Original Article

The Antiepileptic Ketogenic Diet Alters Hippocampal Transporter Levels and Reduces Adiposity in Aged Rats

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Abstract

Nutritional ketosis is induced by high fat/low carbohydrate dietary regimens, which produce high levels of circulating ketone bodies, shifting metabolism away from glucose utilization. While ketogenic diets (KD) were initially introduced to suppress seizures, they are garnering attention for their potential to treat a myriad of neurodegenerative and metabolic disorders that are associated with advanced age. The feasibility and physiological impact of implementing a long-term KD in old animals, however, has not been systematically examined. In this study, young and aged rats consumed a calorically- and nutritionally-matched KD or control diet for 12 weeks. All KD-fed rats maintained higher levels of BHB and lower levels of glucose relative to controls. However, it took the aged rats longer to reach asymptotic levels of BHB compared to young animals. Moreover, KD-fed rats had significantly less visceral white and brown adipose tissue than controls without a loss of lean mass. Interestingly, the KD led to significant alterations in protein levels of hippocampal transporters for monocarboxylates, glucose, and vesicular glutamate and gamma-aminobutyric acid. Most notably, the age-related decline in vesicular glutamate transporter expression was reversed by the KD. These data demonstrate the feasibility and potential benefits of KDs for treating age-associated neural dysfunction.

Keywords: Cognitive decline, Brain aging, Glucose, Metabolism, Neurodegeneration

The past decade has seen a surge in the number of studies investigating the therapeutic potential of ketogenic diets (KD), which shift the body's primary metabolic substrate from glucose to fat, to treat a wide range of neurological disorders. Clinically, KDs have been well documented to improve brain functioning in epilepsy (1,2), as well as reduce cognitive dysfunction in rodent models of seizure disorders (3). While the KD has been used as a treatment for epilepsy for nearly a century (4,5), more recently it has been implicated as an effective therapeutic in several other neurological disease states including Parkinson's Disease, Alzheimer's Disease and dementia (6,7), as well as diabetes, cancer, and cardiovascular disease (7–9). The biggest risk factor for many of the diseases for which the KD is currently being investigated is advancing age. However, a well-controlled, systematic study as to how age affects the ability to enter and maintain a state of ketosis, as well as if this causes any adverse effects over the long term, has yet to be documented.

Currently, most studies involving rodent models of age-related neurodegenerative diseases have tested the effects of nutritional ketosis in young animals (6,10,11). Because of the age-related changes in metabolic function and capacity, there is great potential for differential effects across the life span. Thus, there is a direct need for a KD study in an aged rodent model that examines the physiological and biochemical impact of long-term nutritional ketosis. Moreover, previous studies using KDs in rodents are hard to reconcile and often report conflicting results due to the high degree of inconsistency in dietary implementation across studies (see Table 1). Supplementation with medium chain triglycerides, which quickly enter circulation and are readily converted into ketones by the liver, have been shown to have modest effects on hippocampal morphology (12) and behavior (13,14) in aged animals. While these data demonstrate the feasibility of KD-based therapies, prior medium chain triglyceride (MCT)-based dietary regimes have not been calorically matched across ketogenic and control groups. Moreover, young ketogenic animals

Table 1. Comparison of Variations in Previously Implemented Age- or Neurodegenerative Disease-related Ketogenic Diet Studies

Paper	Summary	Species	Duration of Diet	Macronutrient Composition (%Fat; %Carbs; %Protein kcal)	Fat Source	Calorically Matched? (Y; N)	Age at start
(6)	A KD improved motor performance but did not alleviate cognitive or pathological burden in 2 AD models	Mouse	12–16 wk	77.1; 0.5; 22.4	MCT Oil	No	5 mo
(10)	A KD improved motor performance but did not alleviate or pathological burden in AD model	Mouse	1 mo	79; 1; 8	BioServ Diet (Lard, Butter, Corn Oil)	No	1–2 mo
(11)	A KD reduced pathological burden (amyloid beta) in AD model	Mouse	43 d	79; 1; 8	BioServ Diet (Lard, Butter, Corn Oil)	No	3 mo
(31)	KD alone and with pramipexole enhanced motor function in PD model	Rat	14 d	50% cal from MCT oil; 50% cal from standard chow	MCT Oil	Yes	12–14 wk
(32)	KD protects dopaminergic neurons against 6-OHDA in rat PD model	Rat	2 wk	90.7; 0.3; 9	Crisco, corn oil	No	Not Specified
(13)	MCT oil supplementation resulted in changes in behavior and phosphorylation status	Rat	8 wk	5% cal from MCT oil or control oil; 90% calories from standard diet	MCT Oil	No	21 mo
(12)	KD resulted in changes in CA1 and DG morphology	Rat	2 mo	74.8; 2; 19 + 10% MCT or 7; 38.2; 19 + 20% MCT	MCT Oil	No	19 mo
(33)	No deleterious motor or memory effects of a KD on a Huntington's model	Mouse	10 wk	79; 1; 8	BioServ Diet (Lard, Butter, Corn Oil)	No	6 wk

Note: AD = Alzheimer's disease; KD = Ketogenic diets; MCT = Medium chain triglyceride; 6-OHDA = 6-hydroxydopamine; PD = Parkinson's Disease.

were not included in the experimental designs of previously reported studies, so it is not possible to determine if the reported effects are specific to aged animals or a ubiquitous impact of ketosis.

While little is known regarding the efficacy of KDs in aged animals, these diet regimens have extensively been shown to be effective at treating seizure disorders (1,2,4). Importantly, epilepsy shares biological features with aging, such as aberrant excitation in the hippocampus (15–18). Moreover, the incidence of subclinical seizures is higher in individuals with Alzheimer's disease (19). In fact, antiepileptic medications have been shown to reduce aberrant hippocampal activity and improve cognitive outcomes in aged rats (15,20) and humans with mild cognitive impairment (21). Because the hippocampus is vulnerable in old age (22,23), shows glucose dysregulation (24,25), and plays a major role in epileptogenesis (26), this structure is likely to exhibit neurobiological alterations following a long-term KD. Thus, as a first pass, it is critical to determine how glucose and monocarboxylate transporter expression within the HPC is altered by a KD and if shifting metabolism to using ketone bodies can reverse reported age-related alterations in the expression of metabolism-related transporters within this brain region (27,28). Furthermore, because neurotransmission is metabolically demanding and the levels of vesicular transporter proteins are known to go down with aging (29,30), it is important to examine whether the KD impacts levels of vesicular transporters in the hippocampus of young and aged animals. We hypothesized a KD, which would lead to decreased circulating glucose and elevated ketone body levels, would be associated with increased monocarboxylate transporter expression in the hippocampus, which may compensate for decreased glucose utilization in aged animals. Conversely, we predicted that glucose transporter expression would go down in all animals, reflecting the metabolic shift away from glycolysis as a primary ATP source. Furthermore, we hypothesized that the KD implemented here would alter the expression of ATP-dependent vesicular glutamate and gamma-aminobutyric acid (GABA) transporters in the hippocampus. Finally, we hypothesized that if caloric intake and protein content was equivalent across groups that a KD would not lead to considerable fat accumulation or muscle loss. The current study aimed to test these hypotheses by quantifying the impact of a 12-week KD on transporter expression within hippocampus, body composition and physical performance of young and aged rats.

