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Dietary Impact on Neuronal Autophagy Control and Brain Health

Claudia Ntsapi, Andre du Toit and Ben Loos

Abstract

Autophagy is the major intracellular system which is critical for the removal of harmful protein aggregates and malfunctioning organelles. Dysfunctional autophagy is associated with a multitude of human diseases, such as protein aggregation in Alzheimer's disease and non-successful aging. Major interest exists in the dietary manipulation of the autophagy pathway activity, so as to tune the cell's protein degradation capabilities and to prevent cell death onset. It has recently become clear that the machinery required to degrade protein cargo has a distinct activity level which can be altered through specific dietary modulation. Moreover, this activity may differ from that of the proteinaceous cargo. Overall, brain health and successful aging are characterized by limited protein aggregation, with a distinct molecular signature of maintained autophagy function. However, it is largely unclear how to control autophagy through dietary interventions with a precision that would allow to maintain minimal levels of toxic proteins, preserving neuronal cell viability and proteostasis. In this chapter, we carefully dissect the relationship between autophagy-modulating drugs, including caloric restriction mimetics and their impact on neuronal autophagy, in the context of preserving brain health.

Keywords: autophagic flux, caloric restriction, proteotoxicity, Alzheimer's disease, neurodegeneration, autophagosome, lysosome

1. Introduction

At the beginning of the twentieth century, life expectancy at birth was about 45 years. Today, this figure has markedly increased to nearly 77 years [1]. Recent estimates [1] predict that in the next four decades, the world's proportion of people aged 65 years and older will account for nearly 22% of the total population—from the present 800 million to 2 billion people. Although this increase in life expectancy is reflective of the healthcare achievements [2], the socioeconomic costs associated with a higher chronic disease burden have necessitated the development of robust prevention and management strategies that are both safe and immediately executable.

2. The role of protein aggregation in Alzheimer's disease

Alzheimer's disease (AD) is a debilitating neurodegenerative disease, affecting 40 million people worldwide [3]. The prevalence of AD is strongly correlated with age,

imposing a greater socioeconomic burden as life expectancy continues to increase. Clinically, AD is associated with the progressive loss of essential cognitive functions and progressive hippocampal and cortical brain atrophy [4]. Current AD treatment Food and Drug Administration (FDA)-approved drugs include N-methyl-D-aspartic acid (NMDA) receptor antagonist memantine and cholinesterase inhibitors donepezil, galantamine, and rivastigmine [5]. These drugs augment cholinergic neurotransmission or attenuate excitotoxic neuronal injury. However, they only provide palliative benefits at best, with limited impact on the underlying disease mechanisms. Therefore, there is an urgent need for interventions that not only impact the aging process in favor of sustained brain health but also promote successful brain aging in the context of neurodegenerative diseases. AD is pathologically defined by the widespread brain distribution of amyloid-beta peptide ($A\beta$) plaques, neurofibrillary tangle (NFT) formation, as well as synaptic and neuronal loss [6]. Despite growing understanding of the disease, it remains unclear how these pathological features relate to the specific disease processes. The amyloid cascade hypothesis continues to serve as the predominant model of AD pathology. This hypothesis suggests the overproduction of $A\beta$, particularly an increase in $A\beta$ [42] relative to $A\beta$ [40], as the causal trigger in the disease process [7]. $A\beta$ is derived from the amyloidogenic cleavage of the amyloid precursor protein (APP), protein cleaved by two endoproteases: the beta-site APP-cleaving enzyme 1 (BACE1/ β -secretase) and γ -secretase enzyme. Briefly, APP is cleaved by BACE1, releasing sAPP β and leaving the membrane-bound C99 carboxy-terminal fragment that is subsequently processed by γ -secretase to generate $A\beta$, a nontoxic P3 peptide, and the APP intracellular domain (AICD) [7]. γ -Secretase cleavage results in a C-terminal heterogeneity of the resulting $A\beta$ peptide population. Hence, $A\beta$ peptides of different lengths exist, with $A\beta$ 40 being the most abundant (~80–90%), followed by the more hydrophobic and fibrillogenic $A\beta$ 42 (~5–10%) form which is the principal peptide aggregated in the AD brain [8].

Similar to AD, protein aggregation is also a hallmark of neuronal cell death onset in Parkinson's disease (PD). PD is pathologically defined by the formation of intraneuronal inclusions consisting of aggregated α -synuclein (α -syn) and the presence of Lewy neurites and Lewy bodies (LBs) [9]. This neuropathology is associated with impaired functioning of intracellular protein degradation mechanisms [10]. Thus, strategies to either degrade or prevent the initial accumulation of $A\beta$ oligomers and α -syn may be promising in the treatment or prevention of AD and PD, respectively.

3. Protein quality surveillance machinery

The postmitotic nature of neuronal cells makes them highly susceptible to the accumulation of protein aggregates. Hence, the maintenance of protein homeostasis is critical to maintain neuronal function, particularly with age. Although the etiology and molecular mechanisms underlying the pathological changes in AD are not fully understood, studies suggest that localized deficits in the autophagy pathway are likely to precede the formation of $A\beta$ plaques or NFTs [11]. Autophagy is a highly conserved catabolic process that is critical for the systemic removal of long-lived proteins, protein aggregates, and dysfunctional organelles and serves as a major regulator of longevity in various species [12]. This process is triggered by various stressors, e.g., low nutrient levels, and proteotoxicity [13]. Proteotoxicity is by the of functional conformation as mature proteins misfold due to normal aging, posttranslational modifications, or inherent mutations [14]. In the absence of intracellular corrective mechanisms, this proteotoxicity can lead to uncontrolled protein aggregation, impair the cells' ability to maintain protein homeostasis, and promote cell death onset [14]. Depending on the cargo sequestered, and the mechanism

