**Could Ketogenic Diets Become a Complimentary Therapeutic Strategy for Ganglioside Storage Diseases?**

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Chronic inflammatory disease is emerging as a major health crisis in the US. Many Americans are suffering from a plethora of chronic inflammatory diseases including obesity, Type 2 diabetes, cardiovascular disease, and cancer. Chronic and acute inflammation is also linked to several brain diseases to include epilepsy, Alzheimer's disease, Parkinson's disease, and ganglioside storage diseases. Diet and lifestyle issues contribute to the pathology of those suffering from these and other inflammatory diseases. Hence, the management of inflammation is one means of reducing the health burden from these diseases (1-3).

Humans and other mammals have evolved to function for considerable periods without food (2, 4). While glucose is the primary fuel for the brain under normal fed states, the water-soluble ketone bodies, D-β-hydroxybutyrate (β□OHB) and acetoacetate, can compensate for glucose as a metabolic fuel for brain and other organs under conditions of prolonged fasting (5, 6). The liver makes ketone bodies naturally from fatty acids released largely from stored body fat (triglycerides). Changes in key hormones, insulin and glucagon, and regulatory genes orchestrate the physiological transition between the glucose-dependent fed state and the lipid-dependent fasted state. Although ketoacidosis was linked originally to the pathology of diabetes, physiological ketosis is generally linked to improved health (7). The difference in blood β-OHB levels distinguishes ketoacidosis (> 20 mM) from therapeutic ketosis (1-8 mM). Acetone is a non-enzymatic degradation product of acetoacetate that can be easily detected in the breath of individuals with diabetic ketoacidosis (mostly type 1), or in those who conduct prolonged water-only therapeutic fasting.

Calorie restriction (CR) is a type of therapeutic fasting that has long been recognized as a means to improve general health, to manage a broad range of chronic diseases, and to delay aging (2, 8). Chronic inflammatory diseases produce oxidative stress that ultimately damage tissue biomolecules. Hyperglycemia can contribute to this oxidative stress and inflammation (1, 9). Moreover, oxidative stress accelerates entropy, the thermodynamic basis for the aging process (10). CR reduces oxidative stress, which can delay entropy and the therapeutic effects of CR are associated with the reduction of blood glucose and the elevation of blood ketone bodies within normal physiological ranges. Veech showed that metabolism of ketone bodies can reduce oxidative stress by increasing the redox span of the mitochondrial Coenzyme Q couple, which reduces the amount of the Q semiquinone and thus, oxygen radical production in cells with normally functioning mitochondria (11). We previously showed that CR could target inflammation in the brain of mice with Sandhoff's disease (SD) (12). The reduced inflammation was associated with improved motor behavior and increased survival. It has been difficult to determine, however, if the major health benefits of CR are related to the elevation of ketone

bodies, the reduction of glucose, or to the unique metabolic state arising from the combination of these effects.

Although periodic water-only fasting can improve health (13), prolonged fasting will eventually lead to the pathological state of starvation (14), while prolonged CR can lead to nutritional imbalances unless the nutritional composition of the restricted diet is carefully monitored. Nevertheless, emerging evidence indicates that dietary therapies, which lower glucose and elevate ketone bodies, are safe in children and adults, and are effective for a variety of neurological and neurodegenerative diseases (15).

A ketogenic diet (KD) is a low-carbohydrate, high-fat diet that was designed originally to manage refractory seizures in children with epilepsy (16). The KD mimics the physiological state of fasting, which was known since the time of Hippocrates to reduce seizure susceptibility. An energy transition from carbohydrate metabolism to fat metabolism is thought to contribute to the therapeutic benefits of KDs. As with therapeutic fasting and CR, it remains unclear if the anti-epileptic and anti-convulsant effects of the KD are due to reduced glucose, to elevated ketone bodies, or to some combination of these effects. Circulating ketone levels become higher when KDs are administered in restricted amounts than when administered in unrestricted amounts (17, 18). We recently described how restricted KDs, administered with drugs and hyperbaric oxygen therapy, could help manage cancer (19). We believe a similar therapeutic strategy could be used for managing neurological and neurodegenerative diseases.