Materials and Methods

Subjects and Handling

Young (4 months; $n = 26$) and aged (20 months; $n = 30$) male Fischer 344 × Brown Norway F1 Hybrid rats from the NIA colony at Taconic Farms were used in this study. Rats were housed individually and maintained on a 12-hour light:dark cycle, and all behavioral testing was performed in the dark phase. Rats were given 1 week to acclimate to the facility prior to blood collection. All experimental procedures were performed in accordance with National Institutes of Health guidelines and were approved by Institutional Animal Care and Use Committees at the University of Florida.

Diet

Prior to diet administration, rats were randomly assigned to either a high fat; low carbohydrate KD (Lab Supply; 5722, Fort Worth, TX) mixed with MCT oil (Neobee 895, Stephan, Northfield, IL) or a calorically and micronutrient comparable control diet (Lab Supply; 1810727; see Figure 1 and Supplementary Table 1 for macro and

Figure 1. Dietary composition, timeline of experiments, and glucose and ketone body measurements in young and aged rats. (A) Macronutrient composition of the control and ketogenic diets. Diets were nutrient matched and rats were fed equivalent calories (see Table 1; for micronutrient profile see Supplementary Table 1). (B) Timeline of experiments. Body composition analysis was performed using TD-NMR. Physical testing included body composition analysis as well as rotarod and grip strength tests (Supplementary Figure 1). (C) Diet significantly affected serum glucose levels ($p < .001$), such that they decreased over time in animals on the ketogenic diet regardless of age. (D) Diet significantly affected serum β -hydroxybutyrate (BHB) levels ($p < .001$), such that they increased over time in animals on the ketogenic diet regardless of age, although the rate at which young and aged animals reached asymptotic levels of serum BHB significantly differed ($p < .001$), with the aged rats taking longer. Error bars represent ± 1 standard error of the mean (SEM).

micronutrient composition). There were 13 young and 15 aged rats in each diet group. Because aged animals were used in this study, care was taken to provide adequate protein as well as sufficiently high levels of choline to protect the liver. Rats were weighed daily and given ~ 51 kcal at the same time each day. Although rats consumed all of their food each day, this amount of food was sufficient for continued growth in the young adult rats. That being said, the observation that food bowls were emptied from one day to the next could represent a modest caloric restriction. This small amount of restriction was necessary to ensure that all experimental groups had identical caloric intake and to protect against over consumption, which has been noted in other ketogenic or high fat diet studies (34–36). Access to water was ad libitum and water intake was measured daily in a subset of rats ($n = 3/\text{group}$) to monitor potential effects of the diet on water consumption.

BHB and Glucose Measurements

β -hydroxybutyrate (BHB; mmol/L), the prominent ketone body in the blood, and glucose levels (mg/dL) were determined using the Precision Xtra blood monitoring system (Abbott Diabetes Care, Inc, Alameda, CA). Before starting the control or KD, baseline levels were obtained from each rat while free feeding on standard laboratory chow. Rats either underwent blood collection from the saphenous

vein ($n = 3/\text{group}$) or received a small tail nick to obtain several drops of blood for monitoring glucose and ketone body levels ($n = 10$ young, 12 aged rats; diet group). For saphenous vein collection, rats were briefly anesthetized with isoflurane; the area was shaved and cleaned. A small needle was then used to puncture the vein. For tail nicks, the animal was briefly restrained, the tail was cleaned and a sterile scalpel was used to make a small nick in the tail of the awake animal. Measurements were taken 1 hour after eating at multiple weekly time points between weeks 0 and 12.

Body Composition

Brown and white adipose tissue was isolated ($n = 8$ rats per group) at the time of sacrifice during week 12 by an experimenter blind to diet and age group. Perirenal, retroperitoneal, and epididymal white adipose tissues, as well as interscapular brown adipose tissue (iBAT) were excised and weighed (see Figure 2D). White adipocytes have few mitochondria and are single lipid droplets. In contrast, brown adipocytes consist of many small lipid droplets, are rich of iron and contain mitochondria, causing the identifiable brown discoloration. During the excision of the iBAT, all the muscle and white adipose tissue that were connected to the iBAT were removed, and the remaining tissue was weighed. In three rats/group, fat was only measured at the time of sacrifice.

In the other 10 young and 12 aged rats/group, time domain-nuclear magnetic resonance (TD-NMR) imaging was performed using a Minispec LF series machine (Bruker; Billerica, MA) to obtain lean, fat and fluid mass. Measurements were taken before initiation of the diet, and then after 10 weeks on the diet. Awake rats were weighed, positioned in a clear plastic tube and then placed inside the machine. Each rat was measured twice per session and the average was taken for each rat.

Physical Assessments

Ten young and 12 aged rats from each group were trained on the rotarod test of motor coordination (Rotamex, Columbus Instruments; Columbus, OH) for 2 consecutive days at 4.0 RPM. On the third and fourth days, motor performance was evaluated by an accelerating rotamex test consisting of six total trials across 2 days. The trial began at 4.0 RPM and accelerated 1.0 RPM every 8.0 seconds for a maximum of 300 seconds. The time spent on the rotarod (seconds) and the speed of rotation at the time of fall (RPM) were recorded.

Grip strength (kgF) was tested ($n = 10$ young and 12 aged/group) using a Chatillon Grip Strength apparatus (Columbus Instruments; Columbus, OH). Rats were held by the tail and allowed to place their forelimbs on the machine. Once the rat was able to grip the machine, they were firmly pulled away from the machine to measure grip strength. This was repeated for a total of three trials. The maximum grip strength was then corrected for body weight. The same experimenter performed all grip strength testing for all rats.

Hippocampal Tissue and Blood Collection

The hippocampus was collected from five rats/group. One hour after feeding, rats were anesthetized briefly in a jar of isoflurane and decapitated. Blood collected from the trunk was tested for BHB, glucose, and corticosterone (as described in Supplementary Materials). The brain was extracted and hemisected, and the left hippocampus was extracted for protein quantification. All tissue samples were immediately frozen on dry ice and stored at -80°C .

