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Ketones: Metabolism's Ugly Duckling

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Ketones were first discovered in the urine of diabetic patients in the mid-19th century; for almost 50 years thereafter, they were thought to be abnormal and undesirable by-products of incomplete fat oxidation. In the early 20th century, however, they were recognized as normal circulating metabolites produced by liver and readily utilized by extrahepatic tissues. In the 1920s, a drastic "hyperketogenic" diet was found remarkably effective for treatment of drug-resistant epilepsy in children. In 1967, circulating ketones were discovered to replace glucose as the brain's major fuel during the marked hyperketonemia of prolonged fasting. Until then, the adult human brain was thought to be entirely dependent upon glucose. During the 1990s, diet-induced hyperketonemia was found therapeutically effective for treatment of several rare genetic disorders involving impaired neuronal utilization of glucose or its metabolic products. Finally, growing evidence suggests that mitochondrial dysfunction and reduced bioenergetic efficiency occur in brains of patients with Parkinson's disease (PD) and Alzheimer's disease (AD). Because ketones are efficiently used by mitochondria for ATP generation and may also help protect vulnerable neurons from free radical damage, hyperketogenic diets should be evaluated for ability to benefit patients with PD, AD, and certain other neurodegenerative disorders.

Key words: ketogenic diet, hyperketonemia, Parkinson's disease, Alzheimer's disease, mitochondriopathies, seizure disorders, ketone metabolism

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An Inauspicious Debut

From the very beginning, ketone bodies—β-hydroxybutyric acid (BHB), acetoacetic acid (AcAc), and acetone—suffered the stigma so often inflicted on those discovered in bad company. When ketones first came to the attention of physicians in the latter part of the 19th century, it was because they were found in abundance in the urine of patients in diabetic coma.[1,2] It soon became evident that a vast overproduction of ketones in the body was largely responsible for the devastating clinical manifestations of what is now called diabetic ketoacidosis. Negative views about ketones prevailed and the nature of the relationship between impaired glucose utilization and ketone body metabolism continued to be misunderstood for nearly a half century. In the words of Peters and Van Slyke,[3] "The formation of β-hydroxybutyric and acetoacetic acids instead of $CO_2 + H_2O$ was believed to denote incomplete combustion of fat. It was therefore deduced that complete combustion of fat required simultaneous oxidation of carbohydrate, an opinion vividly expressed by Naunyn in the aphorism, 'Fats burn in the flame of carbohydrate' . . . As analytical techniques have gained in precision and sensitivity, ketone bodies have proved to be normal components of blood, not products that appear only when the metabolism of carbohydrate is disordered."

Physiology and Metabolism

Starting in the 1930s, studies of the physiology of ketogenesis and ketolysis established the hepatic origin of ketone bodies and their rapid utilization by extrahepatic tissues.[4–6] Diabetic animals were found to use ketones as rapidly as normal animals do.[7,8] Early studies involving injection of BHB into laboratory animals showed that its utilization increased as its concentration in the blood rose. As the blood level of BHB continued to rise, however, its utilization slowed and then reached a point at which further BHB administration did not increase peripheral uptake.[9,10] Such observations underlie the currently held view that hyperketonemia results when hepatic production of ketone bodies exceeds the ability of the extrahepatic tissues to remove them.

In 1958, Johnson et al.[11] reported on the normal variations in total ketone bodies measured in serum and urine of 208 healthy young men eating normal diets and engaging in moderate daily physical activity. They found

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that there are *always* measurable concentrations of ketones in serum and urine. Regardless of season, the mean postabsorptive blood serum level for this group was 0.7 mM/L. (It is now evident that the method used by Johnson et al. to measure serum ketones systematically overestimated ketone concentrations. When currently preferred enzymatic methods are used, blood ketone levels after an overnight fast rarely exceed 0.5 mM/L and are usually much lower.)

The postabsorptive serum levels of ketones that Johnson and associates thought normal should be contrasted with the dramatically higher concentrations (in mM/L) of BHB (ca 4.4–5.5) and AcAc (ca 1.0) that occur after a week or more of fasting in non-diabetic obese individuals.[12] Among hospitalized patients in severe diabetic ketoacidosis, initial BHB levels of 23.0 mM/L are not unusual.[13]

By 1949, investigators knew that two molecules of acetyl-CoA (the primary metabolic intermediate arising from β-oxidation of fatty acids) could condense to form AcAc.[14] The principal pathway by which AcAc is formed in the liver from acetoacetyl-CoA remained unclear until 1958, however, when Lynen et al.[15] reported elegant studies indicating that deacylation of acetoacetyl-CoA did not take place directly but involved two steps: (1) condensation of acetoacetyl-CoA with acetyl-CoA to form β-hydroxy-β-methylglutaryl-CoA (HMG-CoA), and (2) cleavage of HMG-CoA to form AcAc and acetyl-CoA. As shown in Figure 1, the pathway proposed by Lynen et al. is the one currently accepted. Formation of HMG-CoA from acetoacetyl CoA is catalyzed by mitochondrial HMG-CoA synthase, a step that is stimulated by starvation, low levels of insulin, and consumption of a low-carbohydrate/low-protein, high-fat diet. AcAc is produced from HMG-CoA by HMG-CoA lyase.[16] Mechanisms by which ketones are formed in the liver and used in extrahepatic tissues are shown in Figure 1.

Ketone Body Kinetics in Humans

As measured by Wastney and associates,[17] the ketone bodies have a turnover time in the blood circulation of approximately 2 minutes. From the circulation, they rapidly enter a compartment (probably the body cell mass) that turns over approximately once a minute. In 1984, Hall et al.[18] reported measurements of postabsorptive plasma concentrations and kinetics of AcAc and BHB release and removal in 11 normal subjects, 6 patients with newly diagnosed and untreated insulin-dependent (type 1) diabetes, and 10 severely obese individuals who were first studied in the postabsorptive state and then again after 1 to 2 weeks of starvation (Table 1).

As shown in Table 1, the mean postabsorptive plasma ketone levels (0.12 mM/L) for normal subjects studied by Hall et al. were well below the mean of 0.7 mM/L reported by Johnson et al.[11] as "normal." Hall et al. measured AcAc and BHB enzymatically, adapting the procedure described by Williamson et al. in 1962.[19] In the normal subjects, mean rates of ketone release and removal were of the order of 100 $\mu mol \cdot min^{-1} \cdot m^{-2}$. In the patients with newly diagnosed, untreated type 1 diabetes mellitus, the mean postabsorptive ketone level was 2.46 mM/L, approximately 20 times higher than that in normal subjects. Mean ketone release rate in the diabetic patients was approximately triple that of the normal subjects. Mean ketone removal rate in the diabetic patients was approximately double that of the normal subjects; however, the mean rate coefficient for ketone removal (the fraction of the total uptake lost by oxidation and excretion) was reduced to 1/3 of normal.

In the 10 severely obese subjects, the mean postabsorptive plasma ketone level (0.42 mM/L) was higher than that in the normal individuals. The obese subjects also exhibited substantially higher than normal mean rates of ketone release and removal (171 vs. 81 and 210 vs. 111 $\mu mol \cdot min^{-1} \cdot m^{-2}$, respectively). Similar to the diabetic patients, the obese subjects showed a lower than normal mean rate coefficient for ketone removal.

In the obese subjects who had been deprived of food for 1 to 2 weeks, the mean postabsorptive ketone level was 7.01 mM/L, almost 60 times normal. At the same time, mean ketone release and removal rates in the food-deprived obese individuals were approximately five times normal, whereas the rate coefficient for ketone removal was reduced to approximately 1/6 of normal. Whenever ketone body levels were elevated in the diabetic and obese subjects, the increase was almost always in the form of BHB.

These observations indicate that ketone bodies are very rapidly cleared from the circulation and metabolized by extrahepatic tissues. In untreated patients with type 1 diabetes, ketone production and removal rates are greatly augmented. In the presence of such high plasma ketone levels, however, the mean rate coefficient for ketone removal is significantly reduced. This reduction in the rate coefficient for ketone removal (also observed in the postabsorptive and food-deprived obese subjects) was found by Hall et al.[18] to be coupled with an increase in the interconversion between the ketones, with a larger fraction of AcAc being converted to BHB than the reverse (we now know that this reflects reduction of the free $[NAD^+]$:[NADH] ratio with which ketones are in near equilibrium).[20]

The biochemical mechanism underlying the increased ketonemia that occurs when food deprivation is prolonged is incompletely understood. Because the lipolysis rate after 5 weeks remains essentially unchanged from that seen during the first few days of food depriva-

tion, and the rate of ketogenesis also remains unchanged,[21] it is evident that the rate of peripheral clearance of ketones must diminish in order to account for the elevated blood ketone levels measured during the food-deprivation period.

