“RESTRICCIÓN CALÓRICA Y SU EFECTO SOBRE LAS CÉLULAS GLIALES ”

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Trabajo de Fin de Grado de Psicología

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Curso académico 2015-2016

ABSTRACT

The main objective of this review has been to investigate the relationship between glial cells and calorie restriction. What we found in current academic literature is that glial cells play a key role mediating inflammatory processes via cytokines and interleukins, preventing synthesis of lipopolysaccharides, mainly in hippocampal regions. Caloric restriction (CR) generally decreases inflammatory response, although it elicits it under Interferon-gamma (IFNγ) activity influence. This diet has also shown neuroprotective qualities in hippocampal glial cells, affecting GDNF. Glial cells, mediated by a calorie restriction, prevent glutamate dysregulation and help the CNS avoid its neurotoxic properties. Lastly, flavonoids, which can be found on cocoa or chocolate, also help in antiinflammatory responses on glial cells, CR-like effects, as well as working as a potent antioxidant.

Keywords Glial cells – Caloric Restriction – Inflammation – GDNF - Hippocampus

RESUMEN

El principal objetivo de esta revisión ha sido investigar la relación entre células gliales y restricción calórica. Se ha encontrado en la literatura académica actual que las células gliales juegan un papel vital en la mediación de procesos inflamatorios vía citoquinas e interleucinas, previniendo la síntesis de lipopolisacáridos, principalmente en regiones hipocampales. La restricción calórica (CR) reduce generalmente la respuesta inflamatoria, aunque la promueve bajo la influencia de la citoquina Interferón-gamma (IFNγ). Esta dieta también ha proporcionado propiedades neuroprotectoras en las células gliales del hipocampo, afectando al factor neurotrófico glial (GDNF). Las células gliales, bajo influencia de la restricción calórica, previenen la desregulación del glutamato, y ayudan al SNC a evitar sus propiedades neurotóxicas. Finalmente, los flavonoides, que pueden ser encontrados en el cacao o en el chocolate, ayudan también en la respuesta antiinflamatoria de las células gliales, un efecto similar al de la CR, además de funcionar como potentes antioxidantes.

Palabras Clave Células gliales – Restricción Calórica – Inflamación – GDNF - Hipocampo

INTRODUCTION

The main aim of this review is to revise actual literature concerning caloric restriction, as well as glial cells, and how they interact with each other in terms of a longer lifespan, quality of life or the aging process by itself. For that purpose, *Pubmed* database was consulted, as well as *Punto Q*, local web browser specialized in the search of academic information, such as articles, magazines, internet websites, and so on. *Google Scholar* was also consulted, and served as a great tool for finding some reviews nearly impossible to track down with other sources.

Although it is a relatively old topic, a diet with low calories and its positive effects have not been thoroughly studied up to date, and it is the purpose of this review to try and gather some of the information available about what has been studied before.

As a general overview to this way of eating, we can assert that caloric restriction is a low-caloric diet that has been proved to increase lifespan and reduce the incidence of age-related diseases across numerous species including primates (Weindruch and Walford, 1988; Colman et al., 2014). Furthermore, caloric restriction has shown to delay cellular death in animal models of neurodegenerative diseases (Maswood et al., 2004). It has continued to be studied to this very day, and the current size of literature concerning this matter makes it the more difficult to cope with the vast quantity of models, so on this review we are primarily focusing on mammal models.

Even in the XV century there had been quite some interest in what was thought to be a “healthy way of eating”, and to which extent this would affect the lifespan and the well-being. Taken as a clear example of this is the Italian Luigi Cornaro, an average man with a poor lifestyle that one day decided to change his habits by just altering the way in which he consumed food. He decided to eat 350 grams of food and 414 ml of wine every day of his life starting with circa 35 years, and he eventually grew up to live 98 years. This is but an example of the

benefits one can extract from a diet consisting on restriction of food intake, otherwise known as caloric restriction.

Health and science have evolved quite a lot in former decades on the other hand, and to prove that we have the increased lifespan we now have, having increased significantly over the past years, especially when compared with the former century. Nevertheless, this increased lifespan does not always necessarily come with a healthier lifestyle. In fact, aging is normally associated with an increased probability of having illnesses such as neurodegenerative diseases, obesity, diabetes type 2 or cancer (Redman and Ravussin, 2011).