Figure 2. The effects of a ketogenic diet on body composition. (A) There was a significant effect of both age and diet ($p < .001$ for both) on the change in body weight after 12 weeks on a ketogenic diet, such that aged rats on a ketogenic diet lost more weight than rats on a calorie-matched control diet. (B) There was a main effect of both age ($p < .04$) and diet ($p < .01$) on the amount of visceral white adipose tissue (WAT) obtained from rats after 12 weeks. (C) Similar to WAT, there was an effect of both age and diet ($p < .001$ for both) on the amount of brown adipose tissue (BAT) recovered. (D) Schematic of brown and white adipose tissue depots quantified. (E) There was a significant main effect of both age ($p < .001$) and diet ($p = .03$) on the change in body fat %, but no significant age by diet interaction ($p = .07$). (F) There was a significant main effect of age ($p = .01$), but not diet ($F_{(3,40)} = 0.06$; $p = .81$), on the change in lean mass %. Error bars represent ± 1 SEM and asterisks indicate a p value $< .05$.

Preparation of Hippocampal Membrane Fractions and Western Blotting

Hippocampal tissues were homogenized, and the membrane fractions were isolated according to previously published procedures (37) (see Supplementary Methods for details). Five micrograms of protein/lane were separated on 4%–15% TGX gels (Bio-Rad Laboratories, Hercules, CA) at 200V for 40 minutes in tris-glycine running buffer (Bio-Rad). Total protein was transferred to a 0.45 μ m pore nitrocellulose membrane at 20 V for 7 minutes using iBlot Gel Transfer Nitrocellulose Stacks (NR13046-01, Invitrogen, Waltham, MA) and an iBlot machine (Invitrogen). All experiments were conducted in triplicate and the loading order of samples was randomized between gels and experiments to control for systematic variation in the electrophoresis and electroblotting procedures.

Immediately after transfer, membranes were stained for total protein using LiCor's Revert total protein stain for 5 minutes (Li-Cor, 926–11011) and scanned using a 685 nm laser on an Odyssey IR Scanner (Li-Cor, Lincoln, NE) to detect total protein levels and then placed into Rockland blocking buffer (Rockland Blocking Buffer, Rockland, Gilbertsville, PA) for 1 hour at room temperature. After blocking, membranes were incubated at 4°C overnight with antibodies raised against MCT1, MCT2, MCT4 (ketone body, lactate and pyruvate transporters); GLUT1, GLUT3, GLUT4 (glucose transporters); VGLUT1 (vesicular glutamate transporter 1), or VGAT (vesicular GABA transporter; see Supplementary Table 2 for expanded description of antibodies). Membranes were washed in tris buffered saline before incubation in donkey anti-rabbit or donkey anti-mouse secondary antibodies conjugated to IRDye800 (diluted 1:15,000; LI-COR). Blots were scanned using a 785 nm laser on an Odyssey IR Scanner. The target protein:total protein ratio was calculated for each

technical replicate and data from each independent biological sample were transformed to percent expression of the young, control diet-fed group (ie, mean of this group is set to 100%) for statistical analysis.

Statistical Analysis

All quantitative data are represented as mean $\pm$ SEM. For all longitudinal data, statistical significance was tested using a repeated measures analysis of variance (ANOVA), followed by paired-samples t tests where appropriate, unless otherwise stated. For endpoint or single time point data, a 2×2 multifactorial ANOVA was used, followed by paired- or unpaired-samples t tests where appropriate, unless otherwise stated. For all factorial ANOVAs, each factor had two levels (for diet: control and keto; for age group: young and aged). For all repeated measures ANOVAs the within subjects factor was weeks on diet. The null hypothesis was rejected at the level of $p > .05$ unless it was Bonferroni corrected for multiple comparisons, as stated in the text. All analyses were performed with the Statistical Package for the Social Sciences v24 (IBM, Armonk, NY).

Results

The Initiation and Physiological Impact of Nutritional Ketosis in Aged Rats

Nutritional ketosis is associated with lower blood glucose and elevated BHB. To demonstrate the efficacy of the KD used here for initiating and maintaining ketosis, glucose, and BHB were measured from the blood on a weekly basis (Figure 1). Prior to diet administration, a 2×2 factorial ANOVA was used to compare the baseline glucose levels across diet and age groups. There was a main effect of age ($F_{(1,52)} = 6.40$; $p < .02$), but not diet ($F_{(1,52)} = 0.01$; $p = .91$),

such that all aged animals had higher blood glucose levels than the young group, suggestive of age-associated insulin dysregulation. Repeated-measures ANOVA (within subjects factor of week and between subjects factors of age and diet) of blood glucose levels during weeks 0–11 indicated a significant change over the duration of the diet ($F_{[9,144]} = 80.34$; $p < .001$; Figure 1C). Moreover, there was a significant main effect of both age ($F_{[1,16]} = 28.467$; $p < .001$) and diet ($F_{[1,16]} = 968.887$; $p < .001$) on overall glucose levels. Alterations in glucose levels from baseline through the diet varied significantly as a function of diet group, as indicated by a significant glucose by diet interaction ($F_{[10,160]} = 15.488$; $p < .001$). Planned contrasts showed that after 1 week on the diet, there was a significant difference in glucose levels between the KD and control diet groups, and this was maintained for the duration of the diet ($p < .01$ for all comparisons). The interaction effect between blood glucose levels and age was also significant ($F_{[10,150]} = 2.675$; $p < .01$). Specifically, the young rats on the control diet had glucose levels that increased reaching similar levels to the aged control rats. Conversely, the aged rats on the KD had blood glucose levels that decreased from baseline and became similarly low to young KD rats.

The levels of BHB, the predominant ketone body in the blood, were obtained from the samples collected at baseline and over diet duration (Figure 1D). At baseline, a 2×2 factorial ANOVA indicated that BHB did not significantly differ with age ($F_{[1,52]} = 0.11$; $p = .74$) or diet ($F_{[1,52]} = 0.23$; $p = .63$). Repeated measures ANOVA for BHB comparing baseline to subsequent weeks indicated a significant change across the 11 weeks ($F_{[9,144]} = 4.95$; $p < .001$). Moreover, there was a significant main effect of diet on overall BHB ($F_{[1,16]} = 467.862$; $p < .001$), but not a significant effect of age ($F_{[1,16]} = 0.749$; $p = .40$), and no significant age by diet interaction ($F_{[1,16]} = 1.642$; $p = .218$). These data demonstrate that the KD was able to significantly increase BHB levels in both young and aged rats.