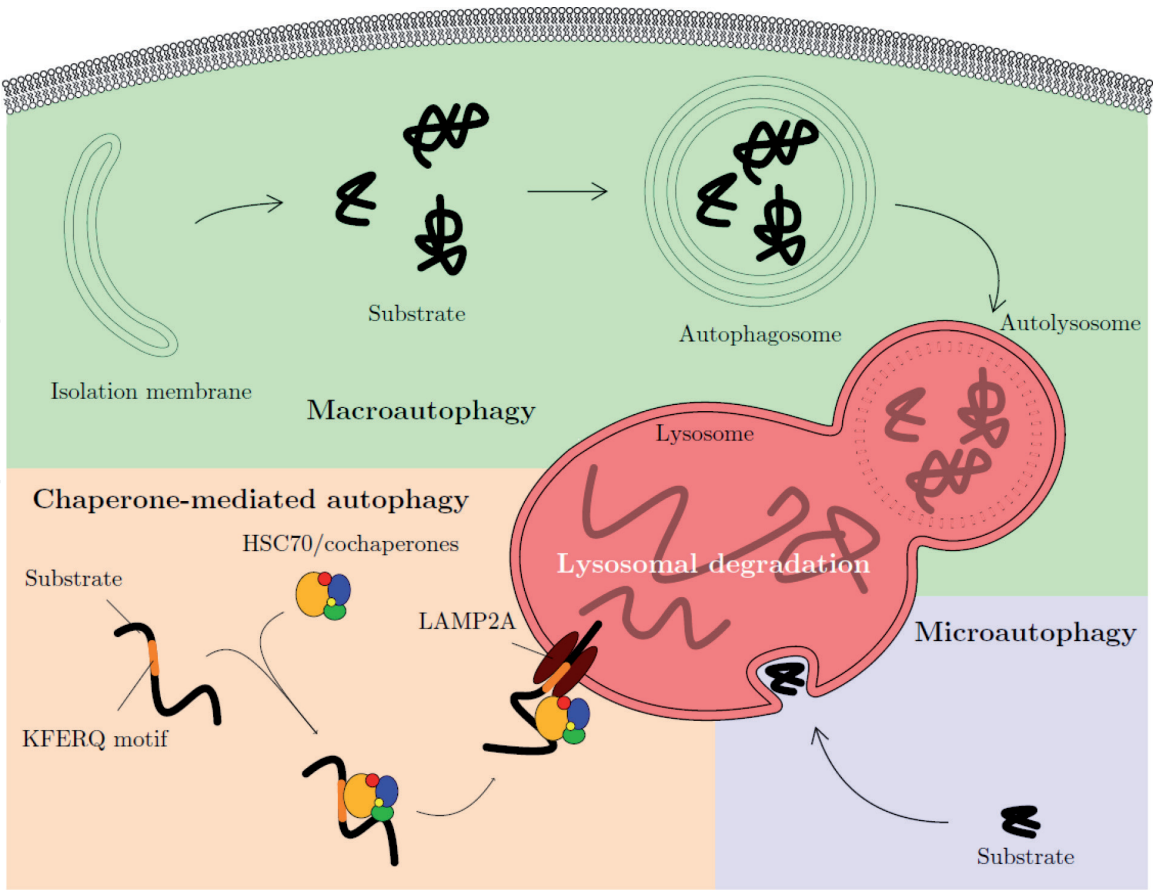


Figure 1.
Schematic model of the three main types of autophagy described in mammalian cells.

through which cargo is delivered to the lysosome, autophagy encompasses at least three subtypes: microautophagy, chaperone-mediated autophagy (CMA), and macroautophagy (**Figure 1**).

In microautophagy, lysosomal membrane invaginations mediate the internalization of cytosolic cargo into small vesicles that detach into the lumen for degradation [15]. CMA refers to a selective form of autophagy, whereby cytosolic proteins containing a CMA targeting motif—an amino-acid sequence biochemically similar to KFERQ—are bound by heat shock-cognate chaperone of 70 kDa (HSC70). HSC70 targets these proteins to the lysosomal membrane, where after binding to the cytosolic tail of lysosome-associated membrane protein type-2A (LAMP2A), proteins are unfolded and translocated across the lysosomal membrane aided by the lysosome-resident form of HSC70 (lys-hsc70) for degradation by the luminal proteases [16]. Of the three pathways, macroautophagy (hereafter referred to as autophagy) is the most extensively characterized and most relevant to AD. Therefore, this review will focus on the role of macroautophagy as the key mechanism which may be exploited to promote brain health and successful brain aging.

4. The tight orchestration of autophagy

Autophagy serves as the cell's principal quality control system which mediates the degradation of entire cytoplasmic materials through a series of stages characterized by the *de novo* formation of double-membraned vesicles, termed autophagosomes, which sequester cytoplasmic cargo and fuse with lysosomes to form autolysosomes. This process culminates in cargo degradation and subsequent recycling of the resulting macromolecules. To date, more than 30 highly conserved

autophagy-related (ATG) genes have been implicated in the core autophagy machinery [17]. The autophagic process is tightly regulated, with distinct sets of Atg proteins forming diverse complexes which control different stages of this pathway under basal and stressful conditions.

The induction of autophagy is primarily mediated by two complexes. Firstly, the initiation complex, which triggers the formation of a phagophore structure (or isolation membrane), comprises the Unc-51-like kinase-1 (ULK1), Atg13, Atg101 and the focal adhesion kinase family-interacting protein of 200 kDa (FIP200) [17]. Secondly, the nucleation complex drives the phosphorylation of phosphatidylinositol (PI) to produce phosphatidylinositol 3-phosphate (PI3P), a membrane-bound lipid which requires the class III phosphatidylinositol 3-kinase (C3PI3K) vacuolar protein sorting 34 (Vps34) to recruit Beclin1, Vps15, Atg14L, or Ambra1 in the region of phagophore formation, termed the omegasome [17]. Autophagosome formation is mediated by two ubiquitin-like conjugation reactions. The first reaction results in the formation of the Atg12-Atg5-Atg16L1 conjugation complex which facilitates the expansion of the phagophore membrane. In the second reaction, Atg12-Atg5 associates with Atg16L1 and localizes to the outer membrane of the pre-autophagosomal structures, in turn catalyzing the recruitment and conjugation of microtubule-associated protein 1 light chain 3 (MAP1LC3/Atg8/LC3) with a membrane phospholipid, phosphatidylethanolamine (PE), through the action of Atg4, Atg7, and Atg3 [18]. Atg4 catalyzes the conversion of the cytosolic form of LC3 (LC3-I) to the autophagosome membrane-associated form (LC3-II), which serves as an indicator of autophagosome pool size at a given time [18]. Mature autophagosomes ultimately fuse with lysosomes to form autolysosomes, in which sequestered cargo is degraded and released back into the cytosol for reuse [18]. Autophagic flux, the rate of protein degradation through the autophagy pathway [19, 20], provides an accurate measure of this dynamic process.

