Biogerontology
https://doi.org/10.1007/s10522-018-9743-y

RESEARCH ARTICLE

Limited daily feeding and intermittent feeding have different effects on regional brain energy homeostasis during aging

Kosara Smiljanic · Smilja Todorovic · Aleksandra Mladenovic Djordjevic · Tim Vanmierlo · Dieter Lütjohann · Sanja Ivkovic · Selma Kanazir

Received: 15 September 2017 / Accepted: 4 January 2018

Abstract Albeit aging is an inevitable process, the rate of aging is susceptible to modifications. Dietary restriction (DR) is a vigorous nongenetic and non-pharmacological intervention that is known to delay aging and increase healthspan in diverse species. This study aimed to compare the impact of different restricting feeding regimes such as limited daily feeding (LDF, 60% AL) and intermittent feeding (IF) on brain energy homeostasis during aging. The analysis was focused on the key molecules in glucose and cholesterol metabolism in the cortex and hippocampus of middle-aged (12-month-old) and aged (24-month-old) male Wistar rats. We measured the impact of different DRs on the expression levels of AMPK, glucose transporters (GLUT1, GLUT3, GLUT4), and the rate-limiting enzyme in the cholesterol synthesis pathway (HMGCR). Additionally, we assessed the changes in the amounts of cholesterol, its metabolite, and precursors following LDF and IF. IF decreased the levels of AMPK and pAMPK in the cortex while the increased levels were detected in the hippocampus. Glucose metabolism was more affected in the cortex, while cholesterol metabolism was more influenced in the hippocampus. Overall, the hippocampus was more resilient to the DRs, with fewer changes compared to the cortex. We showed that LDF and IF differently affected the brain energy homeostasis during aging and that specific brain regions exhibited distinct vulnerabilities towards DRs. Consequently, special attention should be paid to the DR application among elderly as different phases of aging do not respond equally to altered nutritional regimes.

Keywords Aging · Dietary restriction · AMPK · Glucose transporters · Cholesterol metabolism · Brain

K. Smiljanic · S. Todorovic · A. Mladenovic Djordjevic · S. Ivkovic · S. Kanazir (✉)
Department of Neurobiology, Institute for Biological Research "Sinisa Stankovic", University of Belgrade, Belgrade, Serbia
e-mail: selkan@ibiss.bg.ac.rs

T. Vanmierlo
Department of Immunology and Biochemistry, Biomedical Research Institute, Hasselt University, Hasselt, Belgium

D. Lütjohann
Laboratory for Special Lipid Diagnostics/Centre Internal Medicine/UG 68, Institute of Clinical Chemistry and Clinical Pharmacology, University Clinics of Bonn, Bonn, Germany

Introduction

Aging is a gradual, continuous process of natural changes that happens at the molecular, cellular and tissue level (Cummings 2007). During early middle age, many bodily functions begin to gradually decline and the brain is no exception to this phenomenon.

Published online: 16 January 2018

However, the rate of aging is modifiable and one of the environmental factors proven to be very potent in modulating aging is nutrition.

Dietary restriction (DR) is a vigorous nongenetic and nonpharmacological intervention that is known to delay aging and increase healthspan in diverse species from yeast to mammals (Katewa and Kapahi 2010). DR is able to improve learning and memory, enhance synaptic plasticity and neurogenesis, improve behavioral performances of rodents, and counteract age-related molecular and cellular alterations that impair cognition (Duan et al. 2001; Anson et al. 2003; Mattson 2005; Mladenovic Djordjevic et al. 2010; Smiljanic et al. 2015).

Two different DR paradigms have proven effective in ameliorating aging in rodents: limited daily feeding (LDF), with reduced daily food allotment (typically 30–40% of the ad libitum consumption) and intermittent feeding (IF), referring to alternate periods of food intake with periods of fasting (Anson et al. 2003).

Regardless of the regime applied food restriction is altering the energy metabolism. The key molecule that mediates nutrient signaling pathways sensing the cellular energy status is AMP-activated protein kinase (AMPK) (Hardie et al. 2012). It is a highly conserved eukaryotic protein serine/threonine kinase that controls energy homeostasis by switching on catabolic ATP-generating pathways, including glucose uptake (Ha et al. 2015) while switching off anabolic ATP consuming processes. The role of AMPK in the brain is rather complex. Additionally, to perceiving their own energy requisite, neurons integrate neuro-humoral signals to assess organismal energy balance, and AMPK is involved in both circumstances (Ronnett et al. 2009).

Under normal physiological conditions, the brain is absolutely dependent on glucose as a fuel source. The brain comprises about 2% of body mass, but utilizes 25% of the total body glucose (Rossi et al. 2001). The aging is associated with an increased risk of deteriorating systemic control of glucose levels and of the brain glucose uptake (Craft 2006). Several preclinical and clinical studies support a role of lowered brain glucose uptake in age-associated cognitive impairment (Cunnane et al. 2011). As neurons are unable to produce or store glucose they depend on glucose transport across the blood–brain barrier (BBB) which is accomplished by the family of glucose transporters (GLUTs) (Scheepers et al. 2004). There are fourteen GLUT protein isoforms expressed in humans, including transporters for substrates other than glucose, such as fructose, myoinositol, and urate. The well-established glucose transporter isoforms, GLUTs 1–4, have distinct regulatory and/or kinetic properties that reflect their specific roles in cellular and whole body glucose homeostasis (Thorens and Mueckler 2010). GLUT1 is the major GLUT isoform expressed in brain endothelial cells (Koranyi et al. 1991; Simpson et al. 2001; Yeh et al. 2008) and astrocytes (McCall et al. 1996). GLUT3 is the primary mediator of glucose uptake into neurons (Leino et al. 1997), localized in neuropils, mostly in axons and dendrites (Thorens and Mueckler 2010). GLUT4 is expressed in a subset of neurons, especially in cholinergic neurons of the rat forebrain, and is often co-expressed in conjunction with GLUT3 (Apelt et al. 1999).

In addition to glucose, cholesterol is also essential for proper brain functioning. The brain contains 5–10 times more cholesterol than any other organ. The BBB renders homeostasis of brain cholesterol independent of circulating cholesterol and consequently cholesterol in the brain is produced by de novo synthesis (Dietschy and Turley 2001). In the complex process of cholesterol biosynthesis the first steroid intermediate lanosterol can be converted to cholesterol through two alternative pathways: the Bloch pathway via desmosterol, a direct cholesterol precursor, and the Kandutsch-Russell pathway via lathosterol. The disturbance in cholesterol homeostasis underlies many neurodegenerative diseases, and with aging represents a major risk factor for the development of such pathologies (Valenza et al. 2007; Popp et al. 2013; Vanmierlo et al. 2015). Data regarding brain cholesterol metabolism in the course of aging are somewhat inconsistent (reviewed in Martin et al. 2010). Additionally, the cholesterol metabolism in different regions of the brain is not uniform (Smiljanic et al. 2013). The levels of cholesterol precursors were significantly decreased in the hippocampus in the course of aging.

