# Early Motor-Behavioral Outcome of Ischemic Stroke with Ketogenic Diet Preconditioning: Interventional Animal Study

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*Background:* Cerebral stroke, with ischemic stroke being its most common type, is the leading cause of chronic disability. The ketogenic diet has been used for treating seizures for centuries and has been considered to be a treatment for other neurologic diseases in recent years. The goal of this study is to evaluate the effects of ketogenic diet preconditioning on the early motor-behavior outcome of rats with induced cerebral ischemic stroke. *Methods:* Twenty-four rats were surveyed in 3 groups of Main, Control, and Sham. The Main group received a ketogenic diet plus medium chain triglyceride oil starting 3 days prior to stroke induction, while the other 2 groups took a normal diet. Subsequently, Endothelin-1 was injected stereotactically near the middle cerebral artery to induce an ischemic stroke in the Main and Control group. Normal saline was injected to the members of the Sham group with the same technique. The motor-behavior functions of the rats were compared between 3 groups using adjusting step, beam, and cylinder tests. *Results:* After stroke induction, rats on ketogenic diet were able to adjust their steps more efficiently, moved faster on the beam, and used their hands more symmetrically in the transparent cylinder in relation to the rats in the Control group. *Conclusion:* It seems that ketogenic diet preconditioning improves the early motor-behavioral outcome of ischemic stroke.

**Key Words:** Ischemic cerebral stroke—ketogenic diet—medium chain triglyceride—motor-behavior function—cylinder test—adjusting step test—beam test



## Introduction

Cerebral stroke is the leading cause of chronic disability, the second leading cause of dementia, and the fourth leading cause of death in the United States, with ischemic stroke being its most common type.[1] According to an estimation by the WHO, stroke-related incidents and their complications would be increasing due to the rise in the average age of the population.[2]

Despite great developments in medical and interventional strategies, taking control over cerebral ischemic stroke has had its own serious challenges and has brought out many shortcomings regarding major funds for families and societies. Hence, improving treatment strategies and protecting neurons in the early stage of ischemic stroke are considered amongst the most important issues in neurology. Unfortunately, most neuroprotective therapies have not been very successful in clinical studies so far.[3]

Brain's blood feeding is 55 mL/100g/min in the normal state. If decreased to 18 mL/100g/min, neurons get electrically silent and in case of a decrease to less than 8 mL/100g/min, neurons would die. The area between 18 mL/100g/min and 8 mL/100g/min,

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named penumbra, includes a remarkable volume of stroke and is reversible.[4] Protecting penumbra from permanent damage and reversing the electrically silent neurons back to active are a promising endeavor.

Another event that deteriorates the ischemic area state is reperfusion damage; meaning that after the primary damage, when the blood perfusion re-establishes, the secondary damage occurs. This damage seems related to the entrance of more inflammatory cells and more ruining of microvascularization which lead to more delivery of reactive oxygen species (ROS) and more complement activity.[5] There were a lot of forces to protect the ischemic area from reperfusion injury, including inhibition of apoptosis, promotion of angiogenesis, targeting the T-cells of the immune system, inhibiting ROS and modulating the metabolic processing systems.[5,6]

Glucose in a normal situation and ketone bodies (KBs)—especially β-hydroxybutyrate (BHB)—under conditions of glucose deprivation, are the brain's main energy supplies. However, it seems that the role of glucose and BHB in the central nervous system is more than energy supplies. It has been shown that hyperglycemia aggravates ischemic stroke as opposed to improving it[7] and on the other hand, ketogenic diet (KD) and its main product (BHB) have been shown to have protective function in animal models of stroke, epilepsy, Parkinson's Disease, Alzheimer's Disease, Amyotrophic lateral sclerosis, mitochondrial disorders, and even brain tumor.[8-15]

KD (high fat, low carbohydrate diet) mimics the biochemical changes related to fasting or caloric restriction.[16] Administration of KD increases fatty acid β-oxidation, which leads to increased acetyl-CoA, a compound involved in KB formation (acetoacetate, BHB, and acetone) in the hepatic mitochondria.[17] Three types of KD have been developed: Classic (Traditional) KD, Modified Atkin's diet and Low-glycemic-index treatment. These diets are mostly investigated in epilepsy studies and their efficacy was comparable.[18]