5. Key signaling pathways in the regulation of autophagy during nutrient stress

The mammalian target of rapamycin complex 1 (mTORC1) is a component of mTOR, the master regulator of cellular metabolism in response to environmental cues. mTORC1 integrates various signaling networks to promote protein synthesis by suppressing catabolic processes under nutrient-rich conditions [21]. In addition to the class I phosphatidylinositol-3-kinase (PI3K) and the Akt signaling pathway, the tuberous sclerosis (TSC) tumor suppressor complex (TSC1/TSC2) is an important upstream regulator of mTORC1, with loss-of-function mutations in either complex leading to the constitutive activation of mTORC1 [22]. Under conditions of nutrient excess, growth factors such as insulin-like growth factor 1 (insulin/IGF1) activate their cognate receptors, subsequently activating the PI3K/Akt pathway [23]. Activated Akt inhibits TSC1/TSC2, resulting in the activation of mTORC1 [23]. Subsequently, mTORC1 suppresses autophagy activity by phosphorylating (i) components of the ULK1 complex; (ii) Atg14L or Beclin1 regulator (Ambra1); (iii) the Beclin1-binding protein, UV radiation resistance-associated gene (UVRAG); or (iv) the transcription factor EB (TFEB), a key regulator of lysosomal and autophagy gene expression [24]. Therefore, mTORC1 can inhibit autophagy by targeting different components of the core autophagy machinery (**Figure 2**).

Under conditions of nutrient stress, mTORC1 activity is suppressed, resulting in the activation of the ULK1 complex [24]. Cellular energetic sensor, AMP-activated protein kinase (AMPK), positively regulates autophagy to maintain energy

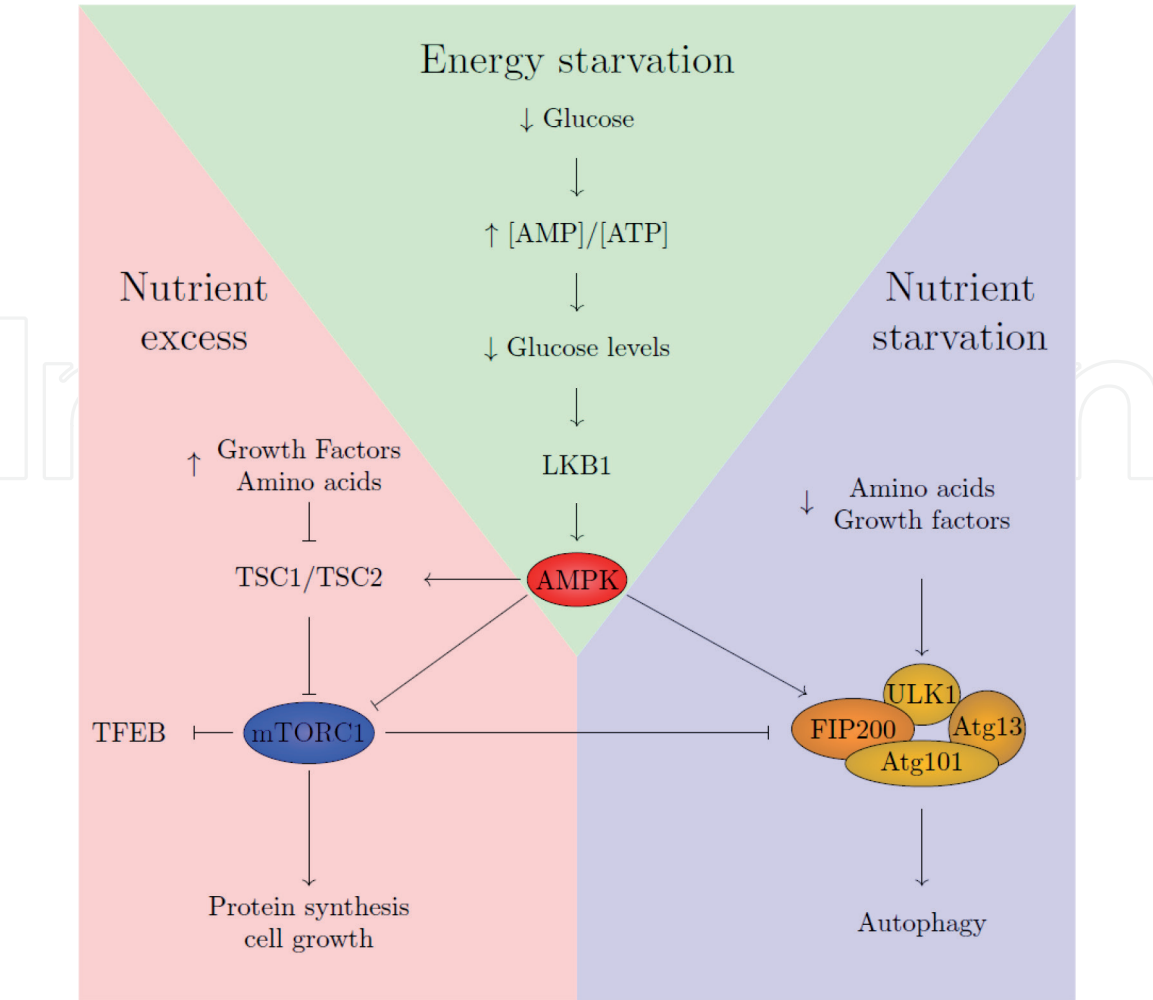


Figure 2.
Regulation of autophagy by cellular nutrient status.

homeostasis under energy depleted conditions. Briefly, AMPK phosphorylates TSC2- and mTORC1-binding partner regulatory-associated protein (Raptor) [25], thereby suppressing mTORC1 activity. Additionally, AMPK can bind and phosphorylate ULK1, freeing this complex to initiate autophagy under both nutrient and ATP deplete conditions [26]. Thus, initiation of autophagy can be jointly regulated by mTORC1 and AMPK, to increase the cell's capacity to adapt to metabolic perturbations.

