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Neuronutrición: Efectos neuroprotectores de la restricción calórica a través de mTOR y sirtuinas

Neuronutrition:

Neuroprotective effects of caloric restriction via mTOR & sirtuins

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“Let food be thy medicine”

Hippocrates

Abstract

Acting as nutrient-sensing, whereas the mechanistic target of rapamycin (mTOR) triggers a specific metabolic response congruent with environmental signals, sirtuins (SIRT) regulates that cellular metabolism by epigenetic mechanisms. Progressive non-functional macromolecules accumulation associated with neurodegenerative disorders, as well as oxidative and DNA damage, resulted in mTOR dysregulation, may be counterbalanced by the antioxidant role of SIRT. Caloric restriction (CR) promotes these neuroprotective benefits, as well as lifespan extension, through SIRT stimulation and by diminishing probabilities of deregulated mTOR signaling, which is highly associated with cancer, obesity, and neurological problems. However, mTOR pathway is also involved in neurogenesis; and nutrient depletion is postulated as one way to boost simultaneously as neurogenesis by brain-derived neurotrophic factors (BDNF) release as the neuroprotective action of SIRT. Understanding mTOR-SIRT interconnection in the nervous system, by considering diverse dietary forms, as well as nutritional values and diet-gene interactions, is a promising challenge for future neuropathology treatments.

Keywords: sirtuins, mTOR, caloric restriction, neuroprotective effects, neuronutrition

Resumen

De acuerdo a señales ambientales, la diana de rapamicina en células de mamífero (mTOR) actúa como detector de nutrientes desencadenando una correspondiente respuesta metabólica específica, mientras que las sirtuinas (SIRT) se encargan de la regulación del ese metabolismo a través de mecanismos epigeneticos. La progresiva acumulación de macromoléculas no funcionales asociadas con trastornos neurodegenerativos, así como del daño oxidativo y genético, resultado de la desregulación de mTOR, puede ser contrarrestado mediante el papel antioxidante de las sirtuinas. La restricción calórica (CR) promueve estos beneficios neuroprotectores, así como la extensión de la vida, estimulando la labor de las sirtuinas y la reduciendo las probabilidades de una desregulación de mTOR, lo cual está altamente asociado con cáncer, obesidad y problemas neurológicos. Sin embargo, la señalización del mTOR también está involucrada en la neurogénesis, siendo el agotamiento de nutrientes una vía para fomentar tanto neurogénesis mediante la liberación de factores neurotróficos

derivados del cerebro (BDNF), como la acción neuroprotectora de las sirtuinas. La comprensión de la interrelación entre mTOR-SIRT en el sistema nervioso, teniendo en cuenta diferentes formas dietéticas, así como los valores nutricionales y las interacciones dieta-gen, resulta prometedor para futuros tratamientos en neuropatología.
Palabras clave: sirtuinas, mTOR, restricción calórica, efectos neuroprotectores, neuronutrición

Contents

Abbreviations

BDNF: brain-derived neurotrophic factors | CR: Caloric Restriction | CRON: Caloric Restriction with Optimal Nutrition | *eNOS:* endothelial nitric oxide synthase | IF: intermittent fasting | IGF: Insulin Growth Factor | mTOR: mechanistic Target of Rapamycin | mtDNA: mitochondrial DNA | NAD: *nicotinamide adenine dinucleotide*| ROS: reactive oxygen species | SIRT: sirtuin | TSC: tuberous sclerosis complex | WAT: white adipose tissue.

1. Introduction

Neuronutrition tell us that every bite of food you eat is a choice that either depletes or nourishes your brain. However, not only can food be responsible for neuroprotective effects, but also exercising or fasting. Since absence or reduced food intake play a role on neurological activity, caloric restriction (CR), widely explained below, becomes a relevant matter of neuronutrition. In this mini-review, attention will be mainly focus on how CR affects the central nervous system through the mechanistic target of rapamycyn (mTOR) pathway and sirtuins (SIRT) activation. For that purpose, in pursuit of the deepest comprehension of this complicated relationship, the following organizational structure has been given.

First at all, it will be explained what caloric restriction is, as well as other dietary forms with similar benefits, and which are CR healthy effects. Secondly, mTOR role will be described in a holistic view, being subsequently explained neuropathologies, as well as neurogenesis and neuroprotective effects under CR, associated with it. Naturally, similar structure will be followed by SIRT section, only adding as extra a small, but remarkable, antioxidant note, related to SIRT activity. As can be expected, both mTOR and SIRT effects caused by reducing calorie consumption effects will be interestingly interconnected for making a complete visualization of the 'big picture' of this neuroprotective relationship. According to all information presented along the manuscript, final considerations for future research, as well as some practical advices for integrating theoretical knowledge in each one lifestyle, will be eventually provided.

2. Search strategy and selection criteria

The keywords exposed above, except '*neuronutrition'*, have been used in order to retrieve the most relevant and newest literature concerning SIRT and mTOR effects under CR; by using respected database, such as Elsevier and Pub Med, as well as library repository. Google Scholar was also consulted, and served as a great tool for finding some reviews nearly impossible to track down with other sources. Only papers written in English, and published from 2010 to April 2016, were considered. Nevertheless, very few, but worthy, exceptions of the two previous years were also taken into the reference list.

The most complete review associated with CR, as well as the principal articles specialized on mTOR and SIRT activity, have been carefully selected. After considering all studies retrieved, only those papers highly associated with any form of **neurological diseases** or **neurodegenerative disorders** in which SIRT or mTOR were involved in, as well as **neuroprotective** or **neurological effects** prompted by any form of dietary restriction via mTOR or SIRT, have been finally considered. Likewise, during the execution of this mini-review, extra articles which allow a better understanding of the mTOR and SIRT interconnection were incorporated as well.

3. Caloric restriction: definition & critical approach

Caloric restriction (CR) consists in the reduction up to 20-40% of food intake, without malnutrition, in comparison to ad libithum feeding (Ma, Dong, Wang, Li, Xu, Zhang & Wang, 2015). Simply by limiting the food access or intake, lifespan has been proved to be extended in several species. The earliest report about the benefits of CR came by Luigi Cornaro about 600 years ago, when at his 30-years-old he decided to change his unhealthy diet and lifestyle, incorporating only the minimum amount of daily calories required; hence his longevity was boosted. However, experimental evidences started lately to be accumulated, since last century (Speakman & Mitchell, 2011), and extended lifespan is not the only positive consequence. CR has been demonstrated to play a preventive role on cancer, autoimmune and cardiovascular diseases, metabolic alterations (e.g. obesity, metabolic syndrome, diabetes II) and neurodegenerative disorders (Speakman & Mitchell, 2011; Corella & Ordovás, 2014).

Besides CR, there are many other different forms of dieting with healthy benefits; for instance, dietary restriction, which has been proved to generate neuroprotective effects by combining CR with intermittent fasting (Pani, 2015). Actually, fasting is carried out in many religious rituals (e.g. Ramadan) and mainly differs in CR in the reduced meal frequency (Longo & Mattson, 2014). Additionally, it should be highlighted the ‘forgotten’ -but very important- difference between nutrients and calories. Protein restriction by 80% replicates CR effects such as lifespan extension, decreased of mitochondrial production of reactive oxygen species (ROS) and reduce insulin, glucose and leptin blood levels (Speakman & Mitchell, 2011). Indeed, only by 40% methionine restriction, likely due to the reduced oxidative mitochondrial damage,

prompts a decreasing amount of respiratory complexes I, III, and IV and apoptosis-inducing factor, reduced oxidative damage to mitochondrial DNA as well as in specific markers of protein oxidation and lipoxidation in brain mitochondria (Caro, Gomez, Sanchez, Naudi, Ayala, López-Torres & Barja, 2009; Speakman & Mitchell, 2011). Likewise, nutrients intake interacts with individual features (e.g. age, sex, physical state, body constitution, etc) and gene idiosyncrasy, modifying gene expression according to changes within nutritional environment, as result of epigenetic mechanisms (Solon-Biet, McMahon, Ballard, Ruohonen, Wu, Cogger & Gokarn, 2014). In spite of all these differences, CR will be mentioned along this paper including all those forms described above -without any difference-, only by sporadically pointing out few specific notions about type of nourishment on nervous system, due to the lack of literature and the limited room allowed.

