The neuroprotective properties of calorie restriction, the ketogenic diet, and ketone bodies

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ABSTRACT

Both calorie restriction and the ketogenic diet possess broad therapeutic potential in various clinical settings and in various animal models of neurological disease. Following calorie restriction or consumption of a ketogenic diet, there is notable improvement in mitochondrial function, a decrease in the expression of apoptotic and inflammatory mediators and an increase in the activity of neurotrophic factors. However, despite these intriguing observations, it is not yet clear which of these mechanisms account for the observed neuroprotective effects. Furthermore, limited compliance and concern for adverse effects hamper efforts at broader clinical application. Recent research aimed at identifying compounds that can reproduce, at least partially, the neuroprotective effects of the diets with less demanding changes to food intake suggests that ketone bodies might represent an appropriate candidate. Ketone bodies protect neurons against multiple types of neuronal injury and are associated with mitochondrial effects similar to those described during calorie restriction or ketogenic diet treatment. The present review summarizes the neuroprotective effects of calorie restriction, of the ketogenic diet and of ketone bodies, and compares their putative mechanisms of action.

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1. Introduction

The anticonvulsant properties of fasting have been recognized since antiquity, strongly suggesting that fasting, and more generally, calorie restriction probably represents the first effective treatment for epileptic seizures in medical history. In addition, more recent evidence suggests that the benefits of calorie restriction, elicited either by daily reduction of energy intake or by intermittent fasting, are not limited to epilepsy, and might in fact include a generalized neuroprotective effect applicable to many acute and chronic neurological diseases. In view of the present obesity epidemic, however, the scientific and medical communities have realized that calorie restriction is often not a practical treatment option. Similarly, the ketogenic diet, a high-fat, low-carbohydrate diet designed to reproduce the biochemical effects of fasting, has been challenging to use in clinical settings despite proven efficacy. Consequently, there is now considerable effort expended to understand the mechanisms underlying the neuroprotective effects of calorie restriction and of the ketogenic diet with the hope of developing alternative therapeutic options. The present article reviews findings supporting the neuroprotective effects of calorie restriction and of the ketogenic diet, summarizes the mechanisms activated by these diets and proposes that ketone bodies could possibly replicate some of their neuroprotective properties by activating common mechanisms at the mitochondrial level.

2. Calorie restriction

2.1. Neuroprotective effects of calorie restriction

2.1.1. Human studies of calorie restriction

Obesity is associated with an increased risk of dementia (Kivipelto et al., 2005). On imaging studies, decreased hippocampal volume and increased white matter hyperintensities—two radiological hallmarks of pathological brain aging—are more common in obese patients (Jagust et al., 2005). In contrast, apart from the well-known effects of fasting on seizure frequency, low dietary energy intake is associated with enhanced longevity (Redman et al., 2008) and decreased incidence of Alzheimer’s and Parkinson’s diseases (e.g., the New York City cohort). Further, calorie restriction for 6 months improves biomarkers associated with longevity, including reduced fasting insulin levels, body temperature and DNA damage (Luchsinger et al., 2002; Heilbronn et al., 2006). Calorie restriction might even reduce disease risk and increase lifespan in normal weight subjects (Johnson et al., 2006). Beneficial effects on mental healthcare reported as well, with improved mood following calorie restriction of obese diabetic patients (Wing et al., 1991). To date, however, clinical trials looking at the effects of calorie restriction on brain aging and neurological disease have not been performed, and all available information is derived exclusively from animal studies.

2.1.2. Animal studies of calorie restriction

Calorie restriction prolongs the lifespan of yeast, roundworms, rodents and monkeys, even when initiated in midlife (Means et al., 1993; Mattson, 2003; Bordone and Guarente, 2005; Guarente and Picard, 2005). Moreover, age-related deficits in learning and motor coordination are diminished by calorie restriction in rodents (Mattson et al., 2003; Mattson and Magnus, 2006). Rats placed on a hypo-caloric diet from the age of 3 weeks, and tested at 2 years of age, perform significantly better than aged-matched controls fed ad libitum on both spatial (i.e., Morris Water Maze, spatial version of the 8-arm radial maze) and non-spatial (non-spatial version of the 8-arm radial maze) learning tasks (Pitsikas et al., 1990; Pitsikas and Algeri, 1992). Calorie-restricted middle-aged and aged mice exhibit similar improvements in learning tasks that also include active and passive avoidance learning (Ingram et al., 1987; Means et al., 1993; Hashimoto and Watanabe, 2005). In parallel, calorie restriction also prevents age-related deficits in hippocampal long-term potentiation (LTP), a cellular correlate of memory (Hori et al., 1992; Eckles-Smith et al., 2000; Okada et al., 2003). In addition to effects on aging, calorie restriction appears beneficial in several models of neurological disease, most notably epilepsy. In EL mice—a mixed genetic-environmental model of stimulus-induced epilepsy, the onset of seizures...
typically occurs in the first few months of life, but is delayed for several weeks by calorie restriction (Greene et al., 2001; Mantis et al., 2004). Calorie restriction also elevates the threshold to seizures elicited by tail-vein infusion of pentyle-nitrazolax, a γ-aminobutyric acid$_A$ (GABA$_A$) receptor antagonist (Eagles et al., 2003). Consistently, rats on a calorie-restricted diet exhibit reduced excitability in the dentate gyrus, as evidenced by higher paired-pulse inhibition and increased threshold, latency and duration of electrophysic seizures following maximal dentate gyrus activation by angular bundle stimulation (Bough et al., 2003). Finally, intermittent fasting prevents spatial learning deficits in rats exposed to excitotoxic injury, and improved cognitive function correlates with decreased neuronal death in the hippocampus (Bruce-Keller et al., 1999).

In animal models of Parkinson’s disease, calorie restriction improves motor function and enhances neuronal survival in the substantia nigra of mice and monkeys exposed to MPTP, a neurotoxin converted to MPP$^+$ in astrocytes. Subsequently, MPP$^+$ is transported into dopaminergic neurons, where it inhibits NADH dehydrogenase and increases reactive oxygen species (ROS) formation at complex I of the mitochondrial respiratory chain (Duan and Mattson, 1999; Maswood et al., 2004). A comparable, neuroprotective effect has been reported in the striatum of mice treated with 3-nitropropionic acid, a succinate dehydrogenase (complex II) inhibitor that causes motor and histological defects similar to those seen in Huntington’s disease (Bruce-Keller et al., 1999). Calorie restriction also attenuates amyloid deposition in monkeys and in transgenic mouse models of Alzheimer’s disease (Patel et al., 2004; Qin et al., 2006a,b), ameliorates cognitive deficits in a mouse model of Alzheimer’s disease (Halagappa et al., 2007), and reduces neuronal loss in neocortex, hippocampus and striatum of rats subjected to a 30-minute, cerebral four-vessel occlusion model of ischemic stroke (Marie et al., 1990). Similarly, feeding rats on alternate days decreases infarct size and improves motor function following middle cerebral artery occlusion for 1 h (Yu and Mattson, 1999). Finally, calorie restriction decreases neuronal loss and improves functional recovery following traumatic brain or spinal cord lesions, even when started after 24 h after the injury (Davis et al., 2008; Plunet et al., in press).

