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Functional Magnetic Resonance Spectroscopy of Lactate in Alzheimer Disease: A Comprehensive Review of Alzheimer Disease Pathology and the Role of Lactate

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Abstract Functional 1H magnetic resonance spectroscopy (fMRS) is a derivative of dynamic MRS imaging. This modality links physiologic metabolic responses with available activity and measures absolute or relative concentrations of various metabolites. According to clinical evidence, the mitochondrial glycolysis pathway is disrupted in many nervous system disorders, especially Alzheimer disease, resulting in the activation of anaerobic glycolysis and an increased rate of lactate production. Our study evaluates fMRS with J-editing as a cutting-edge technique to detect lactate in Alzheimer disease. In this modality, functional activation is highlighted by signal subtractions of lipids and macromolecules, which yields a much higher signal-to-noise ratio and enables better detection of trace levels of lactate compared with other modalities. However, until now, clinical evidence is not conclusive regarding the widespread use of this diagnostic method. The complex machinery of cellular and noncellular modulators in lactate metabolism has obscured the potential roles fMRS imaging can have in dementia diagnosis. Recent developments in MRI imaging such as the advent of 7 Tesla machines and new image reconstruction methods, coupled with a renewed interest in the molecular and cellular basis of Alzheimer disease, have reinvigorated the drive to establish new clinical options for the early detection of Alzheimer disease. Based on the latter, lactate has the potential to be investigated as a novel diagnostic and prognostic marker for Alzheimer disease.

Keywords: aerobic glycolysis, Alzheimer disease, functional proton magnetic resonance spectroscopy, lactate, metabolomics, neuroimaging

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Functional 1H magnetic resonance spectroscopy (fMRS) is a derivative of dynamic MRS imaging. This modality links metabolic response with functional activity, showing spectra of resonances that measure absolute or relative concentrations of metabolites.[1] When a specific functional area of the brain becomes engaged in a task, fMRS can identify the neuronal activity by measuring and recording metabolite alterations, which is a necessary component of effective neuronal firing.[2]

Alzheimer disease (AD) is an irreversible neurodegenerative brain disorder and is the primary cause of dementia worldwide. The central aspect of this disease is cognitive impairment, ranging from memory loss in early phases to distortion of executive functioning in later phases.[3,4] Identifying metabolite changes in biochemical pathways involving different diseases will disclose how these alterations affect interconnected reactions. This will make it possible to follow changes as the disease progresses.[5,6]

Evidence indicates that when glycolysis and mitochondrial metabolic pathways are disrupted in neurons or when the necessary substances for aerobic metabolism are not available, the anaerobic glycolysis cycle will be activated, generating lactate as the final product of the anaerobic pathway. Importantly, in pathological conditions and while the Krebs cycle is deficient, increased lactate levels are identified due to the reduction of oxygen (hypoxia or ischemia).[7,8] This metabolite resonates in 1.32 ppm but shows a significant overlap with lipids/macromolecules, and this makes it challenging to differentiate. To overcome this issue, using echo time (TE) (135–144 msec) displayed to be helpful, so the lactate molecule (CH3COHCOOH) appears inverted and subtracted from lipids/macromolecules.[8]

Meanwhile, J-difference editing as a cutting-edge technique defines the "OFF" and "ON" spectra. ON acquisition includes a single-voxel Point Resolved Spectroscopy (PRESS) sequence.[9] Mescher-Garwood scheme (MEGA)–PRESS is one of the popular protocols because it makes it possible to simultaneously edit the spectra and water suppression.[10] These strategies can measure lactate metabolite in AD individuals. In this article, we discuss various facets of pathophysiologic conditions in AD (low blood flow and mitochondrial dysfunction) that directly or indirectly contribute to lactate imbalance. Furthermore, we will discuss the limitations and benefits of current imaging techniques while considering that the current literature surrounding fMRS imaging in AD is limited, we aim to emphasize on the potential role of lactate imaging in this disease.