While a lot is known about the impact of various DRs on the metabolic pathways in the body, the information regarding their effects in the central nervous system is sparse. Therefore, the aim of our study was to investigate and compare the impact of different chronic restricting feeding regimens on brain energy homeostasis in different aging groups: middle-aged and aged rats (12- and 24-month-old,

respectively). To this end, we analyzed expression patterns of glucose transporters, GLUT1, GLUT3 and GLUT4, and AMPK and pAMPK in the cortex and hippocampus of both age groups. We also expanded our previously published data showing that IF affected the cholesterol metabolism in the aging brain (Smiljanic et al. 2014), with the analyses of the effects of LDF on the concentration of cholesterol and its metabolites in the brain during aging.

Materials and methods

Animals and treatments

Male Wistar rats were used in this study. All animal procedures complied with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Sinisa Stankovic", University of Belgrade. The animals were housed under standard conditions (23 ± 2 °C, relative humidity 60–70%, 12-h light/-dark cycle), and their health status was routinely checked. Food (standard laboratory chow pellets containing 8.34% water, 21.61% crude protein, 2.36% crude fat, 6.68% crude fiber, 6.55% crude ash, 1.95% minerals) was available ad libitum (AL) until the young adult period (3 months). At that time, the average daily food consumption was determined and rats were divided into three groups: the first group continued to receive food ad libitum (AL group), the second group was allowed 60% of determined mean daily intake every day (60% AL-LDF group), and the third group was fed AL every other day (IF group). All experimental groups (n = 5 per group) were maintained on these dietary regimens until 12 (middle-aged) and 24 (aged) months of age when they were sacrificed by decapitation. Brains were quickly removed, and the cortex and hippocampus were dissected on ice, collected for subsequent sterol and protein analysis, and stored at − 80 °C until further use. Blood was collected from the trunk, and the serum was isolated and frozen.

Serum glucose concentration

Serum glucose was determined using a Randox Lab GOD/PAP (4-aminophenazone, glucose oxidase, and peroxidase) Liquid kit, with rat glucose as a standard (INEP, Belgrade, Serbia). Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts after catalysis of peroxidase, with phenol and 4-aminophenazone to form a red-violet quinoneimine dye that serves as an indicator. The absorbance was measured at 500 nm.

Western blot analysis

For Western blot analysis, the tissues were homogenized and sonicated in 10 volumes of RIPA buffer (50 mMTris–HCl pH 7.5, 150 mM NaCl, 1% NP-40, 0.5% Triton X-100, 0.1% SDS, 1 mM EDTA, 1 mM EGTA) containing a complete protease inhibitor cocktail (Roche, Mannheim, Germany) and phosphatase inhibitors (25 mM NaF, 5 mM $Na_4P_2O_7$, 2 mM Na_3VO_4). Protein concentrations were determined using the Micro BCA Protein Assay Kit (Pierce Biotechnology) and bovine serum albumin (BSA) as standard. Fifteen micrograms of proteins per lane were separated by SDS PAGE and blotted onto PVDF membranes. The membranes were blocked with 5% non-fat dry milk/Tris buffered saline with 0.05%Tween 20 (TBST) (150 mM NaCl, 50 mMTris, pH 7.4, and 0.05% Tween 20) for 1 h at room temperature (RT) and incubated with the following primary antibodies: rabbit anti-Glucose Transporter GLUT1 antibody (Abcam, ab 652, 1:2000), rabbit anti-Glucose Transporter GLUT3 antibody (Abcam, ab 41525, 1:3000), rabbit anti-Glucose Transporter GLUT4 antibody (Abcam, ab 654, 1:2000), rabbit anti-AMPKα (Cell Signaling #5831, 1:1000), rabbit anti-phospho-AMPKα (Cell Signaling #4188, 1:2000) and rabbit anti-HMGCR antibody (Millipore, ABS229, 1:1000) overnight at + 4 °C. The antibodies were diluted in TBST. Following several rinses in TBST, the membranes were incubated for 1 h at RT with the appropriate horse radish peroxidase (HRP)-conjugated secondary antibodies (bovine anti-rabbit, 1:5000 from Santa Cruz, sc-2370) diluted in TBST. HRP-immunoreactive bands were visualized by enhanced chemiluminescence (ECL, GE Healthcare) and film (Kodak Biomax) exposure. Ponceau staining

was used as loading control. Signals were quantified densitometrically using Image Quant software (v. 5.2, GE Healthcare) and expressed as relative values (i.e., normalized to the corresponding Ponceau staining). All bends at indicated molecular weight were quantified. Changes in the levels of analyzed proteins in both LDF and IF experimental group were expressed as a fold change relative to the appropriate age-matched control AL group.

Sterol profile determination

The sterol profiles were determined in the cortex and hippocampus samples as previously described (Lütjohann et al. 2002; Jansen et al. 2013; Smiljanic et al. 2013). Briefly, the tissue samples were spun in a speed vacuum dryer (12 mbar; Savant AES 1000) for 24 h. The sterols were extracted from the dried tissue by adding a 1.5-ml mixture of chloroform/methanol (2:1) for 24 h at 4 °C. Fifty micrograms of 5α-cholestane (Serva) (50 µl from a stock solution of 5α-cholestane in cyclohexane; 1 mg/ml) and 1 µg epicoprostanol (Sigma) (10 µl from a stock solution epicoprostanol in cyclohexane; 100 µg/ml) were added to 1.125 ml chloroform/methanol brain extract. One milliliter of NaOH (1 M) in 80% ethanol was added for alkaline hydrolysis over 60 min at 61 °C. Sterols were subsequently extracted with 3 ml of cyclohexane twice. The organic solvents were evaporated, and the residual sterols were dissolved in 160 µl n-decane. Subsequently, 80 µl of the n-decane samples were transferred into micro-vials for GC/MS quantification. The sterols were derivatized to trimethylsilyl (TMSi) ethers by adding 10 µlTMSi reagent (pyridine/hexamethylsisilazane/rimethylchlorosilane; 9:3:1, by volume; all reagents were applied from Merck) and incubated for 1 h at 64 °C. The residual 80 µl of n-decane samples were diluted with 300 µl n-decane and derivatized with 30 µl TMSi reagent preceding analysis of cholesterol by gas chromatography—flame ionization detection (GC/FID), and its precursors and metabolites by GC–MS using epicoprocstanol or deuterium labeled oxysterols, respectively as internal standards. The individual sterol concentrations (cholesterol (µg/mg), 24S-hydroxycholesterol (ng/mg), lanosterol (ng/mg), lathosterol (ng/mg), desmosterol (ng/mg)) were related to dry weight of the brain tissue.