For the first time in the 1950s, a medium-chain triglyceride medium-chain triglyceride (MCT) diet (a variation of traditional KD) was introduced, allowing more use of carbohydrate and protein and making the diet more palatable than the classic KD.[19] Using MCT diet, patients were able to consume more food, have better growth, and require fewer micronutrient supplements compared to the classic KD. Also, in comparison to classic KD, in the MCT diet, fewer incidence of kidney stones, hypoglycemia, ketoacidosis, constipation, low bone density, and growth retardation would be expected.[20] Similarly, in this diet, there is no acidosis[21] or decrease in serum alanine level.[22] However, diarrhea and blowing are its complications which are not consistent findings in all studies.[20] On the other hand, its effectiveness is comparable to the routine KD.[23] The aim of this study is to determine the protective effect of preconditioning KD on the early motor outcome of ischemic stroke.

## Materials

### *Animals*

All procedures were performed in strict accordance with the Iranian revised ethical guideline to work with laboratory animals (revised in 2001. In Persian). We obtained Twenty-four healthy adult male Wistar rats (200-240 g) from Tabriz University of Medical Sciences animal laboratory. Before starting the study, all rats were allowed to acclimate to the new environment with free access to food and water. Before doing the stereotactic procedures, all rats were anesthetized to alleviate the pain. Twenty-four rats were assigned randomly to 3 diet groups (8 rats in each group): Main (KD + stereotactic stroke induction), Control (normal diet + stereotactic stroke induction) and Sham (normal diet + stereotactic normal saline injection).

### *KD Protocol*

The Control and Sham group received the normal diet. Normal diets were prepared in our laboratory based on the American Institute of Nutrition Rodent Diets (AIN-93). In this order, we mixed carbohydrates (cornstarch), fat (soybean oil), protein (casein) and minerals (AIN-93G-MX), vitamins (AIN-93G-VX) and a small amount of sucralose as a sweetener.[24]

For the faster induction of ketosis, the rats in the Main group first were kept hungry for 12 hours and then received the KD with MCT oil during all study days (3 days before stroke induction and 7 days after it). We used classic KD by mixing fat to protein and carbohydrate with a 4 to 1 ratio plus 3.5 cc/day MCT oil (BG, product of CONNOILS).

### *Stereotactic Stroke Induction Technique*

On the fourth day, for both groups of Control and Main, a stereotactic endothelin-1 (ET-1) injection was performed. The rats were anesthetized with ketamine and xylazine and they were transferred to the stereotaxic frame (Fig 1), then stroke was conducted with the injection of 1 μg of ET-1 (400 pmol in 1 μl of saline) adjacent to the left Middle cerebral artery (MCA). First, an incision was performed on the midline of the rat's head and the ET-1 was injected like with Bregma criteria: anteroposterior 1.5 mm and lateral to the midline 7 mm and vertebral to the brain surface .48 mm. The needle was kept for 10 minutes after the injection. The injection angle was 25°. Rats were awake and assessable after 24 hours. Normal saline was injected with the same technique and in the same region for the Sham group.

### *Determination of KB Levels in Blood*

First, basic serum KB level was measured before starting the KD and then it was repeated on the second, fourth, sixth, ninth and eleventh day of the study. All the samples were taken at the same time as every examined day. .2 mL

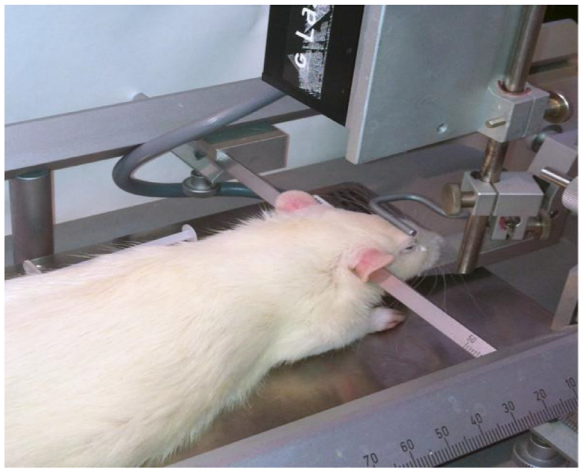

**Figure 1.** *Stereotaxic frame.*

of blood from the tail vein of the rats was collected in heparinized tubes and samples were immediately measured with the enzymatic assay (Ketone Body Assay kit, Sigma-Aldrich).

