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# Role and Function of Dehydrogenases in CNS and Blood-Brain Barrier Pathophysiology

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Additional information is available at the end of the chapter

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## 1. Introduction

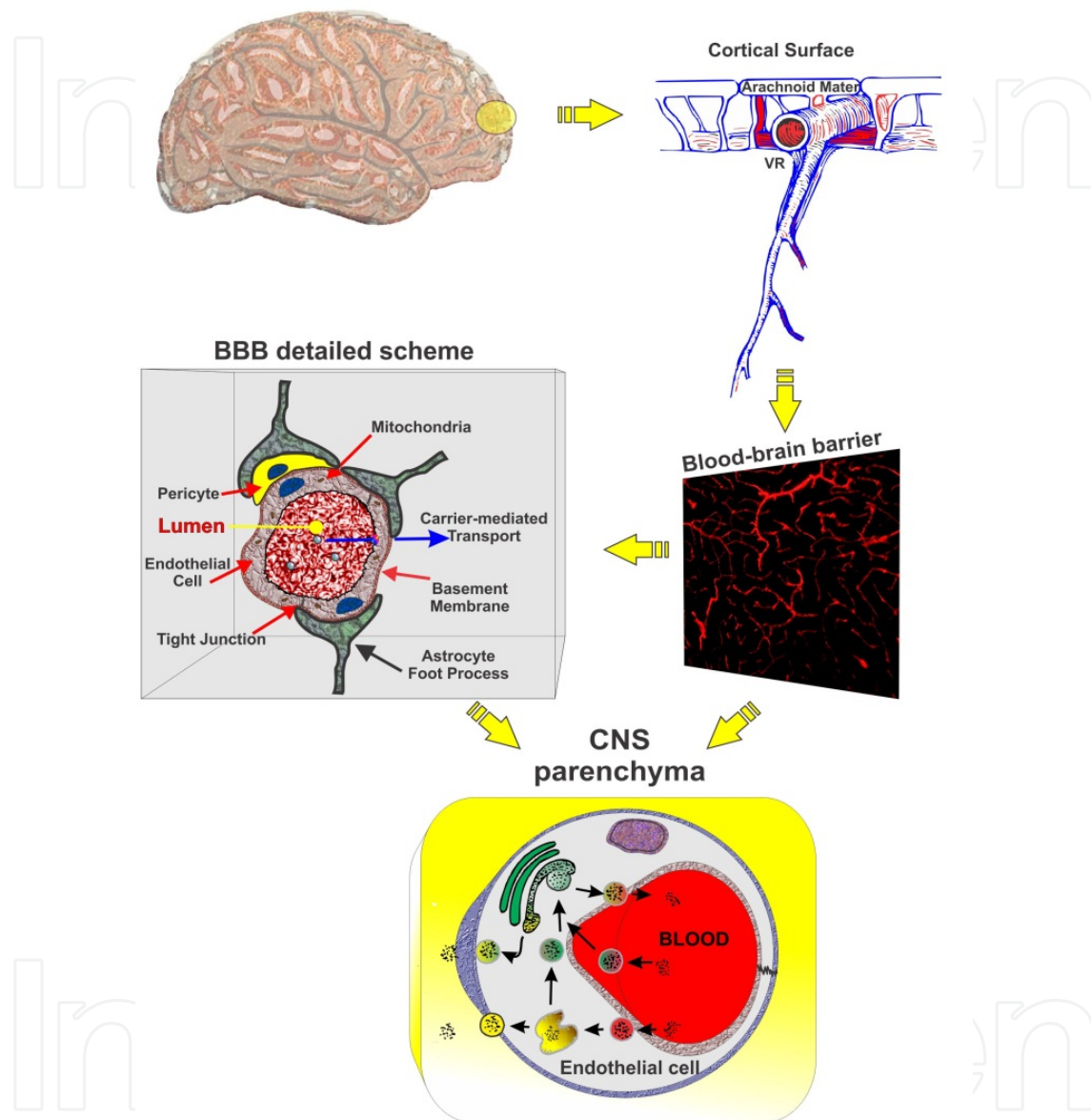
Dehydrogenase (DHO) is one of the most common types of enzyme that is crucial in oxidation reactions. This enzyme oxidizes its specific substrate by a redox reaction in which one or more hydrides ( $H^-$ ) are transferred to an electron acceptor. Apart from energetics and ATP formation, DHOs are associated with both catabolic and anabolic pathways linked to normal functioning and homeostasis. In this chapter, we will cover different aspects of the major DHOs that play a role in the regulation of brain and blood-brain barrier (BBB) physiology starting from their role in bioenergetic metabolism. Inborn errors in metabolism (IEM) due to genetic deficiency in a single specific DHO have strong neurological implications. We will be covering some examples of such IEMs in this chapter. Furthermore, aging processes can impair the function or activity of DHOs. Recent studies show convincing evidence associating altered DHO activity with the pathogenesis and progression of several neurological disorders such as Alzheimer's and Parkinson's disease. Whether they are contributors to the etiology of the disease or symptomatic manifestation of these complex neurological disorders is still debatable; however, this link between DHOs and neurological disorders cannot be overlooked and will be further discussed in this chapter. We will also cover neuronal signaling, neurotransmitter release and degradation emphasizing localized region-specific expression of some brain DHOs in these processes. It is not possible to cover the detailed cerebral physiology and function in this chapter; however to summarize we will discuss the different types of DHOs in central nervous system (CNS) and BBB physiology, their key enzymatic action, their function in crucial metabolic pathways, and thus how their altered activity or expression can be linked to the underlying pathogenesis of various brain disorders.

## 2. Structural and functional complexity of blood brain barrier (BBB) and cerebral physiology – A need for high energy

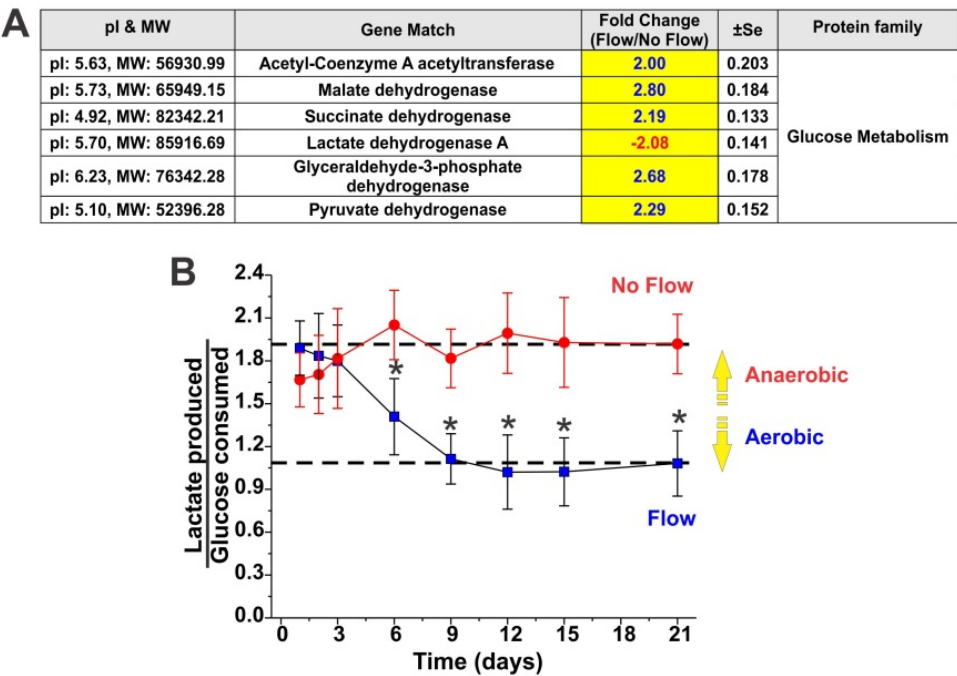
The BBB is a dynamic interface between the peripheral blood and the brain which controls the influx and efflux of substrates and metabolites necessary for normal neuronal function (see **Figure 1**). The BBB is crucial in protecting the brain from harmful substances both endogenous and exogenous in nature. Any alteration in normal BBB functions can play a central role in the pathogenesis and progression of broad variety of CNS disorders such as multiple sclerosis, Alzheimer's disease, neoplasia, hypertension, dementia, epilepsy, infection and trauma (1-4).