Firstly defined by Neel as a decrease between 30%-60% of food intake without malnutrition, it is a non-genetic strategy that has proved to have beneficial metabolic effects, and has successfully increased the lifespan in all those organisms in which it has been proved, ranging from microorganisms to primates (Ortiz-Bautista, Aguilar-Salinas and Monroy-Guzmán, 2013). Such positive effects have been studied recently, in a conducted experiment that provides a good example of a caloric restricted diet and its beneficiary effect measured in rats, in variables such as food consumption, body weight and levels of blood glucose (Sharma and Kaur, 2008). Significant data has been found following this experiment, in which caloric restriction proved to significantly reduce the levels of all three variables, compared to ad libitum-fed rats (ad libitum meaning “at will”) (Sharma and Kaur, 2008).

However inspiring these results may be, it might be interesting to widen our scope and try to cover the topic of caloric restriction taking its impact not only in body weight, blood glucose or other metabolic variables, but in cognitive performance and brain functions. For that purpose we would like to assess glial cells, its implication on central nervous system processes and how can they be influenced by dietary restriction.

WHAT ARE GLIAL CELLS?

Though initially thought to have no function at all, glial cells are now demonstrated to be more than just the net that constitutes the central nervous system (López and Nieto-Sampedro, 2003). They outnumber neurons and play an important role in the central and peripheral nervous systems (Jessen, 2004).

Glial cells share more than just common environment with neurons, as glial and neuronal cells have both a mutual embryonic origin: they derive from the ectodermic neural epithelium that differentiates either into neuroblasts and, subsequently, into adult neurons, or into spongioblasts and, subsequently into adult astrocytes and oligodendrocytes (Timiras, Yaghmaie, Saeed, Thung and Chinn, 2005). The fact that glial cells and neuron share a common origin, deriving both directly from neural epithelium will imply a series of processes that will be commented later on this review.

Neuroglia is believed to be possibly activated via EGF (Epidermal Growth Factor, natural substance that enhances the development and growth of new cells) to promote de-differentiation of the adult cells into multipotent precursor cells, eventually capable of differentiating into neuroblasts (Tsonis, 2004). Some of these studies are investigating the structural and neurochemical characteristics of these glial cells to determine whether they have or not been transformed into neuroblasts and these later on changed into neurons (extracted from Tsonis, 2004).

Studies carried out by Newman have shown that glial cells are mainly taking part in two different processes (Newman, 2003). Neurotransmitters released from pre-synaptic neurons involving Ca2+ are found in greater concentration in adjacent glia. Activated glia also releases ATPases and neurotransmitters such as glutamate. Therefore, glial cells are now considered to be an active part in the synaptic transmission and in the neurotransmitters' regulation process.

Inside glial cells, we have different types of cells, being the astrocytes the most common and notably present in the whole central nervous system (Newman, 2003). These astrocytes are sometimes specialized, as in the cerebellum, in which they cover and envelop synapsis (called Bergmann glia) or helping contacting synapsis in the retina in a radial glia known as Müller cells.

We also have oligodendrocytes, the second most common type of glial cells, whose principal function is to form the myelin sheath, isolating the tissue around the nerve fibers, making the electrical synapsis possible.

Astroglia is also known to be involved in neural plasticity and injury processes, including glutamate uptake, glutamine synthetase (GS) and S100B protein synthesis (Ribeiro et al., 2009). The implication that this process has, and its influences on the central nervous system will be more thoroughly discussed on the “relationship between caloric restriction and glial cells” section.

Finally, the CNS also has the microglia, macrophage cells originated from blood monocytes (Jessen, 2004).

There is actually some debate on the scientific community concerning the role of microglia, inasmuch as, although microglial activation represents an integral part of the CNS response to injury, it is still not clear whether activated microglia promote neuronal survival, or whether these cells further exacerbate the extent of neuronal damage., a large body of data has rather convincingly shown that microglia possess neurotoxic properties, that openly contradict the believed supportive role for microglial cells in the induction of neuroplastic changes after ischemia, stroke or another brain accident (Brown and Neher, 2010).

We also have Ependymal cells, which take part in the synthesis of cerebrospinal fluid, and are found on the spinal cord and in the ventricular system of the brain.

Nevertheless, we also find glial cells outside the central nervous system, in the Schwann cells which take part in neuromuscular junctions as part of the peripheral nervous system (Newman, 2003), as well as satellite cells that also take part in both pathetic and sympathetic activities on the peripheral nervous system.

See the table below for an overview of the different types of glial cells.