Laffel[22] suggested that the inverse relationship between level of ketonemia and rate coefficient for ketone removal could arise from down-regulation of an enzyme involved in ketone utilization in response to a rise in intracellular levels of AcAc during starvation, in association with dietary carbohydrate/protein restriction, or in type 1 diabetes. This explanation seems reasonable; however, the evidence thus far is inconclusive that any such enzyme is sufficiently inhibited or decreased to account for the observed diminution in ketone utilization. It is noteworthy, however, that the brain adapts to starvation with an *increased* rate of ketone utilization and that an increase in 3-oxoacid-CoA transferase (OCT) activity has been demonstrated in the brain of the starved rat. By contrast, *decreased* OCT activity has been described in the skeletal muscle of rats with diabetes of more than 10 days' duration.[16] To complicate matters further, exercise training for a number of weeks appears to increase the capacity of muscle to use ketones. Because exercise also promotes ketogenesis, the muscles presumably accommodate to such increased production by inducing or activating relevant ketone-utilizing enzymes.[16,23]

The physiologic explanation for the increased ketonemia during the early phase of food deprivation appears to lie in data reported by Owen and Reichard,[24] who showed that the rise in blood ketones during the first 2 weeks of starvation results from a decreasing rate of ketone utilization in muscle. As food deprivation continues, a dramatic change occurs in glucose and ketone utilization by the nervous system and muscle. Glucose utilization in the brain drops to 1/3 that in the fed state, with ketones filling the energy gap. A nearly reciprocal relationship exists for muscle, which seems to adapt so fully to prolonged fasting that it virtually ignores ketones as an energy source, drawing instead on fatty acids.[21] As Cahill[25] stated, ". . . early in starvation, ketoacids serve as muscle fuel, but with more prolonged starvation they are less well used. In fact, as acetoacetate is taken up by the muscle, it is returned back to the blood as β-hydroxybutyrate, signifying a more reduced state of the muscle mitochondria—secondary to free fatty acid utilization. Thus fatty acids appear to take preference for oxidation and ketoacid oxidation ceases, sparing the ketoacids for the brain, a superb survival process."

According to Laffel,[22] healthy adult liver can produce as much as 185 g of ketone bodies per day. Ketones supply 2 to 6% of the body's energy needs after an overnight fast and 30 to 40% after a 3-day fast.

Effect of Total Starvation and Carbohydrate Restriction on Blood and Urine Ketones

In the late 19th century, physiologists focused on the striking association between ketonuria and worsening diabetes mellitus. Later, as methods for detecting and quantifying ketones in biologic fluids improved, attention shifted to the effect of food deprivation, changes in energy expenditure, and modifications of dietary composition on the urinary excretion and blood levels of ketone bodies. It was soon evident that the ketonuria associated with total starvation does not become substantial until 3 to 5 days have elapsed. This is the time it takes for liver glycogen to be thoroughly depleted. In 1931, Behre[26] reported that ketonuria begins to increase within a few hours after a meal is skipped. In three subjects studied by Kartin et al.[27] during a 6-day period of total starvation, blood ketones after 2 days of food deprivation varied from 1.1 to 2.5 mM/L. After 6 days blood ketones had risen to 2.3 to 4.0 mM/L. Peters and Van Slyke[3] point out that such rises ". . . are not enough to tax severely the mechanisms for the preservation of acid-base equilibrium; the serum bicarbonate does not fall below 35 to 40 volumes per cent . . . Benedict's subject averaged about 6 grams of β-hydroxybutyric acid in the urine daily for the last 2 weeks of his 31-day fast . . . In diabetic acidosis ketonuria has been known to reach 10 times as much as this."

By accelerating the rate of carbohydrate utilization, human physical exercise,[23,28] administration of dinitrophenol or thyroid hormone to rabbits,[10] and increased bovine lactation[29] can give rise to appreciable ketosis. Pregnant women maintain higher than normal ketone levels and show an exaggerated ketogenic response to fasting.[30] In human subjects undergoing experimental starvation, consumption of relatively small amounts of glucose per day (e.g., 50–75 g) greatly reduces ketone body production.[31]

A low-carbohydrate diet is not necessarily a *ketogenic diet*. This is particularly true of diets with unrestricted content of meat and other protein-rich foods. Heinbecker[32] reported in 1928 that Baffin Island Eskimos subsisting on their usual diet of meat (virtually the only source of carbohydrate in their food was the glycogen in seal muscle) showed minimal ketonuria. (As a reference point, muscle glycogen concentration in resting humans on a mixed diet is approximately 14.4 g/kg.[33]) It is unlikely that these very small amounts of glycogen could have accounted for the absence of appreciable ketonuria. A much more likely explanation is that the glucose derived from catabolism of ingested meat protein was sufficient to prevent ketosis. McClellan and DuBois[34] fed two human volunteers "carbohydrate-free" diets high in meat content (an Eskimo-type diet) for many months in a metabolic ward setting. Their findings led them to conclude that, in persons subsisting on diets

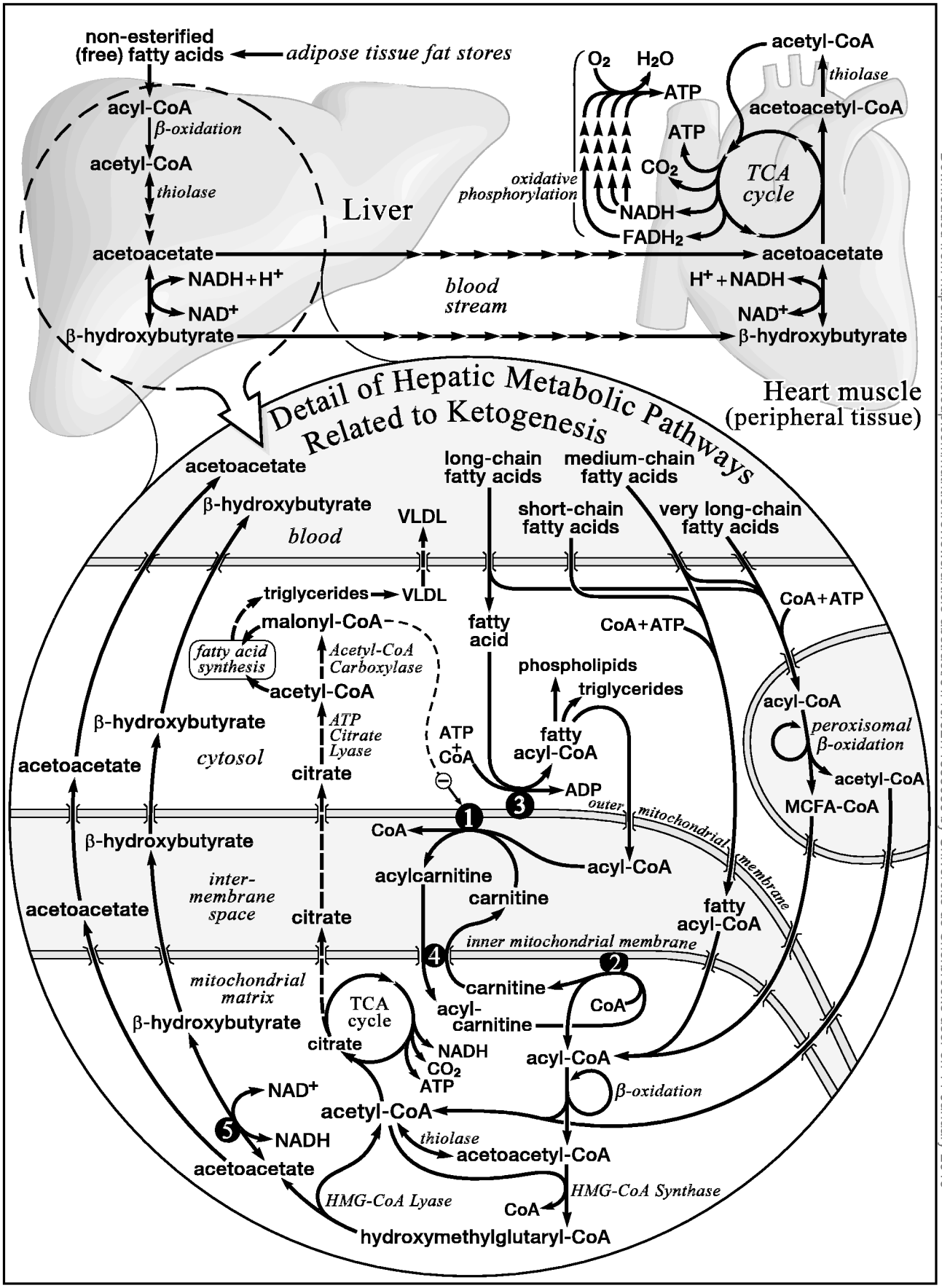

Table 1. Postabsorptive Plasma Concentrations of Acetoacetate (AcAc) and β-Hydroxybutyrate (BHB) and Rates of Ketone Release and Removal, Together with Rate Coefficients for Ketone Removal (mean ± SD) in Normal, Diabetic, and Normally Fed and Subsequently Food-deprived Severely Obese Subjects

Diagnosis	Metabolic State	AcAc (mM/L)	BHB (mM/L)	Ketone Release Rate ($\mu mol \cdot min^{-1} \cdot m^{-2}$)	Ketone Removal Rate ($\mu mol \cdot min^{-1} \cdot m^{-2}$)	Rate Coefficient for Ketone Removal* (min^{-1})
Normal (n = 11)	Postabsorptive	0.05 ± 0.02	0.07 ± 0.04	81 ± 66	110.7 ± 105.9	0.168 ± 0.169
Diabetic† (n = 6)	Postabsorptive	0.67 ± 0.75	1.79 ± 2.63	208 ± 118	218.8 ± 87.4	0.055 ± 0.040
Obese‡ (n = 10)	Postabsorptive	0.15 ± 0.06	0.27 ± 0.12	171 ± 70	210.1 ± 93.2	0.066 ± 0.040
	Food-deprived 1–2 weeks§	1.04 ± 0.26	5.97 ± 1.13	569 ± 286	567.3 ± 256.2	0.027 ± 0.019