At the cellular level, the BBB consists of microvascular endothelial cells (ECs) lining the brain microvessels together with closely associated astrocytic end-feet processes and pericytes (5-8). These associated cells play a major role in EC differentiation and acquisition of morphological and functional characteristics unique to the BBB. At the cellular level, the brain microcapillary endothelium is characterized by the presence of tight junctions (TJ), lack of fenestrations, and minimal pinocytotic vesicles (9;10). In particular, TJs between the cerebral endothelial cells form a diffusion barrier, which selectively excludes most of the blood-borne substances from entering the brain, protecting it from systemic influences mediated by substances which are primarily polar in nature (such as electrolytes). Transport of nutrients (as well as other biologically important substances) from the peripheral circulation into brain parenchyma requires translocation through the capillary endothelium by specialized carrier-mediated transport systems. On the other hand, potentially harmful substances that are lipid soluble are discharged back into the cerebral circulation. This is mediated by specialized active efflux systems belonging to the ATP-binding cassette transporters (ABC-transporter) superfamily (such as P-glycoprotein (P-gp) and Multidrug resistance Protein (MRP)) (11;12). These efflux pumps rely heavily on adenosine triphosphate (ATP) as fuel source. Apart from these ATP dependent pumps, energy independent transporters such as organic ion carriers add to the complexity of BBB transport functions (13). Simultaneously, intake of essential nutrients such as glucose, amino acids, peptides, choline occurs through carrier mediated mechanisms (13-17). Topographic membrane localization of these transporters is indicative of the polarity of the endothelial functions and differentiation that sets apart the BBB endothelium from other vascular beds. The BBB endothelial cytoplasm is richly endowed with enzymes(18) including adenosine triphosphatase, acid and alkaline phosphatases,  $\text{Na}^+/\text{K}^+/\text{ATPase}$ , monoamine oxidase, cytochrome p450s and various dehydrogenases (19-22). The BBB ECs are also characterized by very high density of mitochondria denoting high metabolic activity (23) to support all the specialized cellular activities bestowed upon these highly specialized cells. In addition, previous work from our group has shown that blood flow can modulate the bioenergetic behavior of the BBB endothelial cells favoring the expression of the key metabolic enzyme pyruvate dehydrogenase (switch controller from anaerobic to aerobic pathway) (19). Contrarily, the RNA level of lactate dehydrogenase (switch controller from aerobic to anaerobic pathway) showed decreased expression. In parallel, TCA dehydrogenases such as

acotinase, isocitrate dehydrogenase, succinate dehydrogenase were upregulated. These results clearly emphasize how altered rheological conditions (e.g., hypoperfusion and ischemia) may impact the BBB bioenergetic metabolism and how the BBB is well equipped to respond to such changes (see **Figure 2**).



**Figure 1.** Schematic representation of the brain microvasculature in relation to the brain. Note how the blood vessels start branching in small capillaries while the pia disappears and the endothelium acquires the peculiar characteristics of a tight barrier that regulate the exchange of substances between blood and brain. TJ between adjacent endothelial cells form a diffusion barrier that selectively excludes most blood-borne and xenobiotic substances from entering the brain. In contrast to lipid soluble substances including alcohols, anesthetics and barbiturates, the BBB is highly impermeant to polar molecules or water soluble electrolytes. However, the passage of certain water soluble, but biologically important substances, such as D-glucose or phenylamine are regulated by a variety of specific carrier-mediated transport systems. By contrast, larger vessels (arterioles, small arteries and venous) differ from capillaries by the presence of smooth muscle cells in their walls and a less stringent vascular bed.



**Figure 2.** Effect of flow on BBB glucose metabolism. Comparative analysis of the expression level of key enzymes regulating the glycolytic and TCA pathways strongly supported the gene array data (Panel A). Note that the lactate production/glucose consumption ratio measured in the flow-exposed in vitro BBB modules was  $\approx 1$ . Complete anaerobic metabolism would produce 2 lactates/glucose (ratio = 2) thus, indicating that at least 50% of the glucose consumed underwent aerobic metabolism (Panel B).

The BBB’s ability in maintaining an optimal bioenergetics level at all time is thus crucial to meet the energy demand required by its multiple proprietary functions under normal as well as pathological conditions (e.g., cerebral ischemia). Energetic pathways (such as glycolysis and the tricarboxylic acid cycle -TCA cycle) work in an integrated yet independent manner in the BBB endothelium to respond to physiological (e.g., increased CNS demand in response to changes in neuronal activity) or pathological events that require a prompt BBB response.

The brain on the other hand possesses an incredibly more complex physiology than the BBB vasculature. It is the control center of neuronal as well as hormonal signaling. It is crucial for various functions such as homeostasis, behavior, perception and processing of information, motor control and memory formation. From a physiological stand point, brain functions depend on the ability of neurons to transmit and respond to electrochemical signals. This complex crosstalk is controlled by a wide variety of biochemical and metabolic processes which involve interactions between neurotransmitters and receptors that take place at the synapses. Crucial to this function is the necessity to sustain the bioenergetic demand required to maintain optimal neuronal activity (e.g., generation of action potentials, release of neurotransmitters, restoration of the membrane polarization following an action potential etc). In this respect, the BBB endothelium and glial cells play a major role in brain metabolism, by controlling the influx and distribution of nutrients (e.g., glucose and lactate shuttles as described later) as well as the chemical composition of the extracellular fluids surrounding the neurons.



