The Ketogenic Diet and Brain Metabolism of Amino Acids: Relationship to the Anticonvulsant Effect

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Abstract

In many epileptic patients, anticonvulsant drugs either fail adequately to control seizures or they cause serious side effects. An important adjunct to pharmacologic therapy is the ketogenic diet, which often improves seizure control, even in patients who respond poorly to medications. The mechanisms that explain the therapeutic effect are incompletely understood. Evidence points to an effect on brain handling of amino acids, especially glutamic acid, the major excitatory neurotransmitter of the central nervous system. The diet may limit the availability of oxaloacetate to the aspartate aminotransferase reaction, an important route of brain glutamate handling. As a result, more glutamate becomes accessible to the glutamate decarboxylase reaction to yield gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter and an important antiseizure agent. In addition, the ketogenic diet appears to favor the synthesis of glutamine, an essential precursor to GABA. This occurs both because ketone body carbon is metabolized to glutamine and because in ketosis there is increased consumption of acetate, which astrocytes in the brain quickly convert to glutamine. The ketogenic diet also may facilitate mechanisms by which the brain exports to blood compounds such as glutamine and alanine, in the process favoring the removal of glutamate carbon and nitrogen.

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INTRODUCTION

The development of effective antiepileptic drugs has been a major achievement of neuroscience research. Unfortunately, these medications often fail completely to control convulsions or they cause obnoxious and even incapacitating side effects (13). This therapeutic limitation has prompted a continuing search for new drugs and alternate treatments.

One such intervention is the ketogenic diet, which clinicians commonly recommend when drug therapy proves suboptimal (35, 133, 134, 140). The diet can be remarkably effective—at least 50% of patients experience a reduction in seizure frequency of 50%, and many show complete and sustained remission (36, 55, 61, 78, 117, 123). The diet has been shown effective against different forms of epilepsy and in different patient cohorts, including adolescents and older individuals (56, 57, 65). Even a milder form of a low-carbohydrate diet (e.g., Atkins diet) may have a therapeutic effect (55).

An advantage of the diet is the relative absence of side effects, particularly the obtundation, memory loss, and other cognitive deficits that often accompany administration of antiepileptic drugs. There are no major effects on growth or weight (124). Hyperlipidemia may occur (58), but it is unclear if this perturbation is atherogenic. A recent review (9) of low-carbohydrate diets found no cogent evidence of major adverse effects on blood pressure, blood glucose, serum insulin, or blood lipids.

A ketogenic diet provides 80%–90% of calories as lipid, most frequently long-chain triglycerides, with the remainder deriving primarily from protein (61, 114). An alternate approach is to furnish lipid as medium-chain triglycerides, a more palatable strategy, but one that can cause diarrhea and cramping. Induction of ketosis traditionally is accomplished with a brief (24- to 48-hour) period of fasting, but a recent report suggests that gradual introduction of a high-fat diet results in brisk ketosis (>1.5 mM) and a robust therapeutic effect (4). A diet that provides a greater fraction of fat (>60%) as the polyunsaturated or monounsaturated species affords more intense ketosis (37).

An important and unresolved issue is whether the antiepileptic effect derives from a direct action of ketone bodies on brain physiology or results from a limitation of dietary carbohydrate and/or caloric restriction, both of which lower the blood glucose concentration. Caloric restriction confers seizure control in EL mice, a model of human multifactorial idiopathic epilepsy (43, 67). Dietary caloric restriction lowers neuronal activity in the dentate gyrus (8) and protects rats against the action of pentylenetetrazole, a convulsant (29). Extreme caloric restriction (50%) lowers seizures as effectively as the ketogenic diet and does so without greatly increasing blood ketones (8, 29).

