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Review

The therapeutic implications of ketone bodies: the effects of ketone bodies in pathological conditions: ketosis, ketogenic diet, redox states, insulin resistance, and mitochondrial metabolism

Richard L. Veech*

Laboratory of Membrane Biochemistry and Biophysics, National Institutes of Alcoholism and Alcohol Abuse, 12501 Washington Ave., Rockville, MD 20850, USA

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Abstract

The effects of ketone body metabolism suggests that mild ketosis may offer therapeutic potential in a variety of different common and rare disease states. These inferences follow directly from the metabolic effects of ketosis and the higher inherent energy present in D-β-hydroxybutyrate relative to pyruvate, the normal mitochondrial fuel produced by glycolysis leading to an increase in the $\Delta G'$ of ATP hydrolysis. The large categories of disease for which ketones may have therapeutic effects are:

(1) diseases of substrate insufficiency or insulin resistance,
(2) diseases resulting from free radical damage,
(3) disease resulting from hypoxia.

Current ketogenic diets are all characterized by elevations of free fatty acids, which may lead to metabolic inefficiency by activation of the PPAR system and its associated uncoupling mitochondrial uncoupling proteins. New diets comprised of ketone bodies themselves or their esters may obviate this present difficulty.
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1. Metabolic effects of ketone body metabolism

The therapeutic potentials of mild ketosis flow directly from a thorough understanding of their metabolic effects, particularly upon mitochondrial redox states and energetics and upon substrate availability. The data on metabolic effects of ketone body metabolism presented here has been published previously [1,2]. It presents studies of the isolated working rat heart perfused with 11 mM glucose alone, glucose plus 1 mM acetoacetate and 4 mM D-β-hydroxybutyrate, glucose + 100 nM insulin or the combination of glucose, ketone bodies and insulin. The isolated working perfused heart was studied because of the relative homogeneity of the tissue and the simplicity of its output, the number of parameters which could be accurately measured, particularly O_2 consumption relative to actual hydraulic work output of the heart. In our analysis of disease states, it has been assumed that the effects of ketone metabolism in heart would mimic those in brain, which was not analyzed in this detailed manner for a number of technical reasons, most prominently the inhomogeneous nature of the tissue and its lack of quantifiable outputs.

A detailed metabolic control strength analysis of glycolysis in heart under the four conditions led to several major conclusions [1]. Firstly, the control of flux through the glycolytic pathway was context dependent and shifted from one enzymatic step to another depending upon the conditions. There was not one key "rate controlling" reaction, but rather control was distributed among a number of steps, including some enzymes that were very close to equilibrium. The absence of a single dominant rate controlling step in a pathway calls into question the assumptions on which many pharmaceutical discovery programs have been based [3]. Secondly, when perfused with glucose alone, there was consistent glycogen breakdown, whereas with addition of ketones, insulin or the combination, glycogen synthesis occurred. Addition of either ketones or insulin, increased intracellular [glucose] and the

*Tel.: +1-301-443-4620; fax: 1-301-443-0930.
E-mail address: rveech@mail.nih.gov (R.L. Veech).

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glycolytic intermediates in the first half of the glycolytic pathway from 2 to 8 fold. Thirdly, addition of either ketones or insulin leads to an increase in the measurable hydraulic work of the heart, but a net decrease in the rate of glycolysis. Associated with the increase in hydraulic work and decrease in glycolytic rate addition of ketones or insulin increased the free cytosolic [ATP]/[ADP] × [Pi] ratio three to five fold and a similar change in the [phosphocreatine]/[creatine] ratio showing that both ketones or insulin increased the energy of the phosphorylation state of heart significantly compared to perfusion with glucose alone. These data clearly show that addition of either ketone bodies or insulin, markedly improved the energy status of working perfused heart. How ketone bodies could increase the hydraulic efficiency of heart by 28% could not be explained by the changes in the glycolytic pathway alone, but rather by the changes that were induced in mitochondrial ATP production by ketone body metabolism.

The mitochondrial processes of ATP generation derive their energy from the respiratory electron transport chain, where the electrons from substrates enter at different catalytic centers and travel up through various redox couples within the chain to ultimately combine with H^+ and O_2 to form H_2O. The respiratory chain begins with the NADH multienzyme complex, whose substrate is the free mitochondrial NADH. The redox potential of the free mitochondrial [NAD^+]/[NADH] couple is about −0.28 V [4] while that of the [O_2]/[H_2O] couple is +1.2 V [5]. The total energy available from the movement of electron up the respiratory chain is therefore determined by the difference between the variable redox potential of the mitochondrial NAD couple and the O_2 couple, which is constant at all O_2 concentrations and is given by

$$\Delta G' = -nF\Delta E,$$

where $n = 2$ electrons and $F = 96.485$ kJ/mol/V.

This means 2 electrons traveling up the electron transport system in the redox reaction

$$NADH_{mito} + H^+ + \tfrac{1}{2}O_2 \rightarrow H_2O + NAD^+_{mito}$$

can yield −178 kJ/2 moles of electrons.