PATHOGENESIS OF ALZHEIMER DISEASE: MORE THAN TANGLES

Cerebral Blood Flow and Microcirculation

Cerebral blood flow (CBF) is defined as the blood volume circulating through 100 grams of brain tissue per minute (50 ml/

minute).[11,12] The amount of blood gas and cerebral perfusion pressure are among the factors that determine the overall flow of blood through the brain tissue. CBF can change due to several factors; an increase in intracranial pressure due to the increased hydrostatic pressure results in high vessel outflow.[12–15] Glial fibrillary acidic protein in astrocytes acts as another CBF regulator, which is believed to be reduced in both AD models and AD individuals. Alongside the CBF reduction because of the aging process, Aβ deposition, tauopathy, and reduced glial fibrillary acidic protein are expected, which intensify the deteriorating effect on the microcirculation of the central nervous system (CNS).[16–18] This reduced blood flow can be used as an imaging marker in functional MR techniques, as it will be discussed further.

Energy, Metabolism, and Cell Signaling

Like other parts of the body, the function of neurons is highly dependent on glucose. After entering the brain cells, glucose is used in the Krebs cycle. Thirty-six to thirty-eight ATP molecules are expected to be produced throughout the Krebs cycle.[19–21] This cycle requires oxygen for proper maintenance, and as oxygen supply drops, the anaerobic pathway is inevitably activated to provide the minimum amount of energy needed. In addition to the much lower amount of ATP produced, lactate will be present in the cytoplasm as the ultimate product of this cycle.[22–24] After the increased lactate in neurons and astrocytes, there will be a decrease in pH and more potassium will be transported through the cell membranes, resulting in a disturbance of action potential.[26]

Dysregulation of Mitochondrial Function

Mitochondria have a crucial role in protein synthesis and ATP production in aerobic conditions (Krebs cycle), and mitochondrial dysfunction has been suggested as the main pathology of AD in some studies.[26,27] Chou et al.[26] observed mitochondrial dysfunctions as an early event in AD mouse models.

Hypoxia in various conditions such as decreased CBF as a secondary expected phenomenon in AD results in reduced ATP production, exacerbation of oxidative stress, and the formation of a defective cycle that leads to cell death due to an increase in reactive oxygen species.

Neurotransmitter Dysregulation (GABA/GLU—Lactate)

Neurotransmitters are chemical messengers in the central and peripheral nervous system and are made up of small amino acids or peptides and cause changes in the autonomic system regulation. Gamma-aminobutyric acid (GABA) and glutamate (GLU) are 2 neurotransmitters that are normally in equilibrium. This balance controls individual neurons and complexes of functional neurons in excited and nonexcited states. Any changes in the GABA/GLU balance lead to essential outcomes; in the case of excessive stimulation, GLU increases and GABA decreases, and in the cases of suppression, GABA increases in comparison with GLU, which causes parasympathetic overactivation.[28–32] Acetylcholine levels are claimed to be reduced in AD individuals with consequent memory impairment.[33,34] A higher ratio of GLU to GABA in AD individuals compared with the normal population is expected due to the effect of AD on GLU-GABA balance in the temporal lobe.[28]

Oxidative Stress and REDOX Damage

Oxidative stress is an unfortunate phenomenon that can occur in any cellular and subcellular components because of the imbalance between oxidants and pro-oxidants with antioxidants. In this phenomenon, superoxide is produced, which is highly reactive and can easily cause oxidative damage.[35] It is now widely recognized that oxidative stress contributes to AD pathology. Accumulation of oxidizing cations such as iron and copper, Aβ deposits outside the cell, and hyperphosphorylated tau and mitochondrial dysfunction inside the cell are among the factors that have been known to cause oxidative stress and its exacerbation in AD individuals.[36,37] Glutathione is an antioxidant that plays a vital role in the prevention of a defective cycle of oxidative stress in different parts of the body. Neuronal cells contain small quantities of glutathione, making the neurons susceptible to oxidative damage.[37] Thus, it seems that low CBF in AD individuals result in lactate increase, which occurs because of the activation of the intracellular anaerobic cycle and neuronal cells fall into a defective cycle. This phenomenon leads to severe mitochondrial dysfunction.