The ketogenic diet is today an accepted therapeutic modality, but formidable scientific and ethical obstacles complicate performance of carefully controlled, long-term clinical studies of efficacy. An unresolved issue is the nature of the many physiologic and biochemical responses of brain function that the diet presumably evokes. Research suggests several hypothetical mechanisms: (*a*) A high-fat, low-carbohydrate diet favors production of 3-OH-butyric acid and acetoacetic acid, the "ketone bodies," perhaps acidifying

the brain parenchyma and inhibiting neuronal H^+-sensitive ion channels. However, there is no cogent evidence that such acidification occurs (1). (*b*) Ketosis may hyperpolarize neuronal membranes through an effect of ketone bodies or long-chain fatty acids on adenosine triphosphate (ATP)-sensitive K^+ channels (120). (*c*) Fatty acids may directly inhibit neuronal function (21); (*d*) acetone, which quickly enters brain (40), diminishes seizure threshold and severity (62); (*e*) glucose, even in physiologic concentration, may increase neuronal excitability (11), and hyperglycemia lowers the seizure threshold (103). Conversely, a diminution of blood glucose, which ought to occur with a low-carbohydrate diet, might lower neuronal excitability and thereby attenuate an epileptic diathesis. However, it should be noted that a ketogenic diet enhances brain glucose transport (17), thereby maintaining central nervous system glucose concentrations even though blood levels are diminished. (*f*) Recent investigations in mice lacking dopamine-β-hydroxylase suggest that an intact noradenergic system is necessary to recruit the therapeutic effect (115), suggesting that the diet may alter metabolism and/or function of brain biogenic amines. (*g*) Increased concentrations of fatty acids enhance both the levels and the activity of mitochondrial uncoupling protein (71, 112, 113), a phenomenon that might explain the protective effect of the diet against disorders such as glutamate toxicity (77), traumatic brain injury (96), and Parkinsonism (119).

In addition to these effects, a ketogenic diet alters brain energy metabolism (23, 26, 31, 59, 86, 99, 138–142). Ketone bodies may be a more efficient fuel than glucose in terms of energy produced per mole of oxygen consumed (99). In isolated heart, the administration of 3-OH-butyrate significantly increased (2–10 times) the nicotinamide adenine dinucleotide, reduced form/nicotinamide adenine dinucleotide (NADH/NAD) ratio and diminished (2–4 times) the mitochondrial coenzyme Q couple (99). As a consequence, energy released from oxidation of mitochondrial NADH increased from –53 kJ/mol to –60 kJ/mol. The ability of ketone bodies to increase energy produced per mole of oxygen consumed may favor restoration of membrane potential following depolarization and may diminish free radical production (121, 122).

The partial substitution of ketone bodies for glucose as a fuel also may alter brain handling of amino acids like glutamate and gamma-aminobutyric acid (GABA), the major excitatory and inhibitory neurotransmitters, respectively (91). Thus, the anticonvulsant effect might enhance inhibitory versus excitatory tone in neurons. To understand how a high-fat and low-carbohydrate diet could affect brain amino acid metabolism, we must review interrelationships between cerebral handling of glucose, glutamate, and GABA.

BRAIN METABOLISM OF GLUCOSE

The human brain consumes more than 300 kcal/kg/day. Whole-body energy expenditure in adult humans is about one-tenth this value. The brain satisfies this voracious demand by extracting from blood about 10% of the glucose of the arterial blood, or 310 μmol/kg/min, and about 50% of oxygen in arterial blood, or 1560 μmol/kg/min (53). Since whole-body oxygen use is about 8000 μmol/kg/min, it follows that the brain, which comprises only 2% of body weight, utilizes nearly 20% of overall oxygen consumption. If glucose oxidation is complete, then the stoichiometry of glucose metabolism ($C_6H_{12}O_6 + 6O_2 \rightarrow 6\,CO_2 + 6\,H_2O$) requires that consumption of oxygen must exceed that of glucose by a factor of six rather than the observed ratio (1560/310) of about five. This discrepancy in part reflects the fact that brain glucose oxidation is incomplete, with some glucose being converted to lactate, not CO_2. In addition, a small component of overall oxygen consumption is applied not to glucose oxidation but to the synthesis of macromolecules (19, 66, 106).