The energy gained from mitochondrial electron transport is transferred to the pumping of protons from the mitochondrial to cytosolic phase [6] at sites I, III and IV creating an electrochemical proton gradient between the two phases where the energy of the proton gradient is

$$\Delta G'[H^+]_{cyto}/[H^+]_{mito} = RT\ln[H^+]_{cyto}/[H^+]_{mito} + FE_{mito/cyto},$$

where the major energy component is the electric potential between the mitochondrial and cytosolic phases, which ranges between −120 and −140 mV in working perfused heart [2]. The proton gradient created by the redox energy of the respiratory chain then powers the transport of protons from cytosol back into mitochondria through the F1 ATPase complex in an efficient and reversible process [7,8]. For each electron pair transported up the respiratory chain, 3 ATPs are generated. Since the maximum energy available from the redox reactions of the chain is −178 kJ, the energy available for the synthesis of each of the 3 ATPs can not exceed −59.2 kJ/mol. The energy of the hydrolysis of ATP, $\Delta G'_{ATP}$, in heart, liver and red cell under 6 conditions ranged from −54 to −58 kJ/mol, implying that the overall process of electron transport and oxidative phosphorylation is a remarkably efficient process.

The electrons liberated from mitochondrial NADH by the NADH dehydrogenase complex or complex I, are carried within the mitochondria by co-enzyme Q, where they are transferred to cytochrome C, by $CoQH_2$-cytochrome C reductase, complex III. The difference between the redox potential of the mitochondrial NAD couple and the co-enzyme Q couple, $\Delta E_{Q/NADH}$, determines the energy of the proton gradient generated by mitochondria. This in turn determines the energy of hydrolysis of ATP, $\Delta G'_{ATP}$, which is generated by the mitochondrial F1 ATPase [2].

The ketone bodies, acetoacetate and D-β-hydroxybutyrate are in near-equilibrium with the free mitochondrial [NAD^+]/[NADH] ratio in a reaction catalyzed by D-β-hydroxybutyrate dehydrogenase [9] It would also appear that the [succinate]/[[fumarate] couple is in near equilibrium with the mitochondrial [Q]/[QH2] couple in a reaction catalyzed by succinate dehydrogenase [2]. When ketone bodies are metabolized in heart, the mitochondrial NAD couple is reduced while the mitochondrial Q couple is oxidized increasing the redox span, $\Delta E_{Q/NADH}$, between site I and site III, making more energy available for the synthesis of ATP, and hence an increase in the $\Delta G'$ of ATP hydrolysis. This in turn is observable in the 28% increase in the hydraulic efficiency of the working perfused rat heart.

The fundamental reason why the metabolism of ketone bodies produce an increase of 28% in the hydraulic efficiency of heart compared with a heart metabolizing glucose alone is that there is an inherently higher heat of combustion in D-β-hydroxybutyrate than in pyruvate, the mitochondrial substrate which is the end product of glycolysis (Table 1).

If pyruvate were burned in a bomb calorimeter, it would liberate 185.7 kcal/mole of C_2 units, whereas the combustion of D-β-hydroxybutyrate would liberate 243.6, or 31% more calories per C_2 unit than pyruvate. Metabolizing D-β-hydroxybutyrate in perfused working heart creates a 28% increase in the hydraulic efficiency of heart when compared to the metabolism of the end product of glycolysis, pyruvate.

Table 1
Heats of combustion of common non-nitrogenous energy substrates

Substrate	ΔH^o kcal/mol	ΔH^o kcal/mol C_2 units
$C_{18}H_{32}O_2$ Palmitate	−2384.8	−298
$C_4H_8O_3$ β HOButyrate	−487.2	−243.6
$C_6H_{12}O_6$ Glucose	−669.9	−223.6
$C_3H_4O_3$ Pyruvate	−278.5	−185.7

The mitochondrial processes of electron transport and ATP synthesis appear to be capable of capturing this inherent energy contained in the substrates being metabolized.

The greater energy inherent in D-β-hydroxybutyrate relative to pyruvate derives from the higher ratio of H–C present in each molecule, 2 H per C in the case of D-β-hydroxybutyrate versus 1.3 H per C in the case of pyruvate. Put another way, the ketone body is more reduced than pyruvate. One may then ask, why would there not be even more energy released if the heart were to metabolize the fatty acid palmitate, which has even more inherent energy available during combustion than a ketone body. The reasons why the metabolism of fatty acids does not lead to even a greater increase in efficiency than does the metabolism of ketones are of two types: the architecture of the pathway of fatty acid oxidation and the enzymatic changes induced by elevation of free fatty acids.