LACTATE AND NEURODEGENERATION

Lactate Metabolism and Significance in Glycolysis

Lactate is the conjugate base of lactic acid, a byproduct of energy metabolism in cells. Classically, glucose can be metabolized in 2 main ways. In low-oxygen environment, anaerobic glycolysis is the main route of energy production.[38] Glucose is first phosphorylated to remain in the cytosol. Then, isomerase turns glucose-6-p to fructose-6-p, which itself is again phosphorylated to fructose 1,6-bisphosphate. Then, aldolase and subsequent machinery of enzymes lead to creating 2 pyruvate molecules from a single glucose molecule. Overall, the pathway produces 2 ATP and 2 nicotinamide adenine dinucleotide (NADH) molecules, which can be further used in energy production. NAD+ plays a vital role in various metabolic pathways, and its lack is inconsistent with prolonged cellular physiology. Thus, if NAD+ cannot be generated using the electron transport chain in the mitochondria, it is regenerated through the production of lactate from pyruvate, A step mediated by the lactate dehydrogenase.[38–40]

In oxygen sufficiency, pyruvate is turned into acetyl coenzyme A through pyruvate dehydrogenase, with the latter molecule entering the tricarboxylic acid cycle. For each Acetyl-CoA molecule, the cycle generates 3 NADH, a single guanosine triphosphate molecule, and a single flavin adenine dinucleotide ($FADH_2$) molecule.[41,42] These are then entered into the electron transport chain in the mitochondria and subsequently used to maintain an H^+ gradient along with the spaces of the mitochondria. This gradient is used by an ATP synthesis complex to form ATP from adenosine diphosphate and inorganic phosphate. The completed aerobic pathway creates 36 ATP molecules, and the anaerobic way makes a scant 4 ATPs.[43–45]

Thus, many cells rely on the aerobic method to generate energy. Only a limited number of cells in the human body depended solely on anaerobic glycolysis to survive (namely red blood cells).[46]

Role of Lactate in Energy Metabolism: The Astrocyte-Neuron Lactate Shuttle

Energy metabolism in the CNS is highly dependent on aerobic glycolysis, especially in high neuronal activity. Much of the energy consumed in the neurons is bound to fuel Na/K pumps, which are essential for transmitting signals from one neuron to the other.[47] Conventionally, it was assumed that because of the high rates of aerobic glycolysis, lactate would be scant in intercellular spaces of the CNS. This was refuted by in vivo studies suggesting that lactate had a more than expected presence, even when neuronal activity was not significant.[48] Further light was shed on this issue when histopathologic studies highlighted the cellular heterogeneity of the CNS because cells derived from an embryonic mesodermal origin in the

CNS were not as dependent on aerobic metabolism. Astrocytes and oligodendrocytes processed glycogen glycolytically, yielding lactate and pyruvate from glucose.[49]

Interestingly, astrocytes particularly have multiple mitochondria and would be expected to benefit from the more productive aerobic pathway. However, owing to enzymatic profiles (hyperphosphorylation of pyruvate dehydrogenase) and structural variants in the mitochondrial respiratory chain, complexes cause suboptimal mitochondrial respiration.[50] This phenomenon is similar to the aerobic glycolysis seen in malignant tissue, otherwise known as the Warburg effect.[51] Pellerin L et al. presented a unified model to link the drastically different metabolisms of astrocytes and neurons, further expanding through numerous animal studies.[52] According to this theory (and based on the evidence presented by observational studies), increased neuronal activity leads to increased GLU release to the extracellular space through the excitatory amino acid transporter 3 (EAAT3) transporters, presumed to exist exclusively on neuronal cell membranes. Astrocytes then uptake GLU through EAAT1-2 transporters, driven by a Na gradient. This also causes an increased uptake of glucose, which is entered into the glycolytic pathway. The resulting lactate then trickles out of the astrocyte, into the extracellular fluid, and into neurons, where it is further processed using lactate dehydrogenase, turned into pyruvate, which then can create mediators entering the tricarboxylic acid cycle.[53–56]