Cerebral metabolism is intense for several reasons. Brain cells must maintain extraordinarily high cross-membrane gradients of ions and neurotransmitters. Depolarization of neurons partially dissipates such gradients, the restoration of which obliges vigorous consumption of ATP by astrocytes and neurons. Energy also goes to support the several anabolic functions of brain, including a very active rate of synthesis of proteins and lipids as well as the formation of a key intermediate, glutamine, the synthesis of which consumes ATP.

The oxidation of glucose provides essentially all energy needed to maintain cerebral function. Glycolysis may be relatively more prominent in some cells or in specific subcellular compartments. Thus, the filopodia of astrocytes are too narrow to accommodate mitochondria, and these cells will activate glycolysis (and glycogenolysis) in order to provide the energy that maintains their vital function of removing from the synaptic cleft much of the glutamate and K^+ that presynaptic neuronal terminals release upon depolarization (24, 46). The fate of the pyruvate generated via glycolysis remains a topic of active inquiry and debate. It may be that astrocytes do not immediately oxidize all pyruvate produced via glycolysis. Instead, they may convert some pyruvate to lactate and release the latter to the extracellular fluid, from which neurons extract it and oxidize it as a fuel. Neurons can respire on lactate (102), but they may require glucose as a substrate if they are to maintain large internal pools of glutamate and aspartate (125). Astrocytic release of lactate and subsequent neuronal oxidation may constitute a mechanism by which neuronal and metabolic activity are effectively coupled (33, 66).

Brain glucose metabolism is complex for yet another reason. Extremely rapid transamination of oxaloacetate and 2-oxo-glutarate—key intermediates of the tricarboxylic acid cycle—means that as glucose carbon traverses the cycle, it is in near immediate equilibrium with very large intracellular pools of aspartate and glutamate (72, 143). Extensive compartmentation of these pools has been shown (108, 128). The amino group of glutamate is transferred to glutamine, alanine, and GABA, all of which are present in high concentration in the nervous system. Thus, the flow of glucose carbon seldom, if ever, conforms to the "simple" sequence: glucose → pyruvate → tricarboxylic acid cycle → CO_2. Instead, glucose carbon passes through several amino acid pools, each of which pursues its own idiosyncratic fate, depending upon cell type and metabolic exigencies.

This interaction between metabolism of glucose and that of amino acids suggested to us (23, 31, 75, 138–142) that administration of a ketogenic diet might alter brain handling of neuroactive compounds such as glutamate and GABA. Our rationale was that a dietary regimen that sharply limits carbohydrate intake and obliges a shift in cerebral respiration to fuels other than glucose would have far-reaching implications for the handling of amino acids. Furthermore, we considered that the antiepileptic effect of the ketogenic diet could derive in part from a change in brain metabolism of glutamate. In order better to delineate this hypothetical effect, a short summary of brain glutamate handling is necessary.

BRAIN HANDLING OF GLUTAMATE

Glutamic acid is the major excitatory neurotransmitter (74, 91). In order to maximize the signal-to-noise ratio upon release of glutamate from presynaptic terminals, the glutamate concentration in the synaptic cleft must be maintained at a very low level. Another reason why brain cells keep synaptic glutamate low is that untoward accumulations of this amino acid excessively stimulate postsynaptic neurons, thereby causing the excitotoxicity that plays a role in diverse forms of brain injury, including hypoxia, traumatic brain injury, and epilepsy (82, 101, 131). The role of removing glutamate from the synapse falls primarily to astrocytes (24, 39, 47, 116), which have extremely effective