Although calorie restriction appears to exert beneficial effects in most studies of aging and neurological disease, an absence of such clinical effects (and complications) is reported. First, several studies have failed to reveal any influence of calorie restriction on spatial learning in both rats and mice (Bellush et al., 1996; Markowska, 1999; Hansalik et al., 2006). One study in rats actually found a worsening of cognitive function despite increased longevity (Yanai et al., 2004). Interestingly, cognitive deficits improved with glucose administration. Second, APP transgenic mice became hypoglycemic and died prematurely (within 2–3 weeks) despite a decrease in amyloid deposition (Pedersen et al., 1999). Third, in mice expressing the G93A familial amyotrophic lateral sclerosis (ALS) mutation, age of onset of paralysis was unaffected, and the disease progressed at a faster rate (Pedersen and Mattson, 1999). Reasons behind these discordant findings are not readily apparent, but some studies have suggested that genetic variance among species and amongst the different strains within a single species might influence differential responses to calorie restriction (Willott et al., 1995; Markowska and Savonenko, 2002; Mockett et al., 2006). Additional research is required to identify the factors that determine responsiveness to calorie restriction.

### 2.2. Neuroprotective mechanisms of calorie restriction

There are several mechanisms proposed to explain the neuroprotective effects of calorie restriction (Fig. 1). These mechanistic theories can be grouped into two general categories: 1) improved mitochondrial function, leading to decreased production of ROS and increased energy output; 2) regulation of gene expression, resulting in decreased activity of pro-apoptotic and inflammatory factors and increased levels of neuroprotective factors such as neurotrophins and molecular chaperones. Current hypotheses are generally based, however, on studies of longevity rather than neuroprotection and on data from primitive organisms or non-neuronal mammalian tissue. Moreover, there is considerable variability in the available data. In particular, several mechanistic theories that increase overall longevity in response to calorie restriction might have the opposite effect on neuronal survival and resistance to injury. Nevertheless, we believe that data gathered from research on longevity provide valuable insights into the neuroprotective properties of calorie restriction and generate a useful framework for future studies.

#### 2.2.1. Antioxidant effects

Although ROS are by cytoplasmic oxygenases in response to increased intracellular calcium concentrations, mitochondria are the major source of ROS, particularly complex I in neuronal mitochondrial (Turrens, 2003; Hunt et al., 2006). The superoxide anion radical is normally produced in low concentrations during oxidative phosphorylation, but levels increase substantially following mitochondrial damage—for example, following intracellular calcium overload caused by excitotoxic injury (Balaban et al., 2005; Nicholls, 2004). Superoxide is subsequently converted to hydrogen peroxide, a source of hydroxyl radicals. The resultant oxidative damage to proteins, lipids and DNA leads to manifestations of neurological disease (Keller et al., 2005; Mariani et al., 2005; Moreira et al., 2006; Reddy, 2006). A significant proportion of the neurological deficits that occur following stroke, head trauma, anoxia or even in Alzheimer’s disease can in fact be attributed to secondary injury caused by glutamate excitotoxicity and, consequently, intracellular calcium overload, mitochondrial dysfunction and oxidative stress (Calabrese et al., 2001; Bramlett and Dietrich, 2004; Canevari et al., 2004).

Calorie restriction delays age-related oxidative damage to DNA, proteins and lipids, as evidenced by decreased tissue concentrations of peroxidized lipids, protein carbonyls and damaged bases in nuclear and mitochondrial DNA (Merry, 2004; Hunt et al., 2006). Several mechanisms have been proposed to explain antioxidant properties of calorie restriction. First, some studies suggested that calorie restriction enhances antioxidant defenses, including superoxide dismutase, glutathione peroxidase and catalase (Gong et al., 1997; Sreekumar et al., 2002; Agarwal et al., 2005; Rankin et al., 2006), although others found no significant effects (Sohal
et al., 1994; Deruisseau et al., 2006). Second, a decrease in the mitochondrial production of ROS has been demonstrated, specifically at complex I of the respiratory chain (Sohal et al., 1994; Merry, 2002; Lambert and Merry, 2004; Gredilla and Barja, 2005). Brain mitochondria isolated from aged, calorie-restricted rats produced significantly less hydrogen peroxide than those from controls fed ad libitum in the presence of pyruvate and malate, but not in the presence of succinate, consistent with an effect of calorie restriction at complex I (Sanz et al., 2005). The same conclusion was reached in studies of liver and heart mitochondria (Gredilla et al., 2001; Lopez-Torres et al., 2002).

How calorie restriction actually decreases mitochondrial production of ROS is unclear, but the mechanism may involve uncoupling proteins (UCPs) which span the mitochondrial inner membrane and allow the leakage of protons from the inter-membrane space to the matrix, thereby dissociating the electrochemical gradient (proton motive force) from ATP generation. This uncoupling diminishes the mitochondrial membrane potential and decreases the production of ROS (Harper et al., 2004; Andrews et al., 2005; Bevilacqua et al., 2005; Krauss et al., 2005; Lopez-Lluch et al., 2006). Consistently, enhanced UCP activity has been associated with increased longevity and neuronal resistance to ischemic, toxic, traumatic and epileptic injury (Mattisson et al., 2003; Sullivan et al., 2003; Andrews et al., 2006; Conti et al., 2005, 2006; Liu et al., 2006). Further, mild mitochondrial uncoupling using the protonophore 2,4-dinitrophenol decreases ROS levels and enhances longevity (Caldeira da Silva et al., in press).

2.2.2. Increased metabolic efficiency

Slowing of brain aging in calorie-restricted animals was originally believed to result from reduced metabolic activity and, hence, decreased production of ROS, a natural byproduct of oxidative metabolism (Wolf, 2006). Several studies revealed that calorie restriction was associated with energy conservation (Gonzales-Pacheco et al., 1993; Santos-Pinto et al., 2001) and that mitochondria isolated from calorie-restricted animals produced less ATP than those from controls fed ad libitum, a finding compatible with increased UCP activity (Sreekumar et al., 2002; Drew et al., 2003). However, separate investigations in rodents have suggested that, when adjusted for body weight, metabolic rate does not decrease with calorie restriction (Masoro et al., 1982; McCarter et al., 1985; Masoro, 1993). More importantly, calorie restriction prevents the age-related decline in oxidative metabolism in muscle (Hepple et al., 2005; Baker et al., 2006). These data are supported by recent studies indicating that, in contrast to isolated mitochondria, ATP synthesis in intact myocytes and in vivo does not decrease following calorie restriction (Lopez-Lluch et al., 2006; Zangarelli et al., 2006). Additional support is provided by the finding that, in yeast, oxidative metabolism increases with calorie restriction (Lin et al., 2002).

Although the effects of calorie restriction on ATP generation might appear to contradict those invoking uncoupling proteins, this discrepancy can be explained by the fact that calorie restriction also promotes mitochondrial biogenesis, thereby maintaining total metabolic output per cell while decreasing mitochondrial production of ROS (Diano et al., 2003; Nisoli et al., 2005; Civitarese et al., 2007; Valle et al., 2008).
The neuroprotective benefits of this increased metabolic efficiency, which allows neurons to preserve total metabolic output despite the decrease in calorie intake, have not been directly investigated but would be expected to increase neuronal resistance to injury, especially given that mitochondrial damage and energy failure are central components of many neurological disorders (Green and Kroemer, 2004; Patel et al., 2004; Moro et al., 2005; Martin, 2006; Onyango and Khan, 2006).