6. Decreased autophagy with age

Consistent with the transcriptional downregulation of autophagy during healthy aging in the human brain [27], the impairment of autophagy has been found to decrease life span in various model systems [28]. Screening for chronological aging factors, Matecic et al. [29] published one of the earliest findings implicating impaired autophagy activity in the shortened life span of *S. cerevisiae* mutants. Most compelling findings came from Atg5 [30] or Atg7 [31] knockout mice which revealed that impaired autophagic function led to early postnatal death, the accumulation of intracellular inclusion bodies, and neurodegeneration. Since insufficient/impaired autophagy contributes to aging, it is conceivable that increasing the activity of this process could influence aging, in favor of life span extension. Indeed, it is becoming increasingly clear that modulation strategies that enhance autophagy activity attenuate proteotoxicity, while defects in this pathway have been implicated in increased

risk for cell death onset with age [28]. Autophagy dysfunction has been extensively documented in AD progression, where the accumulation of incompletely degraded cytoplasmic within autophagic vacuoles (AVs) has been shown to be a pathological hallmark of insufficient autophagic induction in AD [32]. This process is further exacerbated by the presence of APP and its processing enzymes within the AVs [33], indicating that autophagy may regulate both A β generation and clearance. Indeed, insufficient expression of autophagy core protein Beclin1 has been shown to increase the expression levels of APP, A β , and the C-terminal fragment (CTF) in cultured neurons, in early AD patients, and mouse models of AD, while Beclin1 overexpression had the opposite effect [34]. Therefore, the modulation of autophagy may ameliorate the loss of proteostasis in AD. Indeed, rapamycin, an mTORC1 inhibitor, has been shown to reduce A β load and tau pathology and improve cognitive function [35], with this reduction being most pronounced when rapamycin was administered prior to the widespread deposition of A β [36]. Therefore, the identification of novel treatment strategies, or repurposing of readily available autophagy-inducing drugs to promote successful brain aging, has attracted considerable attention. Additionally, drugs/strategies with dual-functional capabilities in both the inhibition of A β production and upregulation of its clearance may prove especially beneficial in the attenuation of A β pathology. To this end, the use of calorie restriction (CR) dietary interventions and CR mimetics (CRMs) may offer a relatively simple, safe, and inexpensive avenue to induce autophagy and offset the decline of autophagy activity associated with age. CR, here defined as a reduction in caloric/energetic intake without causing malnutrition, remains not only the most robust and reproducible dietary intervention known to increase life span and delay aging but is also a most potent physiological inducer of autophagy [37]. In fact, short-term fasting in mice has been shown to markedly induce neuronal autophagy, translating in neuroprotection [38].

7. CR effects on aging and neurodegeneration

7.1 CR regimes

Intermittent fasting (IF) is the most studied CR regime in humans. IF involves alternating between periods of *ad libitum* (AL) caloric intake and partial or complete CR in which food intake is restricted for prolonged time periods [39]. The majority of IF animal studies have involved either alternating IF (AIF) or time-restricted intermittent fasting (TRIF), with both resulting in neuroprotection, as evidenced by the enhancement of neuronal plasticity, increased levels of brain-derived neurotrophic factor (BDNF), and increased resistance to metabolic stress [40]. Goodrick and colleagues revealed that rats maintained on a lifelong AIF regime lived nearly twice as long as rats fed AL [41]. More recently, CR regimes have also been shown to attenuate A β neuropathology in the brains of AD mouse models [42]. In agreement, AD mice maintained for 1 year on either AIF or a 40% CR diet beginning from 5 months of age were not found to exhibit the cognitive impairments observed in AL fed AD mice [43]. However, the beneficial effects of CR on aging and maximal life span in humans remain unclear given the ethical controversies associated with long-term survival studies in normal-weight humans, the lack of validated biomarkers of aging, and the limited compliance to prolonged CR regimes [44]. Notably, gender-based differences have been reported in response to CR regimes [45]. For example, work by Martin et al. [46] revealed that while male and female rats maintained on a CR regime for 6 months had similar levels of circulating triglycerides and energy-regulating hormones (insulin, leptin, adiponectin, and ghrelin), the changes were quantitatively greater in males.

The most compelling support for the beneficial effects of CR on longevity stems from epidemiological studies of the older Okinawan population, which is the longest lived population to date [47]. The longevity and apparent rarity of progressive neurodegenerative diseases amongst this population are associated with strict adherence to their traditional Okinawan diet, consisting of soybean-based foods, unrefined carbohydrates, and moderate protein intake with emphasis on root vegetables (sweet potatoes), fish, and lean meats [48]. However, given the paucity of long-term CR studies in humans, there is insufficient data to determine the optimal CR regimen and the degree of CR needed to achieve sustained brain health.