The KD is a metabolic therapy that must be administered with care, as would be the case for any medical therapy. It should be recognized that unrestricted or excessive consumption of KDs, which elevate body weight, could potentially produce hyperlipidemia and insulin insensitivity, thereby, reducing therapeutic benefit (17, 18, 20). The high fat concentration of the KD will usually prevent excess consumption and body weight gain. As with CR, however, evidence indicates that KDs and ketone bodies can have powerful therapeutic benefit for a broad range of neurological and neurodegenerative diseases (15). Recent evidence indicates that the therapeutic effects of β-OHB against oxidative stress can arise through its action as an endogenous histone deacetylase (HDAC) inhibitor (21). These findings reveal how a global shift in energy metabolism from glucose to ketone bodies can regulate gene expression through epigenetic mechanisms. Synthetic ketone esters also appear to replicate several therapeutic features of KDs, but further studies will be necessary to establish these connections (22).

We found an additive interaction of a restricted ketogenic diet (KD-R) and substrate reduction therapy (SRT) using *N*-butyldeoxynojirimycin (*N*B-DNJ) in adult Sandhoff disease (SD) mice (23). This was the first study to evaluate the effects of a dietary treatment and SRT in combination on disease progression in any of the lysosomal storage diseases (LSDs). Total forebrain ganglioside content and GM2 content were lower in mice receiving the KD-R + *N*B-DNJ combination than in mice receiving only *N*B-DNJ. Most interestingly, we found that the content of *N*B-DNJ in brain tissue was significantly (3.5 fold) greater in the KD-R + *N*B-DNJ mice than in the SD + *N*B-DNJ mice, suggesting that the KD-R might facilitate *N*B-DNJ uptake and transport across the blood-brain barrier (BBB). *N*B-DNJ has an acyl side chain of 4 carbon atoms, is freely soluble in water, and is generally believed to cross the BBB to achieve substrate reduction (24, 25). However, if lipid solubility and total polar surface area were used to assess

the potential of *N*B-DNJ for optimal BBB permeability, *N*B-DNJ would be considered marginal (26). On the other hand, ketone bodies (i.e. acetoacetate and β-OHB) are actively transported across the BBB, particularly during fasting and caloric restriction (27-29). We suggest that the active transport of ketone bodies across the BBB might also increase the transport of *N*B-DNJ. The neuroprotective effects of the KD-R, such as reducing reactive oxygen species, could also account for the increased *N*B-DNJ uptake into the brain. The KD-R could allow for facilitated transport of *N*B-DNJ across the BBB, increase ketone bodies directly and thereby, increase ketone-assisted absorption of *N*B-DNJ, or have an indirect effect on *N*B-DNJ transportation by altering transporters or endothelial cells of the BBB. Alternatively, the KD-R could enhance the uptake of *N*B-DNJ by mucosal cells in the intestines and thereby, result in higher concentrations reaching the circulation and being available for transport across the BBB (23). Further studies will be necessary to determine the mechanism by which the KD-R increases *N*B-DNJ transport in cells.

Our discovery of the KD as a novel delivery system for SRT using *N*B-DNJ was recently translated directly to a child with SD (30). The child experienced significant improvement in seizure control, autistic behavior, cardiac function, hepatomegaly, and especially *quality of life*. Although chronic diarrhea has been a debilitating side effect in patients taking *N*B-DNJ, the child's mother reported no diarrhea or gastrointestinal distress suggesting that the KD might ameliorate some adverse effects associated with this SRT (30). The findings with this SD patient indicate that our preclinical findings in mice in combining the KD with imino sugar can have immediate translation to the clinic. Moreover, recent studies showed that the KD could improve psychomotor development in a child with aspartate-glutamate carrier deficiency through an effect on myelination (31). As hypomyelination is also a hallmark pathology in the GM2 gangliosidoses (32), it is possible that the therapeutic action of the KD could involve an effect on myelination. In summary, we suggest that restricted KDs can become a complimentary therapeutic strategy along with SRT therapies for managing Sandhoff Disease and possibly other lipid storage diseases where neuroinflammation is part of the pathology.

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