*Fraction lost by oxidation and excretion.
†Recently diagnosed, untreated patients with insulin-dependent diabetes mellitus.
‡Subjects severely obese.
§Subjects received 50 kcal and 1 multivitamin tablet/day.
Data from Reference 18.

very low in carbohydrate, ketosis varies inversely with the quantity of protein eaten. This occurs because approximately 48 to 58% of the amino acids in most dietary proteins are glucogenic.[35,36] For every 2 grams of protein consumed in a carbohydrate-free diet, somewhere between 1.0 and 1.2 grams are potentially convertible to glucose. Therefore, to obtain a degree of hyperketonemia (approximately 2–7 mM/L) believed to be therapeutically effective in certain important medical conditions such as epilepsy, patients must rigorously restrict protein as well as carbohydrate intake[37] and, when possible, increase their level of physical activity.[23]

Figure 1. Hepatic formation and peripheral utilization of ketone bodies. Mobilization of non-esterified fatty acids (NEFA) from adipose tissue fat stores by the activation of hormone-sensitive lipase is an initial step that provides ample substrate for the hepatic production of ketone bodies (β-hydroxybutyrate and acetoacetate). Once fatty acids enter the hepatocyte from the blood circulation, they are activated to CoA esters by the family of membrane-bound fatty acid acyl-CoA synthetases. The action of one of these synthetases (long-chain acyl CoA synthetase) is identified as (3). After CoA esterification, the metabolic paths diverge based on the fatty acid (acyl) chain length. Short- and medium-chain acyl-CoAs readily cross the outer and inner mitochondrial membranes and undergo β-oxidation into acetyl-CoA and acetoacetate (the terminal 4-carbon fragment). Long-chain acyl-CoAs readily cross the outer mitochondrial membrane, after which they must be converted to long-chain acylcarnitine by membrane-bound carnitine palmitoyltransferase I, or CPT-1 (1), for subsequent transport across the inner mitochondrial membrane. The inner membrane–bound enzyme, carnitine acylcarnitine translocase (4), exchanges one long-chain acyl-CoA from the intermembrane space with one free (unacylated) carnitine from the matrix, thereby permitting carnitine's return to the intermembrane space. The inner membrane–bound enzyme carnitine palmitoyltransferase II, or CPT-II (2), converts the fatty acid back into its CoA ester for subsequent β-oxidation, freeing carnitine for recycling. Very long–chain acyl-CoAs and unsaturated fatty acids of various lengths are transported into peroxisomes, along with long- and medium-chain acyl-CoAs, for chain-length reduction via β-oxidation. Unlike mitochondrial β-oxidation, peroxisomal β-oxidation does not proceed to completion. This results in peroxisomal export of medium-chain acyl and acylcarnitine compounds to the mitochondria for further oxidation. Mitochondrial β-oxidation produces acetyl-CoA, which enters the TCA cycle for further oxidation to produce ATP. Ketogenesis occurs when hepatic energy requirements are met allowing excess acetyl-CoA to be acted upon by thiolase, which condenses two acetyl-CoAs to form acetoacetyl-CoA. Hydroxymethylglutyaryl-CoA synthase (HMG-CoA synthase) condenses acetoacetyl-CoA with acetyl-CoA to form hydroxymethylglutaryl-CoA, which is then dissociated into acetoacetate and acetyl-CoA by hydroxymethylglutaryl-CoA lyase (HMG-CoA lyase). The action of the enzyme β-hydroxybutyrate dehydrogenase (5) reduces much of the acetoacetate to β-hydroxybutyrate using NADH as a reductant. Both acetoacetate and β-hydroxybutyrate are transported back through the mitochondrial membranes for export into the blood circulation. The pathway of lipogenesis, illustrated by dashed lines, becomes activated in the fed state and results in the synthesis of fatty acids and their esterification into triacylglycerol (triglycerides). The first committed intermediary in fatty acid synthesis is malonyl-CoA, which inhibits CPT-1 (enzyme 1). This inhibition is illustrated as a thin dashed line pointing to CPT-1. Inhibition of CPT-1 prevents mitochondrial uptake of fatty acyl-CoA (and its subsequent β-oxidation), and thereby blocks ketogenesis. In the fed (non-ketotic) state, triglyceride is exported from the liver as very low–density lipoprotein (VLDL).

Ketones: Substrates in Search of a Mission

By the early 1960s, a great deal of information had accumulated about the biochemistry and physiology of the ketone bodies, yet their role in human metabolism remained obscure.[38] Nevertheless, five facts were known to medical scientists that, had they been viewed in proper perspective, would have suggested strongly that *ketones have to be the brain's back-up fuel* during starvation. First, scientists recognized that when people are deprived of food for 3 days or longer, they become hyperketonemic and hyperketonuric. Second, catheterization studies showed that the adult human brain uses approximately 100 to 150 g glucose per day.[39] Third, scientists understood that non-esterified fatty acids do not cross the blood-brain barrier (BBB) and are not directly available to the brain as an energy source.[40] Fourth, Benedict[41] showed that during 31 days of total caloric starvation, his "fasting man" excreted an average of 8.72 g/day of nitrogen (N), which corresponds to a rate of body protein catabolism of approximately 55 g/day. Approximately 32 g of this amount (0.58 $\times$ 55.0) could have been available as fuel for the brain—approximately 27% of the brain's daily needs. In another subject deprived of food for 15 days, mean total N excretion was 4.82 g/day, making only 17.4 g of glucose/day potentially available to the brain.[42]

Finally, because glucose was widely assumed to be the exclusive fuel of the adult human brain,[38] one might infer that during continuing starvation (after liver glycogen stores were exhausted) the brain would have to be sustained by glucose derived from intracellular protein. Making 100 to 150 g of glucose/day available to the brain would require the gluconeogenic breakdown of some 172 to 259 g/day of body protein. At this unsustainable rate of protein attrition, death might be expected to occur within approximately 2 weeks—certainly not the 57 to 73 days (mean 61 $\pm$ 2.5 [SEM] days) that it reportedly takes for totally starved young men of average body composition to die.[43] If the nutrition scientists of the time had given more thought to the meaning of this unexplained "prolongation" of life during total starvation, they might have reasoned that during extended food deprivation the brain *is* able to use the ketones generated in such abundance as an alternative energy source. Such a scenario would have explained the greater than expected longevity and the far lower than expected daily nitrogen deficit exhibited by starved individuals.

In 1966, Cahill et al.[39] "connected the dots" and concluded that ketones might provide an alternate fuel for the brain during starvation. To test this hypothesis, the authors performed brain catheterization studies in obese patients undergoing 5 to 6 weeks of therapeutic fasting.[44] This landmark study showed that under conditions of sustained starvation (during which the subjects achieved blood ketone levels close to 7 mM/L and blood glucose concentrations of ca 3.8 mM/L), BHB and AcAc replaced glucose as the predominant fuel(s) for brain metabolism. The "starvation paradox" was thus resolved. It also became clear that during starvation the liver's ability to generate ketones for use by the brain represents a biologic strategy for maintaining strength and prolonging life during conditions of severe nutritional privation. In this way, the burden of sustaining life during starvation is shifted from the body's protein stores to its reserves of fat—a far more effective and efficient adaptation. Thanks to the pioneering studies of Cahill et al., metabolism's ugly duckling finally began to emerge as an incipient swan!

Ketones: Uniquely Efficient Metabolic Fuel

In the mid 1940s, Lardy and associates[45,46] demonstrated that when tested on sperm BHB and AcAc, compared with other metabolic substrates, have the ability to decrease oxygen consumption while increasing motility. For almost half a century this apparent increase in metabolic efficiency remained unexplained. In 1994 and 1995, however, R.L. Veech's group[47–49] at the National Institutes of Health showed that in the working perfused rat heart, when 5 mM ketone bodies/L were added to the glucose-containing perfusate, there was a 25% increase in hydraulic work with a significant decrease in oxygen consumption. Addition of ketones also brought about a reduction of the mitochrondrial NAD couple, with oxidation of the mitochondrial co-enzyme Q couple. The consequence is an increase in the energy of the redox span between site I and site II of the electron transport system.

As Veech et al.[50] described, "An increase in the redox span between two sites will result in an increased energy release by the electron traveling across that span . . . With an increase in redox energy of the respiratory chain, there is a corresponding increase in the energy of the protons ejected from the mitochondria at the energy-conserving sites, which is then reflected in an increase in the energy of ATP hydrolysis."