### *Motor-Behavior Tests*

Motor-Behavior tests were performed by a blind observer. Motor tests were taught to the rats for 3 days, and at the end of the training, we assessed them to make sure that they could perform the tests correctly. The first poststroke induction evaluation was performed after 48 hours. All tests were conducted in the first, fourth, sixth, ninth, and eleventh days of study and the results were compared in 3 groups. In all tests, we tried to avoid making the rats tired, due to negative effects on the results.

#### Adjusting Step Test

This test was initially introduced by Schallert et al (1979) and then refined by Olsson et al (1995) for evaluation of the unilateral animal model of Parkinson's disease. However, it is also considered valid for unilateral stroke animal model evaluation.[25] The number of steps the rats could adjust (when it was on a flat surface and moved by an examiner towards the disabled side at a steady rate) were measured in a specific distance and on a specific time. The results from 3 groups were compared before and after the stroke.

#### Beam Test

Traversal time on a beam was measured (1 m with a flat surface, Fig 2) which was reached to the box with nesting material from home cages (bed). The average time of 2 successful traversal times for each rat was measured. The mean traversal times from 3 groups were compared before and after the stroke. This test is useful in assessing motor coordination and balance in brain-lesioned mice.[26]

**Figure 2.** *Beam test.*

#### Cylinder Test

Animals were placed in a transparent cylinder and recorded for 5 minutes. We assessed the rats during the animals' dark cycle and under red lighting conditions to enhance their expletory behavior. The number of times the rats caught the wall of the cylinder in rearing up position with the unaffected hand, the affected hand, or both hands were recorded for 5 minutes. The percentage of the rat's preferential use of the unaffected hand to hold itself on the inner wall of the cylinder, to the total number of using hands (the number of times the affected hand was used, plus the use of bilateral hands) were compared before and after the stroke induction in the 3 groups. The Cylinder test was even able to detect mild sensorimotor dysfunction between the stroked and control rats, even after several days of repeated testing.[27-29]

### *Exclusion Criteria*

We considered the following as the exclusion criteria: First, if the animal did not reach the moderate results in all 3 tests while the primary training of the rats was being done; second, damage or death for any reason during the study; third, if the rats did not bear the oral diet of MCT oil regarding GI symptoms, and fourth, if the KBs did not increase significantly.

## Statistical Analysis

All the data are described as the mean ± standard deviation. After checking for normal distribution of means and homogeneity of variance, we used repeated measures ANOVA to determine the intra-group means difference in several sets of observations and one-way ANOVA to determine intergroup means differences. Whenever the differences were significant, we ran post hoc tests to highlight exactly where these differences occurred. We used SPSS statistics 22 to analyze our data and significance was considered at $P < .05$.

## Results

During the study and during the stereotactic procedure, we had no mortality or procedure-induced complications

and the rats in all 3 groups were alive without any problem. Also before the study, all the rats could learn how to do the test correctly; therefore we did not exclude any rats.

The KD itself had no significant effect on motor function before stroke induction in all 3 assessed motor-behavior tests (Figs. 3–5). Before the induction of ketosis, the serum ketone levels were statistically the same in all groups (Table 1). From the second day of ketosis induction in the Main group, blood ketone level rose significantly in this group in relation to Control and Sham and it remained consistent during the study (3.39 ± .81 mmol/L, .31 ± .09 and .34 ± .07 mmol/L respectively, $P < .001$).

As shown in Figures 3–5, in all conducted tests, after stroke induction the KD group had more or less better motor-behavior function in comparison to Control group and these findings were consistent in all 3 assessment points (sixth, ninth and eleventh day). Although rats in all 3 groups suffered from the impaired motor function after the stereotactic procedure, it was more prominent in ET-1 injected groups. For instance, in the cylinder test, the difference between the asymmetrical use of hands in the Control and Sham group, before and after the stereotactic procedure, were about 20% and 6%, respectively.