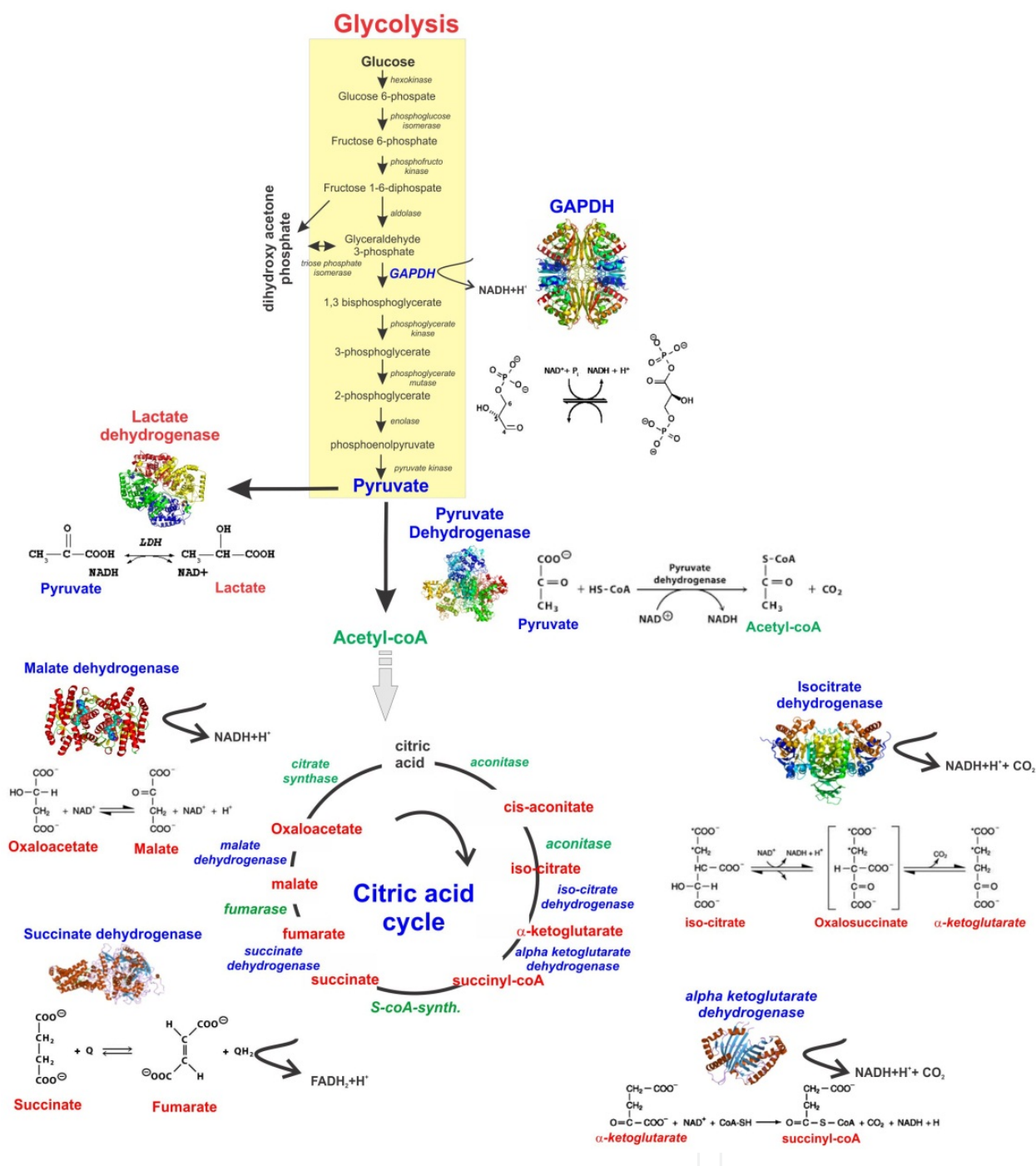
### 3. Pathways related to energy metabolism in BBB and brain: Lactate shuttle: NALS or ALNS?

Before we begin describing the role of DHOs in energy metabolism, it is imperative to understand the various metabolic pathways in the brain involved in energy production. Glucose is one of the primary fuel sources available to the brain.  $\approx 25\%$  of the total glucose intake is utilized by the brain despite accounting for  $\approx 2\%$  of the total body mass (24).

Glucose enters the glycolytic pathway to produce pyruvate along with net production of 2ATP and 2NADH (reduced form of nicotinamide adenine dinucleotide) (see **Figure 3**). Ten intermediate reactions occur until pyruvate is formed in the last step. ATP is first used up in the first part of glycolysis (until formation of glyceraldehyde phosphate-GAP) and is produced later during the second half. The total net ATP gain for each glycolytic cycle is 2 ATP molecules. Another important step of glycolysis involving glyceraldehyde phosphate dehydrogenase (GAPDH), is the conversion of GAP to 1,3-bisphosphate glycerate (1,3- BPG) along with NADH formation. Part of the 1,3-BPG thus formed can be further converted into 2,3-biphosphate glycerate (2,3-BPG) which can bind to hemoglobin enhancing its deoxygenation. The remaining 1,3-BPG undergoes further conversion along the glycolytic pathways and is finally converted into pyruvate.

Pyruvate thus formed can either enter the citric acid chain (or Krebs' cycle or Tricyclic acid cycle-TCA) or get converted to lactate which represents the end product of glycolysis. Pyruvate to lactate conversion is the last step of anaerobic form of respiration which occurs via lactate dehydrogenase and results in 2 molecules of ATP production.

Conversely, pyruvate can be further converted into acetyl co-A and then enter the TCA cycle. This intermediate reaction is crucial in linking glycolysis to the TCA. In this step, pyruvate dehydrogenase (PDH) decarboxylates pyruvate to its acetyl form along with addition of co-enzyme A. Acetyl co-A then combines with oxaloacetate (4C) in presence of water molecule to form citrate (6C). The citrate thus formed cycles through TCA forming oxaloacetate in the last step, which can re-enter the cycle reacting with a new acetyl co-A (see **Figure 3**). The various steps in the TCA cycle involve various oxidation reactions involving various dehydrogenases such as isocitrate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase. Each of these reactions lead to formation of NADH (except for FADH<sub>2</sub> i.e. reduced flavin adenine dinucleotide formation during conversion of succinate to fumarate). These reducing equivalents can then enter the electron transport chain and result in ATP formation or can be used by the cell to counteract the oxidative stress caused by reactive oxygen species (ROS) and free radicals. A complete cycling of two molecules of acetyl co-A (from a single molecule of glucose) results in the production of approximately 10 NADH and 2 FADH<sub>2</sub>. The complete metabolic conversion of a glucose molecule into water and CO<sub>2</sub> (glycolysis and TCA cycles combined) results in the approximate production of 38 ATP molecules (including ATP formed indirectly through NADH and FADH<sub>2</sub> which produce 3 and 2 ATP molecules respectively).



**Figure 3.** Enzymatic pathways of glycolysis and Krebs's cycle. Note the DHOs involved in the different metabolic pathways. (glycolysis and citric acid cycles).

It has been an age old concept that glucose is the major energy source to maintain brain functions and that lactate does not provide further metabolic use for the neuronal activities (25-28). However, it is now accepted that lactate can be produced in the brain under aerobic conditions, this is known as aerobic glycolysis. Aerobic glycolysis under normal conditions contributes to about 10% of the total energy production in the brain, which can increase under ischemic conditions(29). Lactate thus formed can then be shuttled between the

various cell types in the brain, be converted back into pyruvate and be fully reutilized through complete aerobic respiration. Although this concept of lactate shuttle was proposed long ago, it received lot of resistance from the scientific community especially from those who believed that glucose is the sole substrate in the brain (30;31). However now, it is getting accepted that lactose is also used as a main substrate for energy production under normal conditions (32-34). The direction of lactate flow between various cell types in the brain as well as its relative contribution with respect to glucose to the overall energy production is still debatable and not yet fully understood. As far as the lactate flow between the different cell types in the brain there are two current schools of thoughts. One supporting the Astrocyte-lactate-neuron shuttle (ALNS) (33;35-37) and the second advocating a Neuron-Lactate-Astrocyte-shuttle (NLAS) (38-40).