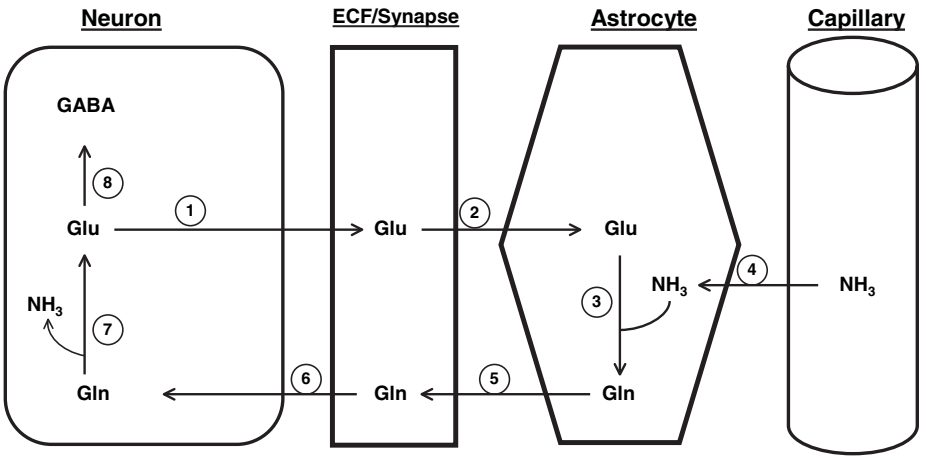

Figure 1

The glutamate-glutamine cycle. Glutamate is released (*1*) from presynaptic terminals into the ECF/synapse, where it stimulates postsynaptic glutamatergic receptors. High-affinity astrocytic transport systems (*2*) quickly remove glutamate from the synapse and convert it to glutamine in the astrocytic glutamine synthetase pathway (*3*). Ammonia diffuses from blood and neurons (*4*) to provide a coreactant for glutamine synthetase. Astrocytes release glutamine (*5*) via specific transport systems. Neurons subsequently take up the glutamine (*6*) via neutral amino acid transporters. Neuronal mitochondria then hydrolyze glutamine to glutamate (7), in the process completing the cycle. ECF, extracellular fluid; GABA, gamma-aminobutyric acid; Gln, glutamine; Glu, glutamate; NH_3, ammonia.

glutamate transporters on the cell surface (2). A relatively high astrocytic membrane potential facilitates uptake of glutamate via Na^+-dependent transport systems (32). In cerebellum, neuronal uptake may be important (84).

A mechanism must exist for the restoration of glutamate to neurons following glial uptake of this amino acid. Astrocytic transfer of glutamate to neurons would invite the risk of depolarization as this neurotransmitter traverses the extracellular fluid. Thus, a strategy evolved that involves astrocytic conversion of glutamate to glutamine via the glutamine synthetase reaction (glutamate + NH_3 + ATP → glutamine + ADP + P_i), an almost exclusively glial enzyme (69, 79). The coreactant ammonia derives from blood (20), although during periods of heightened activity, the oxidation of glutamate can yield ammonia (94). Specific transport systems enable the release of glutamine from astrocytes and subsequent uptake into neurons (16, 51), which hydrolyze this amino acid to glutamate and ammonia via phosphate-activated glutaminase (109).

The cycle starting with neuronal glutamate release, proceeding through glutamine formation in astrocytes and terminating in glutamine hydrolysis in neuronal mitochondria is termed the "glutamate-glutamine cycle" (GGC) (**Figure 1**). This anatomical and biochemical network has long been the central figuration in all formulations of brain amino acid metabolism (104).

A great deal of experimental evidence now supports the main elements of the GGC, but this model oversimplifies important aspects of brain amino acid handling. Thus, the model fails to account for the external sources of nitrogen that must be imported in order to maintain homeostasis. Losses of nitrogen from the system are inevitable, although the mechanisms by which brain exports nitrogen are not well understood. The uptake from blood to brain of either glutamine or glutamate is quite limited (44, 107).

Attention has centered on leucine as a probable external source of nitrogen to replace that lost from oxidative processes.

Leucine readily passes into brain, where it is quickly transaminated to yield glutamate and 2-oxo-isocaproic acid (ketoleucine) (6, 50, 137). Magnetic resonance spectroscopy studies utilizing [^{15}N]leucine as a metabolic probe suggest that leucine is the source of as much as one-third of all brain nitrogen (52). Valine and isoleucine, the other branched-chain amino acids, may increase this contribution to as high as 50%.