As shown in Table 2, Veech and associates[47–49] also observed that the introduction of ketone bodies into the rat heart perfusate produced a 16-fold rise in acetyl-CoA content and increases in TCA cycle intermediates. These stimulatory effects on TCA cycle activity were similar to those obtained with the administration of saturating doses of insulin. Insulin increases glucose transport in the myocardium by promoting translocation of insulin-sensitive GLUT4 to the plasma membrane. Insulin also stimulates the activity of pyruvate dehydrogenase multienzyme complex (PDH)—a rate-limiting step in the mitochondrial transformation of pyruvate to acetyl-CoA. As Veech et al.[50] put it, "The implication was that

Table 2. Insulin and Ketone Effects on Cardiac Efficiency, Acetyl-CoA Production, [NADH]/[NAD^+] Ratio, and Free Energy of ATP Hydrolysis (ΔG_{ATP}) in the Perfused Rat Heart

Substrates Added to Perfusate	Acetyl CoA*†	[NADH]/[NAD^+]‡§ (mitochondrial)	ΔG of ATP Hydrolysis* (cytosolic) kJ/mol	Cardiac Efficiency* (%)	Cardiac Efficiency* (%, relative to glucose)
Glucose	1	0.05	−56.2	10.5	100
Glucose + Insulin	9	0.22¶	−58.9¶	13.4¶	~128
Glucose + Ketones	15	0.62¶	−57.6¶	13.0¶	~124
Glucose + Insulin + Ketones	18	0.43¶	−58.9¶	14.3¶	~136

*Data of Sato K et al.[48] and ‡Kashiwaya et al.[47,49]

†Numbers represent multiples of that measured with perfusate containing only glucose.

§Expressed by Kashiwaya et al.[47,49] as [NAD^+]/[NADH].

¶Indicates a significant difference ($P < 0.05$) from perfusate containing 10 mmol/L glucose; ketones added at 4 mM/L D-β-hydroxybutyrate + 1 mM/L acetoacetate.

ketosis, which is the physiological response to insulin deprivation during starvation, was equivalent in metabolic effects to the actions of insulin . . . (Moreover), ketones by-passed the block in glucose transport caused by lack of insulin . . . (and) also by-passed the block in PDH induced by insulin deficiency by providing an alternative source of mitochondrial acetyl CoA." The pathway by which ketones are able to achieve this effect is shown in Figure 2.

Veech et al.[50] pointed out that the gradients of K^+ and Na^+ (and Ca^{++}) between the extra- and intracellular phases of the cell are normally in virtual equilibrium with the resting electrical potential between these two phases, and with the energy (ΔG) of ATP hydrolysis, which sustains the action of the Na^+ pump. By increasing metabolic efficiency and, thereby, the energy produced by ATP hydrolysis, BHB augments the potential between the extra- and intracellular phases, giving rise to an increase in the energy of the K^+ gradient (ratio of $[K^+]_{out}/[K^+]_{in}$) in muscle and brain. A larger mitochondrial membrane potential would lead to larger energy production because it is the mitrochondrial membrane potential difference that drives the production of ATP. Maintenance of a suitable K^+ gradient in the cell is, of course, indispensable to its proper functioning. As will be discussed below, impairment of ion homeostasis within neuronal cells in certain brain areas might result in a degree of electrical instability sufficient to trigger an epileptic seizure (or, in the heart, a cardiac arrhythmia).

Studies of blood flow and oxygen consumption in the brains of food-deprived obese human subjects revealed values (45 mL · min^{-1} · 100 g^{-1} and 2.7 mL O_2 · min^{-1} · 100 mL^{-1})[44] that were well below the normal levels for adult human brains of 57 and 3.6, respectively.[51] Although these data need confirmation, they suggest an increase in the metabolic efficiency in human brains using ketoacids as their principal energy source in place of glucose.

Examples of mechanisms by which diet-induced hyperketonemia might overcome or circumvent metabolic impediments to utilization of such substrates as glucose and pyruvate are shown schematically in Figure 3.

Use of a "Hyperketogenic" Diet in Treatment of Seizure Disorders

Fasting has long been acknowledged as a method to prevent or reduce seizures in epileptic individuals.[37] Because of the obvious limitations of this treatment, use of a ketogenic diet to mimic the metabolic effects of food deprivation was proposed in 1921 by both Woodyatt[52] and Wilder[53] at the Mayo Clinic. In 1924, Peterman[54] (also from Mayo) reported the clinical application and effectiveness of the regimen suggested by Woodyatt and Wilder. Peterman's diet provided (per day) 1 g of protein/kg body weight (considerably less in adults), 10 to 15 g of carbohydrate, and the remainder of the calories as fat. The Peterman ketogenic diet is sometimes called the 4:1 diet because it provided a ratio of approximately 4 parts (by weight) fat to 1 part (by weight) of a mixture of protein and carbohydrate. This distribution of the major macronutrients is almost identical to that of most anticonvulsant diets used today.[37] The term "hyperketogenic diet" (HKD) will be used in this review to distinguish the 4:1 diet (and certain variations thereof) from the far less drastic, low-carbohydrate, mildly ketogenic diets that have been advocated for weight control in a succession of popular diet books.

In the 1970s, several investigators[55,56] modified the Peterman HKD by substituting medium-chain triglycerides (MCT) in varying amounts for long-chain triglycerides. (MCT's ketogenic effect is not dependent on concurrent restriction of dietary carbohydrate.[57]) Although all such variations of the HKD were approximately equal in reducing seizures, many of the children given MCT-