Types of Glial Cells		
Central Nervous System (CNS)		***Peripheral Nervous System (PNS)***
Microglia	*Astrocytes*	*Satellite Cells*
Oligodendrocytes	*Ependymal Cells*	*Schwann Cells*

RELATIONSHIP BETWEEN CALORIC RESTRICTION AND GLIAL CELLS

Being known and mainly related with the brain in supporting the neurons and supplying them with nutrients and oxygen, as well as destroying pathogens, it is no surprise that glial cells are directly and strongly related with the whole process of aging, neuron apoptosis, and healthy lifespan, as has been said by Lee and Longo and other authors numerous times:

“Caloric restriction is the most effective and reproducible dietary intervention known to regulate aging and increase the healthy lifespan in various model organisms, ranging from the unicellular yeast to worms, flies, rodents, and primates” (Lee and Longo, 2016)

“Caloric restriction affects several nerve growth factors, taking for instance those controlled by the glial cell line-derived neurotrophic factor (GDNF), most prominently in the hippocampal formation, but also in the basal ganglia” (Lee,

Duan, Long, Ingram and Mattson, 2000; Lee, Seroogy and Mattson, 2002; Duan et al., 2003; Maswood et al., 2004; Thrasivoulou et al., 2006).

Glial cell line-derived neurotrophic factor, abbreviated as GDNF, plays a fundamental role on the conservation of neurons in the central nervous system. It has been defined as a protein that promotes the survival and morphological differentiation of dopaminergic neurons and increases their dopamine uptake (Lin, Doherty, Lile, Bektesh and Collins, 1993). However, this mentioned effects were relatively specific; GDNF did not increase total neuron or astrocyte numbers, but their ability to assimilate dopamine. GDNF may have utility in the treatment of Parkinson's disease, which is marked by progressive degeneration of midbrain dopaminergic neurons (Lin et al., 1993).

A ketogenic diet (a way of eating primarily associated with the treatment of epilepsy) is also known to enhance proliferation of glial cells in the CA3 region of the hippocampus (Silva et al., 2005). However, this observed increment of glial cells throughout this region is not related with any functional deficits, but as a conclusion of the aforementioned diet.

Another positive effect that this kind of diet may have on the hippocampus, aside from attenuating such various illnesses as obesity or Parkinson’s disease (Maswood et al., 2004) would also be preventing lipopolysaccharide microglial activation in the hippotalamus (Radler, Hale and Kent, 2014), that is often paired with neurotoxic properties of some elements, as well as activation of protein P53, or can also be seen as a secondary effect from glutamate dysregulations on the brain. Both themes will be commented further, both on Davenport and Ribeiro theories.

Furthermore, a low-fat diet, which normally involves caloric restriction, can have two different impacts on the inflammatory response in the brain:

On the one hand, it is commonly known for improving mitochondrial function, and for the regulation of gene expression, which results in decreasing activity of pro-apoptotic and inflammatory factors, and elicits the synthesis of neuroprotective factors such as neurotrophins (Maalouf, Rho and Mattson, 2009). This is but another positive effect of this particular type of diet, which is in this case believed to have a positive effect on defensive responses on the brain, and mobilization and supervision of glial cells.
On the other hand, it can also have a completely different influence, increasing inflammatory response in the brain, activating a superior number of glial cells, by means of Interferon-gamma (IFNγ) activity (Maalouf et al., 2009; Mascarucci et al., 2002; Lee, Kim, Son, Chan and Mattson, 2006). This is a particular case of inflammatory response in the brain influenced indirectly by caloric restriction, via glial cells (that are as well influenced by IFNγ).

As has been said on previous pages, astroglial cells are responsible for major glutamate transport and for regulating extracellular levels of glutamate (Hertz, 2006). Glutamate is the major excitatory neurotransmitter in the central nervous system and its accumulation is implicated in neurodegenerative disorders (Ribeiro et al., 2009). Its high release or failure in its uptake by astrocytes can lead to excessive and prolonged increases in intracellular free calcium (Ca++) and sodium (Na+), yielding excitotoxicity and often brain cell death by necrosis (Matute, Domercq and Sanchez-Gomez, 2006). Thus, malfunction of astrocytic glutamate transporters will lead to an excessively high extracellular glutamate concentration which may result in neurodegeneration caused by the excitotoxic action of glutamate (Schousboe and Waagepetersen, 2005).

It could also be relevant and interesting to study the possible effect that flavonoids may have on the nervous system. Cocoa products and chocolate have recently been recognized as a rich source of flavonoids, mainly flavanols, potent antioxidant and anti-inflammatory agents with established benefits for cardiovascular health but largely unproven effects on neurocognition and behavior (Sokolov, Pavlova, Klosterhalfen and Enck, 2013). Flavonoids

comprise the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants. Major dietary sources of flavonoids include fruits, vegetables, cereals, tea, wine, and fruit juices (Manach, Scalbert, Morand, Remesy and Jimenez, 2004).