Statistical analysis

All values were expressed as the mean ± SEM. Differences between the experimental groups were tested using nonparametric Mann–Whitney's U test (STATISTICA v. 6.0, StatSoft, Tulsa, OK). Statistical significance was set at $P < 0.05$.

Results

LDF and IF decrease body weight and have the opposite effect on serum glucose levels during aging

As the first step to understanding the effects of different dietary regimes (DRs) on the energy metabolism in middle-aged and aged rats, we assessed the effects of IF and LDF on body weight and serum glucose levels (Fig. 1). Body weight was significantly decreased following applied dietary restriction regimen in comparison to AL-fed controls. In middle-aged rats reduction of body weight was 32 and 40% following LDF and IF, respectively. In old animals, LDF decreased animal's weight by 25%, and IF by 32%. However, all the animals were normoglycemic. Nevertheless, glucose levels in 12-month-old animals were significantly decreased following LDF (25%) when compared to the control AL animals, while IF did not alter glucose levels in this age group. Contrary, in 24-month-old IF significantly lowered glucose levels (32%) and LDF did not have any effect when compared with AL animals of the same age.

AMPK and pAMPK levels are differently regulated following LDF and IF in the cortex and hippocampus

As the AMPK is the main energy sensor, we next examined the impact of different DRs on its expression and phosphorylation, using Western blot analysis (Fig. 2). Only IF elicited the significant decrease in the expression of AMPK in the cortex of both middle - aged and aged rats (51 and 46%, respectively) while LDF had no effect. Similarly, IF, but not LDF, decreased the levels of pAMPK (46 and 57%, respectively) (Fig. 2a). Hippocampal AMPK and pAMPK expression was not affected with either DR regimen in the middle-aged rats. In the aged rats,

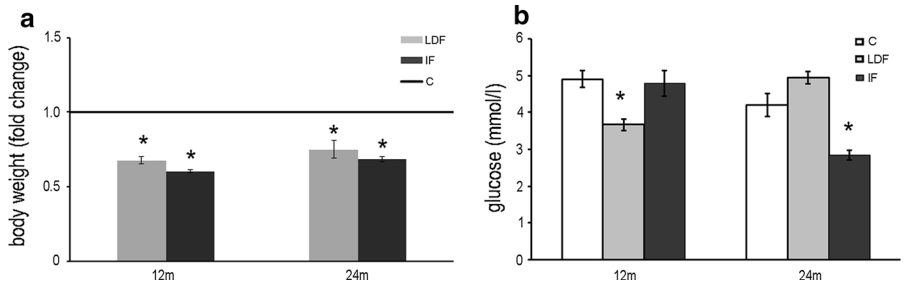

Fig. 1 Body weight and serum glucose concentration. **a** Changes in the body weight in male Wistar rats succeeding LDF (light bars) and IF (dark bars). **b** Glucose concentration in the rat serum following LDF (light bars) and IF (dark bars). The line represents the values of control, AL rats. Data are expressed as the mean ± standard error of the mean. *P < 0.05 versus age-matched control

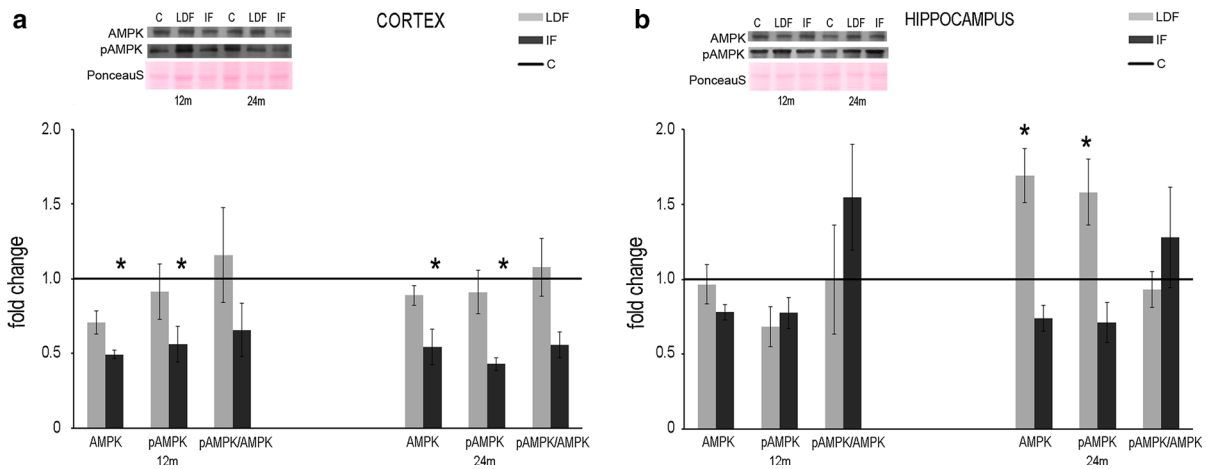

Fig. 2 The effect of long-term DR on the AMPK expression and its phosphorylation. Western blot analysis of the expression of AMPK and its phosphorylated form, as well as pAMPK/AMPK ratio in the rat cortex (**a**) and hippocampus (**b**) following LDF (light bars) and IF (dark bars).Each graph is accompanied by representative immunoblots. Ponceau S staining of the membranes served as a control protein loading. Data are expressed as the mean ± standard error of the mean. *P < 0.05 versus age-matched control

however, LDF caused an increase in AMPK and pAMPK levels (69 and 58%, respectively), while IF did not have any effect (Fig. 2b). However, in all experimental groups pAMPK/AMPK ratio remained unchanged following either LDF and IF in both the cortex and hippocampus (Fig. 2a, b).