7.2 CR and brain health

During the aging process, neuronal cells are exposed to increased oxidative and metabolic stress associated with numerous cellular modifications [49]. These modifications are aggravated in neurodegenerative diseases, where neuronal injury is most pronounced in the hippocampus and cortex region. Strong evidence from animal studies suggests that CR promotes enhanced synaptic plasticity, resulting in increased brain resistance to metabolic stressors, and delays brain aging [50]. Studies suggest that long-term CR, from 3 to 11 months of age, had a survival-promoting effect on newly formed glial cells in the hippocampus region of 2-, 18-, and 24-month-old mice [51]. In AD mouse models maintained on a 6–14 week CR regime, a significant reduction in A β and astrocytic activation was observed [52]. Hence, exploitation of the mechanisms through which CR augments brain health may aid in the development of lifestyle-based therapeutics in the treatment of AD and other neurodegenerative diseases. Although the exact mechanisms through which CR promotes health and life span are not fully understood, nutrient signaling pathways have been implicated (**Figure 3**). Of considerable importance to the CR-induced effects on brain aging is the induction of autophagy following the activation of metabolic energy sensors AMPK and sirtuin-1 (SIRT1) or the inhibition of the insulin/IGF1 pathway and mTORC1 signaling [53].

7.3 SIRT1

SIRT1 is a nicotine amide NAD⁺-dependent histone deacetylase, which exhibits increased expression following CR in many tissues, including the brain [54]. Importantly, SIRT1 overexpression has also been shown to promote autophagy by activating essential Atg proteins [55]. SIRT1 can further stimulate autophagy by deacetylating and activating the Forkhead box (FOXO) family of transcription factors which act as key regulators of longevity under CR conditions [56]. SIRT1 may further promote longevity through FOXO-dependent induction of stress response genes [56].

7.4 AMPK

AMPK is activated in response to low energy levels, e.g., under CR conditions [57], suggesting that AMPK may play a role in CR-induced longevity. Indeed, increased AMPK activity has been found to extend life span in *C. elegans* and *Drosophila* model systems, while its inhibition shortened life span [58]. The mechanism underlying AMPK-induced life span extension under CR is thought to involve the direct phosphorylation of peroxisome proliferator-activated receptor G coactivator-1 α (PGC-1 α), a key regulator of mitochondrial metabolism and biogenesis, and FOXO transcription factors, thereby targeting these components for SIRT1-mediated activation [59]. Importantly, AMPK stimulates autophagy through the direct phosphorylation of ULK1, or the activation of TSC1/TSC2, which in turn inhibits mTORC1 [25], thereby allowing autophagy induction.

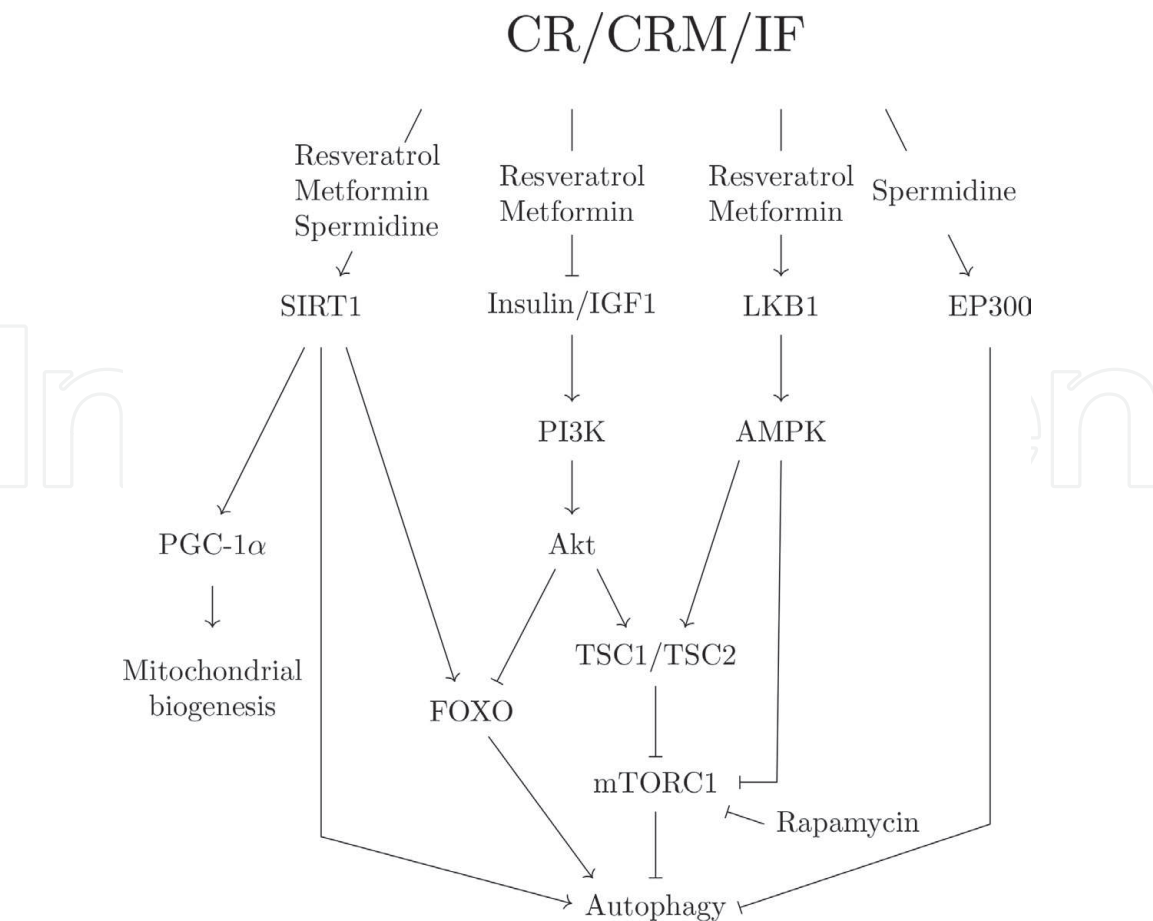


Figure 3.
The targeting of nutrient-triggered pathways by selective calorie restriction mimetics in longevity and the promotion of brain health. CR, calorie restriction; CRM, caloric restriction mimetic; IF, intermittent fasting; SIRT1, sirtuin-1; mTORC1, mammalian target of rapamycin complex 1; AMPK, AMP-dependent protein kinase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-α; insulin/IGF-1, insulin/insulin-like growth factor-1; PI3K, phosphoinositide 3-kinase; FOXO, Forkhead box O; EP300, E1A-binding protein p300.