Physiologic Role of Lactate in Neuronal Functioning

As mentioned, astrocytes may have more critical roles in neuronal physiology than previously thought. Recently, an increased focus has been put on the part of astrocytes in metabolically supporting neurons in instances of improved neuronal functioning. It is now proposed that gluconeogenesis dependent on astrocytic glycogenolysis may be critical in generating neurotransmitters such as GLU and GABA.[57,58] It is postulated that the same could be true for lactate. Empiric evidence suggests that distribution of the lactate transfer from astrocytes to neurons (either by upstream metabolic distributions which reduce lactate synthesis or by downstream loss of function of lactate transfer molecules out of astrocytes) leads to the impairment of memory formation.

Moreover, loss of function of monocarboxylate transporter 2 channels, situated on neurons and responsible for entering lactate into them, leads to the distribution of memory formation even when an excess amount of lactate is present in the extracellular space.[59–62] More studies suggest that lactate may be involved in various forms of learning and decision making, which relies on signal transduction among different anatomic structures such as cortex, amygdala, cingulate gyrus, and hippocampus.[63,64] One interesting observation is that adding lactate to the hippocampus can mitigate the amnesic effects of anti-beta adrenergic such as propranolol.[65,66] β-adrenergic signaling initiated by neurons located in locus coeruleus has a critical role in consolidating inhibitory avoidance memory and is a classic example of how lactate may affect neuronal signaling.[67] Importantly, restoring or injecting excess glucose only marginally substitutes lactate, suggesting that lactate's memory-enhancing and proplasticity effects may be partially energy independent.[68] These effects may be mediated by gene-amplifying characteristics of lactate or due to the shifts in redox balance (with the introduction of NADH after lactate formation). Whatever the cause, studies show that heightened aerobic glycolysis is correlated with increased neuronal plasticity, with direct genomic relations, as the expression of certain neotenic genes ensures plasticity in specific areas of the brain in mammals[69,70] (Figure 1).

Lactate as a Cellular Messenger in Cell Signaling Pathways

Scholars have looked at the possible roles of lactate in neuronal signaling owing to the significant amounts of lactate in the extracellular spaces in the CNS and the demonstrated role of lactate in cell signaling outside of the CNS (such as in cancers).[71,72]

One peculiar association has been proposed to be the role of lactate in locus coeruleus. In vivo studies have found that injection of L-lactate in physiologic concentrations can increase the production of norepinephrine, such as excitatory neurotransmitters including L-GLU. Importantly, these effects are not dependent on neuronal associations of astrocytes, and lactate can exhibit the mentioned effects without ever having to enter neurons.[73] More studies have shown that these effects are not mediated by lactate shunts and are exerted through 2 opposing lactate binders. One of these receptors is the orphan receptor coupled with Gi, G protein–coupled receptor 81, and the other is the G protein–coupled receptors (GPCRs), excitatory receptor, which increases the levels of cyclic adenosine monophosphate by activating protein kinase A.[74]

In vivo studies on mice have also suggested that lactate may have a role in maintaining the sleep-wake cycles. It has been shown that preventing intercellular trafficking of lactate by clocking connexin molecules in mice hippocampus inhibits the excitatory synaptic activity. Similar effects are also seen in other brain regions, such as subthalamic nuclei, pyramidal cells, and CA1 subregion of the hippocampus.[75–77]

Interestingly, other signaling cascades are also affected by lactate. Yang et al. in 2014 showed that lactate can promote neuronal plasticity by potentiating the effects of the NMDA GLU receptor.[68,78] It also increased the intracellular NADH and calcium levels, leading to brain-derived neurotrophic factor signaling potentiation. Brain-derived neurotrophic factor is a mediating neuronal factor that has established a role in exercise-related neuronal plasticity. Many studies suggest that lactate modulates the exact mechanism involved in exercise-related neuronal plasticity by SIRT1 activation, which enhances PGC1α/FNDC5/BDNF signaling.[70,79]