4. mTOR

The mechanistic target of rapamycin (mTOR), known previously as mammalian target of rapamycin, integrates stress, nutrients availability, hormonal and energy fluctuations in order to develop a cellular environmental-consistent metabolic response (Huang & Fingar, 2014; Tee, Sampson, Pal, & Bateman, 2016). This serine/threonine kinase complex is inhibited by rapamycin and its analogues, and depending on the proteins bound to the family TOR, two different complexes can be distinguished (Yuan & Guan, 2016). Both mTORC1 and mTORC2 are responsible for different cellular processes, as well as highly involved in neurogenesis (see *Section 4.2*), described in the next table based on all mTOR literature cited.

	mTORC1	mTORC2
Proteins bound to mTOR	Raptor DEPTOR mLST8 PRAS40	Rictor DEPTOR mSin1 mLST8 PRR5/Protor-1
Functions	Cellular growth by phosphorylating substrates that: -Enhance anabolic processes: lipid, nucleotide & protein synthesis. -Diminishing catabolic processes such as autophagy	Cell proliferation and survival Cytoskeleton organzation Intracellular mechanism related with transport of ions (Ca+) In cancer may activate mTORC1 downstream response

Table 1. Differences between mTOR complexes (Self-elaboration).

When growth conditions are sufficient and favorable, mTOR become active leading a downstream response appropriate for integration of environmental signals, as result cellular homeostasis is maintained by mTOR (Huang & Fingar, 2014; Yuan & Guan, 2016). The most common and simple way to alter cellular homeostasis is by food intake (Swiech, Perycz, Malik & Jaworski, 2008), which triggers several biochemical reactions, illustrated in the following figure (courtesy of Diane C. Fingar).

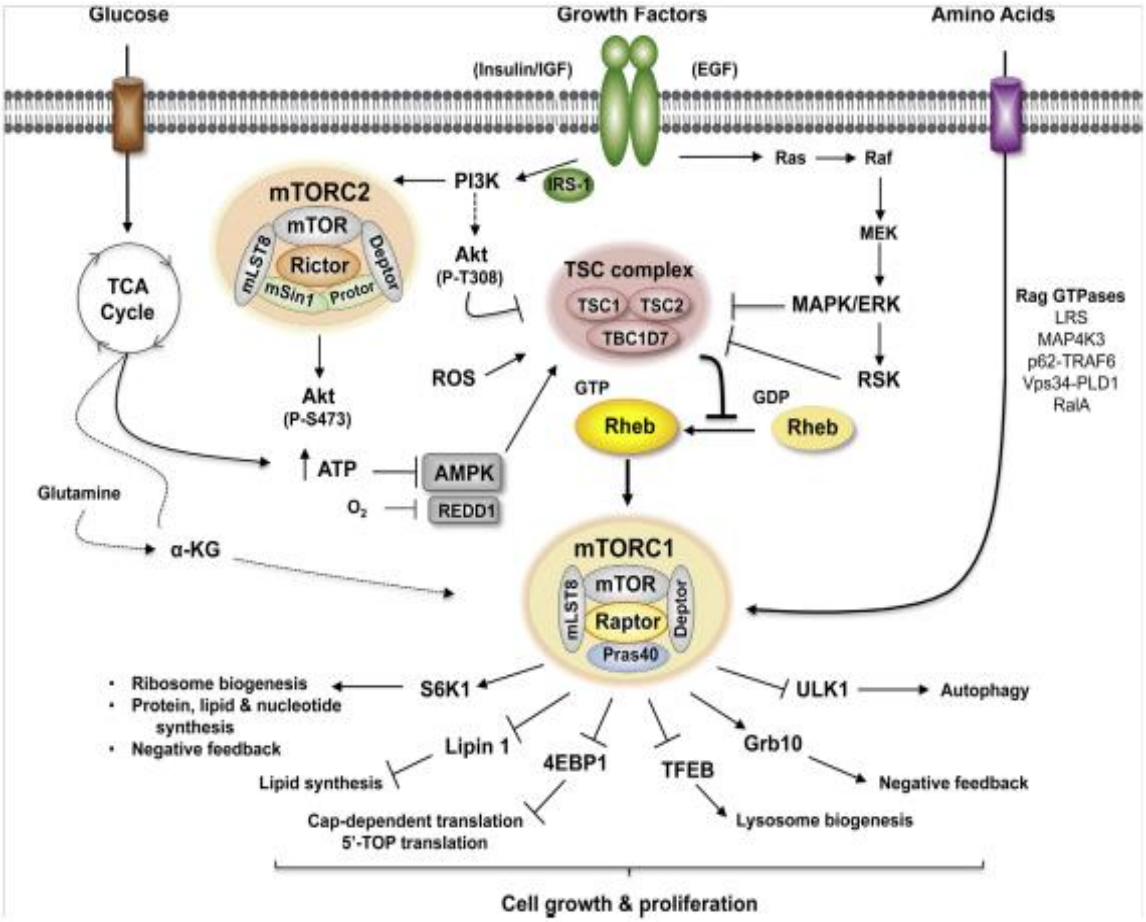

Figure 1. Regulation of mTORC1 and mTORC2 signalling network by upstream inputs. Reprinted from "Growing knowledge of the mTOR signaling network" by K. Huang & D. Fingar, 2014, *Seminars in cell & developmental biology*, *36*, p. 81. Copyright 2014 Elsevier. Reprinted with permission (D. C. Fingar, personal communication, July 3, 2016).

Cancer, obesity, diabetes II, and neurological disorders, such as epileppsy and autism, are involved in mTOR overexpression (Swiech et al., 2008; Speakman & Mitchell, 2011; Curatolo, 2015). As can be expected, dysregulation of mTOR takes part in progressive non-functional macromolecules accumulation, as well as oxidative and DNA damage, since the anabolic processes related to mTOR pathway are energy-

dependent (Huang & Fingar, 2014). Energy is fundamental for any cellular division, and is produced at the mitochondrial respiratory complexes, which consequently leads to oxidative damage (e.g. free radicals, ROS, peroxide) and age-related disorder associated with mitochondrial dysfunction. Moreover, the mutations and telomerase loss associated with the cellular division, triggered by mTOR response, may drive to tumor, which might become cancer if both mTORC1 and mTORC2 become overexpressed (Huang & Fingar, 2014), where mTORC2 overexpression leads to the subsequent mTORC1 downstream signaling. Given these evidences, the more you eat, the more cellular growth and proliferation (fundamental during childhood, but gradually less necessary for along aging), due to increased mTOR activation. Although it is universally known that neurons are unable to be divided by itself (no mitosis) mTOR pathway is indirectly exposing the nervous system to cancer risk because of the mTORC2 cell proliferation role, which is needed as previous essential step before metastasis, when tumor travels from the original site to other parts of the body (e.g. brain). In the following sections will be described how CR affect mTOR pathway.

4.1 Neuroprotective effects of CR via mTOR

Insulin growth factor (IGF) hormone is responsible for keeping cellular go-go mode on, through cellular growth role of PI3K, Akt, mTOR pathway activation, which generate the expected oxidative damage associated with any cellular division. Since mTOR responds to growth factors, energy levels, cellular stress and amino acids deprivation; CR is a perfect way to slow down mTOR downstream signaling and subsequent tumor proliferation, telomere loss and oxidative damage related. In fact, when CR is practiced for a long-term, glucose and adipokines level decrease leading to less IGF and white adipose tissue (WAT) availability in blood flow (Speakman & Mitchell, 2011). So, longevity is boosted and age-related disorders are ameliorated since mTOR negatively regulates autophagy. The housekeep action of autophagy forces to create new nutrients by recycling intracellular components, contributing thus to remove toxic protein accumulation, and oxidative damage produced by aging or overeating, among others (Speakman & Mitchell, 2011), showing neuroprotective effects on Alzheimer, Parkinson and Huntington's disease or neuron death (Yang, Chu, Yin, Liu, Yuan, Niu & Fu, 2014). Independent of total calorie consumption, protein intake can

reverse autophagy benefits, due to amino acid sensing by Rag/Ragulator axis involved in mTOR downstream response (Speakman & Mitchell, 2011; Huang & Fingar, 2014).

