J. M. Vann et al., "A low dose of dietary resveratrol partially mimics caloric restriction and retards aging parameters in mice," *PLoS ONE*, vol. 3, no. 6, Article ID e2264, 2008.

- [254] K. J. Pearson, J. A. Baur, K. N. Lewis et al., “Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span,” *Cell Metabolism*, vol. 8, no. 2, pp. 157–168, 2008.
- [255] R. A. Miller, D. E. Harrison, C. M. Astle et al., “Rapamycin, but not resveratrol or simvastatin, extends life span of genetically heterogeneous mice,” *The Journals of Gerontology A*, vol. 66, no. 2, pp. 191–201, 2011.
- [256] D. L. Smith Jr., T. R. Nagy, and D. B. Allison, “Calorie restriction: what recent results suggest for the future of ageing research,” *European Journal of Clinical Investigation*, vol. 40, no. 5, pp. 440–450, 2010.
- [257] A. Dal-Pan, S. Blanc, and F. Aujard, “Resveratrol suppresses body mass gain in a seasonal non-human primate model of obesity,” *BMC Physiology*, vol. 10, no. 1, article 11, 2010.
- [258] A. Dal-Pan, J. Terrien, F. Pifferi et al., “Caloric restriction or resveratrol supplementation and ageing in a non-human primate: first-year outcome of the RESTRIKAL study in *Microcebus murinus*,” *Age*, vol. 33, no. 1, pp. 15–31, 2011.
- [259] L. Peltz, J. Gomez, M. Marquez et al., “Resveratrol exerts dosage and duration dependent effect on human mesenchymal stem cell development,” *PLoS One*, vol. 7, no. 5, Article ID e37162, 2012.
- [260] J. Mikula-Pietrasik, A. Kuczmarska, B. Rubiś et al., “Resveratrol delays replicative senescence of human mesothelial cells via mobilization of antioxidative and DNA repair mechanisms,” *Free Radical Biology & Medicine*, vol. 52, pp. 2234–2245, 2012.
- [261] J. M. Smoliga, J. A. Baur, and H. A. Hausenblas, “Resveratrol and health—a comprehensive review of human clinical trials,” *Molecular Nutrition & Food Research*, vol. 55, no. 8, pp. 1129–1141, 2011.
- [262] H. Ghanim, C. L. Sia, S. Abuaysheh et al., “An antiinflammatory and reactive oxygen species suppressive effects of an extract of *Polygonum cuspidatum* containing resveratrol,” *The Journal of Clinical Endocrinology & Metabolism*, vol. 95, no. 9, pp. E1–E8, 2010.
- [263] H. Ghanim, S. Abuaysheh, C. L. Sia et al., “Increase in plasma endotoxin concentrations and the expression of toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance,” *Diabetes Care*, vol. 32, no. 12, pp. 2281–2287, 2009.
- [264] H. Ghanim, C. L. Sia, K. Korzeniewski et al., “A resveratrol and polyphenol preparation suppresses oxidative and inflammatory stress response to a high-fat, high-carbohydrate meal,” *The Journal of Clinical Endocrinology & Metabolism*, vol. 96, no. 5, pp. 1409–1414, 2011.
- [265] P. Brasnyó, G. A. Molnár, M. Mohás et al., “Resveratrol improves insulin sensitivity, reduces oxidative stress and activates the Akt pathway in type 2 diabetic patients,” *The British Journal of Nutrition*, vol. 106, no. 3, pp. 383–389, 2011.
- [266] L. M. Vislocky and M. L. Fernandez, “Biomedical effects of grape products,” *Nutrition Reviews*, vol. 68, no. 11, pp. 656–670, 2010.
- [267] A. A. A. Bertelli and D. K. Das, “Grapes, wines, resveratrol, and heart health,” *Journal of Cardiovascular Pharmacology*, vol. 54, no. 6, pp. 468–476, 2009.
- [268] M. M. Dohadwala and J. A. Vita, “Grapes and cardiovascular disease,” *The Journal of Nutrition*, vol. 139, no. 9, pp. 17885–17935, 2009.
- [269] W. R. Leifert and M. Y. Abeywardena, “Grape seed and red wine polyphenol extracts inhibit cellular cholesterol uptake, cell proliferation, and 5-lipoxygenase activity,” *Nutrition Research*, vol. 28, no. 11, pp. 729–737, 2008.
- [270] T. Wallerath, G. Deckert, T. Ternes et al., “Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase,” *Circulation*, vol. 106, no. 13, pp. 1652–1658, 2002.
- [271] T. Wallerath, H. Li, U. Gödtel-Ambrust, P. M. Schwarz, and U. Förstermann, “A blend of polyphenolic compounds explains the stimulatory effect of red wine on human endothelial NO synthase,” *Nitric Oxide*, vol. 12, no. 2, pp. 97–104, 2005.