This aforementioned ability of flavonoids to improve neurological health by helping eliciting antioxidants and anti-inflammatory agents seems to be related to their ability to interact with intracellular neuronal and glial signaling pathways, to influence the peripheral and cerebral vascular system, and to reduce neuronal damage induced by various neurotoxic species and neural inflammation. This inflammation is led by the very microglia, that induces detrimental neurotoxic effects by releasing a diverse set of cytotoxic substances, including proinflammatory cytokine TNF-a (Neumann, 2001), which plays a key role in many physiological and pathological processes including acute and chronic inflammation, and apoptosis (Tuttolomondo et al., 2009).

Flavonoids are also able to exert neuroprotective actions (at low, physiological concentrations) via interactions with critical neuronal and glial intracellular signaling pathways of key importance in controlling neuronal resistance to neurotoxins, including oxidants and inflammatory mediators, neuronal differentiation, long-term potentiation, and memory (Spencer, 2007).

With the whole process of aging, and especially during senescence, the number of neuroglial cells, more particularly astrocytes, increases. The consequent gliosis is viewed as a compensatory response to the progressive functional impairment of neuronal function associated with aging (Brizzee, Sherwood and Timiras, 1968; Brizzee, Cancilla, Sherwood and Timiras, 1969; Bronson, Lipman and Harrison, 1993). Thus, a low-fat diet would improve the significance of this entire process by improving the quality of such mechanism, for instance, activating a superior number of glial cells, or by positively mediating in this activity via GDNF.

Another aspect that would be of interest to explore would be the effect a dietary restriction could have on protein p53, which plays a role in vascular endothelial cell apoptosis and microglia activation (Stempien-Otero et al., 1999).

It is for that relevant to know that p53 expression increased after microglia activation, and blockage of p53 significantly prevented microglia-mediated neurotoxicity (Davenport, Sevastou, Hooper and Pocock, 2010). This increasing p53 expression also leads to accelerated cell death after hypoxia. In fact, 8 in vitro studies have shown how p53 expression was increased after activation of primary rat microglia, and blockage of p53 prevented microglia neurotoxicity (Davenport et al., 2010).

This can be graphically seen on figure 1 below

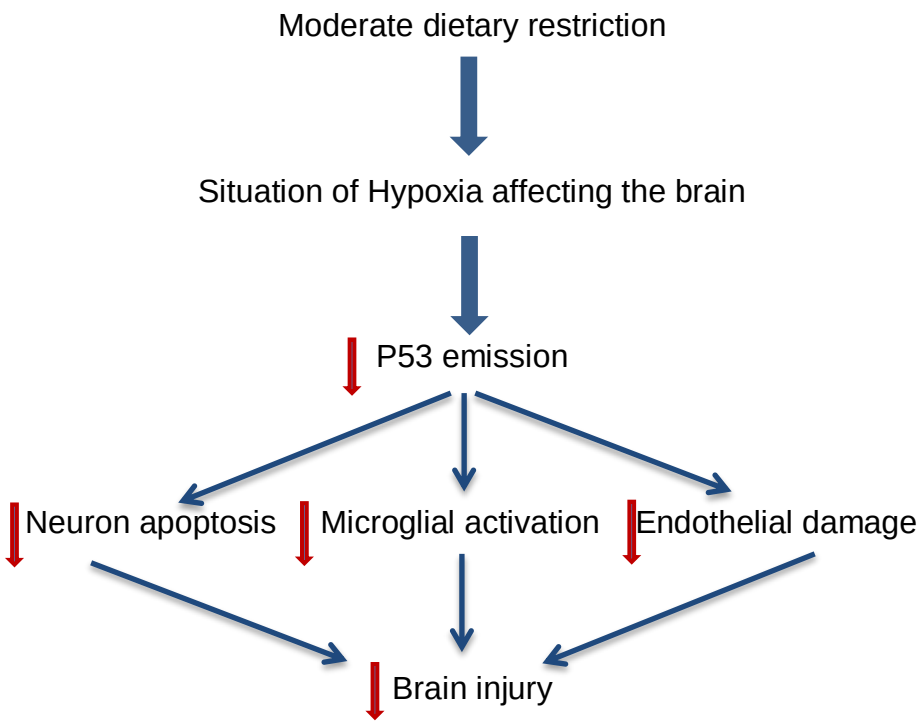

Figure 1

Figure 1 shows how a moderate dietary restriction positively affects brain on an hypoxic situation, specifically decreasing the amount of tumor protein 53 (TP53, or simply P53), and thus mediating on a less quantity of damage on neurons, glial cells

and endothelial tissues, eventually resulting on lesser brain injury (Simplified figure based on Tu, Lu, Huang, Ho and Chou, 2012)

These findings proved how p53 is also involved in endothelial cell injury and microglia activation after brain damage, in this case, as a result of a temporary hypoxia.