LDF and IF predominantly affect glucose transporters expression in the cortex of aging animals

As the brain exclusively uses glucose as the energy source, its proper functioning depends on the expression of GLUT family members. Using Western blot technique we performed a comparative analysis of the expression of GLUT1, GLUT3 and GLUT 4 under LDF and IF in the cortex and hippocampus in middle-aged and aged rats (Fig. 3). GLUT1 expression in the cortex of 12-month-old animals was not altered by either LDF or IF, but in the aged animals (24 months), the expression of GLUT1 was significantly elevated (30%) under LDF and not altered following IF. GLUT3 expression was decreased under both LDF and IF in the cortex of the middle-aged animals (42 and 46%, respectively). However, in the aged animals, LDF increased (31%) and IF decreased (35%) the expression of GLUT3. GLUT4 expression in the cortex of middle-aged rats was affected under LDF only, which decreased its expression (24%). In the cortex of aged rats only IF caused the decrease of

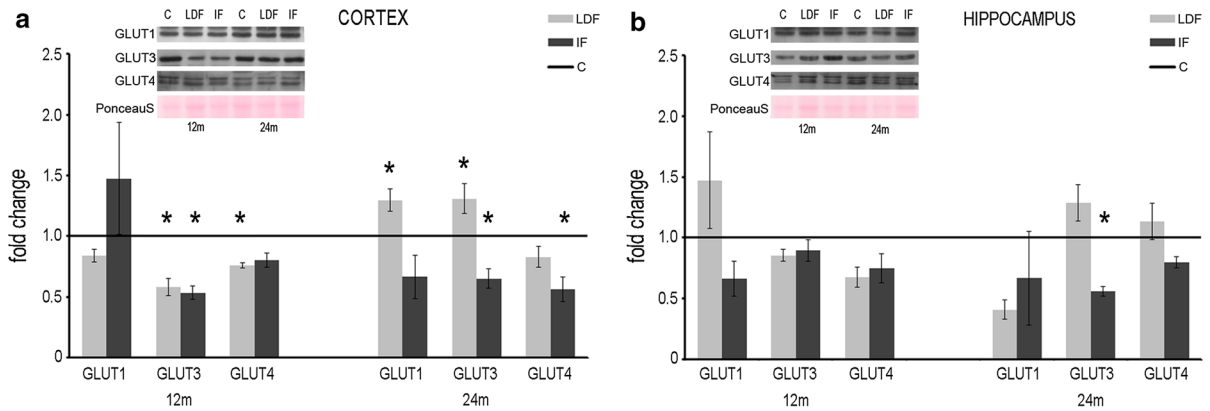

Fig. 3 Expression pattern of GLUT isoforms succeeding long-term DR. Western blot analysis of the expression of GLUT isoforms in rat cortex (**a**) and hippocampus (**b**) following LDF (light bars) and IF (dark bars). Each graph is accompanied by representative immunoblots. Ponceau S staining of the membranes served as a control protein loading. Data are expressed as the mean ± standard error of the mean. *$P < 0.05$ versus age-matched control

GLUT4 (43%) (Fig. 3a). In the hippocampus, GLUT1 and GLUT4 expression was not altered either with LDF or with IF in both middle-aged and aged animals. The expression of GLUT3 in the middle-aged rats was not altered regardless of DR regimens, while in the aged rats only IF had an effect on the GLUT3 expression eliciting its decrease (44%) (Fig. 3b).

LDF and IF differently affect cholesterol biosynthesis and the levels of cholesterol precursors depending on the brain region and age of the animals

In order to understand how these two dietary approaches affected the cholesterol metabolism, we performed Western blot analysis of the expression of the rate-limiting enzyme in cholesterol synthesis, HMGCR (Fig. 4a, b) and assessed the levels of cholesterol, its precursors and specific metabolites (Fig. 4c, d) using GC/MS. The expression level of HMGCR in the cortex remained unaltered in both middle-aged and aged animals regardless of DR protocol applied. In the hippocampus, however, LDF caused a decrease in the HMGCR expression (32%) in the middle-aged rats and an increase in the aged rats (41%). Contrary to LDF, IF decreased the HMGCR expression in the hippocampus of aged animals (28%).

Neither DR regimen altered the levels of cholesterol and its catabolite, 24S-hydroxycholesterol in either cortex or hippocampus regardless of age groups. However, both DR regimens had a profound effect on the concentrations of cholesterol precursors—lanosterol and lathosterol depending on the brain regions and age of the animals. In the middle aged rats IF had an effect only on lanosterol concentration in the cortex eliciting its decrease (33%) as compared to control group. In that same age group, LDF had an effect only on the concentration of lathosterol in the cortex, inducing its decrease (20%). Interestingly, in the cortex of the aged rats, LDF decreased the levels of lanosterol (19%). IF, however, did not affect the concentration of any of the cholesterol precursors in the cortex of aged rats (Fig. 4b). In the hippocampus of middle- aged rats IF decreased both lano- and lathosterol concentrations (32 and 35%, respectively) while LDF downregulated lathosterol only (20%). In the aged rats LDF triggered the decrease in both the lano- and lathosterol concentrations in the hippocampus (20 and 20%, respectively). Interestingly, the only change following IF was an increase (37%) in desmosterol concentration in the hippocampus of aged rats (as we previously reported in Smiljanic et al. 2014) while other metabolites remained unaltered (Fig. 4c).

Discussion

Two different experimental DR paradigms that are widely used are LDF and IF (Anson et al. 2003). A number of studies and reviews suggest that in general, LDF and IF produce similar changes regarding