The GCC oversimplifies the fact that amino acids like glutamate, aspartate, and GABA not only are neurotransmitters, but also are pivotal metabolic intermediates through which glucose carbon must pass during its journey through the tricarboxylic acid cycle. This comes about because the transamination of 2-oxo-glutarate in the aspartate aminotransferase reaction affords a near-immediate articulation between the cycle and the very substantial pools of brain amino acids:

$$\alpha\text{-keto-glutarate} + \text{aspartate} \leftrightarrow \text{glutamate} + \text{oxaloacetate}$$

$$\text{glutamate} \rightarrow \text{GABA} + CO_2$$

We might anticipate that a shift in brain metabolism away from glucose to ketone bodies as metabolic substrate should invite changes in the handling of glutamate and related compounds. As we discuss in the section below, some experimental evidence suggests that precisely such an adaptation may occur.

INTERACTIONS OF METABOLISM OF KETONE BODIES AND OF GLUTAMIC ACID

Ingestion of a diet low in carbohydrate and high (80%–90% of calories) in lipid increases hepatic production of 3-OH-butyrate and acetoacetate from fatty acids. The liver does not consume ketone bodies. Instead, it exports them to peripheral tissues, among them the brain, which utilize ketone bodies as a metabolic substrate. Once the blood concentration of 3-OH-butyrate rises to 2–4 mM, the ketone bodies furnish as much as 70% of cerebral metabolic requirements, thereby replacing glucose as the major fuel (12, 85).

If ketosis is sufficiently intense and longstanding, the brain recruits monocarboxylate transporters that facilitate uptake of ketone bodies from blood (18, 93). Both neurons and glia accumulate and oxidize ketone bodies, with uptake being directly related to the blood concentration. It should be emphasized that at certain stages of development, ketone bodies are utilized not only as a metabolic substrate but also as a source of acetyl-CoA that is a precursor to myelin, especially during early development, when brain myelin synthesis is extremely high (41, 54, 63). It is noteworthy in this regard that the relatively high fat content of maternal milk favors ketosis (28, 76).

The initial step in ketone body oxidation is convertion of 3-OH-butyrate to acetoacetate via β-hydroxybutyrate dehydrogenase, a NAD-dependent enzyme. In the succinyl-coenzyme A (CoA) transferase reaction, a very active pathway in brain (38), acetoacetate becomes converted to acetoacetyl-CoA and succinate, the latter then being oxidized via succinate dehydrogenase of the tricarboxylic acid cycle. A thiolase then hydrolyzes acetoacetyl-CoA to acetyl-CoA, which condenses with oxaloacetate to yield citrate in the citrate synthase pathway.

It might be thought that whether brain consumes glucose or 3-OH-butyrate should make no difference to brain biochemistry and physiology. Oxidation of either fuel leads ultimately to the production of ATP via the electron transport chain. However, there are important differences in the biochemical mechanisms that mediate the oxidation of the two fuels. **Figure 2** illustrates salient features of the impact of ketone body oxidation on brain glutamate handling. Glucose metabolism presupposes flow through glycolysis to yield pyruvate, which mitochondria decarboxylate to acetyl-CoA in the pyruvate dehydrogenase reaction. In addition, glycolysis results in synthesis of NADH, which

the malate-aspartate shuttle transports to mitochondria.

In contrast to the oxidation of glucose, which involves glycolysis in the cytosol, the oxidation of ketone bodies occurs only in mitochondria and directly to acetyl-CoA, the concentration of which is increased in brain of ketotic mice (139). Ketone body metabolism does not involve formation of an intermediate such as pyruvate. As indicated above, when glucose is virtually the sole metabolic substrate of brain, glycolysis is not only a major route of astrocyte glucose metabolism, but the glia may convert some pyruvate to lactate and release the latter to the extracellular fluid (66). Thus, a salient metabolic difference between brain respiring solely on glucose and brain respiring on glucose/ketone bodies is that, in the latter instance, there is (*a*) enhanced formation of acetyl-CoA, (*b*) increased flux through the citrate synthetase reaction (acetyl-CoA + oxaloacetate → citrate), and (*c*) increased flux through the tricarboxylic acid cycle. This phenomenon was reflected in the recent study of Melo et al. (75), who administered [1-^{13}C]glucose and [1, 2-^{13}C]acetate in order to trace neuronal and glial metabolism, respectively, in rats that received a ketogenic diet for 21 days. Flux through glycolysis was diminished in neurons, and consumption of acetate was increased in astrocytes, the major site of brain acetate metabolism (5, 15, 130).