IMAGING IN ALZHEIMER DISEASE

Currently, AD diagnosis is challenging because there are no precise paraclinical criteria, and it is usually made based on the clinical criteria, which themselves are combinations of clinical signs and symptoms and mental status examination results, while biomarkers (e.g., concentrations of Aβ peptides [Aβ1–42:Aβ1–40 ratio]) and neuroimaging modalities, such as MRI and positron emission tomography (PET) scan, are widely used as confirmatory for the primary diagnosis.[80,81] The Food and Drug Administration has approved the florbetapir (Amyvid) PET scan to estimate beta-amyloid neurotic plaque density in AD individuals.[82] Nonetheless, amyloid PET scan is an invasive modality with high radiation dosage, and the cost effectiveness of this modality is still questionable.[83]

MR imaging of the nervous system can facilitate the detection of early changes associated with dementia such as regional atrophy, and some advanced MR techniques have been investigated, mainly in research, for the early detection of AD disease. These techniques, such as functional magnetic resonance imaging (fMRI) and arterial spin labeling (ASL), facilitate the diagnosis by providing dynamic and static imaging, respectively. Nonetheless, some of these methods need further clinical evaluation and represent hypotheses during preclinical studies.[84,85]

MRI in Alzheimer Disease

The traditional MR imaging in AD is classically based on Braak staging.[86] The levels of atrophy are evaluated by an expert

neurologist, in some areas of the brain such as the medial temporal lobe including hippocampus and entorhinal cortex.[87,88] In addition, the loss of limbic gray matter (thalamus, amygdala, and cingulate gyrus), which is associated with memory loss, is another finding in MR imaging.[89,90]

MR imaging could also determine signal intensities related to AD plaques which could be detected on T2-weighted images and fluid-attenuated inversion recovery (FLAIR) images.[91] White matter hyperintensities are also shown to be associated with disease activity and even severity of clinical signs.[84,92] In this regard, white matter high T2-weighted, T2*-weighted with susceptibility-weighted imaging sequence, coronal 2D T2-weighted turbo-FLAIR, coronal 2D T2 turbo/fast spin-echo, sagittal 3D T1 MPRAGE/IR-SPGR postcontrast, and axial spin-echo T1 postcontrast are strongly suggested.[93] Undoubtedly, going through all the noted modalities is not realistic, and it seems that selecting the most suitable shall be according to the local equipment and facilities. Nonetheless, static MRI techniques cannot be used in assessing the proclivity of the condition to deteriorate and even pinpoint the most recent changes, and other advanced dynamic techniques are required for this purpose.

Advanced MR Techniques

Among advanced MR techniques, DTI, ASL, and fMRI are suggested. DTI is based on the anisotropic diffusion and Brownian motion of water molecules of the brain. In this modality, mean diffusivity (MD) and fractional anisotropy (FA), which relate to the average rate of water molecule diffusivity and the variability associated with diffusion, are respectively measured.[84] Increased MD in some brain regions such as the frontal and occipital lobes is shown to be associated with AD. By contrast, FA is decreased in the occipital and parietal lobes. That is because this method is dependent on water motion. Accordingly, artifacts would be more common in this technique. In addition, initially, metabolic changes occur in these tissues and can be detected by DTI if these metabolic changes lead to structural changes.[94,95]