7.5 Insulin/IGF1 signaling

Inhibition of insulin/IGF1 signaling following CR or growth factor removal has been reported to increase life span, delay the onset of age-related diseases, and increase oxidative stress resistance in various species, including humans [60]. CR-induced downregulation of the insulin/IGF1 pathway in turn induces the activation of SIRT1, resulting in the activation of FOXO transcription factors [61].

7.6 mTORC1 signaling

mTORC1 is activated by growth factors, amino acids, or increased glucose levels, and is thought to control life span through various mechanisms, including the regulation of autophagy [62]. Indeed, autophagy has been shown to be essential for life span expansion in mTOR knockout yeast [63] and *C. elegans* model systems in which impaired autophagic function has been shown to abolish the life span extension induced by mTOR inhibition under CR conditions [28]. The convergence of CR-induced signaling pathways on autophagy supports the assertion that this process is intricately involved in aging. Therefore, the precision control of brain autophagy activity could mediate sustained brain health with age.

8. Antiaging pharmacological CR mimetics

Given the challenge to adhere to prolonged CR regimes, as well as selective side effects, such as decreased body temperature [64] and slowed wound healing [65], compounds that elicit similar beneficial effects on aging, health, and life span as CR could be a more practical alternative. This area of research has sparked considerable interest in the use CRM drugs, or CRM supplements as adjuvant therapy to delay the aging process, particularly during mid- to late life. Currently, the most widely studied CRM candidates are resveratrol, rapamycin, 2-deoxy-D-glucose (2DG), metformin, and spermidine (**Figure 3**).

8.1 Resveratrol

Resveratrol is a polyphenol compound isolated from the skins of red grapes, with red wine (5 mg/L on average) being the principal source of this compound [66]. Daily consumption of grape and blueberry polyphenols has also gained interest as CRMs for the prevention and treatment of neurodegenerative diseases. For example, studies indicate that the combined dietary supplementation of grape and blueberry polyphenols may have beneficial effects on age-related cognitive decline, improving episodic memory impairment in elderly subjects [67] and preventing the onset of learning and cognitive deficits in aged mice [68]. However, resveratrol has been found to be the most potent polyphenol compound and is to date the most thoroughly studied CRM, first identified and implicated in life span extension in a yeast model system [69]. Work by Baur et al. [70] revealed that resveratrol significantly increased survival in middle-aged mice on a high-calorie diet compared to that of mice on a standard diet by nearly 31%. In addition, resveratrol increased insulin sensitivity, reduced IGF1 levels, activated AMPK/PGC-1 α signaling, and improved motor function [70]. Similar benefits have been reported in response to resveratrol supplementation in nonhuman primate models fed a high-fat diet [71, 72]. In the latter study, resveratrol improved adipose insulin levels and reduced the inflammatory response caused by the high-fat diet [72], while the former study revealed that resveratrol prevented diet-induced arterial wall inflammation [71].

Naturally sourced resveratrol is poorly absorbed in humans [73]. Hence, high-purity resveratrol-mimetic drugs, such as ResVida™, have been developed to ensure its sustained release [74]. In a recent study, 30-day resveratrol supplementation (150 mg/day ResVida™) in humans led to a decrease in circulating glucose levels, inflammatory markers, triglycerides, and systolic blood pressure [74]. In contrast, others have reported no significant changes in the above parameters in obese men following resveratrol supplementation [75]. Longevinex® is another commercially available resveratrol supplement shown to induce SIRT1 and PGC-1 α and increase mitochondrial biogenesis in the brain [76]. Prolonged Longevinex® supplementation has been shown to result in increased levels of LC3-II, Beclin1, and FOXO transcription factors. Therefore, it appears conceivable that prolonged Longevinex® may influence brain health, in part, by inducing neuronal autophagy. Work by Vingtdeux et al. [77] has also revealed that resveratrol can decrease extracellular A β accumulation by inducing autophagy through the activation of AMPK. Long-term resveratrol treatment is well tolerated in humans [78], with significantly reduced A β levels and improved memory retention observed in AD mouse models [79], making this compound a promising CRM candidate for the treatment of AD.

8.2 Rapamycin

The suppression of mTORC1 activity is associated with a significant improvement in both health and life span in various organisms, while increased activity is associated with old age in humans [80]. Hence, the use of mTORC1 inhibitor rapamycin may have potential applications as a CRM. Currently, rapamycin is clinically used as an immunosuppressant to prevent the rejection of kidney transplants in patients [66]. In AD mouse models, rapamycin treatment has been shown to improve cognitive ability and reduce A β and tau pathology, with these observations being linked to increased autophagic induction [81]. However, it has been reported that prolonged rapamycin treatment in rodents leads to the development of hyperlipidemia, glucose intolerance, and high levels of free fatty acids in skeletal muscle [82]. In contrast, lifelong intermittent administration of rapamycin for 2 weeks/month was found to extend life span in mice [81], suggesting that intermittent rapamycin administration may be more beneficial. In a separate study, adult mice maintained on lifelong rapamycin treatment, starting at 2 months of age, performed significantly better on a task measuring spatial learning and memory compared to age-matched mice on the control diet [83]. However, rapamycin did not improve cognition in adult mice with pre-existing age-dependent cognitive deficits [83], suggesting that rapamycin may have better cognitive outcomes prior to the onset of cognitive deficits.

Rapamycin is unstable in water; thus, different oral preparations such as nanoparticles [84] have been formulated to increase its bioavailability. Rapatar, a rapamycin formulation based on Pluronic block copolymers as nanocarriers, has been shown to have significantly higher bioavailability after oral administration [85]. Rapatar has been shown to increase life span and delay carcinogenesis during lifelong treatment administered at intermittently low doses (0.5 mg/kg) in tumor-prone mice [86]. The advantage of rapamycin is its FDA-approved status for various clinical applications in humans; however, the relevance for longevity in humans has yet to be established given its immunosuppressive effects.