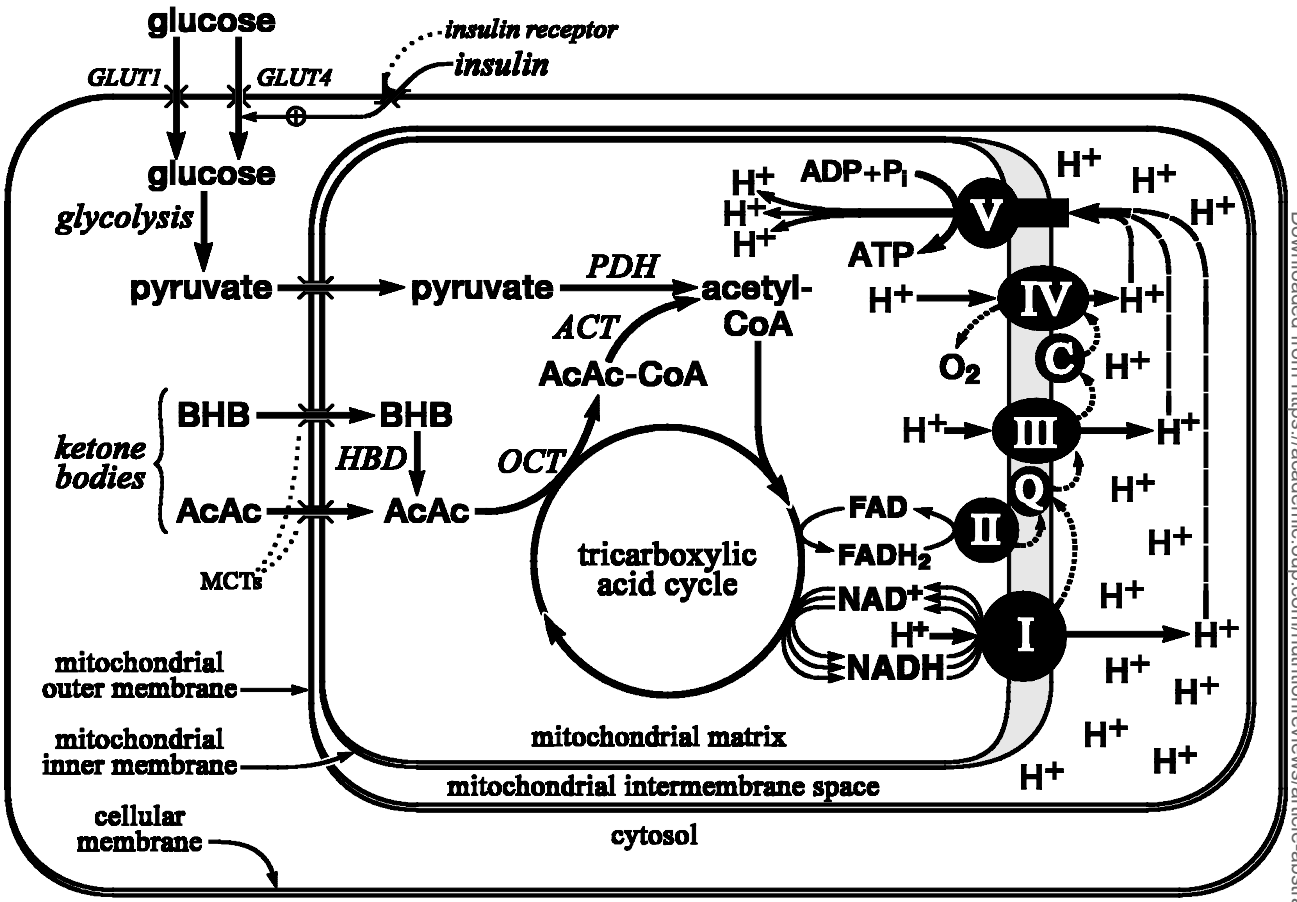

Figure 2. Production of ATP (adenosine triphosphate) in extrahepatic tissues arising from use of ketones and glucose. Glucose is regulated by a family of GLUT transporter proteins. GLUT4 transporters, found in most body tissues, are mediated by insulin. GLUT1 transporters, common in tissues such as brain and erythrocytes, allow glucose uptake without insulin mediation. By contrast, uptake of ketone bodies (shown as BHB and AcAc) occurs via the family of monocarboxylate transporters (MCTs), which are not insulin mediated. MCT proteins enable ketones to pass readily through the blood-brain barrier. Many types of peripheral cells, including brain cells, not only use glucose, but also use ketones to produce acetyl-CoA. Glucose utilization depends on glycolytic enzymes (not shown), which produce pyruvate. Pyruvate, in turn, enters the mitochondrion and is converted to acetyl-CoA by the pyruvate dehydrogenase multienzyme complex (PDH). Ketone substrates enter the mitochondrion in the form of (1) acetoacetate (AcAc) and (2) β-hydroxybutyrate (BHB), which is readily converted to acetoacetate by β-hydroxybutyrate dehydrogenase (HBD). Two enzymes principally control ketolysis. First, 3-oxoacid-CoA transferase (OCT) adds coenzyme-A to AcAc, which is then split into two molecules of acetyl-CoA by acetoacetyl-CoA thiolase (ACT). Acetyl-CoA undergoes oxidative degradation in the TCA cycle to reduce the electron carrriers NAD^+ (nicotinamide adenine dinucleotide) and FAD (flavin adenine dinucleotide) to NADH and $FADH_2$, respectively. Acetyl-CoA is also used for lipid or sterol synthesis (not shown). NADH and $FADH_2$ donate electrons to the protein complexes I and II of the mitochondrial respiratory chain. The flow of these electrons down the electron-transport chain to oxygen (O_2) allows complexes I, III, and IV to pump protons (shown as H^+) out of the matrix and into the intermembrane space, which generates a proton motive force (pmf) between the intermembrane space and the matrix. The pmf provides the energy to drive protons back through complex V (ATP synthase) to produce ATP from ADP (adenosine diphosphate) and inorganic phosphate (P_i).

augmented diets complained of nausea, diarrhea, and occasional vomiting.[58]

Among those patients who adhere to the 4:1 HKD for 12 months, 40% experience greater than 90% reduction in seizures, whereas an additional 40% experience 50 to 90% reduction. Although the precise mechanism of action responsible for the clinical efficacy of the diet has never been clarified, it is likely that the hyperketonemia induced by the 4:1 HKD is the key factor responsible for the regimen's anticonvulsant effect, rather than the accompanying metabolic acidosis[59] or dehydration.[37] In 1972, Uhleman and Neims[60] demonstrated that mice pups fed a high-fat ketogenic diet exhibited significantly increased thresholds against investigator-applied electroshock challenges. This resistance to electroshock disappeared promptly after the animals were shifted to a high-carbohydrate diet. Subsequently, a number of studies have shown an increase in the electroconvulsive threshold in rats maintained on a high-fat diet or deprived of food for 48 hours. De Vivo et al.[61] found that the ratios of ATP to adenosine diphosphate (ADP) were significantly increased in the brains of rats fed the high-

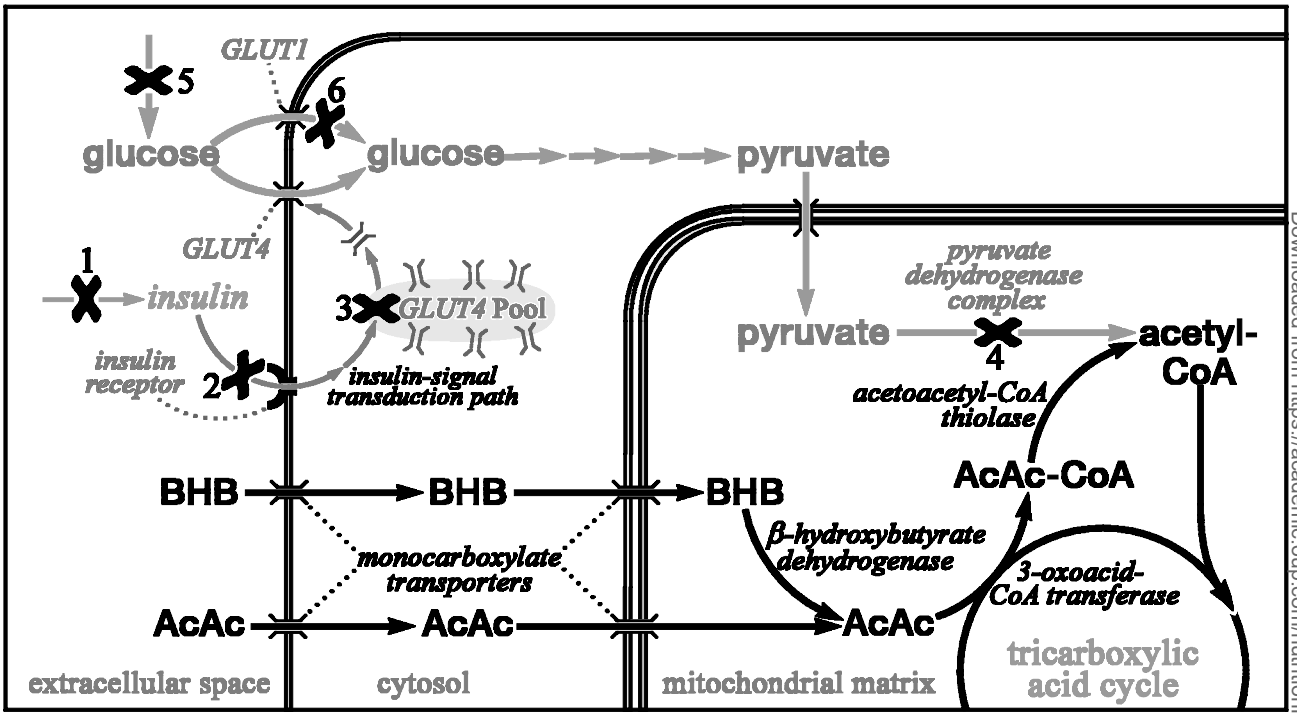

Figure 3. Conditions that can impede acetyl-CoA production from pyruvate and its precursors. Examples include: (1) insufficient insulin; (2) insulin receptor abnormalities; (3) diminished transport of glucose owing to impaired release of GLUT4 transporters associated with insulin resistance [see below]; (4) pyruvate dehydrogenase complex (PDH) deficiency or dysfunction; (5) unavailability of glucose at the cell membrane; and (6) GLUT1 mutations. Provision of ketones to the cell can effectively circumvent or overcome such impediments and compensate for the resulting deficits of glycolytically derived acetyl-CoA. (GLUT1 designates the insulin-independent membrane protein responsible for glucose transport in red blood cells and throughout the blood-brain barrier [BBB]. GLUT4 designates the insulin-dependent glucose transporter protein expressed in most cells. Many forms of insulin resistance entail putative post-receptor defects of insulin-signal transduction resulting in the failure of insulin to release GLUT4 transporters from the GLUT4 pool at a rate sufficient to bind and transport enough extracellular glucose to maintain cellular pyruvate levels.)

fat diet. They concluded ". . . the anticonvulsant property of the ketogenic diet derives from the observed increase in cerebral energy reserve, which in turn reflects the higher ATP/ADP ratio . . . [this] might provide a reasonable explanation for the increased neuronal stability that develops during chronic ketosis." In 1999, Pan et al.[62] carried out ^{31}P magnetic resonance spectroscopic imaging studies in seven patients with intractable epilepsy before and after institution of a ketogenic diet. They measured significant increases ($P < 0.05$) in high-energy phosphates in association with adherence to the ketogenic diet in these subjects. They concluded that enhancement of brain energy metabolism occurs with use of the ketogenic diet in humans as in lab animals.

Thavendiranathan et al.[63] have recently reported studies of the effect of the "classic" ketogenic diet on seizures triggered by two threshold tests—the pentylenetetrazol infusion test and the threshold electroconvulsive shock test. These authors observed small but significant threshold elevations (15–20%) against both chemically and electrically triggered seizures. Similarly protective effects were not seen when suprathreshold stimuli were employed.

Janigro[64] summarized studies indicating that there is a reduction in both glucose uptake and metabolic activity in seizure foci in epileptic patients. In his words, "Diminished ion homeostasis together with increased metabolic demand of hyperactive neurons may further aggravate neuropathological consequences of BBB loss of glucose uptake mechanisms." Therefore, one explanation for the effectiveness of the 4:1 HKD in preventing seizures could be the increased transport of BHB at the BBB and its increased availability as an energy source to the affected neurons. BHB is not only an alternate source of energy for seizure foci in the brain—bypassing an impairment of carbohydrate utilization such as that which can occur from a block in mitochondrial PDH activity—but it may actually promote ketone and glucose uptake in such foci. In rats, diet-induced ketosis produces an eightfold increase in the activity of the monocarboxylate transporter and increases the level of the glucose transporter, GLUT1, in brain endothelial cells and neuropil.[65] The hypothesis put forth by Janigro to explain how ketosis might eliminate or reduce seizures is supported by reports that a suitable ketogenic diet is the treatment of choice for two rare conditions that may

be complicated by seizures—the facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome[66,67] and the PDH complex deficiency syndrome.[68] Treatment with a ketogenic diet reportedly brought about dramatic clinical improvement in a one-year-old boy with Leigh syndrome, which is associated with a deficiency of PDH.[69] In this child, treatment with a ketogenic diet was associated with striking improvement of the cerebral lesions, as measured by neuroimaging.