On the other hand, ASL is a noninvasive and noncontrast MR perfusion technique. ASL labels arterial blood water protons; therefore, it can measure fluctuations in cerebral blood flow as an AD marker. The CBF is reduced significantly in AD individuals in temporal, frontal, parietal, thalamus, hippocampus, and amygdala.[96,97] Furthermore, lower CBF has been shown to be associated with faster cognitive decline in AD individuals.[98] Although this modality is noninvasive and nonionizing, alterations in signal intensity and the low signal-to-noise ratio (SNR) reduce image quality.[99,100] fMRI is a blood-oxygen-level-dependent or BOLD-contrast imaging technique.[101] Individuals can be evaluated for brain activity during the task or resting state. Studies showed that hippocampal and medial temporal lobe have low activation rates in AD individuals.[102]

Working memory, semantic knowledge, attention, motor performance, and visuospatial ability are among the functions included in the task mode.[103,104] The posterior cingulate cortex, entorhinal cortex, and hippocampus were evaluated in this mode, but the most engaged structure was medial temporal lobe.[105,106] Nonetheless, fMRI is not used in routine clinical evaluation, possibly because of its low SNR for neuronal activity.[107]

As discussed, many dynamic modalities encounter quality limitations in clinical diagnostics. However, quantitative imaging techniques have shown promise in recent years. MRS as a quantitative imaging measures CNS metabolite concentrations. For example, N-acetyl aspartate (NAA)/creatine (Cr) and NAA/myo-inositol (mI) can be used as markers for AD.[108] This modality, however, has limited sensitivity for diagnosing AD; therefore, the combination of different modalities could yield a better image in both quantity and quality. In this manner, fMRS has been developed to detect CNS abnormalities in mice and humans.[2,8,108–115]

fMRS PROTOCOL

Repetition Time, Echo Time, and T2* Relaxation

Repetition time (TR) and TE are measured in milliseconds (msec). The degree of longitudinal magnetization recovery between each pulse determines by TR between successive pulse sequences applied to the same slice.[116] The time interval between radio frequency pulse transmissions and the reception of echo signals is called TE. Furthermore, TE regulates the quantity of T2* (transverse relaxation time),[117] during which the gradient echo (GRE) loses signal strength.[118] Magnetic field inhomogeneity can be macroscopic (constant across a voxel) or microscopic (variable across a voxel). The phase shift in a GRE image reflects the average magnetic field of protons in a voxel, which is affected by the tissues' local susceptibility.[119]

For the primary GRE sequence fast low-angle shot (FLASH), the greater flip angles give the image more T1 weighting and the lower flip angles give the image more T2* weighting while TE has increased.[120] In this fast GRE sequence, the gradian is diminished semirandomly after every echo, eradicating transverse magnetization by changing the phase space. In addition, to avoid susceptibility artifact, the amount of TE is set to minimum.[121]

By contrast, there is no 180° refocusing pulse in GRE sequences; thus, dephasing effects are not reduced. As a result, transverse relaxation (i.e., T2* relaxation) in GRE sequences is a mix of "true" T2 relaxation and relaxation produced by magnetic field inhomogeneities. The $1/T2^* = 1/T2 + \gamma\ \Delta B_{inhom}$ equation showed that T2* is shorter than T2; in this equation, ΔB_{inhom} is the magnetic field inhomogeneity across a voxel and γ is the gyromagnetic ratio.[122]

There have been few studies evaluating lactate with 1H-MRS in AD individuals in recent years. In these studies, MRI equipment used 1.5 and 3 Tesla (T), short TE (11–35 msec), and long TR (500–2000 msec) with direct dimension bandwidth (2 and 2.5 kHz). These studies used the PRESS protocol (Table 1).[127–130]

However, the fMRS protocol for lactate in healthy volunteers was run with short and long TE (6–144 msec) and long TR (1500–7500 msec) and using MEGA, LASER, SPECIAL, PRESS, and STEAM protocols (Table 2).[2,8,108–115]

Table 1. Summarize Characteristics of MRS Protocol With Lactate for AD Individuals

Study	MRI Equipment	Coil	MRS Protocol	MRS TE (msec)	MRS TR (msec)	DDB (kHz)
R Mullins et al., 2018[123]	Philips 3T	8-channel SENSE head coil	J-PRESS	35	2000	2
KE Weaver et al., 2015[124]	Philips 3 T	8-channel SENSE head coil	PRESS	24	2000	2
T Ernst et al., 1997[125]	GE 1.5 T	NA	PRESS	11	500	2.5
W. Block et al., 1995[126]	Philips 1.5 T	NA	NA	NA	2000	NA

J-PRESS, J-modulated point-resolved spectroscopy, DDB, direct dimension bandwidth.