During β oxidation, only half of the reducing equivalents released enter the respiratory chain at NADH dehydrogenase while the other half enter at a flavoprotein site with a potential above that of the NAD couple. This results in the loss of the synthesis of about 1 of the 6 possible ATP molecules, for a loss of about 5% in efficiency. The remaining reducing equivalents produced from the acetyl CoA produced during β oxidation, are metabolized in the TCA cycle in the normal fashion. However the redox potential of the Q couple is not oxidized as it is during ketone metabolism, but rather reduced, decreasing the ΔE available for ATP synthesis. In addition, the elevation of free fatty acids, leads to the increased transcription of mitochondrial uncoupling proteins and of the enzymes of peroxisomal β oxidation. Uncoupling proteins allow the proton gradient generated by the respiratory chain to re-enter the mitochondria by pathways which bypass the F1 ATPase generating heat rather than ATP. Fatty acids undergoing β oxidation with peroxisomes have no mechanism for energy conservation and result solely in heat production. Induction of uncoupling proteins, by chronic elevation of free fatty acids or from other causes result not in increased cardiac efficiency, but rather in pathological decreases in cardiac efficiency [10]. Among the common non-nitrogenous substrates for mitochondrial energy generation, ketone bodies deserve the designation of a ''superfuel''.

In addition to their effects on mitochondrial energetics, the metabolism of ketone bodies has other effects with therapeutic implications. Of major significance is the ability of ketone bodies to increase the concentrations of the metabolites of the first third of the citric acid cycle. Next to causing the translocation of GLUT4 from endoplasmic to plasma membrane, one of the most important acute metabolic effects of insulin, is the stimulation of the activity of the pyruvate dehydrogenase multienzyme complex leading to an increased production of mitochondrial acetyl CoA, the essential substrate for the citric acid cycle. The metabolism of ketone bodies or the addition of insulin to working perfused heart both results in increases of cardiac acetyl CoA content 12–18 fold. This increase is associated with increases of 2–8 fold in the cardiac content of citrate and the other constituents of the citric acid cycle down to α-ketoglutarate, and including L-glutamate, an important redox partner of α-ketoglutarate and the mitochondrial NAD couple. Citrate is an important precursor in the generation of cytosolic acetyl CoA for lipid and acetyl choline biosynthesis, while L-glutamate is a necessary precursor for GABA synthesis in neural tissue.

The metabolic effects of ketone bodies are of particular relevance to brain metabolism, where Cahill and his colleagues have established that over 60% of the metabolic energy needs of brain can be supplied by ketone bodies, rather than by glucose [11]. They have further established that mild ketosis with blood levels of 5–7 mM is the normal physiological response to prolonged fasting in man [12]. The 1.5 kg human brain utilizes 20% of the total body oxygen consumption at rest, requiring 100–150 g of glucose per day [13]. Although man can make about 10% of the glucose to supply brain needs during fasting from fat [14], prolonged starvation in man leads to the excretion of 4–9 g of nitrogen per day which is equivalent to the destruction of 25–55 g protein/day. This amount of protein catabolism could supply between 17 and 32 g of glucose per day, far below the 100–150 g/day required. To supply the glucose required to support brain from protein alone would lead to death in about 10 days instead of the 57–73 days required to cause death in a young male of normal body composition during a total fast [15]. Cahill also noted that in his subjects undergoing total starvation for 30 days, hunger subsided on about the third day, coincident with the elevation of blood ketones to 7 mM. During prolonged fasting, the human produces about 150 g of ketone bodies per day [16]. This suggests, that even though the V_{max} of the monocarboxylate transporter in brain is increased during ketosis [17], the K_m at the endothelial cell of approximately 5 mM for ketone body transport of approximately 5 mM would still dominate the ketone effects in brain, so that a blood level only slightly below that of 7 mM ketone bodies would be required to

achieve the effects on brain metabolism were elevation of ketones to be achieved by means to achieve similar effects to those observed during prolonged starvation. These amounts are not pharmacological, but rather nutritional, which changes markedly the traditional pharmaceutical industry approaches to therapy. Although brain has insulin receptors, it has either no or very low insulin levels making ketosis the only practical mechanism for increasing the efficiency of oxidation ATP generation in that organ.

Finally there are broad therapeutic implications from the ability of ketone body metabolism to oxidize the mitochondrial co-enzyme Q couple. The major source of mitochondrial free radical generation is Q semiquinone [18]. The semiquinone of Q, the half-reduced form, spontaneously reacts with O_2 to form free radicals. Oxidation of the Q couple reduces the amount of the semiquinone form and thus would be expected to decrease $O_2^{\bullet -}$ production. In addition, the metabolism of ketones causes a reduction of the cytosolic free [$NADP^+$]/[NADPH] couple which is in near-equilibrium with the glutathione couple [19]. Reduced glutathione is the final reductant responsible for the destruction of H_2O_2.

From the multiple effects of the metabolism on the basic pathways of intermediary metabolism, it is clear that there are a number of disease states in which mild ketosis could offer possible therapeutic benefits. Some of these therapeutic uses of ketosis have been discussed earlier [20].