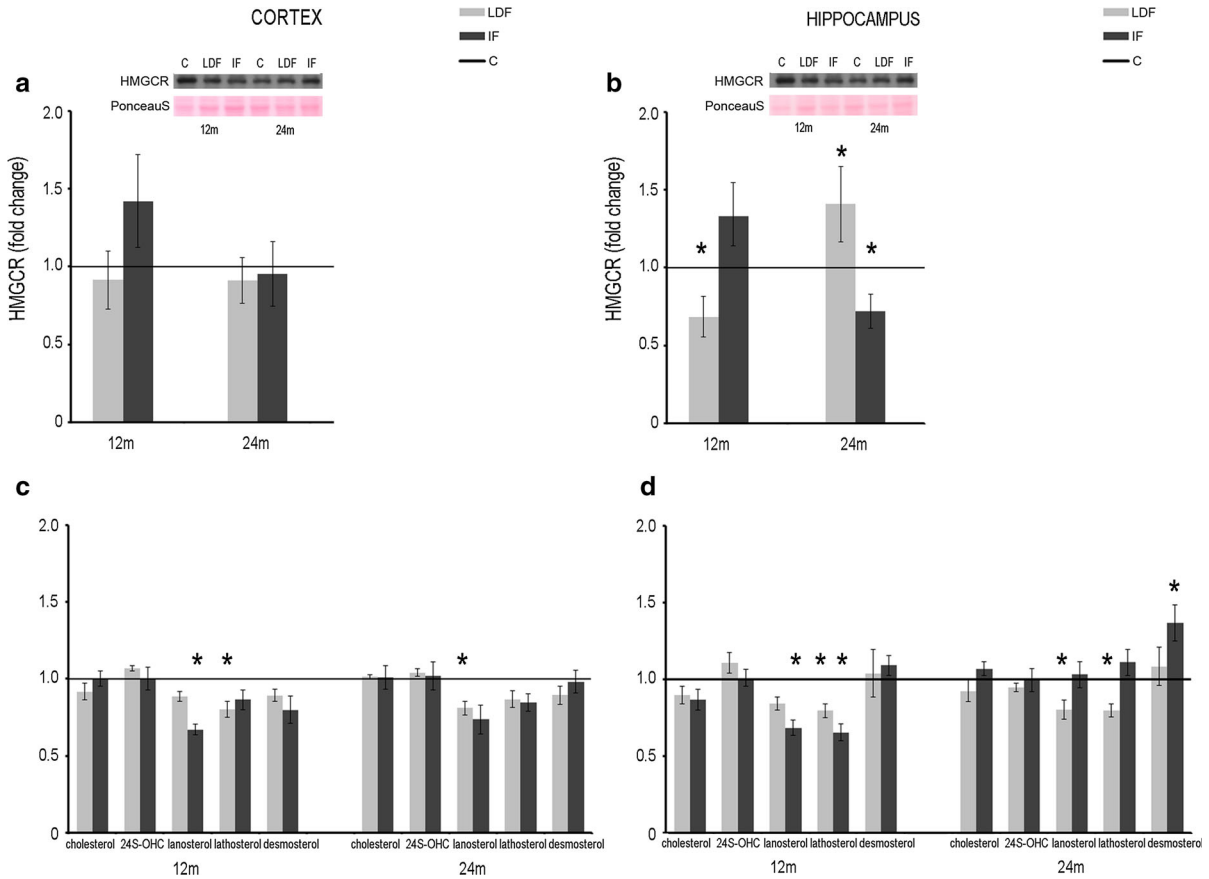

Fig. 4 The effect of long-term DR on the cholesterol metabolism in the cortex and hippocampus. Western blot analysis of the HMGCR expression in the rat cortex (**a**) and hippocampus (**b**) following LDF (light bars) and IF (dark bars). Each graph is accompanied by representative immunoblots. Ponceau S staining of the membranes served as a control protein loading. Gas chromatography/mass spectrometry analysis of the levels of cholesterol metabolites in the rat cortex (**c**) and hippocampus (**d**) following LDF (light bars) and IF (dark bars). The line represents the values of control, AL rats. Data are expressed as the mean ± standard error of the mean. *$P < 0.05$ versus age-matched control

physiological parameters such as body temperature, heart rate, blood pressure, and decreased glucose and insulin levels (Mattson et al. 2003; Yuen and Sander 2014). However, all signals generated by peripheral systems (such as the gut, fat tissue, liver, and pancreas) in response to food ingestion/restriction are transmitted to the brain to stimulate or suppress the "satiety center". These peripheral signals that regulate appetite and satiety can be episodic (short-term, meal to meal) and tonic (long-term, days and weeks). Thus, it is not only that percent of food reduction has a great impact on energy balance, but also the type of reduction, i.e., do animals get food constantly in a reduced manner or they are exposed to food intermittently. Studies in young mice showed that LDF and IF feeding regimes have the similar effect on glucose and insulin levels but affect differently the serum IGF-1 levels (Anson et al. 2003). However, aging significantly alters metabolic processes and the question remains which DR regime could be more beneficial in older age. Therefore, we analyzed how these different feeding paradigms affect the expression of key molecules involved in the regulation of energy homeostasis in the brain during aging.

Glucose is the main energy source in the brain. The glucose level in the CSF is proportional to the one in the blood, corresponding to 60–70% of the blood concentration (Mundt and Shanahan 2010). Although neither LDF nor IF caused hypoglycemia, confirming the absence of malnutrition in our experimental setting, they still significantly altered glucose levels in aging rats. Blood glucose levels in the middle-aged

rats appeared more sensitive to LDF while the old rats were more sensitive to IF. Thus, it is important that subtle changes within the physiological range of different parameters should be taken into consideration when applying different dietary regimes to different age groups.

As neurons are characterized by high metabolic activity and poor nutrient storage capacity (Vilchez et al. 2007), it is easy to understand why neurons are particularly responsive to fluctuations in energy levels. AMPK is the principal energy sensor in eukaryotic cells and functions to maintain cellular energy homeostasis (Hardie et al. 2012) and thus has a critical role in neuronal maintenance and survival. AMPK stimulates energy production from glucose and fatty acids during stress and inhibits energy consumption for protein, cholesterol, and glycogen synthesis (Steinberg and Kemp 2009; Hardie et al. 2012). Dagon et al. (2005) showed that a moderate LDF (60% AL) increased hippocampal AMPK activity, induced neurogenesis, and improved cognition, but that severe LDF (40% AL) over activated AMPK, reduced cognition, and induced neural apoptosis. AMPK is thus a regulatory factor balancing between neuronal growth and death in response to nutritional status and stress.

AMPK is activated by ATP depletion (increased AMP/ATP ratio) caused by various stimuli such as exercise, starvation, hypoxia, cellular pH and redox status, and increased creatine/phosphocreatine ratio. However, AMPK is also activated by certain drugs, hormones, and cellular stressors that do not alter AMP/ATP ratio (reviewed in Kahn et al. 2005). IF decreased the expression of AMPK and its phosphorylation in the cortex of both age groups, while LDF had no impact. As an overall cellular energy crisis can decrease AMPK activity, as occurs in the injured brain for example (Hill et al. 2016), it appears that IF regime is a stressful condition for the cortex of aging animals. However, AMPK downregulation can also be caused by the excess of food/fuel as it was shown that the surplus of glucose induced the decrease in AMPK activity in skeletal muscle and the insulin resistance that accompanies it (Coughlan et al. 2015). It is possible that AMPK regulatory network reads the IF regime as an intermittent abundance of food rather than intermittent fasting, thus reacting with the decreased AMPK expression. Interestingly, the hippocampus was resistant to the stress-inducing effect of IF and responded only to LDF and only in aged animals with increased AMPK and pAMPK levels probably as a result of stimulation other than blood glucose levels. AMPK activation can protect hippocampal neurons against glucose deprivation and glutamate excitotoxicity (Culmsee et al. 2001), and astrocytes from ceramide-induced apoptosis (Blázquez et al. 2001). Moreover, recent findings showed that the activation of AMPK in neurons has the ability to improve tissue homeostasis in the aging intestine through the non-cell autonomous induction of autophagy in the intestinal epithelium (Ulgherait et al. 2014). Additionally, it was shown that up-regulation of AMPK was able to positively affect mitochondrial development and metabolism (Zhang et al. 2018). Aging is accompanied with increased oxidative stress and production of reactive oxygen species (ROS), mainly from dysfunctional mitochondria (Balaban et al. 2005). Different brain regions are not equally vulnerable to oxidative damage by ROS (Hadem et al. 2017). DR is anticipated to lessen the age-related increase in oxidative stress. Our results suggested that LDF could represent a significant protection from oxidative stress in the aged hippocampus since the levels of both AMPK and pAMPK were increased following such treatment. However, this result should be interpreted with caution, since the pAMPK/AMPK ratio remained unaltered as compared to control.