Both acetate consumption and glutamine synthesis are primarily glial functions (5, 15, 69, 79, 130), and blood acetate and acetylcarnitine probably increase in the ketotic individual (100). Thus, ketosis is associated with augmented astrocytic production of glutamine from acetate, as our groups independently demonstrated in studies with [1-^{13}C]acetate and [1, 2-^{13}C]acetate (75, 139).

As indicated above, acetyl-CoA must "enter" the tricarboxylic acid cycle via citrate synthetase, an extremely active reaction in brain with a maximal velocity that exceeds flow through pyruvate dehydrogenase by a factor of 10 (98). In synaptosomes, flux through pyruvate dehydrogenase scarcely equals overall metabolic rate (30). Intensified flow of acetyl-CoA through citrate synthetase (acetyl-CoA + oxaloacetate → citrate) tends to diminish the concentration of oxaloacetate, thereby limiting transamination of glutamate to aspartate, a major route of brain glutamate metabolism (31, 70, 72, 143, 138) (**Figure 2**). Transamination of glutamate to aspartate via aspartate aminotransferase, an equilibrium enzyme, depends in large measure upon the size of the oxaloacetate pool. If the latter if reduced in ketotic brain, then formation of aspartate will be diminished (26, 138).

We probed this hypothesis (138) by incubating cultured astrocytes with [^{15}N]glutamate and following appearance of label in [^{15}N]aspartate. In the presence of acetoacetate (5 mM), we noted a significant reduction in the glutamate → aspartate exchange. No change was observed with regard to the intra-astrocytic glutamate concentration, suggesting that glial uptake of glutamate was unaffected. Reductions in brain aspartate concentration have been seen in suckling mice that were injected with ketone bodies (118) and in rats on a high-fat diet (26). Our recent study of animals ketotic for 21 days did not show a diminution of total brain aspartate, but reduced production of [2-^{13}C]aspartate from [1-^{13}C]glucose (75).

Attenuated transamination of glutamate to aspartate in ketosis might contribute to an antiepileptic effect. Glutamate transamination to aspartate increases after depolarization (72, 90, 126), and extracellular aspartate increases postseizure (14, 27). Aspartate is an excitatory neurotransmitter that may have a role in the pathogenesis of hippocampal epilepsy (34, 68, 73, 97).

Reduced conversion of glutamate to aspartate might favor decarboxylation of glutamate to GABA (glutamate → GABA + CO_2). GABA is the major inhibitory neurotransmitter and a likely antiepileptic factor (25, 42, 48, 60, 64, 73, 83, 92, 132). It might be thought that the brain glutamate concentration (8–10 mM) saturates glutamate decarboxylase

(K_m 0.1–1.2 mM), but the glutamate level may be much lower in GABA-ergic neurons (110, 111), and less than 5% of total brain glutamate is precursor to GABA (87, 88) in GABA-ergic neurons, which constitute less than 20% of neurons. Furthermore, flux through glutamate decarboxylase (~0.5 nmol/min/mg protein) is rigorously controlled and much lower than maximal enzyme activity (3). Thus, small fluctuations of ambient internal glutamate affect GABA synthesis (89). Finally, several factors influence binding of glutamate to the decarboxylase, including Cl^- and aspartate (95, 135).

Studies in synaptosomes (31) showed an increase in GABA concentration as well as GABA synthesis upon exposure to a high (5 mM) concentration of acetoacetate. Thus, incubations with either L-[^{15}N]glutamine or L-[2, 3, 3, 4, 4-d_5]glutamine (0.5 mM each) were associated with sharply higher levels of [^{15}N]GABA or [$^2H_4$]GABA. The intrasynaptosomal level of aspartic acid was diminished in the presence of acetoacetate, suggesting a relative diversion of glutamate carbon from transamination and toward decarboxylation (31).