4.2 Neurogenesis role of mTOR boosted by CR

However, mTOR pathway is needed for neurogenesis: soma size, neuronal guidance, axon guidance, synaptic plasticity, dendrite development and dendritic spine morphogenesis (Swiech et al., 2008; Urbanska, Gozdz, Swiech, & Jaworski, 2012; Tee et al., 2016). But, how can be prompted the mTOR pathway in the nervous system without IFG? By burning fat, during fasting or exercise, ketones bodies are realised to supply cell energy demands (Pani, 2015). As consequence, mTOR pathway is energetically activated by ketones, and it also will be also sensitive to brain-derived neurotrophic factors (BDNF) result of dietary restriction, which encourage neurogenesis (Marosi & Mattson, 2014; Pani, 2015). This neurotrophin increases mTOR activity and dendritic protein synthesis in cultured hippocampal neurons (Marosi & Mattson, 2014; Yang et al., 2014), being proteins synthesis related to neural morphological changes triggered by mTORC1 through translational control. On the other hand, according to the table 1, cytoskeletal reorganization (e.g. dendrite and dendritic spine) will be determined by mTORC2, which is also involved in cellular mechanisms which control ions transportation, like transient receptor potential channel (TRPC)-Ca2+, which enhance synaptic plasticity and memory by BDNF regulation over glutamate receptor (Marosi & Mattson, 2014). However, mice aged brain present low activity of BDNF/Akt/mTOR signalling in hippocampus (Yang et al., 2014). Additionally, rapamycin prevents long-term potentiation LTP in the hippocampus by blocking BDNF, whose absence is highly associated with hyperphagia, high blood glucose and leptin levels, mTOR overexpression, as well as obesity, cancer, neurodegenerative disease, and so on. (Marosi & Mattson, 2014).

Although energy restriction can enhance neurogenesis, mTOR first prioritizes protein synthesis for ongoing function and survival (Marosi & Mattson, 2014). Cellular stress and survival are mediated by mTORC2, whose role may be boosted by intermittent fasting (IF), which have been involved in cellular stress resistant and prevention of neuronal damage and death through glucocorticoid receptors downregulation (Longo & Mattson, 2014). All this might postulate IF as the ideal

neuroprotective diet by incorporating neurogenesis precursors like curcumin during eating days and promoting BDNF, as well as cellular self-recycling through autophagy, during fasting or CR days.

4.3 CR and ketogenic diet

The importance of the mTOR/TSC axis in neuronal morphology and myelination, axonogenesis, dendritic arborization, regulation of neurotransmitter-receptor expression, autophagy and cortical architecture is fundamental in the understanding neurological pathologies associated with tuberous sclerosis complex (TSC), an unusual autosomaldominant genetic disorder whose most common symptom is epilepsy (Curatolo, 2015; Tee et al., 2016). In individuals with TSC, inactive TSC1 or TSC2 was observed to be related to high levels of mTOR signaling, prompting thus global disturbances in the architecture and connectivity of the brain. Dysregulation of mTOR signaling in the brain leads to several different neuronal abnormalities.

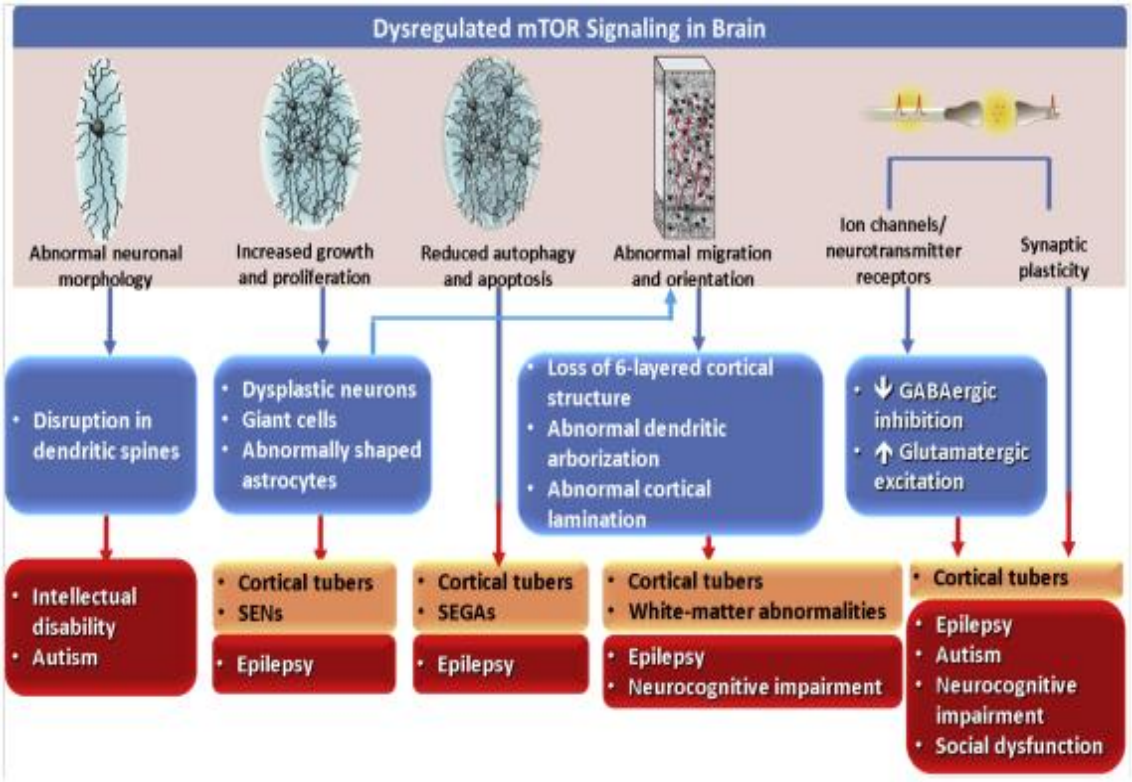

Figure 2. Dysregulated mTOR signaling and TSC-associated neuropathology. Reprinted from "Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy" by P. Curatolo, 2015, *Pediatric neurology, 52(3),* p. 284. Copyright 2015 Elsevier. Reprinted with permission (P. Curatolo, personal communication, September 8, 2016).

Keeping in mind mTOR pathway (see again ***Figure 1***), knockout of TSC1 or TSC2 induce the mTOR downstream response, which might underlie neuronal hyperexcitability responsible of seizures. Indeed, hippocampal TSC1 loss in mice have been involved with seizures since drive to a synaptic transmission imbalance due to aberrant mTOR signaling (Curatolo, 2015). Physiological evidences of dysregulation of excitatory and inhibitory neurotransmission have been provided by AMPA and NMDA glutamate receptor seen abnormal cell types (e.g. giant cell, reactive astrocytes). In addition, ketogenic diet is recommended to reduce seizures in severe epilepsy patients, pharmacoresistant or non candidate for surgery (Marosi & Mattson, 2014). Decreased phosphorylated S6 and Akt levels in the hippocampus were found in animals who followed this diet, supporting thus the idea of aberrant mTOR signaling associated with epileptogenesis, and pointing it out as antiepileptic novel treatment, based on preclinical and clinical studies, for TSC associated epilepsy by using mTOR inhibitors (Curatolo, 2015). Although ketogenic diet may work, its efficacy is limited because of long-term treatment adherence, changes in lipid metabolism and risk of ketoacidosis in prolonged cases.

5. Sirtuins

Silent information regulator are nicotinamide adenine dinucleotide-dependent enzymes with deacetylase activity (Corella & Ordovás, 2014; Poulose & Raju, 2015), whose sirtuin mammalian expression is variable depending on stage of development and cell location (Radak, Koltai, Taylor, Higuchi, Kumagai, Ohno & Boldogh, 2013). The SIRT family, composed by seven mammalian homologs, is involved in inflammation, gene silencing, genomic stability, cell longevity and metabolism through antioxidant, insulin and anti-apoptotic response (Bonda, Lee, Camins, Pallàs, Casadesus, Smith & Zhu, 2011). Based on the recent Poulose & Raju (2015) paper, each mammalian sirtuin enzyme will be described in a brief table in terms of location and function.