2. Ketogenic diets in human subjects

Starvation, with attendant ketosis, has been used as a treatment for refractory epilepsy since the early 20th century. Pierre Marie proposed this treatment on the theory that epilepsy resulted from intestinal intoxication. On this assumption, a diet consisting of water only for 30 days was used to successfully treat some refractory epileptics by Hugh Conklin, a Wisconsin osteopath. The inference that ketone bodies themselves were the effective agent, led Russell Wilder of the Mayo Clinic to propose a high fat, low carbohydrate diet for treatment of epilepsy. High fat, low carbohydrate diets were first used in medicine as a treatment for refractory epilepsy.

2.1. Refractory epilepsy

With the development, by Houston Merritt, of anti-epileptic drugs, use of the ketogenic diet fell into disfavor. This approach to the treatment of drug resistant epilepsy has continued to have its advocates, most notably James Freeman and Elizabeth Vining of Johns Hopkins who have written a comprehensive review of the subject [21]. In a study of 600 patients with a history of 20 seizures per day refractory to over 6 drugs, the Hopkins group reported that the ketogenic diet led to cessation of seizures in slightly less than one third of subjects, a significant decrease in seizure frequency in another third and no effect in one third [22]. Urinary ketone levels correlate poorly with blood levels, so it is difficult to evaluate the effectiveness of the various ketogenic diets. One report of the blood levels of ketone bodies achieved on the diet suggests that blood levels of about 4 mM are required for satisfactory results [23]. In a reverse of the increasing risk of a subsequent seizure after the first one (the Gower effect), Freeman reports that after 2 years on the ketogenic diet, a normal diet can be resumed without recurrence of seizures.

The mechanisms responsible for the therapeutic response in epilepsy remain unclear. Some authors have championed the effects of increased brain glutamate to increase the inhibitory neurotransmitter GABA [24,25]. Others have attributed the therapeutic efficacy of the ketogenic diet to a decrease in circulating blood glucose [26]. It is my hypothesis that the metabolism of ketones in brain is likely to raise the $\Delta G'$ of ATP hydrolysis, and with it the extent of the Na^+ and Ca^{2+} gradients which depend upon $\Delta G'_{ATP}$ [27], thus raising the so-called resting membrane potential and inhibiting the synchronous neuronal discharge characteristic of epilepsy.

A number of variations of the Hopkins ketogenic diet have been reported, including those made up primarily of mid chain length fatty acids [28] or diets containing large amounts of n-3 polyunsaturated fats [29]. In most variations, patient intolerance of the high fat diet is a major contributor to therapeutic failure. Also of concern is the elevation of blood cholesterol which can accompany a high fat diet. Freeman reports that the mean blood cholesterol on the Hopkins form of the diet, the average blood cholesterol rises to over 250, significantly above generally recommended levels. In addition there are reports of dilated cardiomyopathy in patients on the ketogenic diet [30], which are not incompatible with the toxic effects of elevated plasma free fatty acids discussed earlier. Finally an increased incidence in nephrolithiasis in patients on the ketogenic diet [31] as well as increases in serum uric acid secondary to decreased urinary uric acid excretion have been reported in patients on a ketogenic diet.

2.2. Weight loss

In the 1970s the "carbo-caloric diet" became popular, advocating unlimited intake of all foodstuffs save carbohydrates which were kept to specified limits. The high fat low carbohydrate diet produced both mild ketosis and weight loss, but unfortunately also produced high levels of saturated plasma fats. As the public became more conscious of the dangers of elevated

triglycerides and cholesterol, this diet program fell out of favor.

More recently, largely as the result of popularization by Atkins [32], low fat high carbohydrate diets have become popular again as an aid to weight loss. Studies report significant weight loss on a high fat, high protein, low carbohydrate diet without significant elevations of serum cholesterol [33]. Studies comparing the so-called Atkins diet versus more classical caloric restriction have appeared recently and are the subject of significant public interest.

2.3. Adjuncts to cancer chemotherapy

As discussed earlier, elevation of ketone bodies decrease amino acid release from muscle as well as decreasing hepatic gluconeogenesis from amino acids. Feeding a ketogenic diet to mice with implanted tumors decreased tumor size as well as decreasing muscle wasting associated with tumor transplantation [34]. Similar results have been reported in human cancer patients [35,36]. In human patients with advanced astrocytomas, feeding of a ketogenic diet decreased tumor glucose uptake and in part of the group an increase in patient performance [36]. In a surprising report in mice implanted with astrocytoma, a ketogenic diet associated with caloric restriction resulted in an 80% decrease in tumor mass and a decrease in tumor vascularity implying an inhibition of angiogenesis [37].

3. Salts of ketone bodies

The salts of ketone bodies have been examined for their therapeutic effects after either parenteral or oral administration.

3.1. Intravenous and dialysis fluid therapy

Traditional parenteral fluid therapy as currently practiced uses fluids whose compositions are historical and have not been subjected to systematic study of less toxic alternatives. It has been argued that the use of acetate in dialysis fluids and racemic D,L-lactate in both dialysis and parenteral fluid therapy for burns or resuscitation results in significant and unnecessary toxicity [38]. A recent report by the Academy of Medicine suggests a more systematic investigation of the use of Na D-β-hydroxybutyrate as a replacement of the conventional anions in parenteral fluid therapy [39].