Table 2. Summarized Characteristics and Technical Specifications of fMRI Imaging in Studies Aiming to Quantify Lactate Levels

Study	MRI Equipment	Population	Evaluation	MRS Protocol	MRS TE (msec)	MRS TR (msec)	fMRI Paradigm	Outcomes
Y Koush et al., 2021[120]	3 T Siemens Prisma scanner using 64-channel head/neck coil	Twenty healthy volunteers	Cortical areas in the human brain	MEGA	144	2700	Visual stimulation/ cognitive task	Lactate increased on activating the visual cortex but did not change on deactivating the posterior cingulate cortex.
CC Fernandes et al., 2020[119]	7 T Philips, 32-channel receive head coil	Six healthy volunteers	Human visual cortex	MEGA (semi-LASER)	144	2000	Visual stimulation	Lactate increases significantly (~10%).
Ligneul et al., 2020[117]	9.4 T Bruker BioSpec scanner	20 mice (C57Bl6 females, age 3–4 months)	Mouse superior colliculus activity	LASER	28	1500	Visual stimulation	NAAG, PCr, Cr, and GLU were increased measured in the superior colliculus
Y Koush et al., 2019[116]	4 T Bruker spectrometer using a 16-channel transmit-receive head coil	Ten healthy volunteers	Human motor cortex	MEGA	144	3330	Finger-to-thumb tapping	J-edited fMRS has high sensitivity and specificity for task-induced lactate modulation.
R Mekle et al., 2017[127]	7 T Siemens whole-body system with a 60-cm bore and a whole-body gradient coil	Twenty healthy volunteers	Human visual cortex	SPECIAL	30	2000	Visual stimulation	An increase in lactate of 0.04 mmol/L (7%) was the only significant effect.
P Bednařík et al., 2015[118]	7 T/90-cm magnet (Agilent/Magnex Scientific)	Fifteen healthy volunteers	Human visual cortex	Semi-LASER	26	5000	The block-designed paradigm of visual stimulation	BOLD-fMRI signals were a linear relationship positively correlated with lactate concentration changes.
B Schaller et al., 2014[2]	7 T/68-cm scanner Siemens, 2-channel receive coil	Eleven healthy volunteers	Motor activation	SPECIAL	12	7500	Finger-to-thumb tapping	Increases in lactate during motor stimulation are small. The lactate changes may be a general manifestation of the increased neuronal activity.
B Schaller et al., 2013[128]	7 T/68-cm Siemens	Ten healthy volunteers	Human visual cortex	SPECIAL	6	5000	Visual stimulation	The rate of lactate concentration increases.
AL Lin et al., 2010[121]	3 T Trio MRI scanner (Siemens)	Twelve healthy volunteers	Human visual cortex	PRESS	30	2000	The reversing-checkerboard paradigm of visual stimulation	The energy demand of task-induced brain activation is approximately 15%. Increases in CBF positively correlated with lactate production.
S Mangia et al., 2007[122]	7 T/90-cm horizontal bore magnet (Magnex Scientific)	Twelve healthy volunteers	Human visual cortex	STEAM	6	5000	Visual stimulation	The lactate concentration reached the new steady-state level within the first minute of activation and returned to baseline only after the stimulus ended.