2.2.3. Increased sirtuin activity
Sirtuins are a large and diverse family of enzymes that regulate gene expression. The first sirtuin, silent information regulator 2 (Sir2), was described in yeast. Sir2 is a class III histone deacetylase that uses the cofactor nicotinamide adenine dinucleotide (NAD⁺) in a catalytic reaction that releases nicotinamide (a feedback inhibitor) and O-acetyl ADP ribose (Imai et al., 2000; Marmorstein, 2004; Sauve et al., 2006). Increased Sir2 activity lengthens life span and calorie restriction increases Sir2 levels but does not promote longevity in Sir2 knockouts (Kaeberlein et al., 1999; Lin et al., 2000). Calorie restriction may activate Sir2 by increasing NAD⁺ levels (a result of improved mitochondrial function) or by increasing the expression of PNC1, a nicotinaminidase that would alleviate nicotinamide-mediated inhibition (Anderson et al., 2002, 2003; Gallo et al., 2004). These findings only apply, however, to the active replication (i.e., mitotic) phase but not to the chronological (post-mitotic) phase of the yeast life span, even though calorie restriction is known to extend both phases (Bitterman et al., 2003; Fabrizio and Longo, 2003; Longo and Kennedy, 2006).

Before dying, yeast cells undergo a specific number of divisions, and this number is a measure of the replicative life span. Yeast cells actively replicate only when nutrients are abundant. In contrast, chronological life span indicates the amount of time yeast cells survive without dividing following nutrient deprivation. Yeast cells can actually remain viable for weeks (and possibly longer) in a hypo-metabolic state when nutrients are scarce. Therefore, to study chronological life span, yeast cells are usually incubated in a special synthetic medium that promotes a relatively high metabolism while restricting replication. Interestingly, Sir2 deletion does not affect longevity under these conditions. Moreover, if calories are severely restricted, for example by incubating yeast cells in water, Sir2 deletion actually prolongs life span (Fabrizio et al., 2005).

In mammals, calorie restriction increases the expression of Sirt1, the Sir2 mammalian ortholog, in various tissues, including brain. Resveratrol, a natural Sirt1 activator found in red wine, lengthens the life span of mice and prevents the age-related deterioration of their motor function (Cohen et al., 2004; Fabrizio and Longo, 2003). Moreover, resveratrol reduces neuronal dysfunction and death in nematode and murine models of Huntington’s disease. The neuroprotective effects of resveratrol can also be seen in C. elegans by increasing the dosage of Sir2.1, the nematode Sir2 ortholog, and can be blocked by the sirtuin inhibitors nicotinamide and sirtinol (Park et al., 2005).

Slowing of axonal degeneration following peripheral neuronal injury in mice carrying the Wallerian degeneration slow mutation (wldS) has also been attributed to increased Sirt1 activity (Araki et al., 2004). In contrast, Sirt1 deficiency and nicotinamide, a sirtuin inhibitor, prolong the replicative life spans of mouse embryonic and human fibroblasts chronically exposed to sub-lethal oxidative stress (Chua et al., 2005; Lim et al., 2006). Increased Sirt1 activity however prevents apoptosis of mouse embryonic and human fibroblasts following acute administration of hydrogen peroxide at higher doses and following acute DNA damage (Luo et al., 2001; Chua et al., 2005). The reasons behind these seemingly contradictory effects of Sirt1 in fibroblasts remain unclear.

Several mechanistic hypotheses have been advanced to explain the effects of Sirt1 on neuronal survival. Sirt1 exhibits broad deacetylase activity. Identified targets include: the tumor suppressor protein p53; the forkhead transcription factors (class O) FOXO; the DNA repair protein Ku70; the peroxisome proliferator-activated receptor gamma (PPARγ); and the nuclear factor NFκB (Mattson et al., 2003; Hisahara et al., 2005; Anekonda and Reddy, 2006; Martin et al., 2006). Sirt1 represses p53-dependent apoptosis triggered by acute oxidative stress or DNA damage in fibroblasts and mesangial cells (Luo et al., 2001; Vaziri et al., 2001; Kume et al., 2006). To our knowledge, however, the importance of Sirt1-mediated p53 deacetylation has not yet been directly demonstrated in neurons. Sirt1 also prevents FOXO3-mediated apoptosis. Mammalian FOXO factors regulate the transcription of various genes involved in resistance to stress, DNA repair and apoptosis (Furukawa-Hibi et al., 2005). In cerebellar granule cells, fibroblasts and embryonic stem cells, apoptosis triggered by FOXO3 acetylation in response to oxidative stress and DNA damage is inhibited by Sirt1 (Brunet et al., 2004; Motta et al., 2004). Furthermore, Sirt1 inhibits apoptosis by deacetylating the DNA repair protein Ku70, causing it to sequester the proapoptotic factor Bax (Cohen et al., 2004).

Sirt1-mediated deacetylation of the nuclear receptor PPARγ was originally identified as a pathway to longevity by repressing adipocyte formation and fat storage (Picard et al., 2004; Guarente and Picard, 2005). More recent evidence suggests, however, that PPARγ is neuroprotective (Bordet et al., 2006; Sundararajan et al., 2006). PPARγ agonists reduce neuronal death secondary to NMDA excitotoxicity (Zhao et al., 2006a), infarct size following middle cerebral artery occlusion (Pereira et al., 2005, 2006; Shimazumi et al., 2005; Sundararajan et al., 2005), Aβ deposition in the hippocampus and cerebral cortex of Alzheimer’s disease mouse models (Heneka et al., 2005; Sastre et al., 2003, 2006), and dopaminergic cell loss in a Parkinson’s disease model (Breidert et al., 2002). Furthermore, PPARγ agonists improve cognitive function in patients with Alzheimer’s disease (Watson et al., 2005; Risner et al., 2006), enhance motor function and delay death in murine models of amyotrophic lateral sclerosis (Kiaei et al., 2005; Schutz et al., 2005) and reduce clinical severity in experimental allergic
encephalomyelitis, a model of multiple sclerosis (Niino et al., 2001; Feinstein et al., 2002; Natarajan et al., 2003). PPARγ has also been shown to stimulate neural stem cell growth and differentiation (Wada et al., 2006).

The mechanism of action of PPARγ that has received the most attention is decreased expression of inflammatory factors (Daynes and Jones, 2002). This anti-inflammatory effect involves NFκB inhibition (Poynter and Daynes, 1998; Hu et al., 2005; Pereira et al., 2005). It should be noted, however, that Sirt1 also deacetylates NFκB (Yeung et al., 2004). Therefore, NFκB repression by Sirt1 might compensate for the concomitant inhibition of PPARγ by Sirt1. Unfortunately, it is not clear if this combination of changes results in an overall neuroprotective effect because NFκB activation promoted neuronal survival in several studies (Barger et al., 1995; Mattson et al., 1997; Maggirwar et al., 1998; Hamanoue et al., 1999; Middleton et al., 2000; Fernyhough et al., 2005).

Adding to the apparently contradictory effects mentioned above, Sirt1 was recently found to repress UCP2 in pancreatic beta cells, an unexpected finding given that calorie restriction up-regulates UCP2 (Bordone et al., 2006). Moreover, several studies in yeast have recently questioned the role of sirtuins in longevity and have suggested alternative mediators for the effects of calorie restriction on longevity, including the Sir2 homolog Hst2, the kinases TOR and Sch9 and the ribosomal DNA replication fork barrier protein Fob1 (Kaeberlein et al., 2004, 2005; Lamming et al., 2005; Tsuchiya et al., 2006).

In summary, Sirt1 activation is associated with molecular mechanisms that appear contradictory and that might differ from those of calorie restriction. Nevertheless, the dependence of sirtuins on NAD+ provides an important link between the effects of calorie restriction on mitochondrial function and on gene expression. This link is reinforced by the recent discovery that Sirt1 activation promotes mitochondrial biogenesis and that resveratrol exhibits antioxidant properties in various cell types and experimental models of injury (Anekonda, 2006; Anekonda and Reddy, 2006; Guarente, 2008). The underlying molecular pathway probably involves Sirt1-mediated deacetylation of peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α), a transcriptional coactivator of nuclear genes encoding mitochondrial proteins (Nemoto et al., 2005; Rodgers et al., 2008).