In 1973, Buckley and Williamson[70] proposed that neural lipid synthesis preferentially uses AcAc over glucose. Patel and Owen[71] obtained confirmatory data for this hypothesis in 1976. In 1984, Koper et al.[72] compared the rate of incorporation of AcAc and glucose into fatty acids and cholesterol using labeled AcAc and glucose. At saturating levels of glucose the authors observed that the rate at which AcAc label incorporated into fatty acids and cholesterol exceeded that of glucose by a factor of 5 to 10 in both oligodendrocytes and astrocytes. These observations provide a basis for arguing that some of the benefits associated with ketogenic diets may be related to their enhancing effects on lipogenesis and/or sterol synthesis (i.e., damaged neurons might repair more readily in brains given an adequate ketone supply).

Therapeutic Potential of Diet-induced Hyperketonemia in Neurodegenerative and Other Disorders

Veech et al.[50] suggest that maintenance of a suitable degree of diet-induced hyperketonemia (in the range of 2–7 mM/L) has the potential to aid management of several neurologic diseases, including common neurodegenerative diseases like Alzheimer's disease (AD) and Parkinson's disease (PD). In their "hypothesis paper," the authors single out AD, PD, Friedreich's Ataxia, Leprechaunisms, and lesser forms of insulin disorders as examples of illnesses that might benefit from therapeutic ketosis. They also describe experience with one case of LaFora body disease (until now a uniformly fatal genetic disease associated with progressive dementia), in which use of the 4:1 HKD resulted in unprecedented improvement in cognition, gait, and awareness after one month.[73] In this disease, mutations in a gene encoding a novel protein, tyrosine phosphatase, cause progressive myoclonic epilepsy.

Calabrese et al.[74] and DiMauro and Schon[75] have recently called attention to evidence suggesting the mitochondrial genome may play a key role in neurodegenerative diseases. Decreases in respiratory chain complex activities have been identified in AD, PD, amyotrophic lateral sclerosis, and Huntington's disease. Mitochondriopathies (MCPs), possibly associated with oxidant/antioxidant balance perturbation, may underlie defects in energy metabolism and contribute to cellular damage and destruction.

Parkinson's Disease

Although the etiology of PD is complex, involving both genetic and environmental factors, there is growing interest in the role of mitochondrial dysfunction in the pathogenesis of this disorder. Schapira[76] pointed out that a proportion of PD patients exhibit a deficiency in mitochondrial respiratory chain function, most notably a defect of complex I within neurons of the substantia nigra. In his words, "Both mitochondrial mutations and toxic agents (endogenous and exogenous) have been demonstrated to cause a deficiency in complex I function. This is achieved by increasing the degree of, or susceptibility to, oxidative stress, which may contribute to apoptopic cell death."

Finsterer[77] reported metabolic studies indicating that PD can be a manifestation of MCP. In his series, 6.3% of PD patients had features of generalized MCP; however, he used lactate stress testing to detect patients with MCP—an approach that could not be expected to pick up mitochondrial lesions limited to brain sites.

According to Veech et al., "PD appears to result from an acquired defect in the mitochondrial NADH multienzyme complex of the dopaminergic cells of the mesencephalon."[78] They point out, although the disease is treatable for a period by administration of levodopa (the metabolic precursor of dopamine), continuing free radical damage eventually reduces the effectiveness of this therapy. Kashiwaya et al.[79] reported that primary cultures of mesencephalic dopaminergic neurons exposed to the meperidine analogue MPP^+ (a molecule that inhibits NADH dehydrogenase [complex 1] and causes oxygen free radical formation) can be protected from death by addition of 4 mM D-β-hydroxybutyrate. Veech et al.[50] suggest that BHB probably acts by decreasing the source of mitochondrial oxygen radical formation ". . . by oxidizing the coenzyme Q couple while at the same time reducing the redox potential of the NADP couple which, through glutathione, is the final detoxification step of H_2O_2." In a recent review, Schon and Manfredi[80] wrote, "Because complexes I and III are the principal sources of free radicals in the cell, altered complex I function in the substantia nigra pars compacta could be responsible for the increased DNA damage and lipid peroxidation found in PD brains."

To the extent that ketones crossing the BBB could circumvent or compensate for mitochondrial defects associated with PD, or could protect dopaminergic neurons from free radical damage, a suitable ketogenic diet might well have beneficial effects in certain patients suffering from Parkinsonism.

Alzheimer's Disease

In 1996, Ogawa et al.[81] determined regional cerebral blood flow and studied energy utilization in the brains of seven patients with AD by measuring arteriovenous differences of an array of substrates. Single-photon emission computed tomography showed that the patients had significantly decreased regional cerebral blood flow limited to the parietotemporal region. The glucose extraction fraction and global cerebral metabolic rate of glucose were significantly decreased, whereas the global cerebral metabolic rate of oxygen was only slightly decreased. Because the cerebral metabolic rates of the various substrates (ketones, lactate, etc.) did not change, the authors concluded that the markedly elevated oxygen:glucose utilization ratio indicated an altered energy metabolism in AD.

Conceivably the impairment of glucose metabolism in AD patients described by Ogawa et al. is attributable, at least in part, to local insulin resistance. Watson and Craft[82] called attention to emerging evidence that insulin abnormalities and insulin resistance in AD may contribute to the pathophysiology and clinical symptoms of this disorder. In their words, "It has been recently demonstrated that insulin-sensitive glucose transporters are localized in the same regions supporting memory and that insulin plays a role in memory functions. Collectively, these findings suggest that insulin may contribute to normal cognitive functioning and that insulin abnormalities may exacerbate cognitive impairments, such as those associated with AD." The authors suggest that improving insulin effectiveness may have therapeutic benefit for patients with AD. The suggestion of Watson and Craft that insulin abnormalities may play a role in the pathogenesis of AD has to be reconciled with the finding by Molina et al.[83] that cerebrospinal fluid insulin levels in 27 AD patients did not differ from those in 16 matched controls.

In 1996, Hoshi et al.[84] reported that a fragment of the beta chain of amyloid inhibits PDH activity in primary cultures of hippocampal neurons. A year later, the same authors found that the 1–42 fragment of the beta chain of amyloid, $A\beta_{1-42}$, can inhibit acetyl choline synthesis of septal neurons.[85] Veech et al.[50] note that cultured hippocampal neurons die when exposed to $A\beta_{1-42}$. However, addition of 4 mM D-β-hydroxybutyrate protects these neurons from amyloid fragment–induced death. Casley et al.[86] showed that beta-amyloid causes a significant reduction in state 3 and state 4 mitochondrial respiration, which is diminished further by nitric oxide. These authors also found that cytochrome oxidase, α-ketoglutarate dehydrogenase, and PDH activities are inhibited by β-amyloid. They concluded that β-amyloid can directly disrupt mitochondrial function, inhibit key enzymes, and contribute to the impairment of energy metabolism observed in AD.

Veech et al.[50] contend that the foregoing studies provide a rationale for trial of a ketogenic diet in the treatment of AD. Ketones can overcome localized insulin resistance, can bypass a block in PDH, and have been shown to protect dopaminergic neurons from free radical damage and from apoptosis induced by the 1–42 amyloid fragment in tissue-culture experiments.

What Next?

In considering the potential usefulness of therapeutic hyperketonemia in the treatment of AD, PD, and certain other neurodegenerative conditions, it is essential to be clear about what is meant by terms like "ketogenic diet" and "hyperketonemia."

There is widespread confusion among both physicians and lay individuals about what constitutes a ketogenic diet. Similar to glucose, ketones are present in the blood at all times. As shown in Table 3, the range of ketone levels that can be achieved by prolonged food deprivation or by adherence to various diets is quite wide. Hence, it makes no sense to speak of a "ketogenic diet" without also specifying the degree of serum ketone elevation that the diet is intended to achieve.

The serum concentrations sought for treatment of epilepsy range from 2 to 7 mM/L. Such levels can be achieved only by strict adherence to a regimen like the 4:1 HKD—one most patients find burdensome. On the other hand, the kind of low-carbohydrate "ketogenic diet" (e.g., 30% protein, 8% carbohydrate, 61% fat [as proportions of total energy]) that has recently become popular—although much easier to follow—achieves BHB levels of only 0.28–0.40 mM/L.[87] (This "popular" ketogenic diet, at a 2000 kcal/day intake, provides 127 g of presumptive carbohydrate [40 preformed] plus ~87 g derivable from 150 g protein, not including the small quantity of glycerol released in the course of adipocyte triglyceride hydrolysis. At the same energy level, the 4:1 HKD would yield 33 g of presumptive carbohydrate [10 g preformed] plus ~23 g derivable from 40 g protein. Thus, the effective quantity of carbohydrate provided by the popular ketogenic diet is almost four times that supplied by the 4:1 HKD.)