Sirtuin	Location	Function
SIRT1	*Nuclear, cytoplasmic*	*Epigenetic role:* chromatin modification according to metabolic signals (see below Fig. 3) Control inflammation
SIRT2	*Nuclear, cytoplasmic*	*Via FOXO3 (under cellular stress):* antioxidant & pro-apoptotic *VIa FOXO1:* adipocyte differentiation Migration to the nucleus during mitosis
SIRT3	*Nuclear Mitochondrial*	Thermogenesis in brown adipose tissue Mitochondrial homeostasis (SIRT3 is transported to the mitochondria under stress conditions to prevent ROS accumulation) Acting as a tumour suppressor by suppressing HIF-1α activity
SIRT4	*Mitochondrial*	*Cellular metabolism:* secreting insulin & control of glutamine metabolism Prevent tumorigenesis under DNA damage conditions
SIRT5	*Mitochondrial*	Considered dispensable for regulating metabolism, but still unknown
SIRT6	*Nuclear*	Maintenance of genomic stability, DNA repair and longevity *Glucose metabolism*: controlling hepatic gluconeogenesis and suppressing HIF-1α and other glycolytic genes Negative triglyceride synthesis regulation
SIRT7	*Nucleolus*	Ribosome biogenesis: positively regulate ribosomal gene transcription (repressed during mitosis) Protein synthesis Mitochondrial homeostasis

Table 2. Description of mammalian sirtuins (Self-elaboration).

In the yeast Saccharomyces Cerevisiae the overexpression of SIRT2 leads to longevity through telomeres conservation, by interrupting SIRT2 redistribution in the nucleolus and preventing DNA breaks in processes like replication; in mammals, by contrast, the enzyme which plays a major role on lifespan extension is SIRT1(Radak et al., 2013). Oxidative stress is one of the causes of aging, leading to harmful macromolecules damage, as well as a toxic metabolites accumulation, which alters the balance between reactive oxygen species (ROS) and antioxidant defence (Camins, Sureda, Junyent, Verdaguer, Folch, Pelegri & Pallàs, 2010). Mitochondrial biogenesis and ROS sequestration through PGC-1, as well as oxidative stress resistance via FOXO3, are boosted by SIRT1 role, among many other gene deacetylation (Poulose & Raju, 2015).

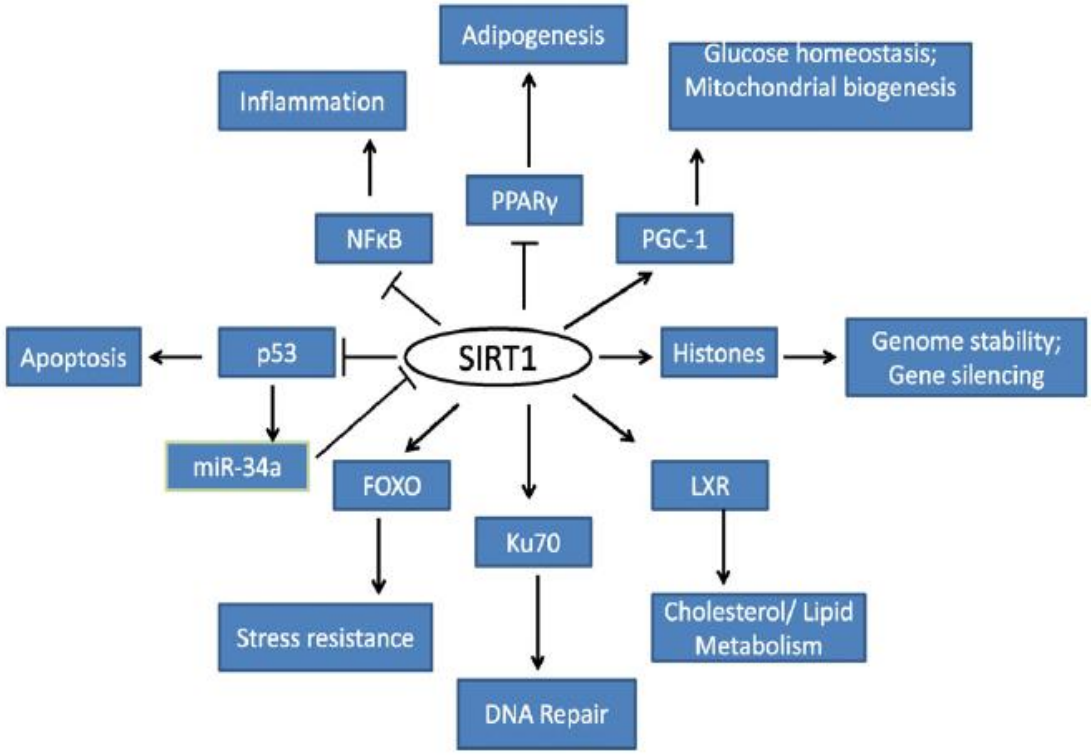

Figure 3. SIRT1 downstream signaling. Reprinted from "Sirtuin regulation in aging and injury" by N. Poulose & R. Raju, 2015, *Biophysica Acta (BBA)-Molecular Basis of Disease, 1852(11),* p. 2444. Copyright 2015 Elsevier. Reprinted with permission (R. Raju, personal communication, September 8, 2016).

5.1 How does CR affect sirtuin family?

Since sirtuins adapts the cells to environmental changes (Poulose & Raju, 2015), the nutrients intake by diet, DNA damage and stressful conditions, such as ischemic preconditioning or CR, automatically activate SIRT1 (Radak et al., 2013). Reduction of intracellular NAD^+ is produced by aerobic glycolysis, one of the main causes of aging; whereas CR boosts its intracellular concentration, fundamental to SIRT1 enzyme activity (Speakman & Mitchell, 2011). Despite controversial findings, likely due to the lack of distinction among different types of restrictions or diets, it has been verified that fasting (nutrient depletion) raises NAD^+ levels (Radak et al., 2013; Pani, 2015). Appart of increasing mitochondria bioavailability, and the beneficial antioxidant action, SIRT1 also plays a role on autophagy and glucose and lipid metabolism regulation during fasting (Poulose & Raju, 2015). According to Camins et al. (2010), SIRT1 decreases insulin resistance and regulate adiponectin secretion, whose function is to turn WAT

into energy, reducing thus adipocytes concentration. Although SIRT1 has been longer and deeper investigated, other sirtuins should be taken into account as well. Not only can SIRT6 carry out similar activities than SIRT1, but also regulate negatively triglyceride synthesis; moreover, under stressful conditions, SIRT2 shoes an antioxidant & pro-apoptotic function via FOXO3 (Poulose & Raju, 2015). Oxidative stress is also diminished under CR by SIRT3 which deacetylates Idh2 (mitochondrial isocitrate dehydrogenase 2), whose activity raises NADH concentration that will be used by glutathione mitochondrial antioxidant defence systems, responsible of turning oxidized glutathione into reduced glutathione (Someya, Yu, Hallows, Xu, Vann, Leeuwenburgh & Prolla, 2010; Speakman & Mitchell, 2011). As can be expected, CR mediates metabolic adaptations via SIRT3 which affects mitochondrial complex I activity and fatty acid oxidation in the mitochondria (Someya et al., 2010). Give all these evidences, sirtuins activity have demonstrated to contribute to lifespan extension in response to CR through deacetylation of genes involved in antioxidant defence, DNA conservation, mitochondrial biogenesis and macromolecules metabolism.