2.2.4. Increased neurotrophic factor activity

The insulin-like growth factor IGF-1 has received much attention as a neuroprotectant, but there have been several contradictory findings similar to those previously described for PPARγ. IGF-1 has been widely shown to be neuroprotective (Cheng and Mattson, 1992; Sonntag et al., 2005; de la Monte and Wands, 2005; Russo et al., 2005; Tang, 2006). IGF-1 reverses age-related declines in spatial memory, prevents hydrogen peroxide and amyloid Aβ-induced neuronal death and promotes neurogenesis in aged brains (Dore et al., 1997; Markowska et al., 1998; Heck et al., 1999; Lichtenwalner et al., 2001). It is therefore quite surprising that IGF-1 levels decrease following calorie restriction in animals and humans, and that inhibition of IGF-1 signaling is associated with increased longevity (Smith et al., 1995; Rincon et al., 2005; Rasmussen et al., 2006). Moreover, the enhanced resistance of cultured fibroblasts to stress conveyed by increasing SIRT1 expression is partially reversed by IGF-1 (Cohen et al., 2004). The reasons behind this apparent discrepancy are not clear.

Unlike the effects on IGF-1, calorie restriction increases the expression of several nerve growth factors, including brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and glial cell line-derived neurotrophic factor (GDNF), most prominently in the hippocampal formation, but also in the in the basal ganglia (Lee et al., 2000, 2002b; Duan et al., 2003; Maswood et al., 2004; Thrasivoulou et al., 2006). In contrast, a diet rich in refined sugars and saturated fats reduces hippocampal BDNF levels and impairs spatial memory (Molteni et al., 2002). Furthermore, decreased BDNF signaling causes hyperphagia and obesity whereas BDNF diminishes food intake (Mattson, 2005; Lebrun et al., 2006). It is intriguing to note, however, that NFκB activation can increase BDNF expression given that calorie restriction represses NFκB (Marini et al., 2004). Nevertheless, BDNF has been shown to mediate the neuroprotective effects of calorie restriction against excitotoxic injury (Duan et al., 2001a,b). BDNF promotes neuronal differentiation of embryonic and adult hippocampal progenitor cells in calorie-restricted animals and facilitates synaptic plasticity, learning and memory (Bramham and Messaoudi, 2005; Lee et al., 2000, 2002a).

Interestingly, Garriga-Canut et al. (2006) found that 2-deoxy-D-glucose (2-DG), a phosphoglucose isomerase inhibitor being investigated as a potential calorie restriction mimetic, reduced seizure progression in the rat kindling model of temporal lobe epilepsy by decreasing hippocampal expression of BDNF and its receptor tyrosine kinase B (TrkB). Consistently, acute infusions of BDNF in the hippocampus accelerate kindling development and deletion of TrkB gene prevents epileptogenesis in that same model (He et al., 2004; Xu et al., 2004). Furthermore, under certain experimental conditions, prolonged exposure to BDNF leads to neuronal necrosis (Kim et al., 2002, 2003), indicating that certain neuroprotective mediators may act as double-edged swords.

2.2.5. Increased protein chaperone activity

Chaperones are highly conserved, ubiquitous proteins that prevent misfolding and aggregation of polypeptides into potentially toxic compounds (Hart and Hayer-Hartl, 2002; Young et al., 2004). Aberrant folding, leading to the formation of insoluble aggregates, has been implicated in the pathogenesis of several neurodegenerative disorders, including Alzheimer’s, Parkinson’s and Huntington’s diseases (Agorogianis et al., 2004; Chaudhuri and Paul, 2006). In addition to their role in polypeptide folding, chaperones— which include several families of heat shock proteins and glucose-regulated proteins—are involved in protein translocation across cellular membranes, targeting of misfolded proteins for degradation and expression of anti-apoptotic and anti-inflammatory factors (Muchowski, 2002; Yenari et al., 2005).

Calorie restriction increases chaperone levels in the brain as well as in several other tissues including the heart, the liver, the intestines, skeletal muscle and macrophages (Heydari et al., 1995; Ehrenfried et al., 1996; Moore et al., 1998; Guo et al., 2000; Frier and Locke, 2005; Selsby et al., 2005; Sharma and Kaur, 2005). In turn, chaperones protect neurons in rodent and Drosophila models of both acute and chronic neurological disorders—such as ischemic injury, glutamate and kainate...
excitotoxicity, oxidative stress and toxicity secondary to phosphorylated tau protein, α-synuclein or proteins with polyglutamine expansions (Lowenstein et al., 1991; Warrick et al., 1999; Yu et al., 1999; Chan et al., 2000; Rajdev et al., 2000; Cummings et al., 2001; Auluck et al., 2002; Giffard et al., 2004; Shimura et al., 2004). Interestingly however, one study found that expression of genes encoding heat shock proteins actually decreased following calorie restriction of aged animals (Park and Prolla, 2005). The reasons behind these seemingly contradictory effects are unknown.

2.2.6. Anti-inflammatory effects
Aging and various neurological disorders are characterized by increased levels of several inflammatory mediators (Chung et al., 2002; Sarkar and Fisher, 2006). NF-κB activation is central component of this inflammatory process. NF-κB activation can be triggered by several sources of injury, such as reactive oxygen or nitrogen species or amyloid Aβ, and causes enhanced transcription of interleukins (IL1β, IL2, IL4, IL6), tumor necrosis factors (TNFα and TNFβ) and the pro-inflammatory enzymes cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in various tissues, including the brain (Gloire et al., 2006; Valerio et al., 2006).

Calorie restriction reduces NF-κB levels (probably through a Sirt1-dependent process), blocks the synthesis of interleukins and TNFα and suppresses the activity of COX-2 and iNOS in animal models and in humans (Spaulding et al., 1997; Clement et al., 2004; Bhattacharya et al., 2006; Kalani et al., 2006; Kim et al., 2006; Ugochukwu and Figgers, 2007). Intermittent fasting results in a reduction of seizure-induced microglial activation in a mouse model of epileptic seizures (Lee et al., 2003). Surprisingly, interferon-gamma (IFNγ), which typically activates microglial cells and promotes an inflammatory response in the brain, is increased by calorie restriction in brain and leukocytes, and pretreatment of hippocampal neurons with low concentrations of IFNγ provides significant protection against excitotoxic injury (Mascarucci et al., 2002; Lee et al., 2006). Under certain experimental conditions, NF-κB, interleukins and tumor necrosis factors exhibit neuroprotective and neurotrophic properties that include the promotion of neuritic outgrowth and the differentiation of progenitor cells into neurons and that possibly involve enhanced transcription of the BDNF gene (Munoz-Fernandez and Fresno, 1998; Mattson and Camandola, 2001; Marini et al., 2004; Widera et al., 2006). These same mechanisms might also underlie the carcinogenic potential of NF-κB (Karin, 2006).

NF-κB exhibits apparently contradictory effects on neuronal survival, similar to many factors regulated by calorie restriction. These contradictory findings may be reconciled, however, by the fact that NF-κB comprises several regulatory subunits that can vary depending on the cell type and the presence of activators and repressors, thereby providing the means to target a large variety of genes (Perkins, 2007). It is therefore highly conceivable that NF-κB can mediate both neuroprotective and apoptotic processes depending on subunit composition (Kaitschmidt et al., 2005).