Following these findings, but taking microglial activation now into account, it has been found that activation of microglia exacerbates neuronal damage, and inhibiting microglial activation reduces injury (Yenari, Xu, Tang, Qiao, Giffard, 2006). This is supported by the discoveries made by Tu et al. (2012), as has been seen on figure 1 above, in which a higher microglial cells activation is directly related, among other processes (neuron apoptosis and endothelial damage) to a greater amount of injuries on the brain, as could have been done by a more notable, well-known neurotoxic factor as protein P53.

Another field covered in former research of caloric restriction and glial cells support us with relevant new data concerning possible benefits that a diet low in calories paired with physical training and exercise may have on our organism. As an interesting paper has shown, “Exercise training, caloric restriction and both combined can decrease oxidative damage in the hippocampus, possibly involving modulation of astroglial function, and could be used as a strategy for the prevention of neurodegenerative diseases” (Santin et al., 2011). This is related with former investigations by Silva and his group, in which a ketogenic diet was related with a proliferation of glial cells in the CA3 region of the hippocampus was noted, but without having any relation with cognitive impairment (Silva et al., 2005).

Another perspective from which caloric restriction has been covered, and that could be of interest in this current review, would be the neurobiological evidence of the correct development and realization of central nervous system’s

functions. For that, neural cell adhesion molecule (NCAM), a neuronal plasticity marker, has been taken into account. Some research has confirmed how a dietary restriction attenuates glial fibrillary acidic protein (GFAP) and enhances altogether levels of neuronal plasticity marker NCAM (Sharma and Kaur, 2008). Trying to explain neural impairment by this scope could be of relevance by knowing how does GFAP interact with the normal, or in this case abnormal, functioning of the central nervous system.

What we know now concerning this matter is that any brain injury is known to exert a glial reaction, which involves the conversion of protoplasmic astrocytes of the gray matter into reactive astrocytes, positive for glial fibrillary acidic protein (GFAP) (Miyazaki et al., 2003). Recent evidences suggest that GFAP markers significantly increase during reactive gliosis, when astrocytes undergo such changes on its normal state as both hypertrophy and hyperplasia (Miyazaki et al., 2003).

Results presented in an experiment carried by Loncarevic-Vasiljkovic et al. showed that, even though a total number of microglial cells around the site of lesion increase significantly in both AL (feed “Ad Libitum”, that is, eating as much quantity of food as at will) and CR (group under “Caloric Restriction” condition) group, the morphology of these cells is strikingly different. Namely, the majority of microglial cells seen early after injury in AL animals displayed large round cell bodies on recovery time, whereas in the group of animals exposed to CR, the microglial cells surrounding the lesion site maintained ramified morphology during the entire recovery period (Loncarevic-Vasiljkovic et al., 2012).

What this result implies is the sustentation of previous findings that indicate that microglial activation and recruitment, rather than proliferation, mediate neurodegeneration following injury (Rogove, Lu and Tsirka, 2002). The role that caloric restriction plays in this theory is direct, since it proved to be neuroprotective by preventing neurons from secondary cell death after injury.

We refer here to secondary injury as it is straightforwardly derived from primary injury that is the direct, mechanical damage to brain tissue that results in the irreversible destruction of neural structures (Loncarevic-Vasiljkovic et al., 2012). However grave this primary lesion may be to the central nervous system, its damage is utterly augmented by the pathological processes that come together with the secondary injury, that evolves over an undetermined period of time that can vary from minutes to weeks following initial trauma (Sande and West, 2010), and that consists of an amount of inflammatory processes that lead to neuroapoptosis (Veenith, Goon and Burnstein, 2009), in which glial cell activation around the injured area plays a major role, as has been discussed above.

Cytokines and interleukins (mainly IL-10) also play a role in the inflammatory response, as activated microglial cells are a predominant source of proinflammatory cytokines (Hanisch, 2002). For example, as has been said above by Neumann (2001), “In response to CNS injury, microglia become active and induce detrimental neurotoxic effects by releasing a diverse set of cytotoxic substances, including proinflammatory cytokine TNF-a“. Given that TNF-a represents one of the main pro-inflammatory cytokines involved in the expansion of a secondary injury, abolishment of TNF-a protein expression following injury may reduce the extent of secondary injury (Loncarevic-Vasiljkovic et al., 2012).