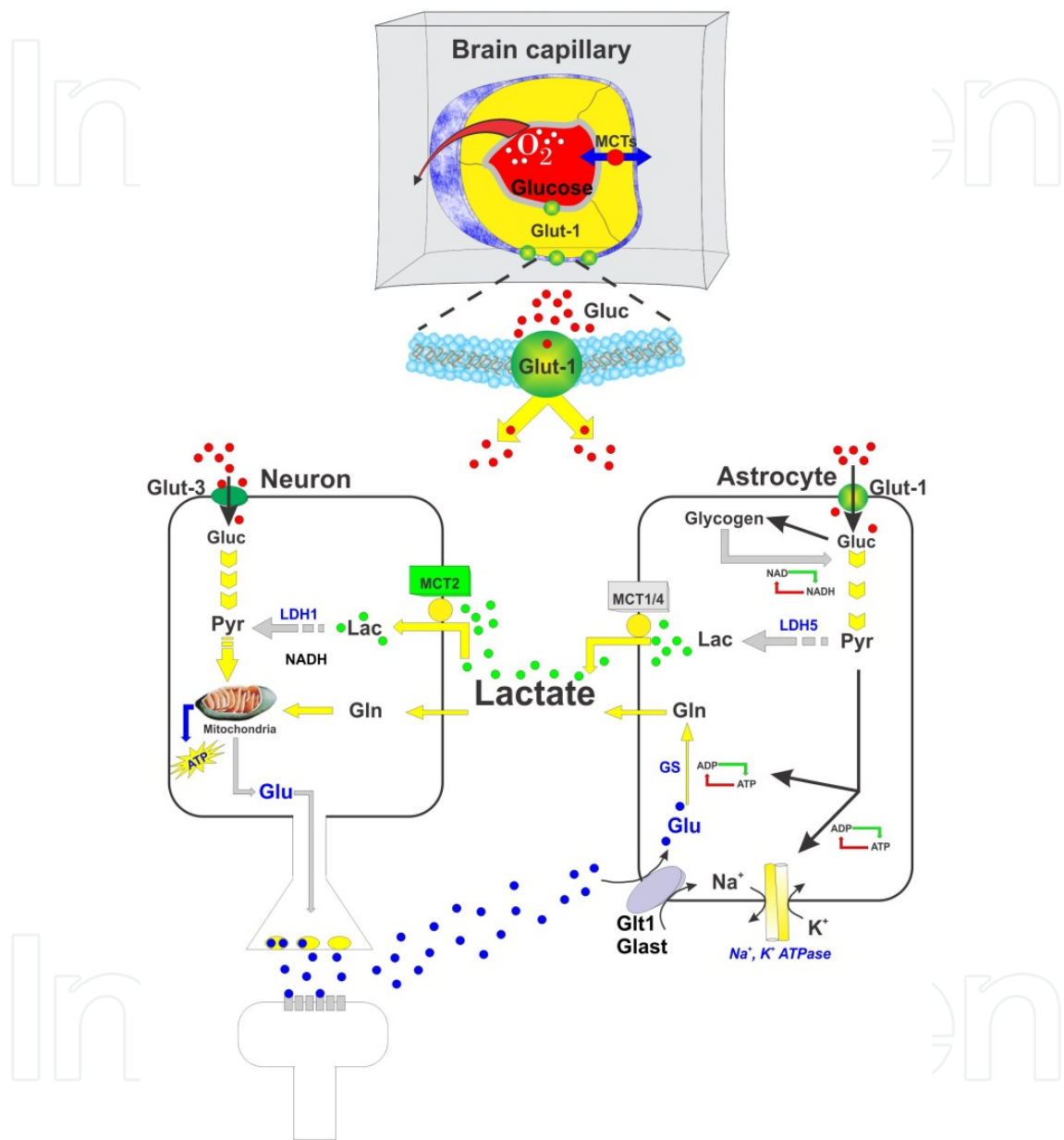
Classically, glucose is transported from the cerebral blood flow into the neurons or astrocytes via glucose transporters such as Glut-1 and Glut-3 respectively (see **Figure 4**). This glucose is then metabolized to produce ATP through complete aerobic respiration (glycolysis and citric acid pathways). In the astrocytes, part of the glucose also gets converted into glycogen, as an energy reserve to be used under critical conditions of low oxygen supply.

Astrocytes can withstand low oxygen tension for a longer period of time than neurons and have proven to be more resilient to hypoxic insults (7;41-43). Based on the concept of lactate shuttle, at the astrocytic level (under resting conditions), glucose can be converted to lactate. Lactate thus produced can shuttle first into the interstitial fluid (through monocarboxylate transporter –MCT-1 & 4). From there lactate can be influxed into neurons by the neuronal specific MCT-2 transporter and converted back into its pyruvate form ready to undertake complete aerobic metabolism (36;44;45). This route is known as the Astrocyte-lactate-neuron shuttle (ALNS shuttle).

Continuous glutamatergic activation of neurons results in a more exhaustive energy expenditure. As pyruvate utilization during the TCA cycle increases and its cytoplasmic levels decrease correspondingly, the condition becomes favorable for increasing both glucose and lactate utilization. By the late phase of activation, glutamate released is taken up by the astrocytes for recycling. Aerobic glycolysis is enhanced in the astrocytes with increased lactate production. The lactate thus formed helps sustain the energy demands of astrocytes as well as replenishing the neurons. During intense and prolonged neuronal stimulation which may occur under certain conditions, energy replenishment becomes crucially important. This is because the continuous glutamate reuptake by the  $\text{Na}^+$ , glutamate co-transporter in astrocytes (GLutamate ASpartate Transporter – GLAST; glutamate transporter 1 –GLT1) must be paired with an equivalent intense activity of the  $\text{Na}^+$ ,  $\text{K}^+$ ATPase to efflux the  $\text{Na}^+$  back in the extracellular space thus continuing the cycle. When the extracellular glucose levels become insufficient to sustain this level of activity then glycogen stored in the astrocyte is mobilized to provide the extra glycosyl units necessary to support the cellular activity. Thus sustained activation of the neurons results in conversion



of the stored energy substrate glycogen to glucose and further lactate production for shuttling to the neurons. This concept of ALNS shuttle during activation has received opposition and few groups suggest that lactate is primarily produced by the neurons and is then transported from the neurons to the astrocytes (38;40).



**Figure 4.** Schematic of neuroenergetics pathways: The astrocyte – neuron lactate shuttle.

In summary whether lactate flux moves from astrocytes to neurons or vice-versa the important point is that glucose is not the sole substrate utilized in the brain. Lactate plays an equally important role especially during activation, neurotransmission and pathological conditions such as under ischemic insults. This further emphasizes the still dismissed importance of lactate dehydrogenase which represents the key switch in the metabolic pathway of glucose in the generation of lactate.

In summary at the microcapillary level, the BBB acts as a functional interface which is charged with the critical task to fuel the brain with energy sources. Whether this is glucose or glucose-derived lactate the BBB is the main fuel distribution system to the brain and a safe for energy storage to which the brain can avail when normal fuel supplies are short.

## 4. IEM

Mutation in single gene leads to enzyme deficiency that results in type of genetic disorders termed as inborn error in metabolism (IEM) (46-50). IEM related deficiencies are generally autosomal recessive or X-linked. IEM linked enzyme deficiencies may be directly related to metabolic pathways of energy metabolism, purines/pyrimidine synthesis or degradation, amino acid synthesis, fatty acid oxidation, etc. Metabolic errors lead to accumulation of toxic or absence of essential products in the brain with neurological implications such as ataxia-motor control, encephalopathy, mental deficits, learning disabilities and mental retardation with structural anomalies. Some examples of DHOs involved include the pyruvate dehydrogenase complex.