2.2.7. Enhanced neurogenesis
Neurogenesis persists in several regions of the adult brain, including the dentate gyrus, a critical region for cognition, but progressively decreases with age (Bernal and Peterson, 2004; Abrous et al., 2005). Newly generated neurons in the hippocampus might play a part in learning and memory but the functions of neural stem cells remain largely unknown (Aimone et al., 2006; Lledo et al., 2006). Calorie restriction promotes neurogenesis in adult rodents, probably by increasing BDNF levels (Lee et al., 2002a). The number of bromodeoxyuridine-positive cells in the dentate gyrus is higher in calorie-restricted animals than in aged-matched controls fed ad libitum, indicating an increased survival of newly generated cells (Lee et al., 2002b; Bondolfi et al., 2004). However, the functional consequences of neurogenesis remain unclear.

2.2.8. Hormesis
Calorie restriction is associated with multiple and apparently independent (or even contradictory) mechanisms. Recent studies have attempted to reconcile these effects by suggesting that many of the benefits of calorie restriction on the nervous system result from a widespread activation of adaptive cellular stress responses, a process called hormesis (Calabrese et al., 2007; Mattson 2008a,b). Calorie restriction imposes a mild stress on cells, which results in the activation of stress response pathways including those involving transcription factors such as CREB, Nrf-2 and NF-κB (Mattson and Cheng, 2006). Cellular stress may result from a combination of the direct consequences of reduced energy intake and an increase in the activity of neuronal circuits secondary to increased hunger. This proposed neuroprotective mechanism of action of calorie restriction is analogous to the beneficial effects of physical exercise on muscle and heart cells, wherein energy demand and oxidative and ionic stress increase during exercise and activate adaptive stress responses in the cells. Indeed, some of the same classes of adaptive stress response proteins have been shown to be up-regulated in neurons in response to calorie restriction and in muscles in response to exercise, including heat-shock protein, growth factors and energy-regulating enzymes (Mattson and Wan 2005; Martin et al., 2006).

3. The ketogenic diet
Calorie restriction in animals is achieved by either daily reduction of food intake or by intermittent fasting. Both protocols induce similar physiological and metabolic changes except for one important difference: intermittent fasting leads to a much larger increase in blood levels of the ketone body β-hydroxybutyrate (Mattson et al., 2003; Mattson 2005). Interestingly, this rise in β-hydroxybutyrate concentration is associated with a more significant reduction in the vulnerability of hippocampal neurons to kainate injections. The ketogenic effect of fasting was appreciated nearly a century ago, and led to the formulation of the high-fat, low-carbohydrate ketogenic diet in the 1920s.

Currently, the anticonvulsant properties of the ketogenic diet are well recognized, but available data suggest that the ketogenic diet is also neuroprotective and that some of the underlying mechanisms are similar to those activated by calorie restriction (Greene et al., 2003; Kossoff 2004; Gasior et al., 2006). It is important to note, however, that the ketogenic
Parkinson’s disease, Unified Parkinson’s Disease Rating Scale
limited to epilepsy. In a small sample of patients with
Lennox–Gastaut syndrome, and certain inherited metabolic
disorders. More than half the patients are experiencing at least
a 50% decrease in seizures (Freeman et al., 1998; Vining et al.,
1998; Lefevre and Aronson 2000; Kossoff et al., 2002; Klepper et
al., 2005; Caraballo et al., 2006; Eun et al., 2006; Kang et al.,
2007). The ketogenic diet may also improve long-term out-
come in epileptic children beyond the dietary treatment
period (Hemingway et al., 2001; Marsh et al., 2006). Similar
favorable outcomes have been reported in teenagers and
adults (Sirven et al., 1999; Freeman et al., 2006). Furthermore,
ketogenic diet use has been associated with improvements in
cognitive function (Nordli et al., 2001; Pulsifer et al., 2001).
Whether these improvements are due to improved seizure
control, reduced medication or an independent, neuroprotective
effect of the diet is unknown.

The anticonvulsant efficacy of the ketogenic diet was
recently confirmed in a prospective, randomized controlled
trial involving children and teenagers with uncontrolled
seizures (Neal et al., 2008b). Both generalized and focal
epilepsies were included. Following 3 months of treatment,
patients on the ketogenic diet exhibited significantly less
seizures than those on a regular diet. Several side-effects were
noted, most importantly height and weight retardation (Neal
et al., 2008a). Growth retardation was not prevented by the use
a medium-chain triglyceride diet, even though the group on
this diet consumed significantly more proteins.

Clinical efficacy of the ketogenic diet has been further
validated in several animal models of epilepsy. The ketogenic
diet increases the threshold for seizures induced by amygdala
kindling, GABA antagonists (such as pentylentetrazole) and
delays the development of seizures in El and in fluroethyl-
treated mice (Hori et al., 1997; Bough and Eagles 1999; Rho et
al., 2002; Todorova et al., 2000; Bough et al., 2002; Mantis et al.,
2004). Moreover, in rats exposed to kainic acid, a model of
temporal lobe epilepsy, the ketogenic diet decreases both the
risk of developing epilepsy and the severity of the seizures
that do occur. These effects are associated with reduced hippocampal excitability and decreased supragranular mossy
fiber sprouting (Muller-Scharze et al., 1999; Stafstrom et al.,
1999; Noh et al., 2003; Xu et al., 2006). Unexpectedly however,
in one study, the ketogenic diet impaired learning and memory in rats (Zhao et al., 2004). Animals experienced a
significant retardation of brain growth as well, but were fed a
diet with a fat-to-carbohydrate plus protein ratio that was
more than twice as high as in the standard diet. Consequently, impaired learning and memory might have been secondary to malnu-
trition and delayed development (Cunnane and Likhodi 2004).

The neuroprotective effects of the ketogenic diet are not
limited to epilepsy. In a small sample of patients with
Parkinson’s disease, Unified Parkinson’s Disease Rating Scale
(UPDRS) scores improved by a mean of 43% following
treatment with the ketogenic diet for 1 month (Vanitallie et
al., 2005). The substitution of unsaturated for saturated fats
was well tolerated and prevented the expected hypercholes-
terolemia in the majority of participants. In addition to the
lack of a control group, the effect of the ketogenic diet on
levodopa absorption was not considered, and the possible
benefits of concomitant weight loss were not investigated.
Notwithstanding these caveats, this study was instrumental
in demonstrating the applicability of the ketogenic diet to a
group of older adults suffering from a neurodegenerative
disorder. Epidemiological observations based on the Rotter-
dam study, a longitudinal study of senior adults, are consist-
tent with these results. Higher intake of unsaturated fatty
acids is associated with a decreased incidence of Parkinson’s
disease (de Lau et al., 2005). Similarly, oral intake of a
ketogenic medium-chain triglyceride diet improves cognitive
function in patients with Alzheimer’s disease (Reger et al.,
2004). Preliminary data also suggest that the ketogenic diet
might be beneficial in autistic children (Evangelio et al.,
2003).

Such clinical observations are supported by animal data. In
mice expressing a mutant form of the human amyloid
precursor protein gene, the ketogenic diet reduced the amount of soluble Aβ in brain homogenates, although performance on
an object recognition task did not improve (Van der Auwera et
al., 2005). The ketogenic diet also decreased contusion volume
following head trauma and neuronal loss secondary to
insulin-induced hypoglycemia (Prins et al., 2005; Yamada et
al., 2005). Similarly, in rats subjected to global cerebral
ischemia secondary to cardiac arrest, neuronal death and
post-ischemic myoclonus and seizures were significantly
reduced by the ketogenic diet (Tai and Truong 2007; Tai et
al., 2008). Finally, in transgenic mice expressing a mutated
superoxide dismutase 1, a model of amyotrophic lateral
sclerosis, the ketogenic diet delayed the progression of
motor deficits and decreased motor neuron loss in the spinal
cord (Zhao et al., 2006b).