Future research must address the problem of matching diet-induced elevation of serum ketone levels with therapeutic benefits. Because the drastic type of hyperketogenic diet—the one used to treat epilepsy and related neurologic disorders—is so onerous and difficult to follow, it is especially important to determine the extent to which such a diet can be liberalized without losing desirable therapeutic effects. Given the daunting nature of the 4:1 HKD, it is encouraging that there exists a potential nutritional stratagem, which eventually may

Table 3. Relationship of Metabolic State and Diet Composition to Plasma Levels of Acetoacetate (AcAc) and β-Hydroxybutyrate (BHB) in Normal-weight, Overweight, and Obese Adults

Metabolic State/ Diet Composition	Comments	AcAc (mM/L)	BHB (mM/L)	References
Postabsorptive	*Males*			
$n = 11$	Normal weight	0.05	0.07	Hall et al.[18]
$n = 12$	Normal weight		0.08	Sharman et al.[87]
$n = 10$	Obese	0.15	0.27	Hall et al.[18]
$n = 6$	Overweight	0.01	0.02	Cahill et al.[39]
*Ketogenic diets**	*Males (normal weight)*			
(eucaloric)	3 weeks on diet ($n = 12$)		0.40	Sharman et al.[87]
C8, P30, F61	6 weeks on diet		0.28	Sharman et al.[87]
	Female (normal weight)			
C2, P8, F90	2 weeks on diet ($n = 1$)	0.70	6.02	VanItallie, Nonas & Heymsfield†
Fasting	*Males*			
$n = 6$	*(overweight)*			
	1-week fast	0.95	3.58	Cahill et al.[39]
$n = 6$	*(obese)*			
	1–2-week fast	1.04	5.97	Hall et al.[18]

*Distribution (percent total calories) of carbohydrate (C), protein (P), and fat (F).
†Unpublished study (St. Luke's-Roosevelt Hospital, 2003).
Where $n > 1$, all values are means.

make it possible to induce therapeutic hyperketonemia in patients without requiring them to adhere to a Draconian regimen. The stratagem entails administration by mouth of substantial quantities of ketones in a form—namely, as esters or small polymers—suitable for ingestion. In their article about the potential therapeutic uses of ketone bodies, Veech et al.[50] suggest that the problems associated with "the very unappetizing, high-fat ketogenic diet" might be obviated by feeding patients up to 100 to 150 g per day of a nutritionally acceptable form of ketone bodies. Unfortunately, neither ketone esters nor oligomers of BHB are presently available in sufficient quantities for the conduct of clinical trials.

The traditional 4:1 HKD is difficult but far from unmanageable. Numerous epileptic children and adults with seizures resistant to anticonvulsant medication have managed to remain on this regimen for years without experiencing serious adverse effects. There are justifiable concerns about its hyperlipidemic effect, however, and the fact that the diet has been reported to increase risk of side effects including nephrolithiasis,[88] depression of platelet function (manifested by easy bruising),[89] and occasionally prolongation of the electrocardiographic QTc interval in children.[90] It should be possible to mitigate, or perhaps prevent, the adverse effects of this very high–fat diet on plasma lipids by modifying its fatty acid content. What is most important now, however, is to get on with the job of determining whether diet-induced hyperketonemia (at any level) will prove clinically beneficial in the management of a number of neurodegenerative disorders, particularly Parkinson's disease and Alzheimer's disease.

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1. Gerhardt J. Diabetes mellitus und aceton. *Wiener Medizinische Presse.* 1865;6:672.
2. Hirschfeld F. Beobachtungen über die Acentonurie und das Coma diabeticum. *Z klin Med.* 1895;28: 176–209.
3. Peters JP, Van Slyke DD. *Quantitative Clinical Chemistry: Interpretations*. Volume 1, 2nd edition. Baltimore: Williams & Wilkins; 1946:439.
4. Cohen PP. Studies in ketogenesis. *J Biol Chem.* 1937;119:333–346.
5. Cohen PP, Stark IE. Hepatic ketogenesis and ketolysis in different species. *J Biol Chem.* 1938;126:97–107.
6. Jowett M, Quastel JH. Fat metabolism III. The formation and breakdown of acetoacetic acid in animal tissues. *Biochem J.* 1935;29:2181–2191.
7. Chaikoff IL, Soskin S. The utilization of acetoacetic acid by normal and diabetic dogs before and after evisceration. *Am J Physiol.* 1928;87:58–72.
8. Barnes RH, Drury DR, Greeley PO, Wick AN. Utilization of the ketone bodies in normal animals and in those with ketosis. *Am J Physiol.* 1940;130:144–150.
9. Mirsky IA, Broh-Kahn RH. The influence of increased metabolism on β-hydroxybutyric acid utilization. *Am J Physiol.* 1937;120:446–450.
10. Waters ET, Fletcher JP, Mirsky IA. The relation between carbohydrate and β-hydroxybutyric acid

utilization by the heart-lung preparation. *Am J Physiol.* 1938;122:542–546.
11. Johnson RE, Sargent F II, Passmore R. Normal variations in total ketone bodies in serum and urine of healthy young men. *Q J Exp Physiol.* 1958;43: 339–344.
12. Cahill GF Jr. Starvation. *Trans Am Clin Climatol Assoc.* 1982;94:1–21.
13. Foster DW. Diabetes mellitus. In: Wilson JD, Braunwald E, Isselbacher KJ, eds. *Harrison's Principles of Internal Medicine.* 12th edition. New York: McGraw-Hill; 1991:1750.
14. Crandall DI, Gurin S. Studies of acetoacetate formation with labeled carbon I. Experiments with pyruvate, acetate, and fatty acids in washed liver homogenates. *J Biol Chem.* 1949;181:829 – 843.
15. Lynen F, Henning U, Bublitz C, Sorbo B, Kröplin-Rueff L. Der chemische Mechanismus der Acetessigsäurebildung in der Leber. *Biochem Ztschr.* 1958;330:269–295.
16. Robinson AM, Williamson DH. Physiological roles of ketone bodies as substrates and signals in mammalian tissues. *Physiol Rev.* 1980;60:143–187.
17. Wastney ME, Hall SEH, Berman M. Ketone body kinetics in humans: a mathematical model. *J Lipid Res.* 1984;25:160–174.
18. Hall SEH, Wastney ME, Bolton TM, Braaten JT, Berman M. Ketone body kinetics in humans: the effects of insulin-dependent diabetes, obesity, and starvation. *J Lipid Res.* 1984;25:1184–1194.
19. Williamson DH, Mellanby J, Krebs HA. Enzymic determination of D(-)β-hydroxybutyric acid and acetoacetic acid in blood. *Biochem J.* 1962;82:90–96.
20. Krebs HA. The physiological role of the ketone bodies. *Biochem J.* 1961;80:225–233.
21. Cahill GF Jr. Starvation in man. *N Engl J Med.* 1970;282:668–675.
22. Laffel L. Ketone bodies: a review of physiology, pathophysiology and application of monitoring to diabetes. *Diabetes Metab Res Rev.* 1999;15:412–426.
23. Courtice FC, Douglas CG. The effects of prolonged muscular exercise on the metabolism. *Proc R Soc.* 1936;119B:381–439.
24. Owen OE, Reichard GAJ. Human forearm metabolism during progressive starvation. *J Clin Invest.* 1971;50:1538–1545.
25. Cahill GF Jr. Starvation in man. *Clin Endocrinol Metab.* 1975;5:397–417.
26. Behre JA. Studies in ketone body excretion. I. Daily variations in the ketone bodies of normal urine and the ketonuria of short fasts, with a note on diabetic ketonuria during insulin treatment. *J Biol Chem.* 1931;92:679–697.
27. Kartin BL, Man EB, Winkler AW, Peters JP. Blood ketones and serum lipids in starvation and water deprivation. *J Clin Invest.* 1944;23:824–835.
28. Johnson RE, Passmore R, Sargent F. Multiple factors in experimental human ketosis. *Arch Intern Med.* 1961;107:43–50.
29. Bach SJ, Hibbitt KG. Biochemical aspects of bovine ketosis. *Biochem J.* 1959;72:87–92.
30. Paterson P, Sheath J, Taft P, Wood C. Maternal and foetal ketone concentrations in plasma and urine. *Lancet.* 1967;1:862–865.
31. Aoki TT, Muller WA, Brennan MR, Cahill GF Jr. The metabolic effects of glucose in brief and prolonged fasted man. *Am J Clin Nutr.* 1975;28:507–511.
32. Heinbecker P. Studies on the metabolism of Eskimos. *J Biol Chem.* 1928;80:461–475.
33. Astrand P-O, Rodahl K. *Textbook of Work Physiology. Physiological Bases of Exercise.* 3rd edition. New York: McGraw-Hill; 1986:317.
34. McClellan WS, DuBois EF. Clinical calorimetry. XLV. Prolonged meat diets with a study of kidney function and ketosis. *J Biol Chem.* 1930;87:651–667.
35. Krebs HA. The metabolic fate of amino acids, In: Munro HN, Allison JB, eds. *Mammalian Protein Metabolism*. New York: Academic Press; 1964:126–176.
36. Jungas RL, Halperin ML, Brosnan JT. Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. *Physiol Rev.* 1992;72: 419–448.
37. Swink TD, Vining EPG, Freeman JM. The ketogenic diet: 1997. *Adv Pediatr.* 1997;44:297–329.
38. Vanltallie TB, Bergen SS Jr. Ketogenesis and hyperketonemia. *Am J Med.* 1961;31:909–918.
39. Cahill GF Jr, Herrera G, Morgan AP et al. Hormone-fuel interrelationships during fasting. *J Clin Invest.* 1966;45:1751–1769.
40. Sokoloff L. Metabolism of the central nervous system *in vivo*. In: Field J, ed. *Handbook of Physiology, Section I. Neurophysiology.* Baltimore: American Physiological Society, Williams and Wilkins; 1960: 1843–1864.
41. Benedict FG. A study of prolonged fasting. Washington, DC: The Carnegie Institute; 1915:Publication 203.
42. Gamble JL, Ross GS, Tisdall FF. The metabolism of fixed base during fasting. *J Biol Chem.* 1923;57: 633–695.
43. Leiter LA, Marliss EB. Survival during fasting may depend on fat as well as protein stores. *JAMA.* 1982;248:2306–2307.
44. Owen OE, Morgan AP, Kemp HG, et al. Brain metabolism during fasting. *J Clin Invest.* 1967;46: 1589–1595.
45. Lardy HA, Hansen RG, Phillips PH. The metabolism of bovine epididymal spermatozoa. *Arch Biochem.* 1945;6:41–51.
46. Lardy HA, Phillips PH. Studies of fat and carbohydrate oxidation in mammalian spermatozoa. *Arch Biochem.* 1945;6:53–61.
47. Kashiwaya Y, Sato K, Tsuchiya S, et al. Control of glucose utilization in working perfused rat heart. *J Biol Chem.* 1994;269:25502–25514.
48. Sato K, Kashiwaya Y, Keon CA, et al. Insulin, ketone bodies, and mitochondrial energy transduction. *FASEB J.* 1995;9:651–658.
49. Kashiwaya Y, King MT, Veech RL. Substrate signaling by insulin: a ketone bodies ratio mimics insulin action in heart. *Am J Cardiol.* 1997;80:50A–64A.
50. Veech RL, Chance B, Kashiwaya Y, Lardy HA, Cahill GF Jr. Ketone bodies, potential therapeutic uses. *IUBMB Life.* 2001;51:241–247.
51. McHenry LC Jr. Cerebral blood flow. *N Engl J Med.* 1966;274:82–91.
52. Woodyatt RT. Objects and method of diet adjust-