### *Adjusting Step Test*

As seen in Figure 3, results from the adjusting step test showed that stroke induction in rats with normal diet could clearly decrease the animal's ability in adjusting steps with the affected limbs ($P < .001$). It's worth pointing out that the mentioned state showed a time-relevant additive effect in the Control group so that the adjusting step had a significant decrease on the ninth and eleventh day in comparison to the sixth day. Interestingly, this trend was inverse in the Main group but the difference was significant ($P < .01$) only on the eleventh day.

### *Cylinder Test*

As seen in Figure 4, stroke induction significantly increased the percentage of priority usage of unaffected side hand in the Control group in relation to the Sham group. However, our results demonstrated that KD lessened the asymmetric use of anterior limbs in the Main group's rats in comparison to Control's rats at any point of the assessment (sixth, ninth and eleventh day) significantly ($P < .01$).

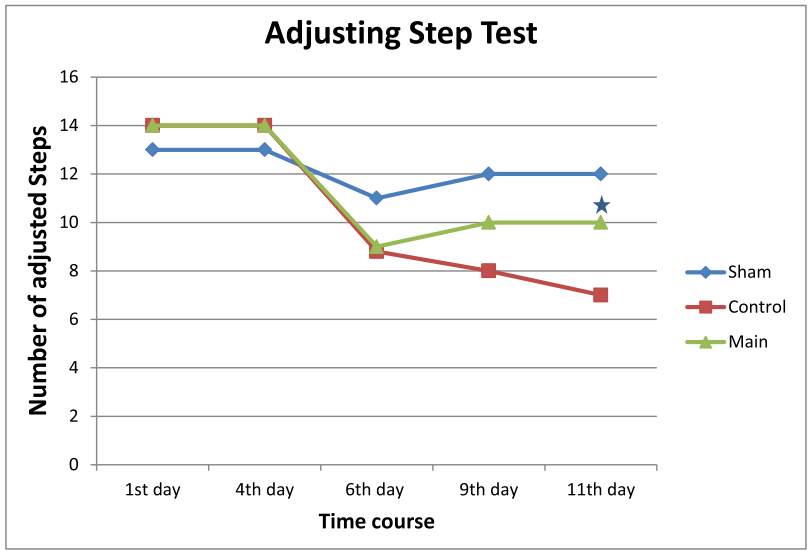


**Figure 3.** *Comparison of motor function based on adjusting step test on different groups of rats. *point of significant difference from control group.*

**Table 1.** *Serum ketone levels in rats before and after ketogenic diet in all assessment points in main, control and sham groups*

| | Basic level (mmol/L) | Second day (mmol/L) | Fourth day (mmol/L) | Sixth day (mmol/L) | Ninth day (mmol/L) | Eleventh day (mmol/L) |
|---|---|---|---|---|---|---|
| Main | .34 ± .03 | 3.39 ± .81* | 3.4 ± .72* | 3.6 ± .51* | 3.9 ± .41* | 3.2 ± .65* |
| Control | .33 ± .07 | .31 ± .09 | .30 ± .02 | .37 ± .06 | .34 ± .06 | .4 ± .04 |
| Sham | .36 ± .04 | .34 ± .07 | .33 ± .04 | .38 ± .04 | .39 ± .04 | .37 ± .05 |

*Significant difference in comparison to both Sham and Control groups.

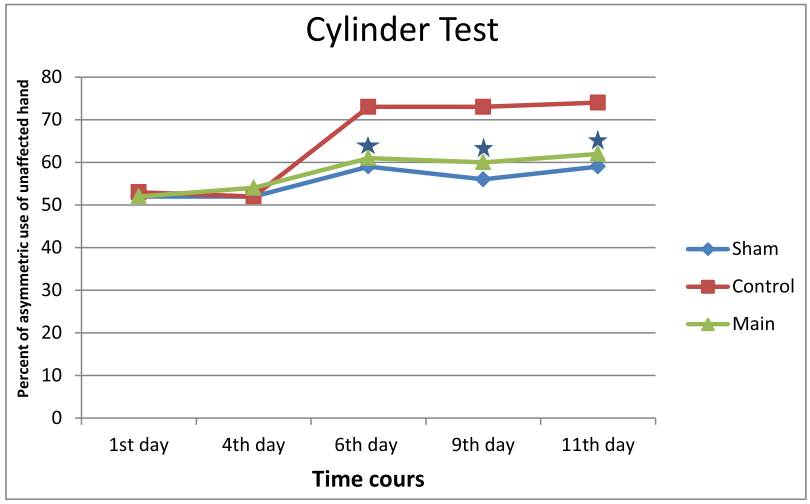


**Figure 4.** *Comparison of motor function based on cylinder test on different groups of rats. *point of significant difference from control group.*