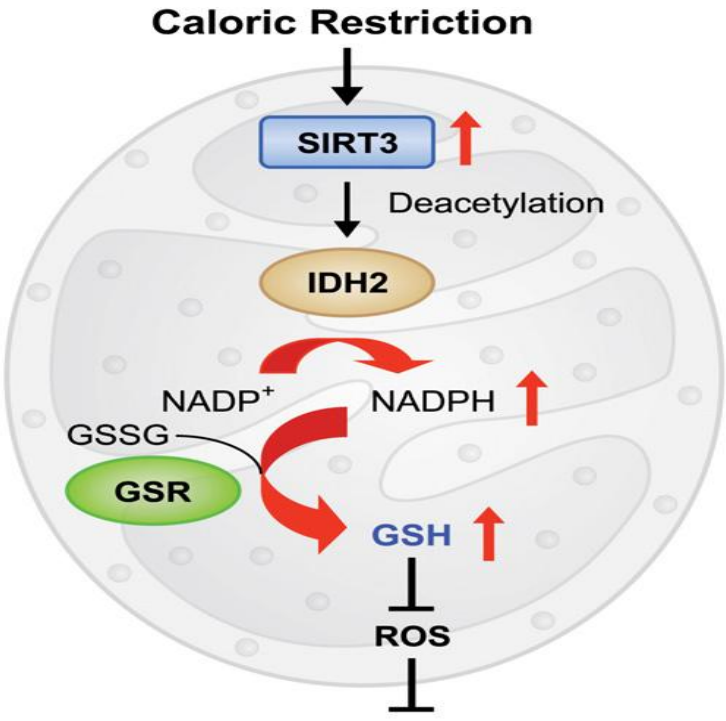

Figure 4. Activation of SIRT3 downstream response under CR. Reprinted from "Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction" by S. Someya, et al., 2010, *Cell, 143(5),* p. 809. Copyright 2010 Elsevier. Reprinted with permission (T. Prolla, personal communication, September 8, 2016).

5.2 SIRT1: Neuroprotective effects

The anti-aging effect of CR by activating sirtuins helps to prevent neurodegenerative disorders like Alzheimer, Parkinson or Huntington (Poulose & Raju, 2015). The parahippocampal region is a highly energy-demanding brain region, supplied of ATP by aerobic glycolysis, which leads to decreasing NAD^+ concentration and consequently SIRT1 becomes hypoactive (Bonda et al., 2011). In spite of elevated SIRT1 expression in hippocampus, low SIRT1 enzymatic activity is found in hippocampal areas of mammalian aged brains because of NAD^+ depletion (Radak et al., 2013). As result, progression of amyloidogenesis takes place in mediotemporal lobe, contributing to Alzheimer's apparition or progression (Bonda et al. 2011).

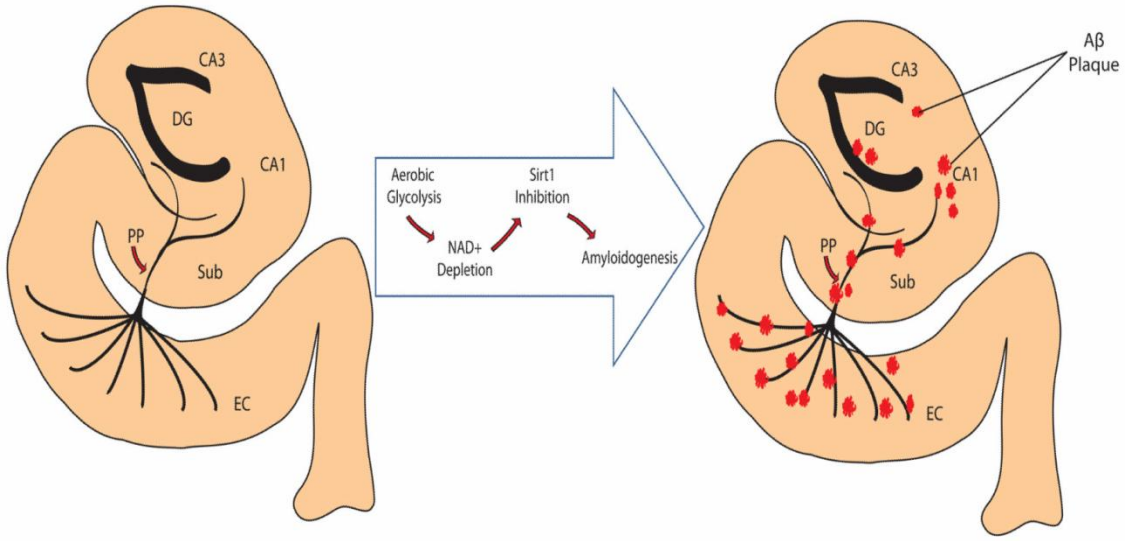

Figure 5. Relationship between lack of Sirt 1 activity and Alzheimer. Reprinted from "The sirtuin pathway in ageing and Alzheimer disease: mechanistic and therapeutic considerations" by D. J. Bonda et al., 2011, *The Lancet Neurology, 10(3),* p. 278. Copyright 2011 Elsevier. Reprinted with permission (X. Zhu, personal communication, September 8, 2016).

However, as it has been described above, NAD^+ levels are elevated by CR by stimulating Namp overexpression, which leads to a neuroprotective action of SIRT1 (Poulose & Raju, 2015), like, for instance, preventing Alzheimer progression. SIRT1 deacetylates RARβ, facilitating thus ADAM10 gene transcription. As result, α-secretase levels, enzyme responsible for reducing pathological accumulation of amyloid-β protein, are raised (Bonda et al., 2011). Supporting these evidences, animal experimental studies have verified beneficial effects of CR reducing amyloid-β content

and tau hyperphosphorylation, above all in the temporal cortex (Camins et al. 2010). In Parkinson disease, mitochondrial dysfunction, as a source of free radical, is considered one of main causes of Lewy bodies, as well as loss of dopaminergic neurons, which can be reversed by SIRT1 (Camins et al., 2010). Autophagy is another way to prevent dopaminergic cellular loss through SIRT1 action, helping to clear intracellular toxic macromolecules accumulation (Speakman & Mitchell, 2011) and MPTP neurotoxicity in Parkinson. The anti-inflammatory and antiapoptotic role of SIRT1 prevents neuronal death during brain damage (trauma, ischemia, hypoperfusion injury) and contribute to vasculoprotective effects by deacetylating brain endothelial nitric oxide synthase (eNOS) (Poulose & Raju, 2015).

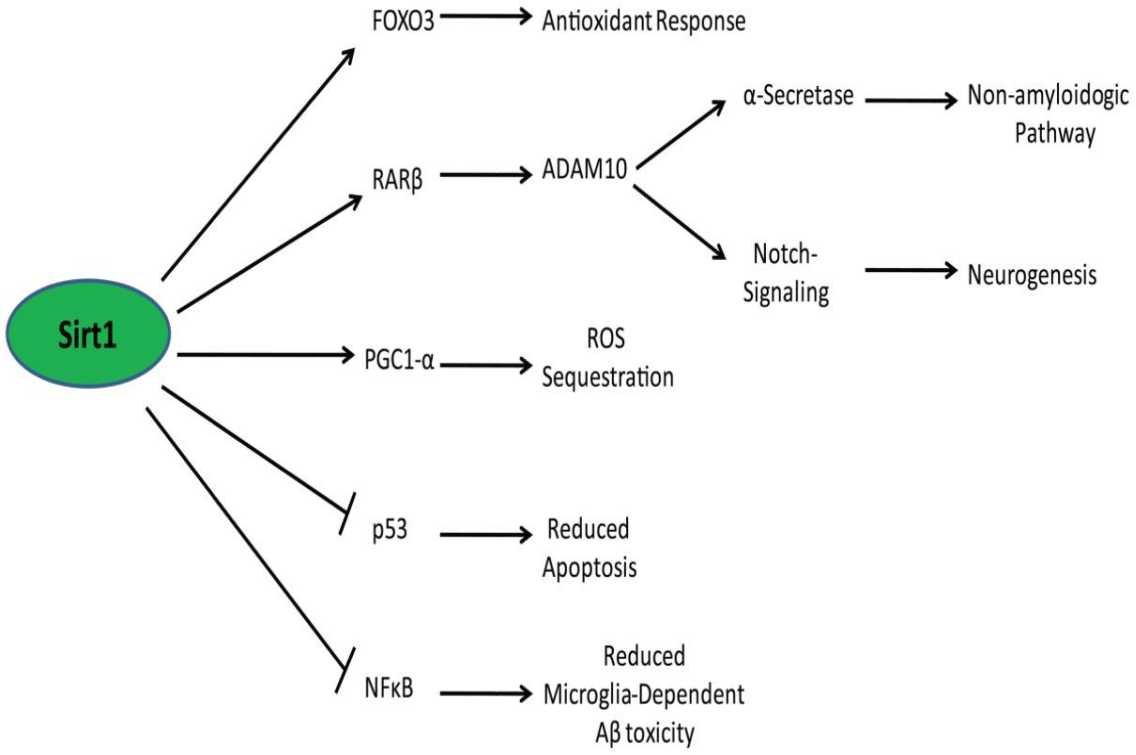

Figure 6. Sirt1 activity related to Alzheimer disease. Reprinted from "The sirtuin pathway in ageing and Alzheimer disease: mechanistic and therapeutic considerations" by D. J. Bonda et al., 2011, *The Lancet Neurology, 10(3),* p. 277. Copyright 2011 Elsevier. Reprinted with permission (X. Zhu, personal communication, September 8, 2016).