### 4.1. Pyruvate dehydrogenase (PDH) deficiency

Pyruvate dehydrogenase is a multi-enzyme complex which catalyzes the conversion of pyruvate (the end product of glycolysis) into acetyl-coA- a substrate that can enter citric acid cycle (for production of ATP and energy equivalents). PDH is a six subunit complex composed of E1-pyruvate dehydrogenase, E2-dihydrolipoyl transacetylase and E3-dihydrolipoyl dehydrogenase, E3BP- E3 binding protein and two regulatory subunits - pyruvate dehydrogenase kinase and pyruvate dehydrogenase phosphatase. Although several mutations in the PDH complex deficiency (such as point mutations, deletions, duplications) have been reported so far; deficiency in PDH E1-alpha subunit (abbreviated as PDHA1) is the most common type (51-53). PDH deficiencies due to mutations in other subunits of the PDH complex are comparatively rare. All mutations leading to PDH deficiency are X linked except, the one in regulatory units, which are autosomally recessive (54-57). Since PDH results in acetyl co-A formation, the most common clinical manifestation of PDH deficiency is severe lactic acidosis. Defects in energy metabolism can cause neurological deficits such as mental retardation, developmental delay as well as psychomotor retardation. Hypertonia/hypotonia, ataxia, motor dysfunction like spasticity are the more common symptoms observed (58). Structural anomalies (such as microcephaly, facial dysmorphism) and epilepsy (focal or generalized seizures- both have been reported) may develop *in utero*. Optic atrophy, nystagmus and strabismus are observed at ocular level whereas peripheral neuropathies such as in nerve conduction have also been reported.

Based on clinical case studies, PDH deficiencies have been classified into four typical neurological patterns (58-60):

- i. Neonatal encephalopathic pattern with facial dimorphic features and cerebral developmental defects, prenatal brain lesions, affecting the females
- ii. Leigh syndrome like presentation with symmetric necrotic lesions of basal ganglia, more common in males

- iii. A chronic relapsing ataxia with prolonged survival
- iv. Static encephalopathy, cerebral palsy like motor deficits associated with paroxysmal dystonia.

Alkali (such as sodium bicarbonate) administration to neutralize severe lactic acidosis and provide immediate temporary relief has been reported to treat acute episodes of severe acidosis. Chronic treatment strategies for PDH deficiency on the other hand, include incorporation of ketogenic diet consisting of high fat, low carbohydrate and low protein. High doses of thiamine can be beneficial in treating thiamine responsive PDH deficiency. Dichloroacetate (DCA) can reduce the inhibition of PDHc phosphorylation and thus can be used to treat severe lactic acidosis to some extent.

#### **4.2. Branched Chain Alpha Ketoacid dehydrogenase (BCKDH) complex Deficiency – Maple syrup urine disease (MSUD)**

Another important dehydrogenase deficiency leading to an inborn error in metabolism is that in the branched chain alpha ketoacid dehydrogenase (BCKDH) complex (61;62). This complex is similar to PDH complex, and autosomal recessive mutations in the different subunits of the complex have been reported for this disease. In this disorder, accumulation of branched chain amino acids (BCAAs like isoleucine, leucine and valine) and branched chain alpha ketoacids (BCKAs) (with maple syrup odor to the urine) is observed along with neurological deficits and developmental disorders. Based on the characteristic manifestations and level of neurological complications, this disease is classified into the following five forms:

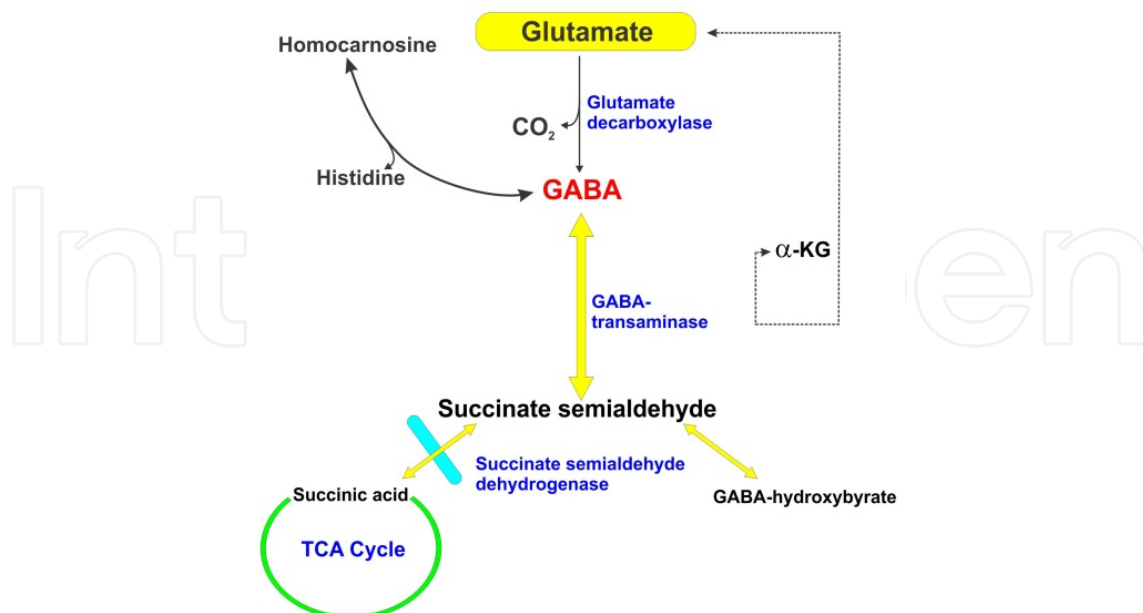
- i. Classical MSUD: This is the most common type of MSUD with early symptoms in neonates. Neonates born normal, within 3-7 days of birth show symptoms such as lethargy, weight loss, metabolic dearrangement, encephalopathy with hypotonia and hypertonia. It is characterized by seizures, coma and even death if not treated.
- ii. Intermediate MSUD: This type shows mild but persistent ketoacidosis and developmental delay with 3-30% less dehydrogenase activity.
- iii. Intermittent MSUD: Episodic ataxia, semicoma, elevated BCAAs and BCKAs occur after episodes of infection or acute illness. Cognitive functions may be affected only in case of repeated episodes of acute illness.
- iv. Thiamine responsive MSUD: Mutation in E2 protein of the BCKDH complex results in reduced affinity of cofactor thiamine pyrophosphate (TPP), thus this form of disorder can be treated with thiamine substitution. Reduced activity of the complex results in hyperaminoacidemia.
- v. E3-deficient MSUD: Combined deficiency of E3 subunit (common component of all three mitochondrial multi enzyme complexes - BCKDH, pyruvate dehydrogenase complex and alpha ketoglutarate dehydrogenase) results in elevated lactate, pyruvate and alpha ketoglutarate along with BCAAs and BCKAs.

Thus, all these forms of IEM are caused due to varying degree of deficiency in the enzyme activity leading to varying levels of neurological complication. Treatment is initiated by high calories leucine free diet rich with BCAA-free formulas and an optimum

supplementation of isoleucine and valine. Hemodialysis or hemofiltration may be used to remove deposited BCAAs and BCKAs from the body. During acute MSUD, brain edema and hyponatremia can also occur but can be immediately treated by administration of mannitol or diuretic drugs. In adolescents and adults, it can lead to depression, anxiety. However, the burden of these pathologies can be decreased by treatment with appropriate standard drugs such as psychostimulants or antianxiety drugs. Although no direct drug is used to treat MSUD, recent studies have shown the role of phenylbutyrate in increasing BCKDH activity, reducing levels of BCAAs and BCKAs and causing relief in MSUD patients (63). However, careful monitoring and routine biochemical testing is key in appropriate treatment in MSUD affected patients.