*Beam Test Results*

As shown in Figure 5, while beam traversal time in the Main and Sham group remained more or less the same, it significantly increased in the Control group. For instance, 48 hours after stroke induction, mean of traversal time was 4.7 ± .5 seconds for the Main group, while it was 7.1 ± .85 seconds for the rats in the Control group ($P < .01$).

## Discussion

In our study, in all motor-behavior function tests, the preconditioning with KD had a significant effect on reduction of the motor dysfunction after stroke induction, and this effect, more or less, was consistent at all assessment points. After the stereotactic procedure, the rats in all 3 groups showed motor function impairment, although it was more obvious in the Main and Control group, which demonstrated the efficacy of stroke induction. Slight impairment of motor function in the saline-injected group could be due to mechanical damage of stereotactic technique and/or saline injection.

In relation to the Main group, motor impairment was severe in the Control group for Beam and Cylinder tests; and more significantly on the second day. However, it was not significant in the Adjusting Step test at this point. The difference between the Main and Control group appeared in all tests from the fifth day after stroke induction (ninth day of the study) and it persisted throughout the rest of the study significantly. In the first few days after stroke (48-72 hours), some factors such as inflammation and swelling in the ischemic area could worsen the motor function[30] and after that, ischemic areas were

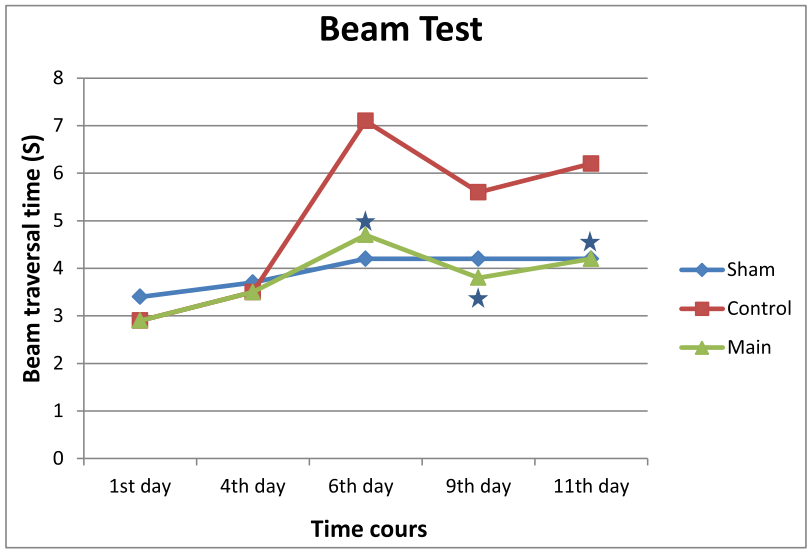


**Figure 5.** *Comparison of motor function based on beam test on different groups of rats. *point of significant difference from control group.*

stabilized gradually. Therefore, this could be a reason why the positive effect of KD appeared a little bit later in some functions.

In different animal studies, a variety of methods are used to induce an ischemic stroke. Some of these methods include vessel occlusion (filament occlusion of the middle cerebral artery or clip occlusion), using microspheres, using a model of glutamate excitotoxicity via application of iodoacetate, and using hypoxic chamber.[31] In this study, using a stereotaxic injection of ET-1 adjacent to the MCA to induce an ischemic stroke, we tried to simulate the most common type of human ischemic stroke pattern with similar gradual reperfusion rates that mimic the reperfusion state in humans more closely.[32]

Two main methods were used in the previous studies regarding the methods to evaluate the ischemic stroke outcome, namely pathological and functional. Among the functional outcomes, we used 3 behavioral-motor tests, ie adjusting step, cylinder, and beam, which we think are more clinically oriented, and impairment in these functions has a major impact on the disability of stroke patients. To our knowledge, there is no similar study to evaluate the total of these functional outcome parameters in 1 study with ketogenic preconditioning in the animal model of ischemic stroke. But as we used a KD before and after stroke induction, this study is preconditioning. Hence, its results cannot be translated into real human stroke situations precisely.