As is shown above, the ADAM10 gene involved in SIRT1 effects on Alzheimer disease is also associated with neurogenesis, due to Notch pathway (Bonda et al., 2011), which might ameliorate (or hide in case of nondiagnostic patients) Alzheimer's symptoms. (Xiao, Han, Shao & Jin, 2009; Bonda et al., 2011). Exercise and fasting raise

BDNF levels in the hippocampus and mitochondrial biogenesis in new neurons, boosting thus neurogenesis as well (Marosi & Mattson, 2014; Pani, 2015). The more mitochondrias, the less oxidative damage and the more number of neurons, which can form and maintain new sinapsis because of BDNF (Marosi & Mattson, 2014); hence, memory and learning abilities are improved. In mice have been demonstrated that BDNF partly attenuates Huntington consequences through SIRT1 overexpression (Radak et al., 2013). Additionally, this neurotrophin also enhances neuron use of ketones (Marosi & Mattson, 2014), which generate fewer free radicals (Speakman & Mitchell, 2011) and prevent seizures and progressive excitotoxic neuronal damage (Marosi & Mattson, 2014; Curatolo, 2015). On the other hand, low glucose levels caused by intermittent fasting or CR reduce IGF production, forcing the cell to switch from 'growth mode' to 'repair mode' via SIRT1, triggering in mice protective effects through higher FOXO transcription, as well as by decreasing hydrogen peroxide (H2O2) production and oxidative stress (Speakman & Mitchell, 2011).

5.3 Natural antioxidants

Under stressful conditions (e.g. CR, ROS concentration, exercise), as well as DNA damage, SIRT1 role have been identified as crucial for the cell survival (Bonda et al., 2011). Besides CR, natural antioxidants are traditionally used to delay aging and prevent age-related pathogenesis like neurodegenerative disorders. The most known natural antioxidants are flavonoids, coenzyme Q10, ginkgo biloba, carnosine, curcumin, carotenoids, lycopene and resveratrol, among others. Despite some findings, which supports that several new synthesized compounds guarantee better results than natural antioxidant, such as resveratrol; this manuscript will be only focus in natural treatments able to reproduce similar effects than CR, as well as by contributing to reduce neurodegenerative risk and oxidative damage. Actually, resveratrol has been investigating for years as a promising treatment for neurodegenerative disorders, because of its neuroprotective effects by reducing amyloid-β secretion and by boosting autophagy (Camins et al., 2010). During neuronal cell injuries, natural components such as tea polyphenols boost PGC-1α levels via SIRT1 which help to suppress ROS production (Poulose & Raju, 2015). Diet can also counteract brain injuries (e.g., omega-3 fatty acids after a traumatic hippocampal damage), and it can also reduce SIRT1

expression in hippocampus and neocortex by high fat diet, or enhance it by vitamin E (lycopene) administration (Poulose & Raju, 2015).

The same beneficial effects showed by SIRT1 are emulated by resveratrol (Speakman & Mitchell, 2011) such as reduced IGF-1 levels, increasing insulin sensitivity, as well as higher activation of AMPK and PGC- 1a, autophagy and mitochondrial biogenesis. In fact, resveratrol administration imitates CR effect by glucose uptake in muscle, and protect the cell against DNA and oxidative damage (Speakman & Mitchell, 2011), showing neuroprotective effects associated with MMT^{+} cytotoxicity in Parkinson disease and amyloid-β aggregation plaques in Alzheimer (Camins et al., 2010). Actually, resveratrol also plays an antioxidant role in mitochondrial energy and free radical metabolism, as well as increasing the plasma antioxidant capacity, by increasing the expression of two antioxidant enzymes such as manganese superoxide dismutase (MnSOD) and glutathione (GSH) (Camins et al., 2010). Despite resveratrol supporting, this natural polyphenol is considered problematic because of its multiple cellular targets and its controversial role about if it acts as SIRT1-activator or directly as antioxidant (Speakman & Mitchell, 2011).

6. Neuroprotective effects of CR via SIRT-mTOR interconnection

Despite the separate description of both nutrient sensing pathways for a clearer understanding, mTOR is involved in protein, carbohydrates and lipid metabolism downstream response which sirtuin expression is responsible of regulating, therefore mTOR and sirtuin are highly interconnected (Corella & Ordovás, 2014). For instance, glucose metabolism is regulated by SIRT3 and SIRT6, via HIF-1α activity, which affects mTOR response, and even cell proliferation since SIRT3 acts as tumour suppressor by inhibiting HIF-1α (Poulose & Raju, 2015). Not only are interconnected because of metabolism issues, but also because of lifespan extend-related activities, therefore, as mTOR response is stimulated by upstream inputs, the cell is pushed into the cell cycle and during mitosis phase, SIRT2 migrates to the nucleus (Poulose & Raju, 2015), which might facilitate telomerase loss and senescence.

Under cellular stress, like CR, SIRT1 is not only involved in deacetylation of transcription and non-transcription factors associated with oxidative issues, but also in genes related to the translational control of mTOR role (e.g.translation initiation factor

eIF-2alpha). So, SIRT1 acts as nutrient-sensitive growth suppressor via TSC2 by regulating stress induced translation control (Ghosh, McBurney & Robbins, 2010). Indeed, on mesanglial cells, rapamycin inhibition requires SIRT1 intervention in order to interrupt mTOR downstream signaling (Ma et al., 2015). Resveratrol, either in a SIRT1 dependent manner or in parallel to it, has also reported similar results as rapamycin by inhibiting mTOR pathway, including reduction of plasma lipid peroxidation (Camins et al., 2010; Ghosh et al., 2010). Indirectly, via OGG1 inhibition, SIRT1 affects Ras/GTPase which may modulate mTORC1 response and reduce peroxidation production (Radak et al., 2013). When mTOR is inhibited by resveratrol, rapamycin, CR or amino acids deprivation, due to negative relationship autophagy switch on, and via SIRT1 (Camins et al., 2010) not only several stress-responsive factors associated with autophagy machinery are promoted (Ghosh et al., 2010), but also intervenes through TSC2–mTOR–S6K1 signaling pathway (Wang, Guan, Du, Zhai, Su & Miao, 2012).