#### 4.3. Succinic semialdehyde dehydrogenase (SSD) deficiency

SSD deficiency is an autosomal recessive disorder of  $\gamma$ -hydroxybutyric acid (GABA) metabolism. In human brain, GABA is the most important inhibitory neurotransmitter. Oxidative conversion of succinate semialdehyde to succinic acid is impaired in this deficiency. This leads to production of  $\gamma$ -hydroxybutyrate (GHB) (see **Figure 5**). ALDH5A1 is the only gene associated with this deficiency. Mild developmental delay, psychomotor retardation, hypotonia, ataxia are observed along with extrapyramidal symptoms such as dystonia, choreoathetosis and myoclonus. More than 50% of affected individuals develop seizures (64-67). Neuroimaging screening generally reveals hyper intensities in globus pallidus, sub cortical white matter, cerebellar dentate nucleus and brain stem (68). Accumulation of  $\gamma$ -amino butyric acid (GABA) and GHB are considered positive indicators for this disease which can be confirmed by testing of SSD enzyme activity in leucocytes.



**Figure 5.** SSD deficiency: In the absence of SSD, transamination of  $\gamma$ -aminobutyric acid (GABA) to succinic semialdehyde is followed by reduction to 4-hydroxybutyric acid ( $\gamma$ -hydroxybutyrate [GHB]). SSADH deficiency leads to significant accumulation of GHB and GABA.

Current therapies are mostly symptomatic, directed at seizure treatment and amelioration of neurobehavioral symptoms. Antiepileptic drugs such as carbamazepine and anti-anxiety drugs may be administered in conjunction with physical and occupational therapy. Early attempts to use Vigabtrin (GABA transaminase inhibitor) did not meet the therapeutic expectations (69).

#### 4.4. IEM related to fatty acid oxidation

Fatty acids are a major source of energy in heart as well as muscle. Fatty acid oxidation (FAO) is a series of four reactions occurring in mitochondria. The first step is catalyzed by four straight chain acyl coA dehydrogenases such as:

- Short chain acyl coA dehydrogenase (C4-C6 fatty acyl coAs)
- Medium Chain acyl coA dehydrogenase (C6-C10 fatty acyl coAs)
- Long Chain acyl coA dehydrogenase (C10-C14 fatty acyl coAs)
- Very Long Chain acyl coA dehydrogenase (C14-C20 fatty acyl coAs)

Medium chain acyl-coA dehydrogenase (MCAD) deficiency is the most common fatty acid oxidation-related disorder (1:10000 to 1:30000 in US) which is inherited in an autosomal recessive fashion (70-72). MCAD is an enzyme that catalyzes breakdown of fatty acids for energy production during long periods of prolonged fasting. Accumulation of octanoylcarnitine with Reye-like syndrome is typical clinical manifestation of this disorder (73;74). Children can exhibit severe hypoglycemia in mild illnesses. It can also lead to sudden infant like death syndrome (75). Symptoms may appear from 2 days to 6.5 years of age, however the patient can also remain asymptomatic for long time. When left undiagnosed MCAD deficiency has a mortality of 20% and 10-15% are severely handicapped. A case study of diagnosis as late as 30 years of age is reported in literature. The 30 year old man exhibited rhabdomyolysis, muscle weakness, acute encephalopathy after exertion in cold and fasting. Urine detection of carnitine led to the diagnosis of MCAD deficiency. Point mutation at position 985 in the coding region of MCAD gene was detected. 449\_452 deletion mutation is also studied. During acute episodes, symptomatic relief to overcome hypoglycemia cerebral edema, seizures or metabolic acidosis is the main line of treatment. Avoiding long periods of fasting is the best preventive measure that can be employed in cases with MCAD deficiency.

Short chain acyl coenzyme A dehydrogenase deficiency (SCAD) is another autosomal recessive disorder in mitochondrial fatty acid oxidation. It is characterized by increased C4-carnitine in plasma and ethylmalonic acid in urine. Clinical symptoms which appear early in life include developmental delay, hypotonia, epilepsy and behavioral disorders along with hypoglycemia and myopathy (76;77). Unlike MCAD deficiency, if neonatally screened and followed up it is found to remain asymptomatic, thus the clinical disease outcome of SCAD deficiency is questionable. Thus, need for treatment is not clear. Avoidance of fasting for longer hours with age appropriate diet is the only recommendation for prevention of primary manifestation. Annual checkup for growth, development is generally suggested.



## 5. Aging: The role of dehydrogenases in metabolic and mitochondrial dysfunction

Aging or growing old is defined as a time related loss and decline in certain morphological, anatomical and functional features of body in comparison to its previous state. Beginning as a maturation process from childhood to young adulthood, it assumes the characteristic of decline through middle and late ages. Accumulation of molecular, cellular, or organ level damage leads to higher vulnerability of disease and eventually death. There have been numerous theories and hypothesis for causes of aging but it is still under investigation and discussion. "The Free Radical Theory of aging (ROS generation), shortening of telomerase, DNA methylation and epigenetics are few main ones. Important to us is the "The Free Radical Theory of aging since it is closely associated with mitochondria, and linked DHOs (78-81).

Broadly, both genetic as well as external environmental factors can be responsible for promoting the age associated decline in functionalities (82). Oxidative stress and dietary restrictions can influence the genes externally. "Oxygen derived species", "Reactive nitrogen species" and "Reactive aldehydic species" can cause changes at cellular level ensuing damage to our natural defense mechanisms affecting repair and elimination processes in the body. In totality, irregularities in function, oxidative changes and the piled up cellular damages can lead to homoeostatic imbalance which finally result in aging as well as age-related diseases. "The Free Radical Theory of aging" suggests generation of superoxide radical, hydrogen peroxide and hydroxyl radical as a side reaction to the electron transport chain at mitochondrial membrane (83). These free radicals can cause enzyme inactivation to different extent with different mechanisms (see **Figure 6**). Studies show that mitochondrial enzymes are resistant to hydrogen peroxide free radical but are fairly affected by hydroxyl free radical. On the other hand oxygen free radical by itself can cause significant oxidative damage with respect to inactivation of mitochondrial enzymes like NADH dehydrogenase, succinate dehydrogenase, NADH oxidase, succinate oxidase and ATPase 2.

Link between aging and various dehydrogenase enzymes is based on the energy demand of our body which involves the participation of different dehydrogenases for production of ATP at cellular level (as elaborated in the earlier section of energy metabolism). Several dehydrogenases involved in energy metabolism can exhibit altered activity or complete inactivation with aging. This can result in hampering energy production as well as accumulation of toxic metabolites in the body.