The effects of the KD have been investigated in some studies and have got some promising results too. Qi Yang et al demonstrated in 2017 that KD increased regional cerebral blood flow during both the ischemic and reperfusion stages by increasing extracellular adenosine levels. Increased cerebral blood flow could increase neuronal activity and metabolism, thus could attenuate ischemic damage. Furthermore, adenosine is an endogenous purine nucleoside that is a potent neuroprotective and promotes vasodilation and angiogenesis and augments plasma level of vascular endothelial growth factor (VEGF).[33] But in a recent review, regarding the effect of VEGF in increasing the permeability of blood-brain barrier which could exacerbate brain edema, the beneficial effect of VEGF in the acute phase of ischemic stroke has been debated. In the end, the authors concluded that its beneficial effect could be in the late phase, ie during collateral formation and reparative angiogenesis.[34]

Suzuki et al showed that BHB reduced cerebral edema formation and infarct size by improving the cerebral energy metabolism during ischemia and by inhibition of lipid peroxidation after reperfusion. Also, they showed that administration of BHB even after stroke induction was neuroprotective too.[35,36] Puchowicz et al mentioned in 2007 that diet-induced ketosis led to an increased vascular density at the blood-brain barrier without changes in blood flow.[37] In the rest of their investigation in 2008, they showed that preconditioning with the KD (3 weeks) or administration of BHB (intraventricular infusion 4 days before stroke induction) led to 55% or 70% reduction in infarct volume, respectively. Also, the KD raised Hypoxia-inducible factor-1α (HIF-1α) and an antiapoptotic protein (Bcl-2) by three fold and BHB increased the amount of these factors to some extent. At the same time Succinate is increased up to 55% by diet-induced ketosis and fourfold by BHB infusion. Of note, Succinate with inhibition of prolyl hydroxylase, the enzyme responsible for the degradation of HIF-1α, could raise the content of HIF-1α.[9]

In fact, KBs are the only circulating substrates in the absence of glucose that are known as the main contributor to cerebral metabolism. It has been mentioned that ketone metabolism is enzymatically simpler and more efficient than glucose or pyruvate metabolism and in fact, D-beta-hydroxybutyrate, physiologic isomers of BHB, presents higher inherent energy in relation to pyruvate, which is potentially important in recovering ATP levels during reperfusion.[38] With reviewing the existent evidence about the effect of KD on ischemic stroke, some bio-mechanisms could be mentioned; First KD upregulate HIF1-α[39] that regulates the cellular response to hypoxia and ischemia and enhances cellular survival pathways by regulating target genes, such as for Erythropoietin and VEGF.[40] Second, KD stimulates mitochondrial biogenesis and increases metabolic efficiency. KD enhances phosphocreatine-creatine ratio in the hippocampus as an indicator of the increase in cellular energy reserves, which enable the neurons to better withstand metabolic challenges such as ischemia, and stabilization of the neuronal membrane potential through an improved mitochondrial function.[41] Third, KD activates the mitochondrial uncoupling proteins which could decrease ROS production.[18] Fourth, by decreasing ROS production on one hand, and reducing production of inflammatory cytokines on the other hand, KD could show anti-inflammatory activity.[42,43] And lastly, KD enhances the extracellular adenosine level which has potent neuroprotective and vasodilatory effects.[33,39]

## Conclusion

Taken together, in this study we evaluated the effect of KD preconditioning on the motor-behavior function of the rate model of ischemic stroke. Using this diet led to a significant reduction in motor-behavior impairment. According to previous studies and ours, it seems that the KD reduce motor complications of ischemic stroke. Several mechanisms have been mentioned in this diet that altogether could lessen the ischemic stroke damage, probably with retrieving penumbra area and attenuating the reperfusion damage which could lead to improving the motor-behavior outcome. Regarding the safety profile and mentioned effectiveness of this diet and its induced

derivatives, ie KBs, on ischemic stroke more human studies are recommended.