8.3 Metformin

Metformin is a first-line drug approved for the treatment of diabetes [87], which also targets the insulin/IGF1 pathway, mTORC1, AMPK, and SIRT1 [88]. Metformin is rapidly distributed to many tissues following partial absorption, whereas the luminal concentration in the gastrointestinal tract remains high after a single oral dose [89]. Patients with type 2 diabetes have an increased risk of developing AD [90], as insulin has been shown to prevent A β oligomer formation in a dose-dependent manner [90]. A 12-year cohort study revealed that metformin supplementation reduced the AD risk in type 2 diabetes patients, with the risk being further reduced when metformin was combined with an antihyperglycemic agent, sulfonylurea [91]. In contrast, a case-control study revealed that long-term metformin-treated type 2 diabetes patients had a slightly higher risk of developing AD [92]. Despite these contradictory findings, metformin remains a promising CRM, but further research is required to unravel its effects on brain health.

8.4 2DG

2DG is a well-established glycolysis inhibitor first identified by Lane et al. [93] as a potential CRM drug. In this study, rats fed with a 2DG supplemented diet at varying weight-dependent doses revealed that 2DG was toxic at a high dosage, while at the lower dosage, 2DG supplementation had beneficial effects, including reduced blood insulin levels [93]. Rodents maintained on a 2DG-supplemented

diet for 2 weeks increased neuronal resistance in AD [94] and PD [95] models. While 7 weeks of 2DG supplementation at a dosage of 0.04% has been shown to attenuate amyloid pathology and increase the levels of BDNF in an AD mouse model [96], toxicity studies revealed that 2DG supplementation at a dosage of 0.2–0.4% may induce cardiotoxicity [97]. 2DG has been linked to the upregulation of CR-related signaling pathways, specifically increased activation of AMPK and SIRT1 [93]. Hence, 2DG remains a viable CRM candidate provided the dose-dependent toxicity can be fully established.

9. Autophagy-inducing agents

Given the cell's declining capacity to sustain efficient autophagic degradation with age, it is not surprising that autophagy dysfunction plays a key role in pathological processes common to aging and neurodegeneration in the elderly [98]. The modulation of autophagic activity may thus be a promising strategy to offset the progression of neurodegenerative processes with age [99]. In addition to adhering to low CR regimes, and the use of CRM drugs, autophagy-induced life extension may also be mediated using the histone acetylase inhibitor, spermidine. Unlike other autophagy-inducing drugs, spermidine has shown no adverse effects during lifelong administration in mice [100], with clinical data indicating good safety and tolerability in elderly subjects during long-term dietary supplementation [101].

9.1 Spermidine

Spermidine is a naturally occurring polyamine that has been shown to decline throughout the aging process in humans [102]. Accordingly, spermidine dietary supplementation in mice (26 weeks) and humans (2 months) has been shown to increase blood polyamine concentrations [103, 104]. Studies reveal that spermidine influences life span, partly, by inducing autophagy through the suppression of E1A-binding protein p300 (EP300), an acetyltransferase that transfers acetyl groups from acetyl coenzyme A to core Atg proteins, thereby inhibiting this pathway [105]. Indeed, He et al. [99] revealed that spermidine's life-extending effects were abolished when autophagy activity was suppressed through *Atg7* or *Beclin1* knockdown *in vivo*, consistent with a causal connection between autophagy induction, neuroprotection, and longevity.

Spermidine's autophagy-inducing potency has been quantified to be equivalent to that of rapamycin [106]. Although dietary supplementation with spermidine has emerged as a promising prevention strategy in aging individuals with an elevated risk of developing AD, the spermidine concentration required for optimal autophagy activity with healthy aging in humans remains unknown. A recent study revealed that spermidine supplementation had no toxic effects even at high concentrations in mice and in older adults at risk for AD [101]. Improved memory performance was reported in the aged subjects after 3 months of spermidine intake compared to the placebo group [107], suggesting that nutritional spermidine may potentially delay memory loss with age.

10. Screening for autophagy-inducing CRMs

Considerable efforts have been made to identify autophagy-inducing drugs which may attenuate the risk for age-associated diseases. Recently, Kaizuka et al. [108] developed an autophagic flux probe which was used to rank

Rank	Drug	GFP/RFP (%)	Pathology	Reference
1	Ciclopirox olamine	17.3	Cancer	Zhou et al. [109]
17	Cladribine (2-CDA)	51.1	AD model	Hayes et al. [110]
27	Sertraline hydrochloride	58.8	Depression	Rainey et al. [111]
29	Loperamide hydrochloride	61.7	Huntington's disease model	Williams et al. [112]
47	Azacitidine	70.0	Hematopoiesis disorders	Abdulhaq and Rossetti [113]

Modified from Kaizuka et al. [108].

Table 1.
Autophagy inducers identified using a sensitive autophagic flux probe.