The source of GABA in these studies was glutamine, which neurons cleave to glutamate via phosphate-dependent glutaminase (109). Glutamine is an efficient GABA precursor that GABA-ergic terminals readily transport. Yudkoff et al. (139) found that ketosis increases brain [^{13}C]glutamine synthesis from injected [1-^{13}C]acetate. Melo et al. (75) reported increased formation of both [4, 5-^{13}C]glutamate and [4, 5-^{13}C]glutamine in epilepsy-prone animals (GAER rats) following intraperitoneal injections of [1, 2-^{13}C]acetate. The augmented ^{13}C-glutamine synthesis in part reflects heightened activity of pyruvate carboxylase, which in brain is a glial enzyme (105) that serves the anaplerotic function of restoring to the tricarboxylic acid cycle the carbon that is "lost" consequent to transamination of α-ketoglutarate to glutamate. Thus, in ketosis, overall astrocyte metabolism appeared to be increased, resulting in increased availability of glutamine, a good precursor to GABA, particularly at moments of heightened neuronal activity.

The latter point deserves some emphasis. The putative relationship between the ketogenic diet, the antiepileptic effect, and brain GABA synthesis may not necessarily imply an increased steady-state GABA concentration but an increased capacity for GABA synthesis. Indeed, Melo et al. (75) observed only a modest (~13%) and statistically insignificant increase of the GABA level in the cerebral cortex of ketotic rats. However, they found that ketosis caused a greater fraction of glutamate carbon to flow into GABA, a finding similar to that noted in synaptosomes (31). It may be that the antiepileptic effect derives from the capacity of the system to generate GABA in response to relatively intense neuronal depolarization.

Recent reports in humans suggest that the ketogenic diet might affect brain GABA metabolism. Wang et al. (129) utilized magnetic resonance spectroscopy to study brain GABA concentrations in three patients before and a few months after administration of a ketogenic diet. In one individual, the level of GABA did not increase and may even have declined by ~12% after three months of therapy. However, in the two other subjects, the brain GABA concentration increased by 52% and 34%, respectively. Dahlin et al. (22) measured amino acid levels in the cerebrospinal fluid of children at approximately four months after initiation of a ketogenic diet. They found a significant (~11%) increase of the GABA concentration. No significant change was observed in the spinal fluid level of either glutamate or aspartate, but the concentration of alanine was diminished significantly (by nearly 25%). Of note is the observation that the increase of GABA level was greatest in subjects who were rated "very good" responders to the diet. In these individuals, the pre- and postdiet levels were 4.47 and 4.8 μM, respectively. Similarly, in "good" responders, the cognate values were 3.24 and 3.77 μM. In contrast, in patients who

failed to respond, the pre- and postspinal fluid GABA concentrations were 2.55 and 2.79 μM, respectively.

KETOGENIC DIET AND BRAIN AMINO ACID TRANSPORT

Ketosis increases the entry of leucine to brain as well as the concentration of branched-chain amino acids (140). These compounds enter brain via the L transporter, which exchanges large neutral amino acids for brain glutamine (7, 10, 49). A high brain glutamine concentration favors this process, although affinity of the transporter for glutamine is not high (136). Importation of leucine and other branched-chain amino acids might be favored in ketosis, when the blood:brain ratio for leucine is increased and that for glutamine may be decreased (139). The exit of glutamine from brain would favor removal of glutamate, especially during heightened neuronal activity, when glial uptake of glutamate and formation of glutamine are intense (45). This "loss" of nitrogen eventually would be compensated by transamination of newly imported leucine (50, 137), but the system would have at its disposal an accessory mechanism for the momentary removal of glutamate when synaptic concentrations of this neurotransmitter are high.

Trafficking of amino acids among brain cells and between brain and capillaries involves a dense and tightly controlled network of transporters (10, 45). Rates of amino acid exchange among these compartments depend on both intrinsic properties of the transporters and relative concentrations in brain and blood. These mechanisms have not been scrutinized in the context of the ketogenic diet, but such nutritional intervention likely alters these relationships. For example, we recently observed a sharp decrease in the brain:blood ratio in the concentration of alanine in animals after a short fast (142). Alanine may be an important shuttle of $-NH_2$ groups among brain cells (127), and sodium-dependent amino acid transporters are present on the abluminal surface of brain capillaries (81). Release of alanine and other neutral amino acids down a "favorable" concentration gradient in ketosis might abet removal from brain of glutamate N. Systems for the direct export of glutamate exist (45, 80) and could assume heightened importance in ketosis if blood levels of glutamate and aspartate are diminished.