Interestingly, changes in BHB levels over the 11 weeks significantly interacted with age group ($F_{[10,160]} = 6.69$; $p < .001$), suggesting that young and aged rats initiate nutritional ketosis at different rates. This idea was supported by the observation that during the second and third week of the diet, there was a significant age by BHB by diet interaction ($p < .01$ for both comparisons). Specifically, the young rats on the KD had elevated BHB relative to aged rats, but levels in young and aged rats on the control diet did not differ. Importantly, this difference between young and aged rats in the KD group was ameliorated by week 4 ($p = .651$), and remained similar between age groups for the duration of the diet. The extended amount of time required for aged rats to reach asymptotic high levels of blood BHB could be due to the elevated blood glucose and increased body mass they show at baseline, which could be associated with larger glycolysis stores. Finally, there were no changes in corticosterone levels or average water consumption (see Supplementary Results and Supplementary Figure 1), indicating that adverse physiological consequences in old animals on the KD were not detected.

Weight was recorded daily for rats and weekly averages were determined. At baseline, a 2×2 factorial ANOVA indicated aged rats were significantly heavier than young (average starting weight: young = 329.4 g; aged = 503.4 g; $F_{[1,52]} = 544.52$, $p < .001$), but weight did not significantly differ between diet groups ($F_{[1,52]} = 0.10$, $p = .76$). Moreover, the interaction between age and diet on weight did not reach statistical significance ($F_{[1,52]} = 0.01$, $p = .91$). After 12 weeks on the KD, a 2×2 factorial ANOVA indicated that there was a significant main effect of both age ($F_{[1,52]} = 30.81$, $p < .001$) and diet ($F_{[1,52]} = 10.76$, $p < .01$) on change in body weight (Figure 2A), but the age by diet interaction was not significant ($F_{[1,31]} = 0.38$, $p = .54$).

Post hoc analysis on the effect of diet on weight change over 12 weeks within each age group revealed that the aged rats on the KD lost significantly more weight than the aged rats on the control diet ($t_{[16]} = 3.40$; $p < .01$ corrected $\alpha = 0.025$), while weight change across diet groups in young animals did not significantly differ ($t_{[15]} = 1.60$; $p = .13$; corrected $\alpha = 0.025$).

The amount of postmortem visceral white adipose tissue (WAT) significantly varied by both age ($F_{[1,28]} = 4.74$, $p < .04$) and diet ($F_{[1,28]} = 9.39$, $p < .01$), but the diet by age interaction did not reach statistical significance ($F_{[1,28]} = 0.16$, $p = .69$; Figure 2B). Additionally, the amount of postmortem brown adipose tissue (BAT) indicated that aged rats had significantly less ($F_{[1,28]} = 16.68$, $p < .001$). Moreover, BAT was significantly reduced by the KD ($F_{[1,28]} = 30.71$, $p < .001$), but there was not a significant age by diet interaction ($F_{[1,28]} = 0.20$, $p = .66$; Figure 2C).

When body composition was measured longitudinally with TD-NMR, there was a significant effect of both age ($F_{[3,40]} = 38.49$; $p < .001$) and diet ($F_{[3,40]} = 4.86$; $p = .03$) on the change in body fat %. While the age by diet interaction did not reach statistical significance ($F_{[3,40]} = 3.60$; $p = .07$; Figure 2E), there was a trend for the aged rats to lose body fat, while the young rats did not show reduced body fat and may actually have had a modest increase over 12 weeks. If fat loss is commensurate with reductions in lean mass, weight loss may not represent improved body conditioning. Importantly, the significant difference on the change in lean mass % ($F_{[3,40]} = 7.18$; $p = .01$) was due to reductions in young animals while the aged rats maintained lean mass. Moreover, there was no significant effect of diet on change in lean mass % ($F_{[3,40]} = 0.06$; $p = .81$), nor was there an age by diet interaction ($F_{[3,40]} = 0.30$; $p = .58$; Figure 2F). These data indicate that the KD does not lead to loss of muscle mass in aged animals and may in fact improve overall body condition.

Rotarod and Grip Strength Measurements

To determine whether overall muscle function was preserved in rats on long-term ketogenic or control diets, coordination and grip strength were tested at week 10. A 2×2 factorial ANOVA indicated a significant main effect of age for max grip strength ($F_{[1,40]} = 18.82$, $p < .001$) but not for diet ($F_{[1,40]} = 0.81$, $p = .37$), and no significant age by diet interaction was detected ($F_{[1,40]} = 2.00$, $p = .17$; Figure 3A). A 2×2 factorial ANOVA indicated a significant main effect of age

Figure 3. (A) There was a significant main effect of age ($p < .001$) but not diet ($p = .34$) on the maximum grip strength after 10 weeks on the ketogenic diets (KD) or control diet. Error bars represent $\pm$ standard error of the mean (SEM). (B) There was a significant main effect of age ($p < .001$) but not diet ($p = .41$) on the maximum latency to fall during a rotarod task following 10 weeks of the KD or control diet.

Figure 4. (A) Glucose and (B) monocarboxylate transporter quantification in hippocampal homogenates from young and aged rats on ketogenic and control diets showed age and diet-related alterations in expression level (see Supplementary Figure 2 for complete gel images). There was a main effect of age on MCT1, MCT2, and GLUT1 ($p \leq .04$ for all comparisons). There was a main effect of diet on MCT2, MCT4, GLUT1, GLUT2, and GLUT3 ($p \leq .05$ for all comparisons). There was also a significant age by diet interaction for MCT4 and GLUT1 ($p < .04$ for all comparisons). See Supplementary Table 3 for complete statistical results. Error bars represent ± 1 SEM, asterisks indicate a p value $< .05$ for age and hashtags indicate a p value $< .05$ for diet. MCT = medium chain triglyceride.

Figure 5. Vesicular (A) glutamate and (B) GABA transporter quantification in hippocampal homogenates from young and aged rats on ketogenic and control diets showed age and diet-related alterations in expression levels (see Supplementary Figure 2 for complete gel images). There was a main effect of age ($p \leq .02$), and a significant age by diet interaction for VGLUT1 ($p < .03$). There was a significant main effect of diet on VGAT ($p \leq .001$). See Table 3 for complete statistical results. Error bars represent ± 1 SEM, asterisks indicate a p value $< .05$ for age and hashtags indicate a p value $< .05$ for diet.

($F_{[1,38]} = 16.87$, $p < .001$), but not diet ($F_{[1,38]} = 0.69$, $p = .41$), on the maximum latency to fall off the Rotamex machine (Figure 3B). Furthermore, there was no age by diet interaction ($F_{[1,38]} = 1.80$, $p = .19$). Thus, the KD did not cause a loss in motor performance on either task in young or aged animals. In fact, it appeared that the aged KD rats may have slightly improved grip strength relative to the age-matched controls.

Transporter Quantification

All glucose transporters examined had a significant main effect of diet on expression within the hippocampus ($p < .01$ for all; see

Supplementary Table 3), such that transporter expression decreased with the KD (Figures 4A and Supplementary Figure 2). GLUT1, which is found on epithelial cells of the blood–brain barrier and some glial cells, was the only glucose transporter in which aged rats had significantly lower expression compared to young ($F_{[1,15]} = 7.181$; $p < .02$). For GLUT1, there was also a significant interaction of age and diet ($F_{[1,15]} = 5.619$; $p < .05$). This interaction occurred because the young KD rats had lower levels of GLUT1 compared to young control rats, while levels in aged rats did not differ across diet.