## 6. Metabolic dysfunction and its link to Alzheimer's disease: The role of dehydrogenases

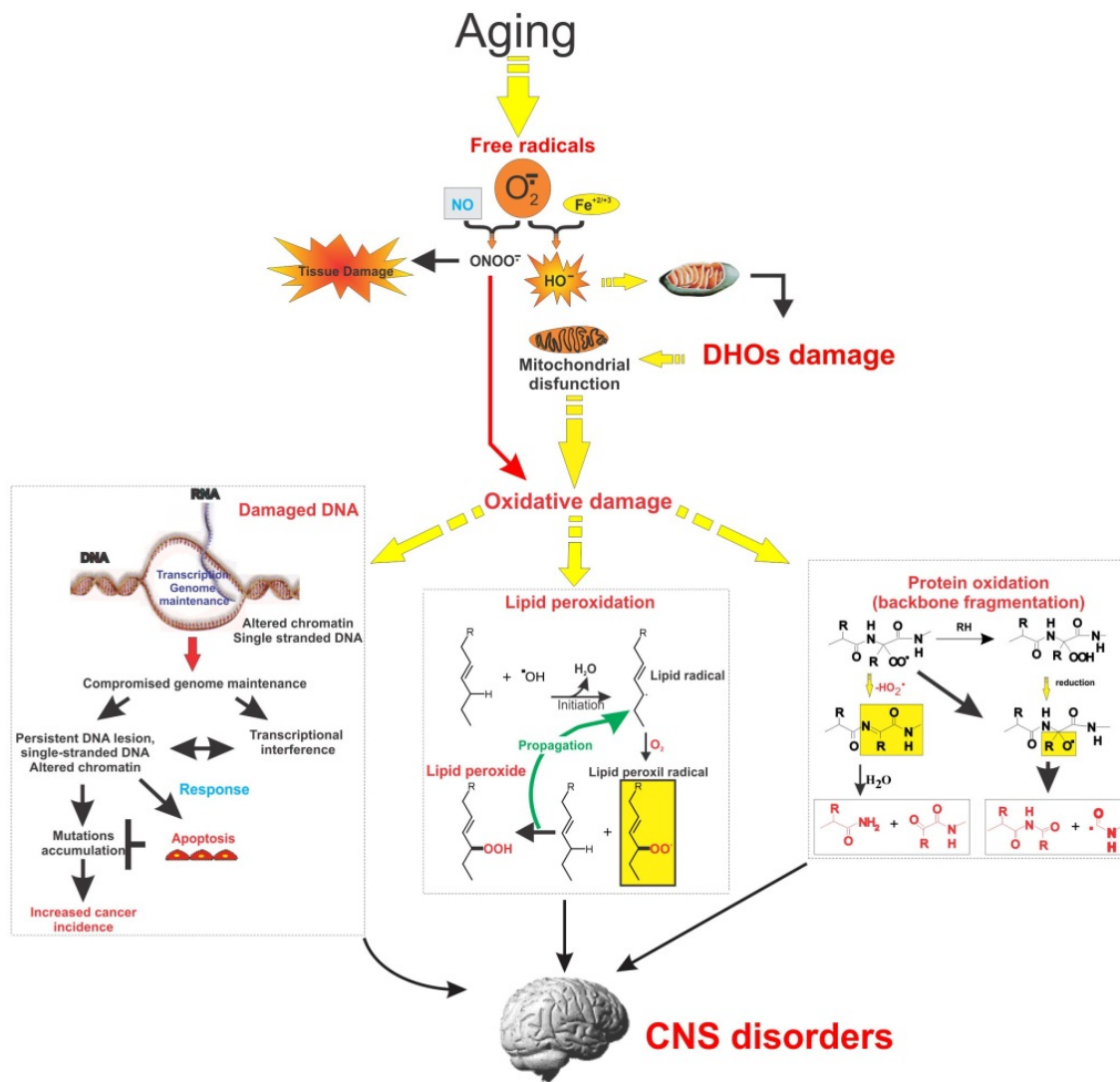
Alzheimer's disease is the most common form of dementia characterized by loss of memory, cognitive decline and change in perception and behavior. Pathological hallmarks include accumulation of amyloid beta protein ( $A\beta$ ) and resulting plaque formation (a cleavage protein of amyloid precursor protein-APP) and formation of neurofibrillary tangles (due to hyper phosphorylation of microtubule associated protein of neurons in the brain). Genetic

mutations in APP protein or ApoE protein (a protein linked to lipid and cholesterol in the body) as well as Down's syndrome are some examples of genetic predispositions that increase the propensity to develop Alzheimer's disease. However, the etiology of the disease is still questionable. Both vascular as well as metabolic dysfunction have been accredited as major factors prodromic to the pathogenesis and progression of Alzheimer's disease (84). Vascular dysfunction includes reduction in cerebral blood flow, reduced glucose uptake, and reduced amyloid beta clearance with cerebral amyloid angiopathy. The patients with atherosclerosis have shown to have an increased risk to develop AD. Vascular structural anomalies in cerebral vessels (like increased pinocytic activity as well as swelling) have been seen in early AD further signifying the importance of vascular dysfunction in AD pathogenesis. Defective glucose utilization has been observed earlier than reduced cerebral blood flow (CBF), indicating the role of glucose metabolism in AD development. What is of primary interest to us is that it is slowly becoming well accepted that metabolic dysfunction, related oxidative stress and mitochondrial deficits can precede AD development (84-86). In one such recent study, triple transgenic mice 3xTg-AD were developed (having mutations in human APP<sub>SWE</sub>, TauP301L, PS1<sub>M146V</sub> genes linked with AD) along with their respective controls. Decreased mitochondrial respiration was observed along with reduced PDH activity. High levels of oxidative stress (via measurement of hydrogen peroxide production and related lipid peroxidation), high A $\beta$  levels with high levels of A $\beta$  binding alcohol dehydrogenase (ABAD) was observed. Decreased respiration was also observed in embryonic neurons, which continued till senescence leading to AD pathogenesis. Thus these studies clearly emphasized how mitochondrial dysfunction and resulting metabolic respiration preceded AD development. Defective enzyme function (of dehydrogenases) in pathways of energy metabolism such as glycolysis, tricarboxylic acid pathway and electron transport chain have been well studied in AD progression. Defective PDH, KGDHC, cytochrome oxidase along with reduced activity of hexokinase, phosphofructokinase are the major enzymes reported in AD so far (87). In the paragraph below we will further discuss some of these DHOs and their mechanism of metabolic dysfunction in AD. We will also look at other DHOs apart from those directly involved in the bioenergetics which have an important role in pathogenesis of AD.

One such DHO as mentioned earlier is A $\beta$  binding alcohol dehydrogenase (ABAD). ABAD is a short-chain alcohol dehydrogenase which is also called type II hydroacylcoA dehydrogenase, 17 $\beta$ -hydrosteroid dehydrogenase type 10 and 2-methyl-3-hydroxybutyrylcoA dehydrogenase. ABAD acts on various substrates such as branched chain fatty acids, alcohols, amino acid catabolites and steroids. In the brain, it is primarily localized in mitochondria of neurons. Previous studies have shown high ABAD expression in the temporal lobe and hippocampus of AD affected patients (88;89).

Animals with transgenic (Tg) APP mice have also demonstrated higher expression of ABAD (88). As the name suggests, ABAD directly binds to A $\beta$  protein which is highly expressed in Alzheimer's patients. These links suggest role of ABAD dehydrogenase in the pathophysiology of Alzheimer's disease. Another crucial factor in causing AD as discussed earlier is mitochondrial dysfunction, related oxidative stress and hypometabolism. In recent

studies, it was hypothesized that ABAD can act as a crucial link between increased  $A\beta$  production and mitochondrial dysfunction in Alzheimer's disease progression (90-92). To test this hypothesis, double transgenic mice with increased levels of ABAD and  $A\beta$  were developed (along with Tg mAP, Tg ABAD, and not Tg littermate controls)(93). Neuron cultures derived from these Tg mice showed increased ROS, oxidative stress and relative decrease in ATP production. Further studies indicated defective activity of mitochondrial Complex IV as the source of the ROS species, also such effect was not observed in single Tg mice with increased ABAD alone (suggesting  $A\beta$  acting as a crucial element linking the two). Lactate dehydrogenase (LDH) was higher in the Tg mAP/ABAD mice as compared to other groups suggesting the reversal to lactate metabolism. Cell apoptosis via caspase 3 activity was observed in *in vitro* studies. Data from the Tg mice also suggested reduced ATP production at 9 months of age along with reduced Complex IV activity. Overall, ABAD acts a crucial enzyme that can lead to mitochondrial dysfunction and disease progression in AD.



**Figure 6.** : Correlation between aging free radicals, DHOs and the onset of CNS disorders.

In another study, ABAD-decoy peptide (ABAD-DP) was introduced in the Tg mAPP mice, which prevented the interaction of ABAD with A $\beta$ . As expected, reduction in ABAD-A $\beta$  complex formation accompanied with attenuated oxidative stress, increased oxygen consumption, increased activity of enzymes associated with mitochondrial respiratory chain, improvement in energy metabolism, and increased spatial memory (89). Thus based on these studies, inhibitors of ABAD-A $\beta$  hold promise as potential targets for the treatment of AD.