## Conflict of Interest

The authors declare that there is no conflict of interest.

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## References

1. Roger VL, Go AS, Lloyd-Jones DM, et al. Heart disease and stroke statistics—2011 update: a report from the American Heart Association. Circulation 2011;123:e18-e209.
2. Truelsen T, Piechowski-Jozwiak B, Bonita R, et al. Stroke incidence and prevalence in Europe: a review of available data. Eur J Neurol 2006;13:581-598.
3. Rajah GB, Ding Y. Experimental neuroprotection in ischemic stroke: a concise review. Neurosurg Focus 2017;42:E2.
4. Olsen TS, Larsen B, Herning M, et al. Blood flow and vascular reactivity in collaterally perfused brain tissue. Evidence of an ischemic penumbra in patients with acute stroke. Stroke. 1983;14:332-341.
5. Eltzschig HK, Eckle T. Ischemia and reperfusion—from mechanism to translation. Nat Med 2011;17:1391-1401.
6. Nour M, Scalzo F, Liebeskind DS. Ischemia-reperfusion injury in stroke. Interv Neurol 2013;1:185-199.
7. Kruyt ND, Biessels GJ, Devries JH, et al. Hyperglycemia in acute ischemic stroke: pathophysiology and clinical management. Nat Rev Neurol 2010;6:145-155.
8. Prins ML. Cerebral metabolic adaptation and ketone metabolism after brain injury. J Cereb Blood Flow Metab 2008;28:1-16.
9. Puchowicz MA, Zechel JL, Valerio J, et al. Neuroprotection in diet-induced ketotic rat brain after focal ischemia. J Cereb Blood Flow Metab 2008;28:1907-1916.
10. Tieu K, Perier C, Caspersen C, Teismann P, Wu DC, Yan SD, et al. D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson disease. J Clin Invest 2003;112:892-901.
11. Lutas A, Yellen G. The ketogenic diet: metabolic influences on brain excitability and epilepsy. Trends Neurosci 2013;36:32-40.
12. Kossoff EH. More fat and fewer seizures: dietary therapies for epilepsy. Lancet Neurol 2004;3:415-420.
13. Seyfried TN, Mukherjee P. Targeting energy metabolism in brain cancer: review and hypothesis. Nutr Metab 2005;2:30.
14. Frey S, Geffroy G, Desquiret-Dumas V, et al. The addition of ketone bodies alleviates mitochondrial dysfunction by restoring complex I assembly in a MELAS cellular model. Biochimica et Biophysica Acta 2017;1863:284-291.
15. Shaafi S, Mahmoudi J, Pashapour A, et al. Ketogenic Diet Provides Neuroprotective Effects against Ischemic Stroke Neuronal Damages. Adv Pharm Bull 2014;4 (Suppl 2):479-481.
16. Gasior M, Rogawski MA, Hartman AL. Neuroprotective and disease-modifying effects of the ketogenic diet. Behav Pharmacol 2006;17:431-439.
17. Hartman AL, Gasior M, Vining EP, et al. The neuropharmacology of the ketogenic diet. Pediatr Neurol 2007;36:281-292.
18. Masino SA, Rho JM. Mechanisms of ketogenic diet action. In: th, Noebels J.L., Avoli M., Rogawski M.A., Olsen R. W., Delgado-Escueta A.V., Editors. Jasper's basic mechanisms of the epilepsies. Bethesda (MD): National Center for Biotechnology Information (US) Michael A Rogawski, Antonio V Delgado-Escueta, Jeffrey L Noebels, Massimo Avoli and Richard W Olsen; 2012.
19. Huttenlocher PR, Wilbourn AJ, Signore JM. Medium-chain triglycerides as a therapy for intractable childhood epilepsy. Neurology 1971;21:1097-1103.
20. Liu YM. Medium-chain triglyceride (MCT) ketogenic therapy. Epilepsia 2008;49(Suppl 8):33-36.
21. DeVivo DC, Pagliara AS, Prensky AL. Ketotic hypoglycemia and the ketogenic diet. Neurology 1973;23:640-649.
22. Schwartz RM, Boyes S, Aynsley-Green A. Metabolic effects of three ketogenic diets in the treatment of severe epilepsy. Dev Med Child Neurol 1989;31:152-160.