3.2. Neuroprotective mechanisms of the ketogenic diet

The ketogenic diet has been associated with antioxidant
effects in several studies (Fig. 2). First, mitochondria from
animals fed a ketogenic diet produced lower amounts of ROS
in isolated mitochondria (Sullivan et al., 2004b). Second, the
ketogenic diet increased glutathione levels and glutathione
peroxidase activity in the hippocampus (Ziegler et al., 2003;
Jarrett et al., 2008). Third, the ketogenic diet increased UCP
expression and activity in the hippocampus, thereby decreasing
mitochondrial membrane potential and, as a result, diminishing the production of ROS (Sullivan et al., 2004b).

The ketogenic diet also stimulated mitochondrial biogenesis and
increased cerebral ATP and phosphocreatine concentra-
tions, suggesting increased metabolic efficiency (De Vivo et al.,
1978; Bough et al., 2006). Furthermore, genes encoding
bioenergetic enzymes are up-regulated by the ketogenic diet
(Noh et al., 2004; Bough et al., 2006). The combination of these
mechanisms suggests that, as with calorie restriction, mito-
chondria are an important target of ketogenic diet action and
that the resultant improvement in mitochondrial function (i.e.
increased energy reserves combined with decreased production of ROS) could account for the ability of the diet to confer neuroprotection in models of neurological disease.

In addition to antioxidant and metabolic effects, anti-apoptotic mechanisms have been implicated, including decreased expression of the pro-apoptotic factors clusterin and caspase-3 in animals exposed to kainic acid, as well as increased activity of calbindin, an intracellular calcium buffer (Noh et al., 2003, 2005a,b). The ketogenic diet also inhibits the dissociation of the pro-apoptotic factor Bad from the chaperone protein 14-3-3, a process implicated in kainate-induced epileptogenesis (Noh et al., 2006b).

The anticonvulsant effects of ketogenic diet might further prevent brain damage by limiting seizure-induced neuronal hyperexcitability. In rodent brain, the ketogenic diet increases the levels of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the expression of glutamic acid decarboxylase (GAD), the rate-limiting enzyme in GABA synthesis (Cheng et al., 2004; Dahlin et al., 2005). Consistently, the ketogenic diet has been associated with diminished transamination of glutamate to aspartate and increased decarboxylation of glutamate to GABA (Yudkoff et al., 2005). It remains unclear, however, whether the observed changes result in an anticonvulsant effect, particularly since GABA levels were elevated in non-seizure-prone areas of the brain (e.g., the superior colliculi and the cerebellum) and increases in GAD levels do not necessarily translate to increased GABA production (Rimvall and Martin 1992; Rimvall et al., 1993; Rimvall and Martin 1994). Nevertheless, stimulation of Schaffer collaterals in hippocampal slices of ketogenic diet-fed rats triggers fewer population spikes than in slices from control animals, a process most probably mediated by enhanced GABA inhibition (Bough et al., 2003).

Additional mechanisms by which the ketogenic diet might limit neuronal hyperexcitability involve purinergic and noradrenergic signaling. First, Masino and Geiger (2008) have proposed that, by enhancing ATP production, the ketogenic diet ultimately increases adenosine levels. Adenosine A1 receptor activation subsequently decreases glutamate release and hyperpolarizes neurons. Second, blocking the synthesis of norepinephrine (by knocking out the gene for dopamine β-hydroxylase) eliminates the anticonvulsant effect of the ketogenic diet, whereas elevating synaptic levels of that neurotransmitter (by knocking out the norepinephrine transporter) potentiates the anticonvulsant effect (Szot et al., 2001; Martillotti et al., 2006). However, the contribution of norepinephrine to the neuroprotective effects of the ketogenic diet has yet to be confirmed. In fact, it should be emphasized that none of the anticonvulsant properties of the ketogenic diet have been shown to be neuroprotective, but such an association is conceivable based on the hypothesis that controlling seizures prevents excitotoxic injury.

4. Ketone bodies

Despite mounting evidence supporting the anticonvulsant and neuroprotective effects of ketogenic diets, the difficulty of adhering to a restrictive diet and the risk of systemic complications such as growth retardation, nephrolithiasis
and hyperlipidemia preclude more widespread implementation (Kwitterovich et al., 2003; Kang et al., 2004; Hartman and Vining 2007). Therefore, investigators have sought a safer, yet efficacious alternative. Within this context, ketone bodies have become the subject of growing interest and research. Under conditions of reduced glucose availability such as fasting, energy is derived from the conversion of fats to ketone bodies, mainly β-hydroxybutyrate and acetocetate, and, to a lesser extent, acetone (Lafel 1999). The liver is the main site of ketone body synthesis, although astrocytes can also produce ketone bodies from fats (Guzman and Blazquez 2004).

Following a day of fasting or consumption of a ketogenic diet, ketone bodies reach low millimolar concentrations in the blood, with cerebrospinal levels being moderately lower (Haymond et al., 1982; Lamers et al., 1995; Seymour et al., 1999; Thavendiranathan et al., 2000). Ketone bodies cross the blood–brain barrier through proton-linked, monocarboxylic acid transporters and then enter neurons by diffusion or through monocarboxylic acid transporters (Nehlig 2004; Morris 2005). Fasting and the ketogenic diet increase the permeability of the blood–brain barrier to ketones and enhance the expression of monocarboxylic acid transporters. The ketogenic diet also enhances glial proliferation in the CA3 region of the hippocampus (Silva et al., 2005). The observed gliosis constitutes an additional means of increasing ketone body synthesis and is not associated with any functional deficits.

### 4.1 Neuroprotective effects of ketone bodies

Similar to calorie restriction and to the ketogenic diet, ketones exhibit both anticonvulsant and neuroprotective properties. Acetocetate and acetone exert anticonvulsant effects in vivo, but not β-hydroxybutyrate. Both acetocetate and acetone decrease the incidence of seizures triggered by loud auditory stimuli in Frings audiogenic-susceptible mice (Rho et al., 2002). Acetone also suppresses seizures induced by amygdala kindling, by maximal electroshock and by the epileptogenic compounds pentyleneetetrazole, AY-9944 (a cholesterol synthesis inhibitor) and 4-aminopyridine (Likhodii et al., 2003; Hartman et al., 2007).

In animal models of Parkinson’s disease, chronic subcutaneous infusion of β-hydroxybutyrate in mice confers partial protection against dopaminergic cell loss and motor deficits induced by the mitochondrial complex I inhibitor MPTP (Tieu et al., 2003). β-hydroxybutyrate also protects cultured mesencephalic dopaminergic neurons from the toxic effects of MPTP and rotenone, another inhibitor of mitochondrial complex I (Kashiwaya et al., 2000; Imamura et al., 2006). In patients with Alzheimer’s disease, administration of medium-chain triglycerides improved memory and the degree of improvement correlates with blood levels of β-hydroxybutyrate (Reger et al., 2004). Furthermore, direct application of β-hydroxybutyrate protects cultured hippocampal neurons against Aβ toxicity (Kashiwaya et al., 2000). Finally, exogenous administration of either β-hydroxybutyrate or acetocetate reduces neuronal loss and improves neuronal function in animal models of hypoxia, hypoglycemia and focal ischemia (Suzuki et al., 2001, 2002; Massieu et al., 2001, 2003; Masuda et al., 2005).