MCT1, which is found on epithelial cells on the blood brain barrier as well as some glial cells, had a significant main effect of age ($F_{[1,15]} = 5.650$; $p < .05$), such that aged rats expressed higher transporter protein levels (Figure 4B). However, there was no main effect of diet ($F_{[1,15]} = 1.42$; $p = .18$), nor was there an age by diet interaction ($F_{[1,15]} = 0.65$; $p = .34$). MCT2, which is found on cell bodies and postsynaptic terminals of neurons, also had a main effect of age ($F_{[1,15]} = 12.446$; $p < .01$). Additionally, there was a main effect of diet ($F_{[1,15]} = 8.473$; $p = .01$), such that rats on the KD had decreased transporter levels, but no age by diet interaction. MCT4, located on astrocytes, had a significant main effect of diet ($F_{[1,15]} = 5.008$; $p = .04$) such that rats on the KD had decreased transporter levels. Although there was no significant age effect ($F_{[1,15]} = 1.66$; $p = .19$), there was a significant age by diet interaction ($F_{[1,15]} = 6.920$; $p < .02$). In fact, the KD restored MCT4 expression levels in the aged rats to the levels observed in young controls.

Vesicular transporter expression in the hippocampus for glutamate and GABA were also examined (Figure 5). There was a significant main effect of age on VGLUT1 expression ($F_{[1,15]} = 7.423$; $p < .02$), but not of diet ($F_{[1,15]} = 3.302$; $p = .089$). However, the interaction between age and diet was significant ($F_{[1,15]} = 6.011$; $p < .03$). Critically, this was due to the reversal of age-related declines in VGLUT1 expression present in the control rats by a KD, such that aged rats in nutritional ketosis had levels of hippocampal VGLUT1 that were comparable to young control animals. Lastly, there was a significant main effect of diet for VGAT ($F_{[1,15]} = 19.802$; $p < .001$), with greater protein levels being observed in the KD rats. There was no age effect ($F_{[1,15]} = 2.23$; $p = .11$) or age by diet interaction ($F_{[1,15]} = 1.12$; $p = .29$) for VGAT expression, indicating that animals on a KD expressed higher protein levels than rats on a control diet, irrespective of age.

Discussion

Aged individuals are at an increased risk for neurodegenerative diseases, cardiovascular disease, diabetes, cancer, increased oxidative stress, and the accumulation of damaged proteins and nucleic acids (8). Recently, there has been a call for nutritional cognitive neuroscience research, advocating a dietary intervention for global neurological deficits affecting older adults (38). KDs may be a potential intervention strategy for aged individuals, as glucose metabolism deficits are observed in this population (39,40), and glucose utilization during cognitive testing is impaired in aged rats (41). Although there are many ongoing studies of the effects of KDs and diets high in oils/fats on age-related disease states, additional data are necessary to establish the safety and efficacy of a KD in older adults.

The current study examined the impact of implementing a long-term KD on several markers of peripheral health and hippocampal biochemistry in a rat model of old age. As varied composition and implementation of KDs complicates the interpretation of existing literature and reconciliation of information regarding health outcomes (see Table 1), the current study took care to ensure that both diets

were calorically and nutritionally equivalent, including protein and micronutrient content in young and aged rats. Furthermore, rather than the commonly used long chain fatty acids, such as lard or butter, the current study used MCT oil as the fat source, as these are more readily digested and converted into ketone bodies (42).

A critical finding from these data are that when protein and caloric intake are matched between control and KD animals, KD-fed aged rats had decreased glucose and increased ketone body levels, demonstrating the efficacy of the diet at inducing a state of nutritional ketosis at advanced ages. While the levels of ketone bodies in aged rats matched young by week 4, there was significant age by diet interaction for weeks 2 and 3 on the diet. This indicated that aged rats take longer to initiate ketosis. While the mechanism of delayed ketosis in aged rats is unknown, this finding demonstrates that short-term dietary intervention may be insufficient to produce measurable physiological changes in old animals.

One criticism of the KD is the potential for decreased muscle mass and increased muscle fatigue from inadequate protein intake (43), which could be particularly problematic for older individuals at risk for sarcopenia. Importantly, the high BHB levels in all ketogenic rats with normative dietary protein content (~20%) show that drastic decreases in protein are not necessary for the induction of ketosis. Moreover, the current data show a preservation of strength, coordination and no signs of muscle mass deterioration in aged rats. This finding is consistent with other data showing that ketosis prevents muscle loss in starved individuals (44), and promotes weight loss, with no declines in strength or muscle mass in humans (45). Aged rats on the KD also had larger changes in body weight and composition compared to aged rats on the calorie-matched control diet, which was marked by a decrease in visceral fat and body fat percentage. Importantly, these results were achieved without a reduction in caloric intake relative to controls. Thus, profound calorie restriction is not necessary for weight loss on a KD. These positive changes on body composition are especially important for aged individuals, as they are at increased risk of developing metabolic syndrome, which is linked to cognitive decline (46). In fact, high amounts of WAT in aged individuals correlate with lower scores on several types of memory tasks in humans (47), and there is a strong link between unhealthy eating habits and poorer performance on hippocampal dependent tasks in rodents (for review see ref. (44)). Furthermore, it is well established that obesity in mid- to late-life increases the risk for dementia and Alzheimer's disease (AD) (48).

While reductions in WAT and improved body composition in aged animals are likely to benefit cognition by several possible mechanisms, it is notable that the KD lead to distinct biochemical alterations in the hippocampus of young and aged rats. Consistent with previous data (27), the current study found that aging significantly reduced the levels of GLUT1 expression in the hippocampus, while not affecting levels of GLUT2 and GLUT3. Reductions in GLUT1, and likewise decreased glucose utilization, can lead to disrupted cellular metabolism and altered signaling properties of the cell. Data from hippocampal slices in a solution containing glucose, pyruvate, lactate and BHB have shown that neurons preferentially utilize BHB as the major source of acetyl-CoA over other substrates (49), suggesting that utilization of an alternative ATP source, such as ketones, may correct for age-related changes in cell physiology. Interestingly, the KD mitigated age-related differences in GLUT1 expression by lowering hippocampal levels of this protein in the young ketogenic animals. The KD also reduced expression of GLUT2 and 3 in the hippocampus of both age groups. While declines in GLUT expression across the brain may be associated with dysfunction in animals on

a standard diet, in KD fed rats this likely reflects the lack of reliance on glucose/insulin signaling for neuronal metabolism. Importantly, this is ubiquitous across age groups and matched declines in blood glucose levels. In line with the current data, the link between declining regional cerebral glucose metabolism in old age and cognitive dysfunction (50), as well as reduced gait speed (51) has been reported to be reversed by the restoration of neuronal metabolic processes. A KD may be one means by which metabolic function can be approved in older adults.