Another dehydrogenase that is implicated in AD progression is aldehyde dehydrogenase (ALDH) (89;94). Aldehyde dehydrogenase is observed as a key enzyme in the brain involved in metabolism and degradation of biogenic aldehydes, monoamine neurotransmitters such as norepinephrine, dopamine, diamines and GABA. Recent studies have also shown that patients with Down's syndrome have reduced activity of ALDH enzyme (95). Two dimensional analysis of proteins extracted from brain samples of nine aged patients with Down's syndrome and nine controls showed that ALDH was down regulated in the patients with Down's syndrome. This resulted in accumulation of aldehydes and further formation of tangles and plaques as observed in aged patients with Down's syndrome.

Oxidative stress and generation of ROS species has been implicated in Alzheimer's disease as elaborated earlier. These oxygen species modify proteins, nucleic acids as well as lead to lipid peroxidation. Lipid peroxidation produces toxic aldehydes such as 4-hydroxy-2-nonenal (HNE) in several disorders such as Alzheimer's as well as Parkinson's disease. In the brain, normally ALDH2- an isoform of aldehyde dehydrogenase oxidizes and degrades end product of lipid peroxidation such as HNE. The role of ALDH in oxidative stress and age dependent memory loss and decline in cognitive function was studied using a transgenic mouse model with defective ALDH2 (96). A dominant negative form of ALDH2 mice was produced and its effect on the metabolic pathways as well as accumulation of toxic products was tested. As expected HNE accumulation was observed in such transgenic mice compared to controls. Further testing of cognitive capability was performed using object recognition and water maze test. Decreased cognitive function in the transgenic mice was observed along with accumulation of tau phosphorylation (a typical pathological sign of Alzheimer's disease).

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## **7. Metabolic dysfunction and its link to Parkinson's disease (PD): The role of dehydrogenases**

PD is a neurological disorder characterized by typical motor features such as tremor bradykinesia, rigidity, slowness of movement and postural instability. Reduction in number of dopaminergic (DA) neurons located in substantia nigra pars compacta is the pathological



cause of PD. It is also characterized by accumulation of  $\alpha$ -synuclein into inclusions called Lewy bodies. 60% of DA neurons are dead and 70% responsiveness of DA is lost. Mostly PD is idiopathic, however specific genetic mutations have shown to increase the risk to develop PD. Mutations in genes such as  $\alpha$ -synuclein, Parkin, PINK1 have been reported so far. After diagnosis of PD based on its classical symptoms and neuroimaging, treatment is usually done using levodopa (L-DOPA). L-DOPA is converted to dopamine in the brain and can temporarily alleviate the motor symptoms. Dopamine receptor agonists as well as selective monoamine oxidase-B (MAO-B) inhibitors are also administered along with L-DOPA (97;98). Treatment thus helps to partially reduce the symptoms of PD, since the actual underlying cause of this disease is still unknown. Altered enzyme activity and mitochondrial dysfunction has been linked to PD as well.

Aldehyde dehydrogenase plays an important role in detoxifying aldehydes in brain. Reduced expression of isoforms of ALDH such as ALDH1A1 and ALDH2 is reported in PD patients. In addition impaired Complex I activity is documented in PD which can reduce the availability of NAD<sup>+</sup> cofactor required by ALDHs to remove toxic biogenic aldehydes. Thus decreased ALDH function could be the underlying factor preceding the development of PD. Using transgenic mice null for both ALDH1A1 and ALDH2, the risk to develop PD was tested (99). Such mice exhibited deficits in motor performance typical of PD. Loss of DA with increased accumulation of biogenic aldehydes such as HNE was observed. L-DOPA administration alleviated the motor deficits suggesting a role of ALDHs in the pathophysiology of PD.

Another DHO implicated in PD is glutamate dehydrogenase (GDH). GDH is a key enzyme involved in interconversion of glutamate to alpha-ketoglutarate and ammonia using NADP(H) and NAD(H) as co factors. It plays an important role in homeostasis by interconnecting amino acid and carbohydrate metabolism pathways. Present in two isoforms in humans, the GDH isoform 2 (hGDH2) is overexpressed in the brain astrocytes and the sertoli cells in testis. ADP levels act as positive regulators for this enzyme and unlike the other isoform it is not inhibited by GTP. Important in recycling glutamate in the brain astrocytes, this enzyme works in concert with glutamine synthetase (GS) providing ammonia as well as ATP for GS activity. Two parallel studies have shown that increased levels of glutamate prepones the onset of the disease by 6 to 13 years (100). Hemizygous individuals with a rare variation in hGDH2 (substitution of Ala for Ser445) was detected in these individuals. GDH deficiencies have also been linked to the onset of epilepsy. All together these results highlight the role of hGDH2 in the maintenance of brain homeostasis.

ABAD associated with Alzheimer's disease has also shown to play some role in PD disease. ABAD expression is seen to be downregulated in PD patients (101). In mouse models of PD generated by administration of neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) ABAD expression is significantly reduced. By contrast, overexpression of ABAD in transgenic mice is shown to attenuate MPTP-induced dopaminergic neurodegeneration. This strongly suggests that ABAD may contribute to the fate of DA neurons during the onset of PD.



## 8. Conclusion

The brain as well as the BBB have complex structural and functional physiology which demands a continuous supply of high energy. Bioenergetic pathways in the brain utilize multiple pathways (such as glycolytic metabolism, TCA cycle etc) to ensure that the energy requirements of the different cell types in the brain are fulfilled at all time. The BBB acts as a critical interface to buffer and influx energy substrates into the brain. Shuttling of multiple substrates such as glucose, lactose as well as glycogen derived lactate/glucose commonly occurs between the neurons and the astrocytes. Various DHOs are a critical part of these bioenergetic pathways and occurrence of DHO defect can lead to inborn errors in the metabolism followed by strong neurological complications. PDH is an important IEM which is directly linked to bioenergetic pathways such as TCA cycle and aerobic respiration. Apart from energy metabolism, BCKDH and SSD are IEMs that correlate to other pathways in the brain such as amino acid metabolism and neurotransmitter degradation. DHOs (such as ALDHs) also play an important role to further degrade the biogenic aldehydes derived from the degradation pathways of neurotransmitters such as for epinephrine, norepinephrine and GABA which are commonly synthesized in the brain. Furthermore, DHOs play an important role in oxidation of fatty acids as an energy supply. Although this does not occur in the brain, IEMs affecting these dehydrogenases have shown to correlate with at least one reported neurological complication (such as Reye-like syndrome).

Aging naturally promotes alterations and/or reduction in DHOs' activity which can alter mitochondrial functions leading to hypometabolism other metabolic dysfunction. This can ultimately facilitate the onset and progression of various neurological disorders such as Alzheimer's disease and PD. Specifically, altered expression/function of ABAD and ALDH2 have been associated with the pathogenesis of Alzheimer's disease whereas alteration of ALDH1A1, ALDH2, GDH2 and ABAD have been linked to PD.

In summary, DHOs play a critical role in supporting neuronal and BBB functions. They constitute an integral part of various metabolic pathways in the brain associated with energy metabolism, as well as synthesis and degradation of neurotransmitters. Their optimal functioning facilitates neuronal signaling and homeostasis. Inborn as well as acquired defects in DHOs have been shown to correlate with various CNS and BBB pathophysiologies.

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