In response, studies funded by the military have suggested that resuscitation fluids containing Na D-β-hydroxybutyrate instead of the traditional D,L-lactate can prevent apoptosis in lung which occurs 24 h after severe hemorrhage in a rodent model [40]. DaNang lung was a leading cause of morbidity and mortality in combat casualties during the Viet Nam war when resuscitation with large volumes of conventional Ringer's lactate became standard therapy. These studies in animals suggest that fluids containing D,L-lactate are associated with significant toxic effects which can be avoided if D-β-hydroxybutyrate replaced the lactate. Progressive heart block ending in cardiac arrest can be induced in rats by administration of Na D,L-lactate and similar forms of heart block are observed in children receiving up to 20 l of Ringer's D,L-lactate during the initial phases of burn therapy [41]. Investigation of the use of ketone containing fluids would seem indicated.

3.2. Acyl CoA dehydrogenase deficiency

Perhaps the most dramatic therapeutic response to therapy with Na salts of ketone bodies has been the report of the response of 3 patients with the rare genetic disease caused by acyl CoA dehydrogenase deficiency. This disease is associated with an inability to metabolized fatty acids by β oxidation lead to leukoencephalopathy and cardiomyopathy, of which the later form is more common. It is conventionally treated with a low fat diet and increased carnitine to bind free fatty acids. Three patients who became refractory to this therapy were treated with very small doses in the order of 5 g/day of Na D,L-β-hydroxybutyrate and all showed dramatic improvement in cardiac function and in the extent of leukodystrophy in the brain as judged by MRI [42]. One patient with quadriplegia showed a clearing of symptoms, certainly a dramatic response. The L-form of β-hydroxybutyrate is a product of β oxidation, while the D-form is the product of ketone body synthesis through HMG CoA. Fatty acid synthesis for myelin production requires the D-form for fatty acid synthesis, whereas there would clearly be a deficiency of the L-form in this genetic condition. Which isomeric form is therapeutic in this condition is unknown, since the administration of the D,L-form was dictated purely on the basis of cost of the material from Sigma, which is dependent not on inherent cost, but rather on commercial availability.

An important observation in this and earlier studies was that the administration of Na D,L-β-hydroxybutyrate lowered markedly the blood levels of free fatty acids from 0.6 mM to less than 0.1 mM. Oral administration of Na D,L-β-hydroxybutyrate raised blood levels of total ketones to 0.4 mM at 1 h, a very low level relative to the K_m for brain endothelial transport of 5 mM, but similar to the K_m for neuronal or mitochondrial transport of 0.5 mM. The K_m for adipocyte transport remain unknown, but is clearly a pressing issue if one is to understand how such low levels of ketone bodies can lower the blood levels of free fatty acids by over 75%. The lowering of release of free fatty acid from adipocytes into blood, cannot be simply ascribed to simple "end product" inhibition since it is not reasonable to

believe that an adipocyte process of free fatty acid release is simply enzymatically related to the hepatic process of ketone body production. Other factors such as the effects of ketosis upon glycerol kinase activity or the activity of the adipocyte hormone sensitive lipase must be at play here. Elucidation of the effects of ketones upon free fatty acid release from adipocytes is an important and unexplored area.

4. Animal and cellular studies of ketone bodies in disease models

4.1. Insulin resistant states

The excessive production of ketone bodies during diabetic ketoacidosis is a life threatening condition usually seen in type I diabetics after some intercurrent event. It is characterized by profound hyperglycemia with insulin resistance and elevated blood ketone bodies approaching 25 mM, blood HCO_3^- approaching 0 and blood pH approaching 7 causing hyperventilation and a compensatory low pCO_2. Death occurs from the low pH and vascular collapse secondary to urinary loss of Na^+ and K^+ in an osmotic diuresis. It is not surprising that physicians view elevation of blood ketone bodies with alarm.

However, our data on the acute effects of insulin and ketone bodies in the perfused working heart suggest that ketosis, within limits, mimic the acute effects of insulin [43]. Insulin has two major acute effects in heart. It increases intracellular glucose concentrations by causing the movement of the glucose transporter, GLUT4, from endoplasmic reticulum to the plasma membrane while at the same time increasing the mitochondrial acetyl CoA concentrations by increasing the activity of the pyruvate dehydrogenase multienzyme complex. Ketone bodies increase the intracellular glucose concentration by providing an alternative metabolic substrate and they increase mitochondrial acetyl CoA concentration by by-passing the PDH complex and instead providing acetyl CoA from acetoacetyl CoA. Both insulin and ketones have the same effects on the metabolites of the first third to the citric acid cycle, on mitochondrial redox states and both increase the hydraulic efficiency of the working perfused heart. Viewed in this light, mild ketosis provides the same metabolic effects as insulin, but at the metabolic or primitive control level which by-passes the complex signaling pathway of insulin. During prolonged fasting, when insulin levels approach 0, mild ketosis compensates metabolically for the absence of insulin effects. It follows that the induction of mild ketosis would be therapeutic in insulin resistant states.