Prior to the current findings, there was a dearth of knowledge regarding age-related transporter expression level alternations within the hippocampus. Here, we report that MCT1 and MCT2 also had significant changes in hippocampal expression levels with age, such that expression of MCT1 was increased and MCT2 was decreased in old control rats. The age-related increase in MCT1 expression may be the result of improper glucose utilization and represent a shift with age towards increased use of monocarboxylates as a fuel source, which has been reported for middle-aged mice and is associated with white matter degeneration (27,28). This difference is ameliorated by a KD, such that expression of MCT1 in young and aged animals on the KD did not differ. The KD increased MCT4 levels in aged rats in a restorative fashion, to be equivalent to levels observed in young control rats. Together these data suggest that nutritional ketosis shifts the expression of transporters for different energy substrates in the hippocampus. Conceivably, this could be beneficial to the aged brain, but future cognitive testing in aged rats on a KD will be necessary to test this hypothesis.

Synaptic transmission is particularly demanding of cellular metabolism and vulnerable in advanced age. Therefore, we also measured levels of VGLUT1 and VGAT in the hippocampus. Similar to previous studies (52), there was a significant decrease in VGLUT1 expression with age in control animals. Importantly, the age-associated decline in VGLUT1 was ameliorated by the KD. Because higher levels of VGLUT1 expression are associated with better learning and memory performance (29), this increase suggests that KDs may be able to restore proper neuronal function on old animals.

To the best of our knowledge, no study has shown effects of age on hippocampus VGAT. The current novel finding suggests that while there were no changes in VGAT expression with age, this transporter was significantly increased following KD administration in both young and aged rats. Increasing the transport of GABA into vesicles may increase the inhibitory signaling potential of neurons, thus conferring antiepileptic properties unto cells within the hippocampus. Increased quantal release of GABA may serve as one of the mechanisms by which the KD is efficacious in the treatment of refractory epilepsy. Other groups have postulated how increased GABA may be, at least partially, responsible for the effects of a KD (5,53).

In addition to the positive effects, a KD can have on energy metabolism and transporter expression; there are several other mechanisms that may work synergistically to restore normal cognitive function. The moderate reduction in glucose levels in the blood has been suggested to be one possible mechanism of seizure prevention of the KD (54). In fact, a shift towards the utilization of ketone bodies for ATP production has been documented to improve brain functioning during epilepsy, which shares biological features with aging. KDs have also been shown to induce the enhancement of energy reserves within the HPC, which can maintain synaptic transmission under periods of metabolic stress (55). Furthermore, it has been suggested the KD reduces excitability within the HPC (55–59), which could be particularly beneficial in older adults and patients

with AD with memory impairments known to have hyperexcitability (17,60). While it is currently not clear how these changes in HPC transporters and signaling map onto behavioral output, future experiments will assess cognition in aged rats, and recent data have suggested that a KD implemented in middle-aged mice improved object recognition and place avoidance task performance, as well as extended the median life span (35,36).

Finally, KDs may be particularly suitable for treating AD, as demonstrated by several instances of clinical efficacy in human studies (61). The onset of mild cognitive impairment and Alzheimer's disease often correlates with epileptiform activity (19), which has shown to be remediated in many studies of epilepsy patients on a KD (1,2). Furthermore, 80% of patients with AD also present with symptoms of diabetes type II or impaired fasting glucose levels (62), which are also positively affected by KDs. The connection with impaired glucose signaling in AD has led to the suggestion that the disease could be reclassified as type III diabetes (63). Furthermore, studies of AD brains have proven that while glucose metabolism is impaired in these patients, ketone body metabolism is not (64,65). Prior studies using young rodent models have shown the feasibility of doing this type of research in animal studies, and here we demonstrate the translational potential of a KD to improve somatic and brain health in an aged population.

Supplementary Material

Supplementary data is available at *The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences* online.

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Conflict of Interest

None reported.