In addition, IGF-1 serves as activator of mTOR pathway whereas acts as SIRT1 blocker (Ma et al., 2015), being observed mTOR hyperactivation and SIRT1 insufficiency under high glucose levels (Speakman & Mitchell, 2011). What is more, increased glucose uptake in muscle and fat tissue leads to a severe hypoglycemia, causing SIRT6 loss and subsequent impairments on genomic stability, DNA repair and longevity (Radak et al., 2013; Poulose & Raju, 2015). In spite of individual variations (such as genotype, age, sex, stress, exercise or nutritional levels), CR usually retards IGF-1 hormone synthesis, counterbalancing thus the negative relationship between SIRT1 and mTOR (Solon-Biet et al., 2014) and promoting augment of intracellular NAD^+ levels. Aerobic glycolysis, promoted by mTOR activity, generates NAD^+ depletion in high glucose-dependent brain areas (Bonda et al., 2011), decreasing SIRT1 activity was found hippocampus of aged brains (Radak et al., 2013). This finding might underlie why neuroprotective effects of SIRT1 seems to be more beneficial in aged-brains than in younger mices (Ma et al., 2015), but it does not explain why there is no more neurogenesis if over the time SIRT1 become hypoactive and mTOR hyperactive in the hippocampal areas. Oxidative stress and macromolecules accumulation probably impedes newborn neuron integration into the neural network and it may be DNA damage, resulted in telomerase loss and cellular division, involved in neurogenesis

transcription factors, unable to be repaired due to the low hippocampal SIRT1 enzyme activity. Although low hippocampal SIRT1 enzymatic activity was observed, because of depleted intracellular NAD^+ levels, it has been controversially demonstrated that aging boost increasing SIRT1 expression in hippocampus, whereas in parietal lobe is reduced (Radak et al., 2013). This evidence might be caused because brain SIRT1 is primordially found in neocortex, hippocampus and cerebellum (Camins et al., 2010); but surprisingly, even though exercise increases SIRT1 levels in hippocampus, it seems ineffective for cerebellar concentration (Radak et al., 2013). Despite being forgotten, cerebellum also play a role on cognition and emotion, likely because its evolution was parallel to neocortex development, and dysregulated mTORC1 expression may lead to progressive accumulation of oxidative damage, which might be related to cerebellum-related psychopathological disorders associated with neurotransmitter release (e.g. dopamine in depression, psychosis, ADHD, anxiety) (Hoppenbrouwers, Schutter, Fitzgerald, Chen & Daskalakis, 2008; Schutter, 2016). All this data supports the importance of considering SIRT cell location in future research, as well as a possible connection with the oxidative and neurogenesis role of mTOR activity.

On the other hand, mTORC1 positively correlates with glutamine consumption, being cell proliferation stimulated via GDH activation and CREB2 destabilization by suppressing the anti-tumorigenesis role of SIRT4 activity (Csibi, Fendt, Li, Poulogiannis, Choo, Chapski & Henske, 2013). Glutamine acts as signaling molecule by regulating mTORC1 assembly and by boosting mTORC1 downstream response through leucine uptake and lysosomal localization (Csibi et al., 2013). Glutaminase is responsible for glutamine conversion into glutamate; whose elevated neurotransmission takes part into neurological stress, inducing excessive intracellular calcium concentration through the ions transportation role of mTORC2 (Marosi & Mattson, 2014; Curatolo, 2015). As result, cell death caused by post ischemic events or epileptic seizures occurs (Marosi & Mattson, 2014), being mitochondrial glutamine metabolism essential for obtaining energy under insufficient glucose levels and cell proliferation by inducing TSC2 and PTEN death (Csibi et al., 2013). Indeed, NAD^+ depletion contributes to glutamate-induced excitotoxicity, supporting the negative correlation between mTOR and sirtuin activity (Radak et al., 2013).

Nevertheless, it should be importantly pointed out that mTOR does not mean cataclysm. Actually, mTOR role is fundamental for neurogenesis (Urbanska et al., 2012; Tee et al., 2016); the problem comes when mTOR pathway is overexpressed. Dietary restriction and exercise may trigger BDNF release, so that several synaptic plasticity actions are enhanced via mTOR (see again *section 2.2*) because of its glutamate receptor association (Marosi & Mattson, 2014) and BDNF/Akt/mTOR pathway, despite signaling is declined by aging in mice brain (Yang et al., 2014). Furthermore, BDNF enhances neuronal ketones use, facilitating thus mTOR neurogenesis role under low glucose levels and reduced oxidative damage (Speakman & Mitchell, 2011), and counterbalancing glutamate excitotoxicity responsible for epileptic seizures (Marosi & Mattson, 2014; Curatolo, 2015). So, by incorporating progressively healthy habits to our lifestyle and diet, an appropriate mTOR-sirtuin balance which might become a future neuroprotective factor for aged brains due to the neurogenesis role of mTOR and antioxidant SIRT1 action.

7. Future directions

Studies have demonstrated that CR failed to extend lifespan when specific genes encoding mTOR and S6K were deleted from yeast genome, being similar results found in C. elegans under DR (Corella & Ordovás, 2014). This finding supports how, through epigenetic mechanisms, nutrition affects aging-disease processes and in a nutrient-dependent manner, dominant-negative alleles of mTOR and S6K prolong lifespan. In fact, TCF7L2 gene has been involved in determining fasting glucose and lipids, suggesting a sort of relationship between its expression and CR (Corella & Ordovás, 2014). So, environmental changes (such as diet, social eating context, physical activity or smoking) modifies individual genetic expression, being CR another factor involved in epigenetics. However, CR studies only talk about calorie, not specific nourishment (Speakman & Mitchell, 2011) and nutrients are *alla fine* the real responsible for downstream environment-genes signaling and diverse physiological responses. According to Solon-Biet et al. (2014) study, diverse nutritional macromolecules proportion affects differently animal longevity, which must be verified in humans in future years.

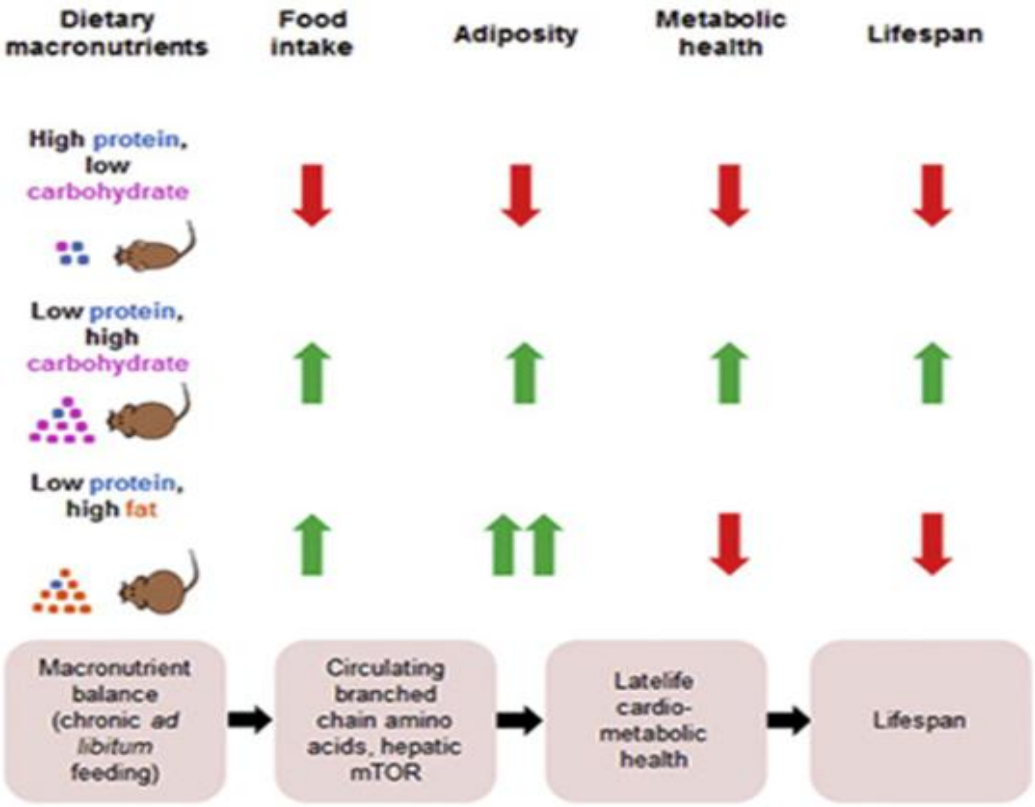

Figure 7. Health consequences of different types of dietary macronutrients. Reprinted from "The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice" by S. M. Solon-Biet et al., 2014. Retrieved September 10, 2016, from http://dx.doi.org/10.1016/j.cmet.2014.02.009. Copyright 2014 Elsevier. Reprinted with permission (S. J. Simpson, personal communication, September 8, 2016).