Insulin resistant states are extremely common. Insulin resistance is the hallmark of type II diabetes and the so-called "metabolic syndrome" where it is associated with visceral obesity and hypertension [44]. Insulin resistance is present in obese subjects and occurs during "stressful" conditions characterized by elevated adrenal steroids and catechol amines. Insulin resistance also occurs in conditions in which inflammatory cytokines are elevated. Acute insulin resistance is seen in alcohol abusers where elevation of either 2,3 butandiol or 1,2 propandiol inhibits insulin action on adipocytes [45] and impair whole body glucose utilization [46]. Given the metabolic effects of insulin, it is reasonable to suppose that mild ketosis might offer a therapeutic potential which acts directly on the primitive metabolic pathways themselves without requiring the action of the complex insulin signaling pathway.

The most extreme example of insulin resistance is Leprechaunism and Rabson-Mendenhall syndrome, rare genetic diseases where a mutation in the insulin receptor gene results in the loss of insulin binding [47]. Some therapeutic success has resulted from treatment with insulin like growth factor [48], but this material is not widely available. Currently these children are treated with huge doses of insulin which, without effective insulin receptors, are without effect, and merely reflect the physician's impotence in the face of a devastating condition. Death usually occurs in these children in, or before late adolescence. In the absence of effective available treatment, mild ketosis might offer therapeutic benefits to these children who are currently without effective therapy.

4.2. Genetic defects in glucose transport and PDH activity

While insulin resistance is associated with hormonally induced defects in both glucose transport and PDH activity, specific genetic defects in both of these activities, while rare, have been extensively studied. Glucose transport across endothelial cells forming the blood brain barrier is accomplished by GLUT1. GLUT1 is also present in glia other than microglia which express GLUT5, while transport into neurons is a function of GLUT3 [49]. Autosomal dominant genetic defects in GLUT1, usually associated with infantile epilepsy, result in a generalized energy deficit in brain and are successfully treated with the ketogenic diet [50].

Leigh's syndrome is the most frequent metabolic disorder in infancy characterized by lactic acidosis and bilateral encephalopathy usually involving the white matter of the substantia nigra and medulla oblongata. Classically, this condition was associated with defects in pyruvate dehydrogenase multienzyme complex. As such, it should be treatable with mild ketosis. Unfortunately the clinical phenotype of Leigh's syndrome can occur with multiple defects in the electron transport system and in the F1 ATPase. The therapeutic response of these

heterogeneous types of defects would naturally be mixed.

4.3. Hypoglycemic episodes

A major limitation in achieving "tight control" of diabetics, is the risk of increased episodes of hypoglycemia. This consequence of robust insulin therapy is particularly frequent in type I diabetics. Because of the potentially serious consequences of cognitive impairment associated with hypoglycemia, physicians are reluctant to keep blood glucose within ranges which are thought to be optimum for the prevention of long-term vascular disease. Ketone bodies are an alternative to glucose as a supplier of the metabolic energy needs for brain. Cahill has shown [51] that during prolonged fasting, when total blood ketone bodies are in the 5–7 mM range, blood glucose concentrations can be decreased to below 1 mM without either convulsions or any discernable impairment of cognitive function. At these concentrations, ketone bodies can provide essentially all of the energy demands in brain to maintain function. The induction of mild ketosis therefore offers a method for obtaining tighter control of blood glucose in brittle diabetics without the induction of the physiological consequences of hypoglycemia on cerebral function.

4.4. Hypoxic states

The metabolism of ketone bodies results in a 28% increase in the hydraulic work produced by perfused heart per unit of O_2 consumed when compared with the metabolism of glucose alone. There are many conditions where limitation of O_2 supply results in pathology. Ketone bodies would, of course, be expected to have no effect in conditions of total anoxia, since they require mitochondrial electron transport to exert their effects. However in conditions of hypoxia, or relative O_2 lack the induction of ketosis might be expected to offer some benefit.

One non-medical use in this category would be the use of mild ketosis to improve physical performance in settings where extreme exertion is required. Such situations would exist in the military when troops are under extreme combat stress and in certain civilian settings involving emergency personnel. Extreme exertion leads not only to exhausting but also to impairment of cognitive and motor skills under conditions of caloric restriction [52]. Mild ketosis may offer a way to increase muscle and brain function without elevation of free fatty acids which will decrease muscle and cardiac metabolic efficiency through the induction of mitochondrial uncoupling protein.

Obvious in this category would be the penumbral damage in heart after coronary occlusion or in brain following a stroke. In areas of tissue, where O_2 supply is limited, the provision of ketone bodies might provide benefit in limiting the area of damage. In a neonatal rodent model of hypoxia, induction of ketosis has been reported to limit brain damage in comparison to control animals after exposure to 3 h of hypoxia [53]. Similar effects of ketone bodies in limiting the area of brain damage have been reported in rodent bilateral carotid occlusion models [54].