References

- Barañano KW, Hartman AL. The ketogenic diet: uses in epilepsy and other neurologic illnesses. *Curr Treat Options Neurol*. 2008;10:410–419.
- Bough KJ, Rho JM. Anticonvulsant mechanisms of the ketogenic diet. *Epilepsia*. 2007;48:43–58. doi:10.1111/j.1528-1167.2007.00915.x
- Jiang Y, Lu Y, Jia M, et al. Ketogenic diet attenuates spatial and item memory impairment in pentylenetetrazol-kindled rats. *Brain Res*. 2016;1646:451–458. doi:10.1016/j.brainres.2016.06.029
- Freeman JM, Kossoff EH, Hartman AL. The ketogenic diet: one decade later. *Pediatrics*. 2007;119:535–543. doi:10.1542/peds.2006–2447
- Hartman AL, Gasior M, Vining EP, Rogawski MA. The neuropharmacology of the ketogenic diet. *Pediatr Neurol*. 2007;36:281–292. doi:10.1016/j.pediatrneurol.2007.02.008
- Brownlow ML, Benner L, D'Agostino D, Gordon MN, Morgan D. Ketogenic diet improves motor performance but not cognition in two mouse models of Alzheimer's pathology. Borchelt DR, ed. *PLoS ONE*. 2013;8:e75713. doi:10.1371/journal.pone.0075713
- Stafstrom CE, Rho JM. The ketogenic diet as a treatment paradigm for diverse neurological disorders. *Front Pharmacol*. 2012;3:59. doi:10.3389/fphar.2012.00059
- Balietti M, Casoli T, Di Stefano G, Giorgetti B, Aicardi G, Fattoretti P. Ketogenic diets: an historical antiepileptic therapy with promising potentialities for the aging brain. *Ageing Res Rev*. 2010;9:273–279. doi:10.1016/j.arr.2010.02.003
- Paoli A, Rubini A, Volek JS, Grimaldi KA. Beyond weight loss: a review of the therapeutic uses of very-low-carbohydrate (ketogenic) diets. *Eur J Clin Nutr*. 2013;67:789–796. doi:10.1038/ejcn.2013.116
- Beckett TL, Studzinski CM, Keller JN, Paul Murphy M, Niedowicz DM. A ketogenic diet improves motor performance but does not affect β -amyloid levels in a mouse model of Alzheimer's disease. *Brain Res*. 2013;1505:61–67. doi:10.1016/j.brainres.2013.01.046
- Van der Auwera I, Wera S, Van Leuven F, Henderson ST. A ketogenic diet reduces amyloid beta 40 and 42 in a mouse model of Alzheimer's disease. *Nutr Metab (Lond)*. 2005;2:28. doi:10.1186/1743-7075-2-28
- Balietti M, Giorgetti B, Fattoretti P, et al. Ketogenic diets cause opposing changes in synaptic morphology in CA1 hippocampus and dentate gyrus of late-adult rats. *Rejuvenation Res*. 2008;11:631–640. doi:10.1089/rej.2007.0650
- Wang D, Mitchell ES. Cognition and synaptic-plasticity related changes in aged rats supplemented with 8- and 10-carbon medium chain triglycerides. Abreu-Villaça Y, ed. *PLoS ONE*. 2016;11:e0160159. doi:10.1371/journal.pone.0160159
- Pan Y, Larson B, Araujo JA, et al. Dietary supplementation with medium-chain TAG has long-lasting cognition-enhancing effects in aged dogs. *Br J Nutr*. 2010;103:1746–1754. doi:10.1017/S0007114510000097
- Robitsek J, Ratner MH, Stewart T, Eichenbaum H, Farb DH. Combined administration of levetiracetam and valproic acid attenuates age-related hyperactivity of CA3 place cells, reduces place field area, and increases spatial information content in aged rat hippocampus. *Hippocampus*. 2015;25:1541–1555. doi:10.1002/hipo.22474
- Wilson IA, Ikonen S, Gallagher M, Eichenbaum H, Tanila H. Age-associated alterations of hippocampal place cells are subregion specific. *J Neurosci*. 2005;25:6877–6886. doi:10.1523/JNEUROSCI.1744-05.2005
- Yassa MA, Lacy JW, Stark SM, Albert MS, Gallagher M, Stark CE. Pattern separation deficits associated with increased hippocampal CA3 and dentate gyrus activity in nondemented older adults. *Hippocampus*. 2011;21:968–979. doi:10.1002/hipo.20808
- Haberman RP, Koh MT, Gallagher M. Heightened cortical excitability in aged rodents with memory impairment. *Neurobiol Aging*. 2017;54:144–151. doi:10.1016/j.neurobiolaging.2016.12.021
- Vossel KA, Beagle AJ, Rabinovici GD, et al. Seizures and epileptiform activity in the early stages of Alzheimer disease. *JAMA Neurol*. 2013;70:1158–1166. doi:10.1001/jamaneurol.2013.136
- Koh MT, Haberman RP, Foti S, McCown TJ, Gallagher M. Treatment strategies targeting excess hippocampal activity benefit aged rats with cognitive impairment. *Neuropsychopharmacology*. 2010;35:1016–1025. doi:10.1038/npp.2009.207
- Bakker A, Krauss GL, Albert MS, et al. Reduction of hippocampal hyperactivity improves cognition in amnesic mild cognitive impairment. *Neuron*. 2012;74:467–474. doi:10.1016/j.neuron.2012.03.023
- Rosenzweig ES, Barnes CA. Impact of aging on hippocampal function: plasticity, network dynamics, and cognition. *Prog Neurobiol*. 2003;69:143–179.
- Burke SN, Barnes CA. Neural plasticity in the ageing brain. *Nat Rev Neurosci*. 2006;7:30–40. doi:10.1038/nrn1809
- Gage FH, Kelly PA, Björklund A. Regional changes in brain glucose metabolism reflect cognitive impairments in aged rats. *J Neurosci*. 1984;4:2856–2865.
- Gold PE. Glucose and age-related changes in memory. *Neurobiol Aging*. 2005;26(suppl 1):60–64. doi:10.1016/j.neurobiolaging.2005.09.002
- Bertram EH. Temporal lobe epilepsy: where do the seizures really begin? *Epilepsy Behav EB*. 2009;14(suppl 1):32–37. doi:10.1016/j.yebeh.2008.09.017
- Ding F, Yao J, Rettberg JR, Chen S, Brinton RD. Early decline in glucose transport and metabolism precedes shift to ketogenic system in female aging and Alzheimer's mouse brain: implication for bioenergetic intervention. *PLoS One*. 2013;8:e79977. doi:10.1371/journal.pone.0079977
- Klosinski LP, Yao J, Yin F, et al. White matter lipids as a ketogenic fuel supply in aging female brain: implications for Alzheimer's disease. *EBioMedicine*. 2015;2:1888–1904. doi:10.1016/j.ebiom.2015.11.002