What is more, caloric restriction also suppresses lipopolysaccharide (LPS)-induced release of pro-inflammatory cytokines, therefore blocking LPS-induced fever and shifting hypothalamic signaling pathways to an anti-inflammatory response. This is made by attenuating microglial activation in the hypothalamic arcuate nucleus (ARC), a brain region containing neurons that synthesize neuropeptide Y (NPY), which has anti-inflammatory properties regulated by a calorie restriction (Radler, Wright, Walker, Hale and Kent, 2015).

It has been demonstrated how CR increases hypothalamic expression of anti-inflammatory signaling molecules including: suppressor of cytokine signaling, interleukin (IL)-10, and neuropeptide Y (NPY) (MacDonald, Radler, Paolini and Kent, 2011). Even though these data suggest that CR may alter immune-system function through decreasing pro-inflammatory signaling and enhancing anti-inflammatory signaling, the effects of CR on neuroinflammatory processes remain relatively unexplored (Radler et al., 2014).

CONCLUSION

Although we know quite a lot about brain and how a lot of its mechanisms actually work, we might as well point out that a lot more study is needed in this field. A lot of works on glial cells have been conducted up to this date, but the relationship between this particular cells and a diet based on caloric restriction is not, unfortunately, clearly known. Up to this point numerous investigations have discovered some positive effects for a low-fat diet and its effect on glial cells, such as regulation of glutamate levels, mediating the nerve-growth factor or the reduction of neuronal impairment with age, but unfortunately we cannot establish further relationships between the two. The effects of physical exercise, GDNF, protein P53, flavonoids or microglial activation have been to some extent studied, but there is still a lot to know. Furthermore, most of these elements have been studied in mammal models, mainly rodents. Thus, a more comprehensive point of view trying to comprise more species in the study of both these neurological processes and the way in which how and what is eaten influences them would be very relevant to try to extrapolate those results found to humans.

Hence, the recommendation extracted from this analysis would be to encourage future research upon the field of caloric restriction and its effects on the whole nervous system, to a wider extent than just glial cell (that are, of course, of major importance on the nervous system, but so are another matters, such as neurogenesis, mTOR or epigenetics), so as to accurately portray what we have always strived for comprehending, the human brain.

REFERENCES

Brizzee, K.R., Sherwood, N., & Timiras, P.S. (1968). *A comparison of cell population at various depth levels in cerebral cortex of young adult and aged Long-Evans rats. J. Gerontol. 23*, 289–297.

Brizzee, K.R., Cancilla, P.A., Sherwood, N., & Timiras, P.S. (1969). *The amount and distribution of pigments in neurons and glia of the cerebral cortex. Autoflourescent and ultrastructural studies. J. Gerontol. 24*, 127– 135.

Bronson, R.T., Lipman, R.D., & Harrison, D.E. (1993). *Age-related gliosis in the white matter of mice. Brain Res. 609*, 124–128.

Brown, G.C., Neher, J.J. (2010). *Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Mol Neurobiol. 41*: 242–247.

Colman, R.J., Beasley, T.M., Kemnitz, J.W., Johnson, S.C., Weindruch, R., & Anderson, R.M. (2014). *Caloric restriction reduces age-related and all-cause mortality in rhesus monkeys. Nat Commun 5*:3557.

Davenport, C.M., Sevastou, I.G., Hooper, C., & Pocock, J.M. (2010). *Inhibiting p53 pathways in microglia attenuates microglial-evoked neurotoxicity following exposure to Alzheimer peptides. J Neurochem.112*: 552–563.

Duan, W., Guo, Z., Jiang, H., Ware, M., Li, X.J., & Mattson, M.P. (2003). *Dietary restriction normalizes glucose metabolism and BDNF levels, slows disease progression, and increases survival in huntingtin mutant mice*. Proc. Natl. Acad. Sci. U. S. A. 100, 2911–2916.

Hanisch, U.K. (2002). *Microglia as a source and target of cytokines. Glia 40(2):* 140–155.

Hertz, L. (2006). *Glutamate, a neurotransmitter—and so much more. A synopsis of Wierzba III. Neurochem. Int. 48*, 416–425.

Jessen, K. R. (2004). Glial cells. *The international journal of biochemistry & cell biology*, *36*(10), 1861-1867.