No studies have been undertaken to determine if ketosis might be effective in the treatment of angina pectoris or in increasing the walking distance in the claudication associated with peripheral vascular disease. From a theoretical point of view, one might expect benefits from ketosis in these common conditions. Unfortunately, aside from parenteral infusion or oral ingestion of ketone and their salts, it is not possible at present to elevation ketone bodies, which might improve metabolic efficiency without increasing free fatty acids which would be expected to decrease metabolic efficiency for reasons discussed previously.

4.5. Muscle wasting

During starvation, ketosis prevents the destruction of muscle mass and decreases the export of alanine and glutamine from muscle by decreasing the gluconeogenic demands to provide glucose to brain. One might therefore expect that ketosis would prevent muscle wasting following surgery or trauma. Unfortunately existing evidence of the effects of the infusion of Na D-β-hydroxybutyrate in patients with septic shock found no effect on the degree of muscle wasting [55]. The reason for this apparently inexplicable observation may be found in observations by Murad and his group [56] where they showed that administration of endotoxin results in nitration and inactivation of 3-oxoacid CoA transferase, the enzyme required to activate ketones making their metabolic utilization possible. An increase in NO production and hence tyrosine nitration of this enzyme may account for a number of conditions where ketone body utilization may be decreased.

4.6. Genetic myopathies

Friedreich's ataxia is the most common hereditary limb and gait ataxia associated with hypertrophic cardiomyopathy. It is an autosomal recessive disease resulting from GAA repeats in the gene's first intron which impairs the transcription of the nuclear encoded mitochondrial protein frataxin leading to dysregulation of iron homeostasis with impairment of the activity of mitochondrial iron–sulfur containing proteins such as aconitase and some of the respiratory chain complexes. While yeast frataxin binds iron, the human form of this protein does not [57]. The precise mechanism whereby

decrease in frataxin impairs mitochondrial function is not precisely known. It is known however that cardiac phosphocreatine is decreased and inorganic phosphorus increased in the hearts of patients with Friedreich's ataxia indicating a deficiency in cardiac ATP production [58]. It also is known that aconitase activity is impaired and that over expression of frataxin in mammalian cells results in increased TCA cycle flux, increased mitochondrial membrane potential and increased ATP content [59]. In our studies of the effects of ketone bodies in heart, ketone body metabolism increased the heart citrate content 3 fold which would be expected to decrease a block in aconitase activity which converts citrate to isocitrate. Ketones also increase the $\Delta G'$ of ATP and thus should ameliorate the defects observed in the hearts of these patients. Antioxidant therapies of various sorts have been reported to improve cardiac function in these patients, but so far have had no effect on the neurological impairment. One might expect mild ketosis to improve the function in both heart and brain.

4.7. Diseases of free radical toxicity

Parkinson's disease is a common, generally acquired movement disorder characterized by bradykinesia, rigidity and tremor resulting from destruction of over 80% of the mesencephalic dopaminergic neurons when clinical symptoms of the disease appear. Dopaminergic neurons decline in numbers in all aging humans, but the rate of destruction is accelerated in patients with clinical Parkinsonism, with symptoms most commonly appearing in the 6th decade. It is thought to result from continued free radical damage to dopaminergic neurons, which with high iron content, are particularly subject to this toxicity. The disease is treatable for a time by dopa administration, but as damage and death of dopaminergic neurons continues, dopa therapy becomes either ineffective or limited by its toxicity. Since there is little genetic loading in this disease, except in the rare early onset forms, it is clear that non-genetic therapies are essential.

The major sources of free radical production is the partially reduced co-enzyme Q semiquinone [18]. The semiquinone spontaneously reacts with O_2 to form superoxide anion in the reaction: $QH^{\bullet -} + O_2 \rightarrow O_2^{\bullet -} + H^+$. A reaction catalyzed by superoxide dismutase, can form H_2O_2: $O_2^{\bullet -} + O_2^{\bullet -} + 2H^+ \rightarrow O_2 + H_2O_2$. Hydrogen peroxide can either be destroyed by glutathione: $2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$, or in the presence of iron, as occurs in dopaminergic neurons can form the more toxic hydroxyl radical in the so-called Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow HO + OH + Fe^{3+}$. The metabolism of ketone bodies oxidizes the Q couple and therefore should decrease the amount of Q semiquinone, $QH^{\bullet -}$, thereby decreasing free radical production. In addition the metabolism of ketones, reduces the cytosolic $[NADP^+]/[NADPH]$ couple [43] whose near equilibrium substrate glutathione is the terminal distructant of H_2O_2. An instant form of Parkinsonism can be induced in humans, animals or cells by administration of the meperidine analogue and free radical generator MPP^+ [60,61]. Administration of 4 mM D-β-hydroxybutyrate to mesencephalic dopaminergic primary neuronal cultures rescues these neurons from death induced by MPP^+ [62]. These findings suggest that mild ketosis might be an effective therapy in this disease.