Supporting these findings, 40% of methionine restriction mimic CR effects (Caro et al., 2009; Speakman & Mitchell, 2011), which seems reasonable since mTOR response requires as essential condition amino acids for Rag machinery activation (Huang and Fingar, 2014). Indeed, vegan diet, poor in methionine, shows the same CR benefits, reducing ROS production and DNA damage because of fewer mTOR activation (Speakman & Mitchell, 2011). What is more, ketogenesis results can also be reproduced by his high-fat, adequate- but low-protein, low-carbohydrate, which promotes the metabolism of fatty acids (Curatolo, 2015), and consequently ketones bodies release, invalidating some previous limitations like long-term treatment adherence. Given all these evidences, future CR studies should incorporate diet-gene interaction and macromolecules consideration to obtain clearer results of how nutrients,

through epigenetic mechanisms, affect mTOR and sirtuin responses associated with longevity and neuroprotective effects.

From a practical perspective, both nutrient maximaxization and calorie restriction have converged in a promising healthy lifestly called CRON: Caloric Restriction with Optimal Nutrition (CRON: http://optimal.org/voss/cron_overview.html). Based on psychology of eating tecniques, CRON success may be achieve by becoming (nutritionally) aware about what are we eating and by discovering new tastes and habits, through an enjoyable way and according to personal food likes and preferences. Moreover, taking also into account that NAD^+ levels and autophagy declines by aging, provoking thus SIRT1 deficiency and mTOR hyperactivation, which leads to neurodegenerative disorders, telomerase loss and others age-dependent consequences; sirtuins activation, through diet (e.g. resveratrol and natural antioxidants) or stressful conditions (e.g exercise, fasting, CR), should be boosted but keeping at the same time the neurogenesis role of mTOR pathway. So, since the 'perfect diet' will vary throughout individual lifetime, depending on cultural eating environment, stage of human development and mTOR genes (Corella & Ordovás, 2014), the maximum neuroprotective effects might be obtained through several different ways by personalizing diet according to each one gene-diet interaction, age and CRON.

Note: *All figures have been formally included in this paper after asking for the corresponding permission to each article contact researcher by e-mail. Personally, the author would like to publicly acknowledge all them again for their collaboration on this mini-review with their respective consents.*

References

Bonda, D. J., Lee, H. G., Camins, A., Pallàs, M., Casadesus, G., Smith, M. A., & Zhu, X. (2011). The sirtuin pathway in ageing and Alzheimer disease: mechanistic and therapeutic considerations. *The Lancet Neurology*, *10*(3), 275-279.

Camins, A., Sureda, F. X., Junyent, F., Verdaguer, E., Folch, J., Pelegri, C., … & Pallàs, M. (2010). Sirtuin activators: designing molecules to extend life span.*Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1799*(10), 740-749.

Caro, P., Gomez, J., Sanchez, I., Naudi, A., Ayala, V., López-Torres, M., … & Barja, G. (2009). Forty percent methionine restriction decreases mitochondrial oxygen radical production and leak at complex I during forward electron flow and lowers oxidative damage to proteins and mitochondrial DNA in rat kidney and brain mitochondria. *Rejuvenation research*, *12*(6), 421-434.

Corella, D., & Ordovás, J. M. (2014). Aging and cardiovascular diseases: The role of gene–diet interactions. *Ageing research reviews*, *18*, 53-73.

Csibi, A., Fendt, S. M., Li, C., Poulogiannis, G., Choo, A. Y., Chapski, D. J., ... & Henske, E. P. (2013). The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. *Cell*, *153*(4), 840-854.

Curatolo, P. (2015). Mechanistic target of rapamycin (mTOR) in tuberous sclerosis complex-associated epilepsy. *Pediatric neurology*, *52*(3), 281-289.

Ghosh, H. S., McBurney, M., & Robbins, P. D. (2010). SIRT1 negatively regulates the mammalian target of rapamycin. *PloS one*, *5*(2), e9199.

Hoppenbrouwers, S. S., Schutter, D. J., Fitzgerald, P. B., Chen, R., & Daskalakis, Z. J. (2008). The role of the cerebellum in the pathophysiology and treatment of neuropsychiatric disorders: a review. *Brain research reviews*, *59*(1), 185-200.

Huang, K., & Fingar, D. C. (2014, December). Growing knowledge of the mTOR signaling network. In *Seminars in cell & developmental biology* (Vol. 36, pp. 79-90). Academic Press.

Longo, V. D., & Mattson, M. P. (2014). Fasting: molecular mechanisms and clinical applications. *Cell metabolism*, *19*(2), 181-192.

Ma, L., Dong, W., Wang, R., Li, Y., Xu, B., Zhang, J., … & Wang, Y. (2015). Effect of caloric restriction on the SIRT1/mTOR signaling pathways in senile mice. *Brain research bulletin*, *116*, 67-72.

Marosi, K., & Mattson, M. P. (2014). BDNF mediates adaptive brain and body responses to energetic challenges. *Trends in Endocrinology & Metabolism*,*25*(2), 89-98.

Pani, G. (2015, April). Neuroprotective effects of dietary restriction: evidence and mechanisms. In *Seminars in cell & developmental biology* (Vol. 40, pp. 106-114). Academic Press.

Poulose, N., & Raju, R. (2015). Sirtuin regulation in aging and injury. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1852*(11), 2442-2455.

Radak, Z., Koltai, E., Taylor, A. W., Higuchi, M., Kumagai, S., Ohno, H., … & Boldogh, I. (2013). Redox-regulating sirtuins in aging, caloric restriction, and exercise. *Free radical biology and medicine*, *58*, 87-97.

Schutter, D. J. (2016). A cerebellar framework for predictive coding and homeostatic regulation in depressive disorder. *The Cerebellum*, *15* (1), 30-33.

Solon-Biet, S. M., McMahon, A. C., Ballard, J. W. O., Ruohonen, K., Wu, L. E., Cogger, V. C., ... & Gokarn, R. (2014). The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell metabolism*, *19*(3), 418-430.

Someya, S., Yu, W., Hallows, W. C., Xu, J., Vann, J. M., Leeuwenburgh, C. & Prolla, T. A. (2010). Sirt3 mediates reduction of oxidative damage and prevention of age-related hearing loss under caloric restriction. *Cell*, *143*(5), 802-812.

Speakman, J. R., & Mitchell, S. E. (2011). Caloric restriction. *Molecular aspects of medicine*, *32*(3), 159-221.

Swiech, L., Perycz, M., Malik, A., & Jaworski, J. (2008). Role of mTOR in physiology and pathology of the nervous system. *Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics*, *1784*(1), 116-132.

Tee, A. R., Sampson, J. R., Pal, D. K., & Bateman, J. M. (2016, April). The role of mTOR signalling in neurogenesis, insights from tuberous sclerosis complex. In *Seminars in cell & developmental biology* (Vol. 52, pp. 12-20). Academic Press.

Urbanska, M., Gozdz, A., Swiech, L. J., & Jaworski, J. (2012). Mammalian target of rapamycin complex 1 (mTORC1) and 2 (mTORC2) control the dendritic arbor morphology of hippocampal neurons. *Journal of Biological Chemistry*,*287*(36), 30240-30256.

Wang, P., Guan, Y. F., Du, H., Zhai, Q. W., Su, D. F., & Miao, C. Y. (2012). Induction of autophagy contributes to the neuroprotection of nicotinamide phosphoribosyltransferase in cerebral ischemia. *Autophagy*, *8*(1), 77-87.

Xiao, M. J., Han, Z., Shao, B., & Jin, K. (2009). Notch signaling and neurogenesis in normal and stroke brain. *International journal of physiology, pathophysiology and pharmacology*, *1*(2), 192.

Yang, F., Chu, X., Yin, M., Liu, X., Yuan, H., Niu, Y., & Fu, L. (2014). mTOR and autophagy in normal brain aging and caloric restriction ameliorating age-related cognition deficits. *Behavioural brain research*, *264*, 82-90.

Yuan, H. X., & Guan, K. L. (2016). Structural insights of mTOR complex 1. *Cell research*.