29. Cheng XR, Yang Y, Zhou WX, Zhang YX. Expression of VGLUTs contributes to degeneration and acquisition of learning and memory. *Neurobiol Learn Mem.* 2011;95:361–375. doi:10.1016/j.nlm.2011.01.010
30. Zhang X, Li G, Guo L, et al. Age-related alteration in cerebral blood flow and energy failure is correlated with cognitive impairment in the senescence-accelerated prone mouse strain 8 (SAMP8). *Neurol Sci Off J Ital Neurol Soc Ital Soc Clin Neurophysiol.* 2013;34:1917–1924. doi:10.1007/s10072-013-1407-8
31. Shaafi S, Najmi S, Aliasgharpour H, Mahmoudi J, Sadigh-Etemad S, Farhoudi M, Baniasadi N (2016) The efficacy of the ketogenic diet on motor functions in Parkinson's disease: A rat model. *Iran J Neurol* 15:63–69.
32. Cheng B, Yang X, An L, Gao B, Liu X, Liu S (2009) Ketogenic diet protects dopaminergic neurons against 6-OHDA neurotoxicity via up-regulating glutathione in a rat model of Parkinson's disease. *Brain Res* 1286:25–31.
33. Ruskin DN, Ross JL, Kawamura M, Ruiz TL, Geiger JD, Masino SA (2011) A ketogenic diet delays weight loss and does not impair working memory or motor function in the R6/2 1J mouse model of Huntington's disease. *Physiol Behav* 103:501–507.
34. Kubant R, Poon AN, Sánchez-Hernández D, et al. A comparison of effects of lard and hydrogenated vegetable shortening on the development of high-fat diet-induced obesity in rats. *Nutr Diabetes.* 2015;5:e188. doi:10.1038/nutd.2015.40
35. Newman JC, Covarrubias AJ, Zhao M, et al. Ketogenic diet reduces midlife mortality and improves memory in aging mice. *Cell Metab.* 2017;26:547–557.e8. doi:10.1016/j.cmet.2017.08.004
36. Roberts MN, Wallace MA, Tomilov AA, et al. A ketogenic diet extends longevity and healthspan in adult mice. *Cell Metab.* 2017;26:539–546.e5. doi:10.1016/j.cmet.2017.08.005
37. McQuail JA, Bañuelos C, LaSarge CL, Nicolle MM, Bizon JL. GABA(B) receptor GTP-binding is decreased in the prefrontal cortex but not the hippocampus of aged rats. *Neurobiol Aging.* 2012;33:1124.e1–1124.12. doi:10.1016/j.neurobiolaging.2011.11.011
38. Zamroziewicz MK, Barbey AK. Nutritional cognitive neuroscience: innovations for healthy brain aging. *Front Neurosci.* 2016;10:240. doi:10.3389/fnins.2016.00240
39. Barzilai N, Banerjee S, Hawkins M, Chen W, Rossetti L. Caloric restriction reverses hepatic insulin resistance in aging rats by decreasing visceral fat. *J Clin Invest.* 1998;101:1353–1361. doi:10.1172/JCI485
40. Gabriely I, Ma XH, Yang XM, et al. Removal of visceral fat prevents insulin resistance and glucose intolerance of aging: an adipokine-mediated process? *Diabetes.* 2002;51:2951–2958.
41. McNay EC, Gold PE. Age-related differences in hippocampal extracellular fluid glucose concentration during behavioral testing and following systemic glucose administration. *J Gerontol A Biol Sci Med Sci.* 2001;56:B66–B71.
42. Ramírez M, Amate L, Gil A. Absorption and distribution of dietary fatty acids from different sources. *Early Hum Dev.* 2001;65 Suppl:S95–S101.
43. Lucas PA, Meadows JH, Roberts DE, Coles GA. The risks and benefits of a low protein-essential amino acid-keto acid diet. *Kidney Int.* 1986;29:995–1003.
44. Veech RL. The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism. *Prostaglandins Leukot Essent Fatty Acids.* 2004;70:309–319. doi:10.1016/j.plefa.2003.09.007
45. Paoli A, Grimaldi K, D'Agostino D, et al. Ketogenic diet does not affect strength performance in elite artistic gymnasts. *J Int Soc Sports Nutr.* 2012;9:34. doi:10.1186/1550-2783-9-34
46. Mozumdar A, Liguori G. Persistent increase of prevalence of metabolic syndrome among U.S. adults: NHANES III to NHANES 1999–2006. *Diabetes Care.* 2011;34:216–219. doi:10.2337/dc10-0879
47. Isaac V, Sim S, Zheng H, Zagorodnov V, Tai ES, Chee M. Adverse associations between visceral adiposity, brain structure, and cognitive performance in healthy elderly. *Front Aging Neurosci.* 2011;3:12. doi:10.3389/fnagi.2011.00012
48. Kivipelto M, Ngandu T, Fratiglioni L, et al. Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. *Arch Neurol.* 2005;62:1556–1560. doi:10.1001/archneur.62.10.1556
49. Valente-Silva P, Lemos C, Köfalvi A, Cunha RA, Jones JG. Ketone bodies effectively compete with glucose for neuronal acetyl-CoA generation in rat hippocampal slices. *NMR Biomed.* 2015;28:1111–1116. doi:10.1002/nbm.3355
50. Seetharaman S, Andel R, McEvoy C, Dahl Aslan AK, Finkel D, Pedersen NL. Blood glucose, diet-based glycemic load and cognitive aging among dementia-free older adults. *J Gerontol A Biol Sci Med Sci.* 2015;70:471–479. doi:10.1093/gerona/glu135
51. Sakurai R, Fujiwara Y, Yasunaga M, et al. Regional cerebral glucose metabolism and gait speed in healthy community-dwelling older women. *J Gerontol A Biol Sci Med Sci.* 2014;69:1519–1527. doi:10.1093/gerona/glu093
52. Martisova E, Solas M, Horrillo I, et al. Long lasting effects of early-life stress on glutamatergic/GABAergic circuitry in the rat hippocampus. *Neuropharmacology.* 2012;62:1944–1953. doi:10.1016/j.neuropharm.2011.12.019
53. Yudkoff M, Daikhin Y, Nissim I, Lazarow A, Nissim I. Ketogenic diet, brain glutamate metabolism and seizure control. *Prostaglandins Leukot Essent Fatty Acids.* 2004;70:277–285. doi:10.1016/j.plefa.2003.07.005
54. Greene AE, Todorova MT, Seyfried TN. Perspectives on the metabolic management of epilepsy through dietary reduction of glucose and elevation of ketone bodies. *J Neurochem.* 2003;86:529–537.
55. Bough KJ, Wetherington J, Hassel B, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. *Ann Neurol.* 2006;60:223–235. doi:10.1002/ana.20899
56. Nylen K, Velazquez JL, Likhodii SS, et al. A ketogenic diet rescues the murine succinic semialdehyde dehydrogenase deficient phenotype. *Exp Neurol.* 2008;210:449–457. doi:10.1016/j.expneurol.2007.11.015
57. Kawamura M Jr, Ruskin DN, Geiger JD, Boison D, Masino SA. Ketogenic diet sensitizes glucose control of hippocampal excitability. *J Lipid Res.* 2014;55:2254–2260. doi:10.1194/jlr.M046755
58. Simeone TA, Samson KK, Matthews SA, Simeone KA. In vivo ketogenic diet treatment attenuates pathologic sharp waves and high frequency oscillations in vitro hippocampal slices from epileptic Kv 1.1α knockout mice. *Epilepsia.* 2014;55:e44–e49. doi:10.1111/epi.12603
59. Dai Y, Zhao Y, Tomi M, et al. Sex-specific life course changes in the neuro-metabolic phenotype of Glut3 null heterozygous mice: ketogenic diet ameliorates electroencephalographic seizures and improves sociability. *Endocrinology.* 2017;158:936–949. doi:10.1210/en.2016-1816
60. Palop JJ, Mucke L. Epilepsy and cognitive impairments in Alzheimer disease. *Arch Neurol.* 2009;66:435–440. doi:10.1001/archneur.2009.15
61. Bredesen DE. Reversal of cognitive decline: a novel therapeutic program. *Aging (Albany NY).* 2014;6:707–717. doi:10.18632/aging.100690
62. Janson J, Laedtke T, Parisi JE, O'Brien P, Petersen RC, Butler PC. Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes.* 2004;53:474–481.
63. Gupta A, Bisht B, Dey CS. Peripheral insulin-sensitizer drug metformin ameliorates neuronal insulin resistance and Alzheimer's-like changes. *Neuropharmacology.* 2011;60:910–920. doi:10.1016/j.neuropharm.2011.01.033
64. Castellano CA, Nugent S, Paquet N, et al. Lower brain 18F-fluorodeoxyglucose uptake but normal 11C-acetoacetate metabolism in mild Alzheimer's disease dementia. *J Alzheimers Dis.* 2015;43:1343–1353. doi:10.3233/JAD-141074
65. Ogawa M, Fukuyama H, Ouchi Y, Yamauchi H, Kimura J. Altered energy metabolism in Alzheimer's disease. *J Neurol Sci.* 1996;139:78–82.