Hypoglycemia and activated AMPK were shown to regulate the expression of glucose transporters in the brain (Cheng et al. 2003). We analyzed the expression of GLUT1, GLUT3 and GLUT4 isoforms following IF and LDF in the cortex and hippocampus of aging animals. As the blood glucose levels were significantly lower in 12-month-old animals under LDF we expected the increase in GLUT isoforms expression. However, in the cortex of middle-aged animals the expression of GLUT3, and to a lesser extent GLUT4, was decreased. In contrast, in spite of the unaltered glucose in 24-month-old LDF animals, GLUT3 and GLUT1 cortical expression was significantly increased suggesting that cortical requirement for glucose intake is stimulated with the chronic restriction of the food consumption at that age. However, the expression of GLUTs was downregulated under IF in aging rats in spite of the decreased glucose blood levels. This is probably the result of the decreased expression and phosphorylation of AMPK. On the other hand, GLUT3 translocation could represent a

potential mechanism for sustaining glucose uptake into neurons, even in the conditions when GLUT3 levels were reduced (Ximenes da Silva et al. 2002). As AMPK downregulation was shown to inhibit the translocation of GLUT3 to the membrane (Weisová et al. 2009), the reduced oxidative phosphorylation as a consequence of reduced food intake could function as another signal to neuronal cells to mobilize GLUT3 at the neuronal membrane surface. It has been shown that AMPK activation in BBB-derived endothelial cells directs the trafficking of GLUT1 intracellular pools to the plasma membrane, thereby increasing the endothelial sugar transport capacity. It is possible that the observed down regulation of AMPK in the cortex of aged rats can induce the opposite effect on the localization of GLUT1. Interestingly, the expression of GLUT isoforms in the hippocampus was mostly unaffected except the slight decrease of GLUT3 in 24-month-old rats subjected to the IF.

Although the overall cholesterol content remains stable following different dietary manipulations (Pallottini et al. 2003; Mulas et al. 2005; Hayakawa et al. 2007; Fon Tacer et al. 2010; Smiljanic et al. 2014), a significant decrease in the levels of cholesterol precursors was detected even after short-term fasting (20 h) (Fon Tacer et al. 2010). Similarly, we have shown that cholesterol content remained unchanged in the brain following both LDF and IF. Stable cholesterol levels could be attributed to the fact that the major pool of cholesterol in the brain resides in the myelin membranes and has quite slow turnover (Anson et al. 2003). However, lack of the changes in overall cholesterol content can blur changes in the smaller, but more active pool of cholesterol in the membrane microdomains such as lipid rafts. The influence of both DR protocols on cholesterol metabolism is observed on the level of cholesterol precursors. These alterations are important since intermediates in cholesterol biosynthesis pathway are also biologically active molecules with a plethora of regulatory functions. Among other roles, they are able to activate nuclear receptor X, which further affects the regulation of genes involved in lipid metabolism and inflammation (Fon Tacer et al. 2010). This study showed that LDF and IF elicited significant and specific changes in the levels of cholesterol precursors. These changes vary depending on the age of the rats and brain region analyzed.

Both DR protocols decreased the levels of cholesterol precursors, lano- and lathosterol, suggesting the consequent reduction of cholesterol synthesis. Lanosterol is the first steroid intermediate in cholesterol synthesis. Interestingly we observed the increase in the levels of desmosterol in the hippocampus of the aged animals under IF. In all other experimental groups, desmosterol was unaltered. This change could point to the ability of IF to promote an age-related adaptive mechanism. The fact that desmosterol is a direct cholesterol precursor produced in the Bloch cholesterol synthesis pathway prevailing in young individuals (Lütjohann et al. 2002) suggests a switch to a less energy-consuming synthesis pathway in the old animals exposed to IF (Smiljanic et al. 2014). Thus, IF, but not LDF had the potency to promote energy-saving mechanism in the hippocampus. Our previous study (Smiljanic et al. 2013) showed that desmosterol is the most abundant cholesterol precursor in the brain during the entire aging process. Additionally, its level in the hippocampus is notably higher than in the cortex. This difference could be attributed, at least partially, to the process of neurogenesis that takes place in the adult dentate gyrus (Couillard-Despres et al. 2011) and to the role of cholesterol in the myelination process (Smith 1968). Obtained data illustrated that the cortex and hippocampus responded differently to DRs applied regarding the changes in glucose and cholesterol metabolism. The summary of all the changes observed under these two different DRs is outlined in Fig. 5. Glucose metabolism estimated through the expression levels of glucose transporters is more susceptible to DRs in the cortex, while more pronounced changes in cholesterol metabolism following DRs were observed in the hippocampus. Because glucose is obtained exclusively from the blood, the density of brain capillaries that varies significantly within the brain depending on the location and energy needs can have the role in these different sensitivities to specific DRs (Klein et al. 1986). Additionally, various pathological, physiological, and environmental factors are able to influence changes in capillary density. Cholesterol, on the other hand, is produced in situ, but the demands for cholesterol are region specific (Dietschy and Turley 2001; Vanmierlo et al. 2010). The hippocampus, as the site of adult neurogenesis, appears more sensitive to the changes in cholesterol metabolism and

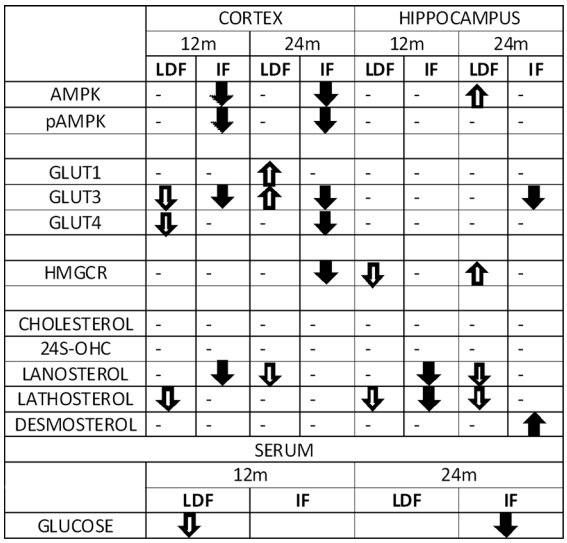

Fig. 5 Summary of changes in glucose and cholesterol metabolism in the cortex and hippocampus of middle-aged and aged rats

adaptively responds to DR effects with altered cholesterol synthesis pathway.