Lee, J., Duan, W., Long, J.M., Ingram, D.K., & Mattson, M.P. (2000). *Dietary restriction increases the number of newly generated neural cells, and induces BDNF expression, in the dentate gyrus of rats*. *J. Mol. Neurosci. 15*, 99–108.

Lee, C., & Longo, V. (2016). *Dietary restriction with and without caloric restriction for healthy aging*. *F1000Research*, *5*.

Lee, J., Seroogy, K.B., & Mattson, M.P. (2002). *Dietary restriction enhances neurotrophin expression and neurogenesis in the hippocampus of adult mice*. J. Neurochem. 80, 539–547.

Lee, J., Kim, S.J., Son, T.G., Chan, S.L., & Mattson, M.P. (2006). *Interferon-gamma is up-regulated in the hippocampus in response to intermittent fasting and protects hippocampal neurons against excitotoxicity. J. Neurosci. Res. 83*, 1552–1557.

Lin, L. F., Doherty, D. H., Lile, J. D., Bektesh, S., & Collins, F. (1993). *GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons.* Science*, 260*(5111), 1130-1132.

López, B. C., & Nieto-Sampedro, M. (2003). *Glial cell function* (Vol. 132). Gulf Professional Publishing.

Loncarevic-Vasiljkovic, N., Pesic, V., Todorovic, S., Popic, J., Smiljanic, K., Milanovic, D. & Kanazir, S. (2012). *Caloric restriction suppresses*

microglial activation and prevents neuroapoptosis following cortical injury in rats. PloS one, 7(5).

Maalouf, M., Rho, J. M., & Mattson, & M. P. (2009). *The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies. Brain research reviews, 59(2),* 293-315.

MacDonald, L., Radler, M., Paolini, A.G., & Kent, S. (2011). *Calorie restriction attenuates LPS-induced sickness behavior and shifts hypothalamic signaling pathways to an anti-inflammatory bias. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301 (1)*, R172–184.

Manach, C., Scalbert, A., Morand, C., Remesy, C., & Jimenez, L. (2004). *Polyphenols: food sources and bioavailability. Am. J. Clin. Nutr. 79*:727–747.

Mascarucci, P., Taub, D., Saccani, S., Paloma, M.A., Dawson, H., Roth, G.S., Lane, M.A., & Ingram, D.K. (2002). *Cytokine responses in young and old rhesus monkeys: effect of caloric restriction. J. Interferon Cytokine Res. 22*, 565–571.

Maswood, N., Young, J., Tilmont, E., Zhang, Z., Gash, D.M., Gerhardt, G.A., Grondin, R., Roth, G.S., Mattison, J., Lane, M.A., Carson, R.E., Cohen, R.M., Mouton, P.R., Quigley, C., Mattson, M.P., & Ingram, D.K. (2004). *Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease*. *Proc. Natl. Acad. Sci. U. S. A. 101*, 18171–18176.

Matute, C., Domercq, M., & Sanchez-Gomez, M.V. (2006). *Glutamate-mediated glial injury: mechanisms and clinical importance. Glia 53*, 212–224.

Miyazaki, T., Miyamoto, O., Janjua, N. A., Hata, T., Takahashi, F., & Itano, T. (2003). Reactive *gliosis in areas around third ventricle in association with epileptogenesis in amygdaloid-kindled rat. Epilepsy Res 56*:5–15.

Neumann, H. (2001) *Control of glial immune function by neurons. Glia 36(2):* 191–199.

Newman, E. A. (2003). *New roles for astrocytes: regulation of synaptic transmission*. *Trends in neurosciences*, *26*(10), 536-542.

Ortiz-Bautista, R. J., Aguilar-Salinas, C. A., & Monroy-Guzmán, A. (2013). *Restricción calórica: efectos metabólicos positivos e impacto celular*. *Cir Cir*,*81*, 459-464.

Radler, M. E., Hale, M. W., & Kent, S. (2014). *Calorie restriction attenuates lipopolysaccharide (LPS)-induced microglial activation in discrete regions of the hypothalamus and the subfornical organ. Brain, behavior, and immunity*, *38*, 13-24.

Radler, M. E., Wright, B. J., Walker, F. R., Hale, M. W., & Kent, S. (2015). *Calorie restriction increases lipopolysaccharide-induced neuropeptide Y immunolabeling and reduces microglial cell area in the arcuate hypothalamic nucleus. Neuroscience, 285*, 236-247.