23. Neal EG, Chaffe H, Schwartz RH, et al. A randomized trial of classical and medium-chain triglyceride ketogenic diets in the treatment of childhood epilepsy. Epilepsia 2009;50:1109-1117.
24. Reeves PG, Nielsen FH, Fahey Jr. GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939-1951.
25. Trueman RC, Diaz C, Farr TD, et al. Systematic and detailed analysis of behavioural tests in the rat middle cerebral artery occlusion model of stroke: tests for long-term assessment. J Cereb Blood Flow Metab 2017;37:1349-1361.
26. Brooks SP, Dunnett SB. Tests to assess motor phenotype in mice: a user's guide. Nat Rev Neurosci 2009;10:519-529.
27. Li X, Blizzard KK, Zeng Z, et al. Chronic behavioral testing after focal ischemia in the mouse: functional recovery and the effects of gender. Exp Neurol 2004;187:94-104.
28. Schaar KL, Brenneman MM, Savitz SI. Functional assessments in the rodent stroke model. Exp Transl Stroke Med 2010;2:13.
29. Hua Y, Schallert T, Keep RF, et al. Behavioral tests after intracerebral hemorrhage in the rat. Stroke 2002;33:2478-2484.
30. Miyamoto N, Tanaka R, Ueno Y, et al. Analysis of the usefulness of the WORSEN score for predicting the deterioration of acute ischemic stroke. J Stroke Cerebrovasc Dis 2017;26:2834-2839.
31. Gibson CL, Murphy AN, Murphy SP. Stroke outcome in the ketogenic state—a systematic review of the animal data. J Neurochem 2012;123(Suppl 2):52-57.
32. Ansari S, Azari H, Caldwell KJ, et al. Endothelin-1 induced middle cerebral artery occlusion model for ischemic stroke with laser Doppler flowmetry guidance in rat. J Visualized Exp 2013.
33. Boison D. Adenosine kinase, epilepsy and stroke: mechanisms and therapies. Trends Pharmacol Sci 2006;27:652-658.
34. Shim JW, Madsen JR. VEGF signaling in neurological disorders. Int J Mol Sci 2018;19. https://doi.org/10.3390/ijms19010275. pii: E275.
35. Suzuki M, Suzuki M, Sato K, et al. Effect of beta-hydroxybutyrate, a cerebral function improving agent, on cerebral hypoxia, anoxia and ischemia in mice and rats. Jpn J Pharmacol 2001;87:143-150.
36. Suzuki M, Suzuki M, Kitamura Y, et al. Beta-hydroxybutyrate, a cerebral function improving agent, protects rat brain against ischemic damage caused by permanent and transient focal cerebral ischemia. Jpn J Pharmacol 2002;89:36-43.
37. Puchowicz MA, Xu K, Sun X, et al. Diet-induced ketosis increases capillary density without altered blood flow in rat brain. Am J Physiol Endocrinol Metab 2007;292:E1607-E1615.

38. 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.
39. Yang Q, Guo M, Wang X, et al. Ischemic preconditioning with a ketogenic diet improves brain ischemic tolerance through increased extracellular adenosine levels and hypoxia-inducible factors. Brain Res 2017;1667:11-18.
40. Freeman RS, Barone MC. Targeting hypoxia-inducible factor (HIF) as a therapeutic strategy for CNS disorders. Curr Drug Targets CNS Neurol Disord 2005;4:85-92.
41. Bough KJ, Wetherington J, Hassel B, et al. Mitochondrial biogenesis in the anticonvulsant mechanism of the ketogenic diet. Ann Neurol 2006;60:223-235.
42. Julio-Amilpas A, Montiel T, Soto-Tinoco E, et al. Protection of hypoglycemia-induced neuronal death by beta-hydroxybutyrate involves the preservation of energy levels and decreased production of reactive oxygen species. J Cereb Blood Flow Metab 2015;35:851-860.
43. Achanta LB, Rae CD. Beta-Hydroxybutyrate in the brain: one molecule, multiple mechanisms. Neurochem Res 2017;42:35-49.