- [272] J. F. Leikert, T. R. Räthel, P. Wohlfart, V. Cheynier, A. M. Vollmar, and V. M. Dirsch, “Red wine polyphenols enhance endothelial nitric oxide synthase expression and subsequent nitric oxide release from endothelial cells,” *Circulation*, vol. 106, no. 13, pp. 1614–1617, 2002.
- [273] P. Greslele, P. Pignatelli, G. Guglielmini et al., “Resveratrol, at concentrations attainable with moderate wine consumption, stimulates human platelet nitric oxide production,” *The Journal of Nutrition*, vol. 138, no. 9, pp. 1602–1608, 2008.
- [274] D. O. Kennedy, E. L. Wightman, J. L. Reay et al., “Effects of resveratrol on cerebral blood flow variables and cognitive performance in humans: a double-blind, placebo-controlled, crossover investigation,” *The American Journal of Clinical Nutrition*, vol. 91, no. 6, pp. 1590–1597, 2010.
- [275] R. H. X. Wong, P. R. C. Howe, J. D. Buckley, A. M. Coates, I. Kunz, and N. M. Berry, “Acute resveratrol supplementation improves flow-mediated dilatation in overweight/obese individuals with mildly elevated blood pressure,” *Nutrition, Metabolism and Cardiovascular Diseases*, vol. 21, no. 11, pp. 851–856, 2011.
- [276] B. Agarwal and J. A. Baur, “Resveratrol and life extension,” *Annals of the New York Academy of Sciences*, vol. 1215, no. 1, pp. 138–143, 2011.
- [277] J. L. Crespo and M. N. Hall, “Elucidating TOR signaling and rapamycin action: lessons from *Saccharomyces cerevisiae*,” *Microbiology and Molecular Biology Reviews*, vol. 66, no. 4, pp. 579–591, 2002.
- [278] I. Bjedov, J. M. Toivonen, F. Kerr et al., “Mechanisms of life span extension by rapamycin in the fruit fly *Drosophila melanogaster*,” *Cell Metabolism*, vol. 11, no. 1, pp. 35–46, 2010.
- [279] N. Anisimov, M. A. Zabezhinski, I. G. Popovich et al., “Rapamycin increases lifespan and inhibits spontaneous tumorigenesis in inbred female mice,” *Cell Cycle*, vol. 10, no. 24, pp. 4230–4236, 2011.
- [280] S. Majumder, A. Caccamo, D. X. Medina et al., “Lifelong rapamycin administration ameliorates age-dependent cognitive deficits by reducing IL-1 $\beta$  and enhancing NMDA signaling,” *Aging Cell*, vol. 11, no. 2, pp. 326–335, 2012.
- [281] G. Payne, A. Laporte, R. Deber, and P. C. Coyte, “Counting backward to health care’s future: using time-to-death modeling to identify changes in end-of-life morbidity and the impact of aging on health care expenditures,” *The Milbank Quarterly*, vol. 85, no. 2, pp. 213–257, 2007.
- [282] A. Yazdanyar and A. B. Newman, “The burden of cardiovascular disease in the elderly: morbidity, mortality, and costs,” *Clinics in Geriatric Medicine*, vol. 25, no. 4, pp. 563–577, 2009.
- [283] J. Mesterton, A. Wimo, Å. By, S. Langworth, B. Winblad, and L. Jönsson, “Cross sectional observational study on the societal costs of Alzheimer’s disease,” *Current Alzheimer Research*, vol. 7, no. 4, pp. 358–367, 2010.

- [284] G. Atzmon, C. Schechter, W. Greiner, D. Davidson, G. Rennert, and N. Barzilai, "Clinical phenotype of families with longevity," *Journal of the American Geriatrics Society*, vol. 52, no. 2, pp. 274–277, 2004.
- [285] G. Atzmon, M. Rincon, P. Rabizadeh, and N. Barzilai, "Biological evidence for inheritance of exceptional longevity," *Mechanisms of Ageing and Development*, vol. 126, no. 2, pp. 341–345, 2005.
- [286] N. Barzilai and I. Gabriely, "Genetic studies reveal the role of the endocrine and metabolic systems in aging," *The Journal of Clinical Endocrinology & Metabolism*, vol. 95, no. 10, pp. 4493–4500, 2010.
- [287] M. C. Haigis and B. A. Yankner, "The aging stress response," *Molecular Cell*, vol. 40, no. 2, pp. 333–344, 2010.
- [288] D. Nipič, A. Pirc, B. Banič, D. Šuput, and I. Milisav, "Preapoptotic cell stress response of primary hepatocytes," *Hepatology*, vol. 51, no. 6, pp. 2140–2151, 2010.