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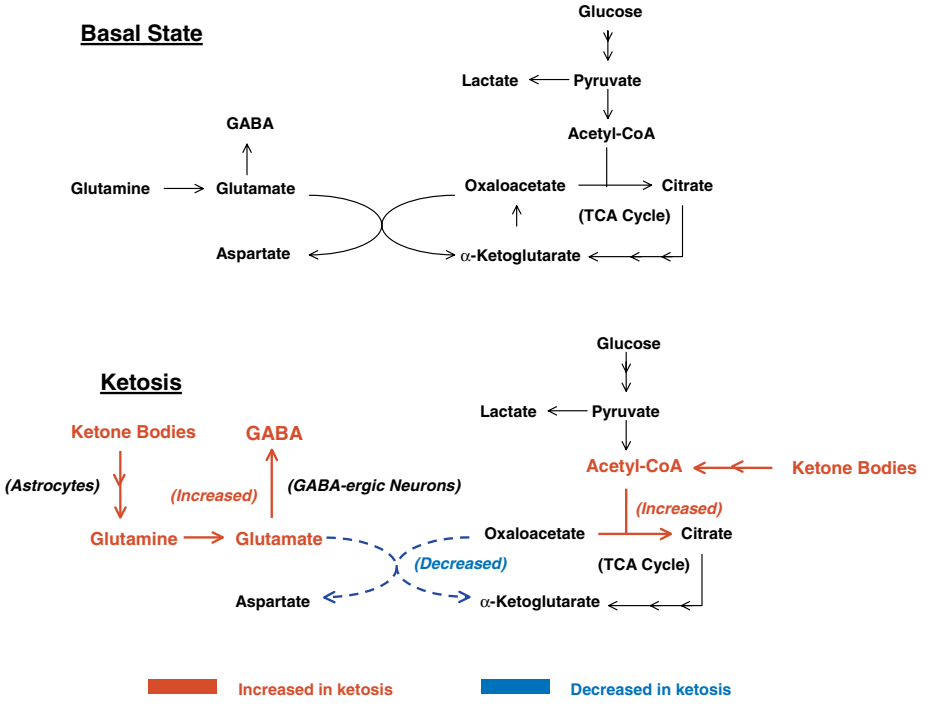

Figure 2

The impact of ketone body metabolism on brain handling of glutamate. Under basal conditions, glucose is the sole fuel of brain metabolism (*upper half of figure*). Glucose is converted to pyruvate via glycolysis, and pyruvate is converted to acetyl-CoA, which enters the tricarboxylic acid cycle. Glutamic acid is formed via transamination of α-ketoglutarate with aspartate. When an individual consumes a ketogenic diet and blood levels of 3-OH-butyrate and acetoacetate increase, the brain will consume these compounds as well as glucose (*lower half of figure*). Unlike glucose, the metabolism of which yields energy during conversion to pyruvate and lactate via glycolysis, the ketone bodies must be converted to acetyl-CoA, which is metabolized via citrate synthetase (acetyl-CoA + oxaloacetate → citrate + CoA), thereby diminishing the availability of oxaloacetate for transamination of glutamate to aspartate. More glutamate is then available to the glutamate decarboxylase pathway for the synthesis of GABA in GABA-ergic neurons. In addition, in ketosis there is increased blood acetate (or acetylcarnitine). Astrocytes, the major site of acetate consumption, convert this substrate to glutamine, which can be exported to GABA-ergic neurons, which convert this precursor to GABA, the major inhibitory neurotransmitter. The red lettering indicates pathways that are relatively more intense in ketosis, and the blue lettering indicates pathways that become relatively more attenuated.

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Errata

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