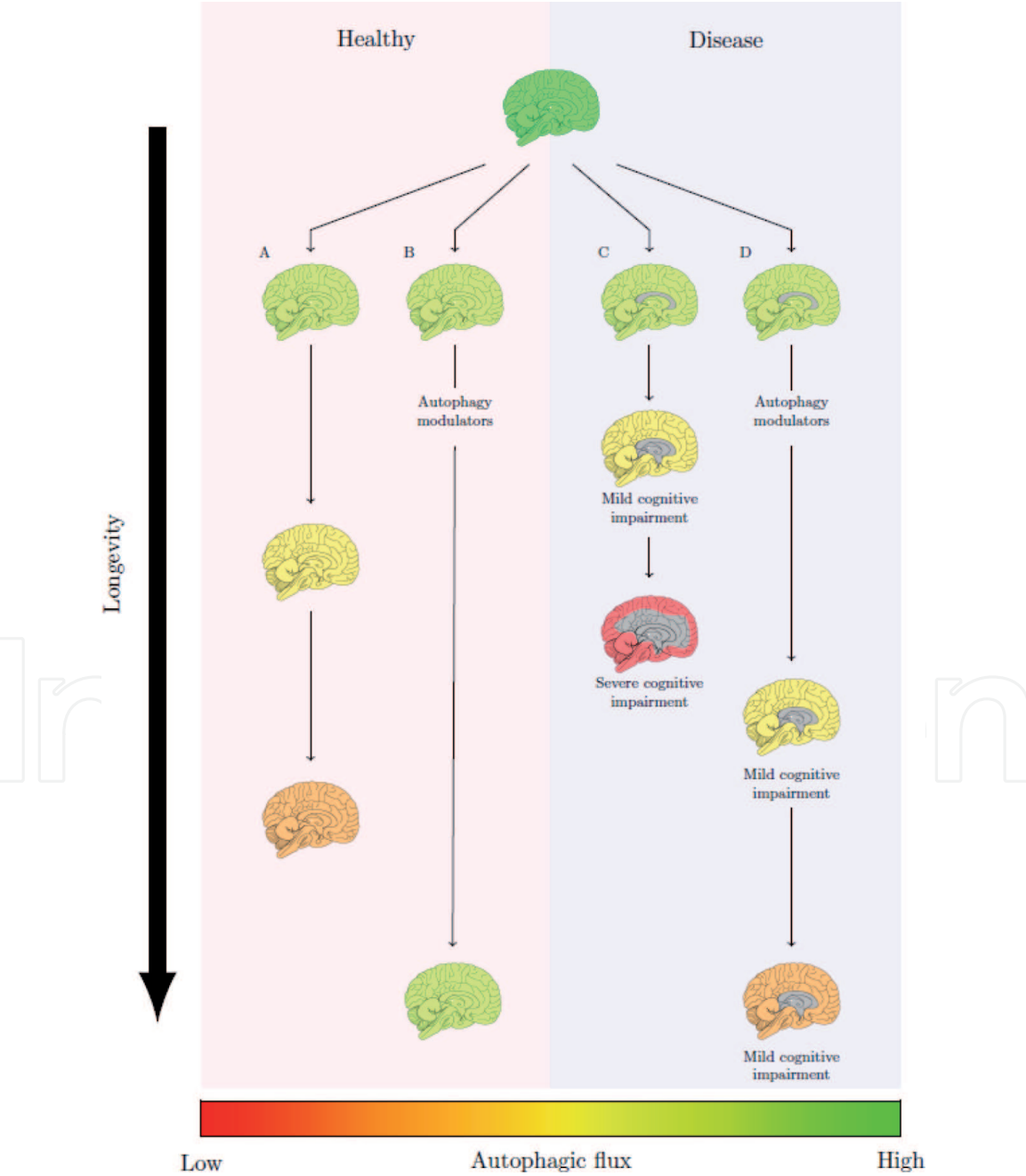


Figure 4.
Matching autophagy induction with autophagic flux decline and dysfunction in brain health and pathology associated with cognitive impairment.

autophagy-inducing drugs according to their level of potency by screening an approved drug library. The autophagic flux probe, i.e., GFP-LC3-RFP-LC3ΔG, is a fusion protein consisting of GFP-LC3 and RFP-LC3, with the C-terminal glycine of RFP-LC3 being deleted. An equal amount of GFP-LC3 and RFP-LC3ΔG is generated in the cytosol, and upon autophagy induction, GFP-LC3 is degraded within autolysosomes, while RFP-LC3ΔG remains in the cytosol and serves as an internal control. GFP-LC3-RFP-LC3ΔG-expressing cells were treated with candidate drugs at varying concentrations for 24 hrs under both nutrient-rich and starvation conditions. The resulting GFP/RFP signal ratio was measured using a microplate reader, with a low GFP/RFP ratio indicating a robust autophagy inducer (**Table 1**). Caution is recommended during cell transfection, as homologous recombination can occur between the two LC3 proteins of the probe. Thus, the isolation of properly expressing GFP-LC3-RFP is recommended. Expression levels of the probe may also vary among different cells/tissues; thus, cells/tissues with similar RFP expression levels should be compared. Lastly, the probe has a relatively low time resolution, making it more ideal for the detection of basal autophagy [108].

Of the 47 autophagy inducers identified, 3 were of relevance to neuroprotection. Importantly, these data indicate firstly, that autophagy activity can be measured accurately and hence standardized and, secondly, that neuronal autophagy decline in aging or neurodegeneration may be matched with an autophagy inducer that is suitable to offset autophagy dysfunction at the respective levels of autophagy activity (**Figure 4**). Further studies using these drugs in the context of healthy brain aging as well as AD pathology are required.

Other antiaging nutrients identified to date, including antioxidants (vitamins A, C, D, and E; quercetin; and coenzyme Q10), and phytochemicals, such as curcumin and epigallocatechin-3-gallate, have been shown to enhance autophagy activity [114]. However, there is a paucity of studies on their overall health benefits in humans.

11. Healthier dietary patterns for successful aging?

Research on the “nutrition transition” reveal that urban areas of developing north and sub-Saharan African countries, Asia, Latin America, and the Middle East share similar dietary pattern shifts [115]. One commonality of this shift is the increased consumption of fat and sugar-laden foods associated with increased risk for age-associated and lifestyle-based diseases. The consumption of nutritionally dense CRM foods, such as marine-based carotenoid-rich food, sweet potatoes, legumes, low-GI grains, fruits, and various flavonoids used in the Okinawan diet, is thought to be the most beneficial food choices for successful aging [48].

12. Future considerations for successful brain aging

Should we restrict our calories/frequently consume CRMs in order to preserve brain health and maintain a sufficiently high neuronal autophagic flux with age? Cumulative evidence from over 70 years of CR research provides compelling support for the role of CR-induced autophagic activity in brain health and longevity [116]. Therefore, a CR regime, alone or in combination with the dietary supplementation of a potent autophagy-inducing CRM, could contribute substantially to successful brain aging, delaying the onset of detrimental effects associated with neuronal proteotoxicity.

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