4.8. Neurodegenerative diseases

Alzheimer's disease is a common form of dementia affecting 3% of those 65–74 years old, 18.7% of those 75–84, and 47.2% of those over 85 years of age [63]. Approximately 20–30% of Alzheimer's disease results in defects in 6 different genes. Defects in chromosome 1 and 14, encoding presenilin 1 and 2; in chromosome 21, encoding amyloid precursor protein, lead to early onset Alzheimer's disease. Defects in chromosome 21, encoding α_2 microglobulin; or in chromosome 19, encoding apolipoprotein E is associated with late onset Alzheimer's disease. All of the genetic defects result in excessive accumulation of amyloid protein. The remaining 70–80% of Alzheimer's disease have the same pathological findings of amyloid plaques, phosphorylated microtubular proteins and loss of hippocampal neurons associated with memory loss and increasing dementia, but from causes which include ischemia [64] mild trauma [65] elevated plasma homocystine [66] insulin resistance [67] and impaired brain energy metabolism [68]. The clinical phenotype of Alzheimer's disease is clearly a complex multifactoral disease.

A major finding in Alzheimer's disease is the decrease in brain acetyl choline [69]. This finding has led to the widespread use of acetyl cholinesterase inhibitor in the treatment of the disease, but with only very limited if any improvement [70]. However this pharmacological approach fails to address the underlying pathophysiology of the disease. The common feature of Alzheimer's disease is the elevated levels of proteolytic fragments of β amyloid both extra and intracellularly [71]. It is reported that the 1–42 fragment of β amyloid, $A\beta_{1-42}$, stimulates a mitochondrial isoform of glycogen synthase kinase 3β [72] which phosphorylates and inactivates the pyruvate dehydrogenase multienzyme complex [73] and results in the decrease in acetyl choline synthesis [74] characteristic of the Alzheimer's disease phenotype. Since under normal conditions brain is entirely dependent on glucose as an energy source, inhibition of PDH would be expected to decrease mitochondrial acetyl CoA formation and hence citrate formation, a necessary precursor of acetyl choline. Blockade of PDH is characteristic of insulin lack in heart and ketone bodies

are the physiological mechanism which overcomes this inhibition. If this were to occur in neurons, administration of ketone bodies should by-pass this block. We tested this hypothesis in primary rat hippocampal neuronal cultures exposed to 5 μM $A\beta_{1-42}$ and found that the addition of 4 mM Na D-β-hydroxybutyrate protected against $A\beta_{1-42}$ toxicity [62]. Induction of mild ketosis would therefore seem a reasonable potential therapy in Alzheimer's disease.

4.9. Methods of induction of ketosis

Mild ketosis can be induced in man by either prolonged fasting or by feeding a high fat, low carbohydrate diet. Total starvation is not a therapeutic option, since death results in a normal weight man in 68–72 days. Feeding a high fat, low carbohydrate diet in adults can result in elevation of triglyceride or cholesterol or both. The vascular pathology accompanying these changes limits the use of such diets, particularly in the elderly. Attempts to design high fat diet enriched with polyunsaturated or short chain fatty acids may avoid elevation of cholesterol levels, but suffer from poor patient tolerance of the diet.

Oral administration of Na D,L-β-hydroxybutyrate in doses from 80 to 900 mg/kg/day was sufficient to achieve peak blood levels of total D-β-hydroxybutyrate + acetoacetate of 0.19–0.36 mM producing a therapeutic response in children with acyl CoA dehydrogenase deficiency [42]. In a 70 kg man to achieve these levels of ketosis would require the feeding of between 5.6 to 63 g/day at a present cost of \$3 g^{-1} or \$17 to \$189 day^{-1}. In normal fasting man achieving blood levels of 5–7 mM total ketone bodies, the daily production of ketone bodies is about 150 g per day [16]. It is believed that to achieve a therapeutic response in refractory epilepsy, the level of blood ketone should be above 4 mM [23] which is compatible with a Km for ketone body transport by the monocarboxylate transporter into brain of about 5 mM. Leaving aside the effects of such a large Na^+ load, the present costs of the administration of salts of D-β-hydroxybutyrate to achieve ketosis makes this approach unlikely.

Poly D-β-hydroxybutyrate occurs at up to 90% dry weight in certain microbial species [75] and has been produced in commercial quantities as a biodegradable substitute for polyethylene at a cost of less than \$2 per kg. Poly D-β-hydroxybutyrate also occurs in mammalian species in both blood plasma and in mitochondrial membranes [76]. The bacterial poly D-β-hydroxybutyrate contains 200–500,000 monomeric units and is not readily degradable in mammalian gut. However, preparation of hydrolysis products of this material into metabolizable forms offers promise that oral or parenteral administration of ketone esters may achieve therapeutic blood levels without either the gastric intolerance or hyperlipidemic effects of high fat diets.

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