This study showed that the effects of LDF and IF vary greatly depending on the age and brain region analyzed. Therefore, the age should be considered as an important factor when choosing the specific DR regime among elderly as different phases of aging have different energy demands. Further studies are necessary in order to achieve optimal effects of DR as a nongenetic and nonpharmacological intervention in order to delay aging and to increase active and healthy lifespan.

Acknowledgements This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grant ON173056; the Fogarty International Research Award, NIH (R03AG046216).

Compliance with ethical standards

Conflict of interest The authors have no conflicts of interests.

References

Anson RM, Guo Z, de Cabo R, Iyun T, Rios M, Hagepanos A, Ingram DK, Lane MA, Mattson MP (2003) Intermittent fasting dissociates beneficial effects of dietary restriction on glucose metabolism and neuronal resistance to injury from calorie intake. Proc Natl Acad Sci USA 100:6216–6220

Apelt J, Mehlhorn G, Schliebs R (1999) Insulin-sensitive GLUT4 glucose transporters are colocalized with GLUT3-expressing cells and demonstrate a chemically distinct neuron-specific localization in rat brain. J Neurosci Res 57(5):693–705

Balaban RS, Nemoto S, Finkel T (2005) Mitochondria, oxidants, and aging. Cell 120(4):483–495

Blázquez C, Geelen MJ, Velasco G, Guzmán M (2001) The AMP-activated protein kinase prevents ceramide synthesis de novo and apoptosis in astrocytes. FEBS Lett 489(2–3):149–153

Cheng CM, Kelley B, Wang J, Strauss D, Eagles DA, Bondy CA (2003) A ketogenic diet increases brain insulin-like growth factor receptor and glucose transporter gene expression. Endocrinology 144(6):2676–2682

Coughlan KA, Balon TW, Valentine RJ, Petrocelli R, Schultz V, Brandon A, Cooney GJ, Kraegen EW, Ruderman NB, Saha AK (2015) Nutrient Excess and AMPK Downregulation in Incubated Skeletal Muscle and Muscle of Glucose Infused Rats. PLoS ONE 10(5):e0127388

Couillard-Despres S, Iglseder B, Aigner L (2011) Neurogenesis, cellular plasticity and cognition: the impact of stem cells in the adult and aging brain-a mini-review. Gerontology 57(6):559–564

Craft S (2006) Insulin resistance syndrome and Alzheimer disease: pathophysiologic mechanisms and therapeutic implications. Alzheimer Dis Assoc Disord 20:298–301

Culmsee C, Monnig J, Kemp BE, Mattson MP (2001) AMP-activated protein kinase is highly expressed in neurons in the developing rat brain and promotes neuronal survival following glucose deprivation. J Mol Neurosci 17(1):45–58

Cummings SR (2007) The biology of aging. J Musculoskelet Neuronal Interact 7:340–341

Cunnane S, Nugent S, Roy M, Courchesne-Loyer A, Croteau E, Tremblay S, Castellano A, Pifferi F, Bocti C, Paquet N, Begdouri H, Bentourkia M, Turcotte E, Allard M, Barberger-Gateau P, Fulop T, Rapoport SI (2011) Brain fuel metabolism, aging, and Alzheimer's disease. Nutrition 27:3–20

Dagon Y, Avraham Y, Magen I, Gertler A, Ben-Hur T, Berry EM (2005) Nutritional status, cognition, and survival: a new role for leptin and AMP kinase. J Biol Chem 280(51):42142–42148

Dietschy JM, Turley SD (2001) Cholesterol metabolism in the brain. Curr Opin Lipidol 12(2):105–112

Duan W, Guo Z, Mattson MP (2001) Brain-derived neurotrophic factor mediates an excitoprotective effect of dietary restriction in mice. J Neurochem 76:619–626

Fon Tacer K, Pompon D, Rozman D (2010) Adaptation of cholesterol synthesis to fasting and TNF-alpha: profiling cholesterol intermediates in the liver, brain, and testis. J Steroid Biochem Mol Biol 121:619–625

Ha J, Guan KL, Kim J (2015) AMPK and autophagy in glucose/glycogen metabolism. Mol Aspects Med 46:46–62

Hadem IKH, Majaw T, Kharbuli B, Sharma R (2017) Beneficial effects of dietary restriction in aging brain. J Chem Neuroanat. https://doi.org/10.1016/j.jchemneu.2017.10

Hardie DG, Ross FA, Hawley SA (2012) AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol 13(4):251–262

Hayakawa K, Mishima K, Nozako M, Hazekawa M, Aoyama Y, Ogata A, Harada K, Fujioka M, Abe K, Egashira N, Iwasaki K, Fujiwara M (2007) High-cholesterol feeding aggravates cerebral infarction via decreasing the CB1 receptor. Neurosci Lett 414:183–187

Hill JL, Kobori N, Zhao J, Rozas NS, Hylin MJ, Moore AN, Dash PK (2016) Traumatic brain injury decreases AMP-activated protein kinase activity and pharmacological enhancement of its activity improves cognitive outcome. J Neurochem 139(1):106–119

Jansen M, Wang W, Greco D, Bellenchi GC, di Porzio U, Brown AJ, Ikonen E (2013) What dictates the accumulation of desmosterol in the developing brain? FASEB J 27(3):865–870

Kahn BB, Alquier T, Carling D, Hardie DG (2005) AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism. Cell Metab 1(1):15–25

Katewa SD (2009) Kapahi P (2010) Dietary restriction and aging. Aging Cell 9(2):105–112

Klein B, Kuschinsky W, Schröck H, Vetterlein F (1986) Interdependency of local capillary density, blood flow, and metabolism in rat brains. Am J Physiol 251(6 Pt 2):H1333–H1340

Koranyi L, Bourey RE, James D, Mueckler M, Fiedorek FT Jr, Permutt MA (1991) Glucose transporter gene expression in rat brain: pretranslational changes associated with chronic insulin-induced hypoglycemia, fasting, and diabetes. Mol Cell Neurosci 2(3):244–252

Leino RL, Gerhart DZ, van Bueren AM, McCall AL, Drewes LR (1997) Ultrastructural localization of GLUT 1 and GLUT 3 glucose transporters in rat brain. J Neurosci Res 49(5):617–626

Lütjohann D, Brzezinka A, Barth E, Abramowski D, Staufenbiel M, von Bergmann K, Beyreuther K, Multhaup G, Bayer TA (2002) Profile of cholesterol-related sterols in aged amyloid precursor protein transgenic mouse brain. J Lipid Res 43:1078–1085

Martin M, Dotti CG, Ledesma MD (2010) Brain cholesterol in normal and pathological aging. Biochim Biophys Acta 1801(8):934–944