Redman, L.M., & Ravussin, E. (2011). *Caloric Restriction in Humans: Impact on Physiological, Psychological, and Behavioral Outcomes. Antioxidant Redox Signal 14*:275-287.

Ribeiro, L. C., Quincozes-Santos, A., Leite, M. C., Abib, R. T., Kleinkauf-Rocha, J., Biasibetti, R. & Gottfried, C. (2009). *Caloric restriction increases hippocampal glutamate uptake and glutamine synthetase activity in Wistar rats.Neuroscience research*, *64*(3), 330-334.

Rogove, A.D., Lu, W., & Tsirka, S.E.(2002). *Microglial activation and recruitment, but not proliferation, suffice to mediate neurodegeneration. Cell Death Differ 9(8)*: 801–806.

Sande, A., & West, C. (2010). *Traumatic brain injury: a review of pathophysiology and management. J Vet Emerg Crit Care (San Antonio) 20(2)*: 177–190.

Santin, K., da Rocha, R. F., Cechetti, F., Quincozes-Santos, A., de Souza, D. F., Nardin, P. & Gonçalves, C. A. (2011). *Moderate exercise training and chronic caloric restriction modulate redox status in rat hippocampus. Brain Research*, *1421*, 1-10.

Schousboe, A., & Waagepetersen, H.S. (2005). *Role of astrocytes in glutamate homeostasis: implications for excitotoxicity. Neurotox. Res. 8*, 221–225.

Sharma, S., & Kaur, G. (2008). *Dietary restriction enhances kainate-induced increase in NCAM while blocking the glial activation in adult rat brain*.*Neurochemical research*, *33*(7), 1178-1188.

Silva, M.C., Rocha, J., Pires, C.S., Ribeiro, L.C., Brolese, G., Leite, M.C., Almeida, L.M., Tramontina, F., Ziegler, D.R., & Gonçalves, C.A. (2005). *Transitory gliosis in the CA3 hippocampal region in rats fed on a ketogenic diet. Nutr. Neurosci. 8*, 259–264.

Sokolov, A. N., Pavlova, M. A., Klosterhalfen, S., & Enck, P. (2013). *Chocolate and the brain: neurobiological impact of cocoa flavanols on cognition and behavior. Neuroscience & Biobehavioral Reviews*, *37*(10), 2445-2453.

Spencer, J.P. (2007). *The interactions of flavonoids within neuronal signalling pathways. Genes Nutr. 2*:257–273.

Stempien-Otero, A., Karsan, A., Cornejol, C.J., Xiang, H., Eunson, T., & Morrison, R.S. (1999). *Mechanisms of hypoxia-induced endothelial cell death. Role of p53 in apoptosis. J Bio Chem. 19*:8039–8045.

Thrasivoulou, C., Soubeyre, V., Ridha, A., Giuliani, D., Giaroni, C., Michael, G.J., Saffrey, M.J., & Cowen, T. (2006). *Reactive oxygen species, dietary restriction and neurotrophic factors in age-related loss of myenteric neurons*. Aging Cell 5, 247–257.

Timiras, P. S., Yaghmaie, F., Saeed, O., Thung, E., & Chinn, G. (2005). *The ageing phenome: caloric restriction and hormones promote neural cell survival, growth, and de differentiation*. *Mechanisms of ageing and development*, *126*(1), 3-9.

Tsonis, P.A. (2004). Stem cells from differentiated cells. Mol. Interventions 4, 81–83.

Tu, Y. F., Lu, P. J., Huang, C. C., Ho, C. J., & Chou, Y. P. (2012*). Moderate dietary restriction reduces p53-mediated neurovascular damage and microglia activation after hypoxic ischemia in neonatal brain. Stroke, 43*(2), 491-498.

Tuttolomondo, A., Di Sciacca, R., Di Raimondo, D., Renda, C., Pinto, A., & Licata, G. (2009) *Inflammation as a therapeutic target in acute ischemic stroke treatment. Curr Top Med Chem 9(14):* 1240–1260.

Veenith, T., Goon, S.S.H., & Burnstein, R.M. (2009). *Molecular mechanisms of traumatic brain injury: the missing link in management. World J Emerg Surg 2*: 4–7.

Weindruch, R., & Walford, R.L. (1988). *The retardation of aging and disease by dietary restriction. Springfield,* IL: Charles C. Thomas.

Yenari, M.A., Xu, L., Tang, X.N., Qiao, Y., & Giffard, R.G. (2006). *Microglia potentiates damage to blood– brain barrier constituents: Improvement by minocycline in vivo and in vitro. Stroke 37*:1087–1093.