ment in diabetics. *Arch Intern Med.* 1921;28:125–141.
53. Wilder RM. The effect of ketonemia on the course of epilepsy. *Mayo Clin Bull.* 1921;2:307–8.
54. Peterman MG. The ketogenic diet in the treatment of epilepsy. A preliminary report. *Am J Dis Child.* 1924;28:28–33.
55. Huttenlocher PR, Wilbourn AJ, Signore JM. Medium-chain triglycerides as a therapy for intractable childhood epilepsy. *Neurology.* 1971;21:1097–1103.
56. Gordon N. Medium-chain triglycerides in a ketogenic diet. *Dev Med Child Neurol.* 1977;19:535–544.
57. Bergen SS Jr, Hashim SA, Vanltallie TB. Hyperketonemia induced in man by medium-chain triglyceride. *Diabetes.* 1966;15:723–725.
58. Schwartz RH, Eaton J, Bower BD, Aynsley-Green A. Ketogenic diets in the treatment of epilepsy. Short-term clinical effects. *Dev Med Child Neurol.* 1989; 31:145–151.
59. Al-Mudallal AS, LaManna JC, Lust WD, Harik SL. Diet-induced ketosis does not cause cerebral acidosis. *Epilepsia.* 1996;37:258–261.
60. Uhleman ER, Neims AH. Anticonvulsant properties of the ketogenic diet in mice. *J Pharmacol Exp Ther.* 1972;180:231–238.
61. DeVivo DC, Malas KL, Leckie MP. Starvation and seizures. *Arch Neurol.* 1974;32:755–760.
62. Pan JW, Bebin EM, Chu WJ, Hetherington HP. Ketosis and epilepsy: ^{31}P spectroscopic imaging at 4.1 T. *Epilepsia.* 1999;40:703–707.
63. Thavendiranathan P, Chow C, Cunnane S, McIntyre BW. The effect of the 'classic' ketogenic diet on animal seizure models. *Brain Res.* 2003;959:206–213.
64. Janigro D. Blood-brain barrier, ion homeostasis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. *Epilepsy Res.* 1999;37:223–232.
65. Leino RL, Gerhart DZ, Duelli R, Enerson BE, Drewes LR. Diet-induced ketosis increases monocarboxylate transporter (MCT1) levels in rat brain. *Neurochem Int.* 2001;38:519–527.
66. Seidner G, Garcia Alvarez M, Yeh J-I, et al. GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood-brain-barrier hexose carrier. *Nat Genet.* 1998;18:166–191.
67. Klepper J, Voit T. Facilitated glucose transporter protein type 1 (GLUT1) deficiency syndrome: impaired glucose transport into brain—a review. *Eur J Pediatr.* 2002;161:295–304.
68. Wexler ID, Hemalatha SG, McConnell J, et al. Outcome of pyruvate dehydrogenase deficiency treated with ketogenic diets. Studies in patients with identical mutations. *Neurology.* 1997;49: 1655–1661.
69. Wijburg FA, Barth PG, Bindoff LA, et al. Leigh syndrome associated with a deficiency of the pyruvate dehydrogenase complex: results of treatment with a ketogenic diet. *Neuropediatrics.* 1992;23:147–152.
70. Buckley BM, Williamson DH. Acetoacetate and brain lipogenesis: developmental pattern of acetoacetyl-coenzyme A synthetase in the soluble fraction of rat brain. *Biochem J.* 1973;132:653–656.
71. Patel MS, Owen OE. Lipogenesis from ketone bodies in rat brain. Evidence for conversion of acetoacetate into acetyl-coenzyme A in the cytosol. *Biochem J.* 1976;156:603–607.
72. Koper JW, Zeinstra EC, Lopes-Cardozo M, van Golde LM. Acetoacetate and glucose as substrates for lipid synthesis by rat brain oligodendrocytes and astrocytes in serum-free culture. *Biochim Biophys Acta.* 1984;796:20–26.
73. Minassian BA, Lee JR, Herbrick JA, Huizenga J, et al. Mutations in a gene encoding a novel protein tyrosine phosphatase cause progressive myoclonic epilepsy. *Nat Genet.* 1198;20:171–174.
74. Calabrese V, Scapagnini G, Giuffrida Stella AM, Bates TE, Clark JB. Mitochondrial involvement in brain function and dysfunction: relevance to aging, neurodegenerative disorders and longevity. *Neurochem Res.* 2001;26:739–764.
75. DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. *N Engl J Med*. 2003;348:2656–2668.
76. Schapira AH. Causes of neuronal death in Parkinson's disease. *Adv Neurol.* 2001;86:155–162.
77. Finsterer J. Parkinson's disease as a manifestation of mitochondriopathy. *Acta Neurol Scand.* 2002; 105:384–389.
78. Veech GA, Dennis J, Keeney PM, et al. Disrupted mitochondrial electron transport function increases expression of anti-apoptotic bcl-2 and bcl-XL proteins in SH-SY5Y neuroblastoma and in Parkinson's disease cybrid cells through oxidative stress. *J Neurosci Res.* 2000;61:693–700.
79. Kashiwaya Y, Takeshima T, Mori N, Nakashima K, Clarke K, Veech RL. D-β-Hydroxybutyrate protects neurons in models of Alzheimer's and Parkinson's disease. *Proc Natl Acad Sci U S A.* 2000;97:5440–5444.
80. Schon EA, Manfredi G. Neuronal degeneration and mitochondrial dysfunction. *J Clin Invest.* 2003;111: 303–312.
81. Ogawa M, Fukuyama H, Ouchi Y, Yamauchi H, Kimura J. Altered energy metabolism in Alzheimer's disease. *J Neurol Sci.* 1996;139:78–82.
82. Watson GS, Craft S. The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs.* 2003;17:27–45.
83. Molina JA, Jimenez-Jimenez FJ, Vargas C, et al. Cerebrospinal fluid levels of insulin in patients with Alzheimer's disease. *Acta Neurol Scand.* 2002;106: 347–350.
84. Hoshi M, Takashima K, Noguchi M, et al. Regulation of mitochondrial pyruvate dehydrogenase activity by tau protein kinase I/glycogen synthase kinase 3beta in brain. *Proc Natl Acad Sci U S A*. 1996;93: 2719–2723.
85. Hoshi M, Takashima M, Murayama K, et al. Nontoxic amyloid beta peptide 1–42 suppresses acetylcholine synthesis. *J Biol Chem.* 1997;272:2038–2041.
86. Casley CS, Canevari L, Land JM, Clark JB, Sharpe MA. Beta-amyloid inhibits integrated mitochondrial respiration and key enzyme activities. *J Neurochem.* 2002;80:91–100.
87. Sharman MJ, Kraemer WJ, Love DM, et al. A ketogenic diet favorably affects serum biomarkers for

cardiovascular disease in normal-weight young men. *J Nutr.* 2002;132:1879–1885.
88. Furth SL, Casey JC, Pyzik PL, et al. Risk factors for urolithiasis in children on the ketogenic diet. *Pediatr Nephrol.* 2000;15:125–128.
89. Berry-Kravis E, Booth G, Taylor A, Valentino LA. Bruising and the ketogenic diet: evidence for diet-induced changes in platelet function. *Ann Neurol.* 2001;49:98–103.
90. Best TH, Franz DN, Gilbert DL, Nelson DP, Epstein MR. Cardiac complications in pediatric patients on the ketogenic diet. *Neurology.* 2000;54:2328–2330.