Mattson MP (2005) Energy intake, meal frequency, and health: a neurobiological perspective. Annu Rev Nutr 25:237–260

Mattson MP, Duan W, Guo Z (2003) Meal size and frequency affect neuronal plasticity and vulnerability to disease: cellular and molecular mechanisms. J Neurochem 84:417–431

McCall AL, Van-Bueren AM, Nipper V, Moholt-Siebert M, Downes H, Lessov N (1996) Forebrain ischemia increases GLUT1 protein in brain microvessels and parenchyma. J Cereb Blood Flow Metab 16:69–76

Mladenovic Djordjevic A, Perovic M, Tesic V, Tanic N, Lj Rakic, Ruzdijic S, Kanazir S (2010) Long-term dietary restriction modulates the level of presynaptic proteins in the cortex and hippocampus of the aging rat. Neurochem Int 56:250–255

Mulas MF, Demuro G, Mulas C, Putzolu M, Cavallini G, Donati A, Bergamini E, Dessi S (2005) Dietary restriction counteracts age-related changes in cholesterol metabolism in the rat. Mech Ageing Dev 126:648–654

Mundt KA, Shanahan K (2010) Graff's textbook of routine urinalysis and body fluids. Lippincott Williams & Wilkins, Philadelphia, p 237. ISBN 1582558752

Pallottini V, Marino M, Cavallini G, Bergamini E, Trentalance A (2003) Age-related changes of isoprenoid biosynthesis in rat liver and brain. Biogerontology 4:371–378

Popp J, Meichsner S, Kölsch H, Lewczuk P, Maier W, Kornhuber J, Jessen F, Lütjohann D (2013) Cerebral and extracerebral cholesterol metabolism and CSF markers of Alzheimer's disease. Biochem Pharmacol 86(1):37–42

Ronnett GV, Ramamurthy S, Kleman AM, Landree LE, Aja S (2009) AMPK in the brain: its roles in energy balance and neuroprotection. J Neurochem 109(Suppl 1):17–23

Rossi S, Zanier ER, Mauri I, Columbo A, Stocchetti N (2001) Brain temperature, body core temperature, and intracranial pressure in acute cerebral damage. J Neurol Neurosurg Psychiatry 71:448–454

Scheepers A, Joost HG, Schürmann A (2004) The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. J Parenter Enteral Nutr 28(5):364–371

Simpson IA, Vannucci SJ, DeJoseph MR, Hawkins RA (2001) Glucose transporter asymmetries in the bovine blood-brain barrier. J Biol Chem 276(16):12725–12729

Smiljanic K, Vanmierlo T, Mladenovic Djordjevic A, Perovic M, Loncarevic-Vasiljkovic N, Tesic V, Rakic L, Ruzdijic S, Lütjohann D, Kanazir S (2013) Aging induces tissue-specific changes in cholesterol metabolism in rat brain and liver. Lipids 48(11):1069–1077

Smiljanic K, Vanmierlo T, Mladenovic Djordjevic A, Perovic M, Ivkovic S, Lütjohann D, Kanazir S (2014) Cholesterol metabolism changes under long-term dietary restrictions while the cholesterol homeostasis remains unaffected in the cortex and hippocampus of aging rats. Age (Dordr) 36(3):9654

Smiljanic K, Pesic V, Mladenovic Djordjevic A, Pavkovic Z, Brkic M, Ruzdijic S, Kanazir S (2015) Long-term dietary restriction differentially affects the expression of BDNF and its receptors in the cortex and hippocampus of middle-aged and aged male rats. Biogerontology 16(1):71–83

Smith ME (1968) The turnover of myelin in the adult rat. Biochim Biophys Acta 164(2):285–293

Steinberg GR, Kemp BE (2009) AMPK in health and disease. Physiol Rev 89(3):1025–1078

Thorens B, Mueckler M (2010) Glucose transporters in the 21st Century. Am J Physiol Endocrinol Metab 298(2):E141–E145

Ulgherait M, Rana A, Rera M, Graniel J, Walker DW (2014) AMPK modulates tissue and organismal aging in a non-cell-autonomous manner. Cell Rep 8(6):1767–1780

Valenza M, Carroll JB, Leoni V, Bertram LN, Björkhem I, Singaraja RR, Di Donato S, Lütjohann D, Hayden MR, Cattaneo E (2007) Cholesterol biosynthesis pathway is disturbed in YAC128 mice and is modulated by huntingtin mutation. Hum Mol Genet 16(18):2187–2198

Vanmierlo T, Bloks VW, van Vark-van der Zee LC, Rutten K, Kerksiek A, Friedrichs S, Sijbrands E, Steinbusch HW, Kuipers F, Lütjohann D, Mulder M (2010) Alterations in brain cholesterol metabolism in the APPSLxPS1mut mouse, a model for Alzheimer's disease. J Alzheimers Dis 19(1):117–127

Vanmierlo T, Bogie JF, Mailleux J, Vanmol J, Lütjohann D, Mulder M, Hendriks JJ (2015) Plant sterols: friend or foe in CNS disorders? Prog Lipid Res 58:26–39

Vilchez D, Ros S, Cifuentes D, Pujadas L, Vallès J, García-Fojeda B, Criado-García O, Fernández-Sánchez E, Medraño-Fernández I, Domínguez J, García-Rocha M, Soriano E, Rodríguez de Córdoba S, Guinovart JJ (2007) Mechanism suppressing glycogen synthesis in neurons and its demise in progressive myoclonus epilepsy. Nat Neurosci 10(11):1407–1413

Weisová P, Concannon CG, Devocelle M, Prehn JH, Ward MW (2009) Regulation of glucose transporter 3 surface expression by the AMP-activated protein kinase mediates tolerance to glutamate excitation in neurons. J Neurosci 29(9):2997–3008

Ximenes da Silva A, Lavialle F, Gendrot G, Guesnet P, Alessandri JM, Lavialle M (2002) Glucose transport and utilization are altered in the brain of rats deficient in n-3 polyunsaturated fatty acids. J Neurochem 81(6):1328–1337

Yeh WL, Lin CJ, Fu WM (2008) Enhancement of glucose transporter expression of brain endothelial cells by vascular endothelial growth factor derived from glioma exposed to hypoxia. Mol Pharmacol 73(1):170–177

Yuen AW, Sander JW (2014) Rationale for using intermittent calorie restriction as a dietary treatment for drug resistant epilepsy. Epilepsy Behav 33:110–114

Zhang H, Liu B, Li T, Zhu Y, Luo G, Jiang Y, Tang F, Jian Z, Xiao Y (2018) AMPK activation serves a critical role in mitochondria quality control via modulating mitophagy in the heart under chronic hypoxia. Int J Mol Med 41:69–76