More recently, the neuroprotective effects of ketone bodies have been demonstrated in two experimental models relevant to several neurological diseases—glutamate excitotoxicity and oxidative stress. Glutamate excitotoxicity is a pathogenic process that can lead to calcium-mediated neuronal injury and death by generating ROS and by impairing mitochondrial bioenergetic function (Emerit et al., 2004; Mattson and Magnus 2006). Oxidative stress subsequently damages nucleic acids, proteins and lipids and potentially opens the mitochondrial permeability transition (mPT) pore which, in turn, can further stimulate ROS production, worsen energy failure and release pro-apoptotic factors such as cytochrome c into the cytoplasm (Kowaltowski et al 2001; Nichols 2004).

A combination of β-hydroxybutyrate and acetocetate (1 mM each) increases the survival of acutely dissociated rat neocortical neurons exposed to glutamate or hydrogen peroxide for 10 min or more (Kim et al., 2007; Maalouf et al., 2007). Increased survival is associated with the inhibition of electro-physiological signs of neuronal injury, specifically an irreversible depolarization associated with a significant decrease in membrane resistance. Acetocetate (also in millimolar concentrations) has a similar effect in primary hippocampal cultures (Noh et al., 2006a). In addition, the combination of β-hydroxybutyrate and acetocetate prevents oxidative impairment of long-term potentiation in the CA1 region of the hippocampus, indicating that ketone bodies not only limit neuronal loss but also preserve synaptic function (Maalouf and Rho, in press).

### 4.2 Neuroprotective mechanisms of ketone bodies

Although certain ketone bodies exhibit anticonvulsant properties, they do not affect neuronal excitability or synaptic transmission in the hippocampus (Erecinska et al., 1996; Thio et al., 2000). Millimolar concentrations of β-hydroxybutyrate and acetocetate reduce, however, the spontaneous firing of neurons in the substantia nigra pars reticulata, another subcortical structure that may be critically involved in modulation of seizure activity (Ma et al., 2007). This effect requires the opening of ATP-sensitive potassium (K<sub>ATP</sub>) channels, but its significance remains unclear because ketone bodies are known to raise ATP levels, which would shut down K<sub>ATP</sub> channels.

In contrast to the ambiguous effects of ketone bodies on neuronal excitability, their antioxidant and metabolic properties have been consistently demonstrated (Fig. 3). A combination of β-hydroxybutyrate and acetocetate (1 mM each) decreases the production of ROS by complex I of the mitochondrial respiratory chain (Maalouf et al., 2007). Specifically, in acutely isolated rat neocortical neurons, pretreatment with ketone bodies inhibits increases in intracellular levels of superoxide following prolonged exposure to glutamate. Ketone bodies also decrease ROS concentrations in isolated mitochondria overloaded with calcium. In a similar study, increased survival of HT22 hippocampal cell lines treated with acetocetate was associated with decreased production of ROS (Noh et al., 2006a).

In the study by Maalouf et al. (2007), ketone bodies decreased NADH levels in intact neurons and in isolated mitochondria, but did not affect glutathione levels. Furthermore, ketone bodies prevented the inhibition of mitochondrial respiration by calcium in the presence of pyruvate and malate,
but not succinate. Given that NADH oxidation correlates with decreased mitochondrial formation of ROS (Duchen, 1992; Kudin et al, 2004; Sullivan et al, 2004a) and that pyruvate and malate drive mitochondrial respiration through complex I, the source of ROS in neurons (Turrens 2003), these findings strongly suggest that ketone bodies decrease the production of ROS by enhancing complex I-driven mitochondrial respiration rather than increase antioxidant factors such as glutathione.

Consistent with the observed improvements in mitochondrial respiration, β-hydroxybutyrate increases ATP production substantially in isolated brain mitochondria and in brain homogenates (Suzuki et al., 2001; Tieu et al., 2003). Similarly, acetoacetate increases phosphocreatine levels in cardiac myocytes (Squires et al., 2003). These findings provide further support for the hypothesis that ketone bodies improve mitochondrial function and explain how ketone bodies increase myocardial hydraulic work and sperm motility (Veech et al., 2001; Veech 2004). Moreover, these findings, which suggest that ketone bodies and the ketogenic diet enhance mitochondrial function through similar mechanisms, might explain why the ketogenic diet is helpful in patients with respiratory chain complex defects (Kang et al., 2006). The ketogenic diet and ketone bodies might either improve the function of the impaired mitochondria or, as suggested by Santra et al. (2004), allow healthy mitochondria to compensate for the deleterious effects of the defective ones.

In addition to the antioxidant and metabolic properties of ketone bodies, available data suggest that anti-apoptotic mechanisms might be involved. Ketone bodies prevent neuronal injury and death caused by hydrogen peroxide or by the thiol oxidant and mPT activator, diamide (Kim et al., 2007). Inhibitors of mPT replicate the neuroprotective effects of ketone bodies. In addition, ketone bodies elevate the threshold for calcium-induced mPT in isolated brain mitochondria. Mitochondrial permeability transition can be triggered by various pathological mechanisms, most notably oxidative stress, causing the cytoplasmic release of cytochrome c and the subsequent induction of caspase-mediated apoptosis (Mattson et al., 2003; Nicholls 2004; Balaban et al., 2005).

Consistent with an anti-apoptotic effect, ketone bodies block the activation of protein phosphatase 2A by oxidative stress (Maalouf and Rho, in press). Protein phosphatase 2A is a serine-threonine protease enzyme that can trigger apoptosis by inactivating the anti-apoptotic factor Bcl2, an inhibitor of mitochondrial permeability transition (Virshup 2000; Janssens and Goris 2001; Dagda et al., 2003; Kroemer et al., 2007). Protein phosphatase 2A activation occurs following the conversion of sphingomyelin, a cytoplasmic membrane constituent, to ceramide in a series of biochemical reactions facilitated by ROS and inhibited by antioxidants such as glutathione (Zabrocki et al., 2002; Sultan et al., 2006; Won and Singh 2006). In turn, ceramide activates protein phosphatase 2A, inducing Bcl-2 dephosphorylation and cytochrome c release from mitochondria (Richter and Ghaforifar 1999; Ruvolet al., 1999, 2002). In addition to triggering apoptosis, activation of protein phosphatase 2A, either by oxidative stress or...
directly by ceramide analogues, inhibits LTP (Fukunaga et al., 2000; Kang-Park et al., 2003). Ketone bodies prevent the impairment of LTP by oxidative stress and ceramide and this effect is associated with the reduction of protein phosphatase 2A activity (Maalouf and Rho, in press).

In summary, current data suggest that mitochondria are the main target of ketone bodies. Improving mitochondrial function presents several obvious advantages. First, oxidative stress decreases. Second, ATP synthesis increases. Third, opening of permeability transition pore and the ensuing apoptotic cascade are inhibited. Increasing the NAD+/NADH ratio potentially provides an additional advantage, the activation of Sirt1, which, in turn, inhibits several apoptotic and inflammatory factors and promotes mitochondrial biogenesis. Ongoing studies are attempting to determine whether ketone bodies affect sirtuin activity.

5. Comparison of the neuroprotective properties of calorie restriction, of the ketogenic diet and of ketone bodies

Our review of the cellular and molecular mechanisms associated with calorie restriction, the ketogenic diet ketone bodies has identified several similarities between these neuroprotective interventions, most importantly the prevention of disease related-mitochondrial dysfunction. Another similarity is the inhibition of apoptosis, although the specific mechanisms underlying the anti-apoptotic effects of each intervention might differ amongst the interventions discussed. Interestingly, recent findings indicate that calorie restriction, the ketogenic diet and ketone bodies exhibit anti-neoplastic properties as well (Seyfried et al., 2003; Mavropoulos et al., 2006; Hurstings et al., 2007; Zhou et al., 2007). Although prevention of carcinogenesis probably occurs independently of neuroprotection, elucidation of the underlying mechanisms might provide valuable insights into the neuroprotective aspects of either calorie restriction, the ketogenic diet or ketone bodies. For instance, Seyfried and Mukherjee (2005) have suggested that, unlike normal cells that can oxidize glucose as well as ketone bodies for energy, neoplastic cells are largely dependent on glucose. Therefore, the metabolic environment created by calorie restriction and by the ketogenic diet – decreased glucose and increased ketone bodies – is detrimental to neoplastic cells. The critical role of ketone bodies is further highlighted by preliminary data indicating that a combination of β-hydroxybutyrate and acetoacetate inhibits the growth of glioblastoma cells maintained in physiological concentrations of glucose (Marthaler et al., 2006).

<table>
<thead>
<tr>
<th>Table 1 – Summary of the similarities and differences between calorie restriction, the ketogenic diet and ketone bodies</th>
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<tbody>
<tr>
<td><strong>Metabolic characteristics</strong></td>
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<tr>
<td>Glucose level</td>
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<td>Normal</td>
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<tr>
<td>Normal</td>
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<tr>
<td>Normal</td>
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<tr>
<td>Ketone body levels</td>
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<tr>
<td>Normal</td>
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<tr>
<td>Increased</td>
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<tr>
<td>Increased</td>
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<tr>
<td><strong>Neuroprotective mechanisms</strong></td>
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<tr>
<td>Production of reactive oxygen species</td>
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<td>Decreased</td>
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<tr>
<td>Decreased</td>
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<tr>
<td>Decreased</td>
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<tr>
<td>Antioxidant defenses</td>
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<tr>
<td>Possibly increased</td>
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<tr>
<td>Increased</td>
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<tr>
<td>Increased</td>
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<tr>
<td>Synthesis of ATP</td>
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<tr>
<td>Relatively increased</td>
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<tr>
<td>Increased</td>
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<tr>
<td>Increased</td>
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<tr>
<td>Sirtuin activity</td>
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<td>Unknown</td>
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<td>Unknown</td>
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<tr>
<td>Apoptotic factor activity</td>
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<td>Decreased</td>
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<td>Decreased</td>
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<tr>
<td>Neurotrophic factor activity</td>
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<tr>
<td>Variable</td>
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<tr>
<td>Unknown</td>
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<td>Unknown</td>
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<tr>
<td>Protein chaperone activity</td>
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<tr>
<td>Increased</td>
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<td>Decreased</td>
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<td>Decreased</td>
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<tr>
<td>Inflammatory factor activity</td>
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<td>Unknown</td>
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<tr>
<td>Neurogenesis</td>
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</tbody>
</table>

Although many cellular and molecular mechanisms have been associated with calorie restriction, it has not been established which of these mechanisms underlies the neuroprotective effects of the diet. In contrast, less is known about the molecular mechanisms associated with ketone bodies. Available data suggests however that ketone bodies, similarly to calorie restriction and to the ketogenic diet, improve mitochondrial function, leading to decreased production of reactive oxygen species and increased (or at least maintained) ATP synthesis.

a Glucose levels are lower on average in calorie restricted and ketogenic diet treated individuals than in those eating ad libitum but remain within normal limits despite a decreased intake of carbohydrates.

d Intermittent fasting raises ketone body levels but ketosis is interrupted during periods of food intake.

d Some studies have suggested that calorie restriction enhances antioxidant enzyme activity whereas others did not find any significant effects.

f Measurements in mitochondria isolated from calorie restricted animals suggest a decrease in ATP production but ATP synthesis in intact cells and in vivo has been reported as normal, despite the decrease in calorie intake.

Although ketone bodies have not been shown to affect apoptotic factors directly, inhibition of protein phosphatase 2A activation and of mitochondrial permeability transition pore opening is consistent with an anti-apoptotic effect.

f Calorie restriction increases the expression of the nerve growth factors BDNF, NT-3 and GDNF but decreases IGF-1 levels.

h Allowing most studies reported an increase in protein chaperone activity following calorie restriction, one particular study (Park and Prolla 2005) suggested that calorie restriction decreases the expression of genes encoding heat shock proteins.

i Calorie restriction increases IFN-γ activity in contrast to its overall anti-inflammatory effect.
Despite various similarities between calorie restriction, the ketogenic diet and ketone bodies, several differences exist (Table 1). When Bough et al. (2003) compared the antiepileptic effects of calorie restriction and of the ketogenic diet in the maximal dentate activation (MDA) protocol, they found that, although both diets significantly elevated MDA threshold and prolonged MDA latency compared with controls fed ad libitum, only the ketogenic diet slowed the prolongation of MDA duration. Similarly, the ketogenic diet was neuroprotective in animal models of familial ALS whereas calorie restriction accelerated disease progression (Pedersen and Mattson 1999; Zhao et al., 2006b).

From a metabolic standpoint, only the ketogenic diet increases brain and serum levels of polyunsaturated fatty acids (e.g., docosahexaenoic acid) that exhibit anticonvulsant and neuroprotective properties (Fraser et al., 2003; Taha et al., 2005; Bazan 2007). Polyunsaturated fatty acids and cerebrospinal fluid from children treated with the ketogenic diet appear to activate voltage-gated Shaker potassium channels expressed in Xenopus oocytes (Xu et al., 2008). Polyunsaturated fatty acids also decrease the excitability of hippocampal neurons and increase neuronal survival following traumatic, ischemic and excitotoxic injuries (Xiao and Li 1999; Lauritzen et al., 2000; Young et al., 2000; Strokin et al., 2006; King et al., 2006). It is interesting to note, however, that ketone bodies are products of fatty acid catabolism and can therefore mediate some of the neuroprotective effects of polyunsaturated fatty acids. Finally, Noh et al. (2004) reported that the ketogenic diet decreases the expression of heat shock proteins whereas all but one study involving calorie restriction described an increase in brain levels of heat shock proteins and other chaperones.

6. Concluding remarks

Calorie restriction and the ketogenic diet share two metabolic characteristics: reduced carbohydrate intake and a compensatory rise in ketone bodies. The neuroprotective effects of reduced carbohydrates per se are being investigated by several research groups (Mattson et al., 2003; Ingram et al., 2006). In this review, we have examined the possibility that ketone bodies might replicate some of the neuroprotective properties of calorie restriction and of the ketogenic diet. In support of this hypothesis, an expanding body of evidence indicates that ketone bodies are indeed neuroprotective. Moreover, the underlying mechanisms appear similar to those associated with calorie restriction and the ketogenic diet at the mitochondrial level. However, several important questions remain unanswered. First, the effects of ketone bodies on gene expression have not been investigated. Second, the neuroprotective effects of ketone bodies in vivo have not been thoroughly examined. It is imperative to demonstrate that the neuroprotective effects of ketone bodies prevent disease-related impairment of clinically relevant functions such as cognition. Future research will attempt to answer these critical questions and further our understanding of the mechanisms underlying calorie restriction, the ketogenic diet and ketone bodies.

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