Spin-echo full-intensity acquired localized (SPECIAL), Mescher-Garwood scheme (MEGA)

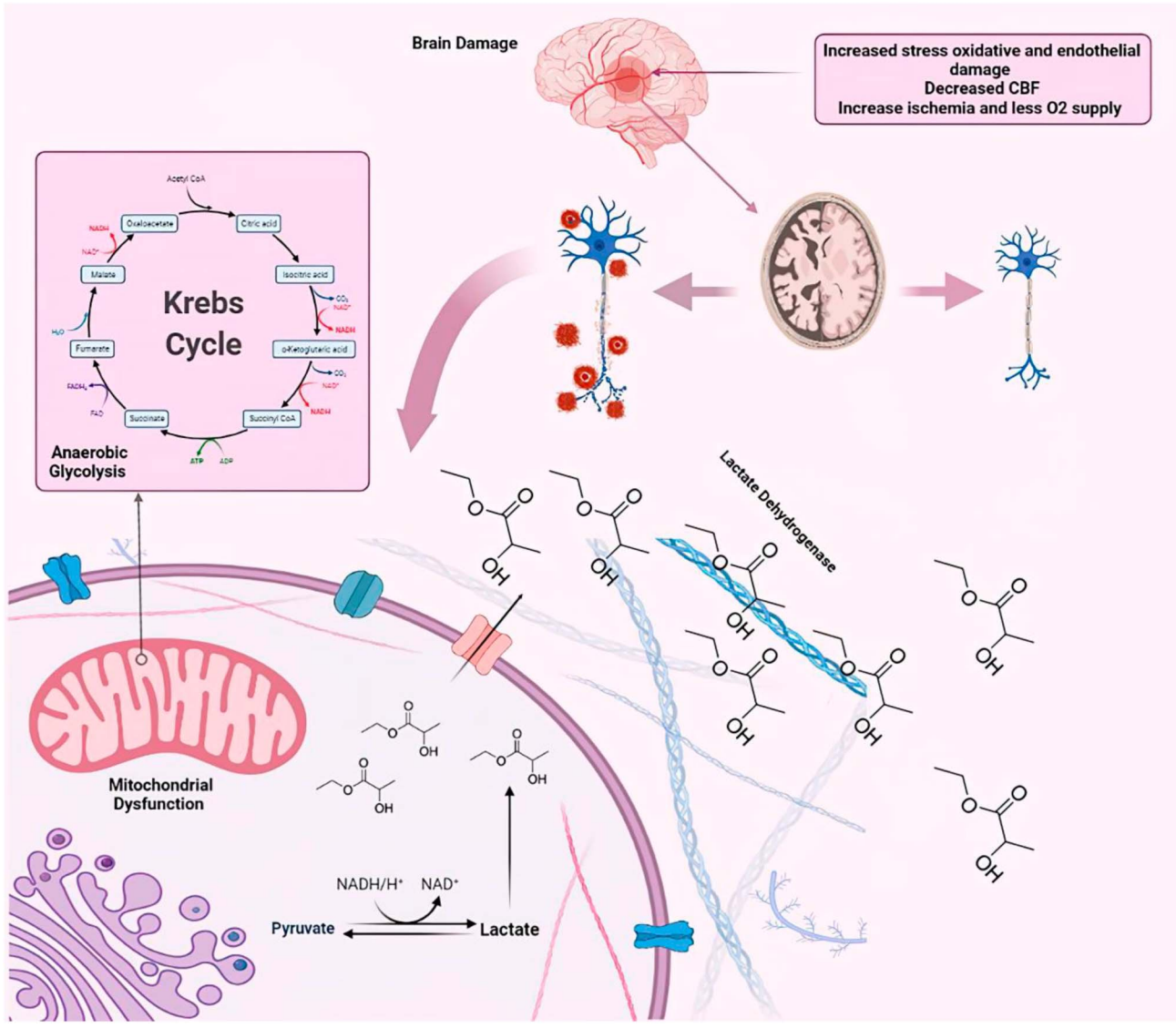

FIGURE 1. Physiologic role of lactate in neuronal functioning. Note that the figure was created by using the website BioRender.

Metabolites such as lactate in pathologies could be recorded with edited 1H-MRS protocol if the expected levels are higher. In addition, long TE (144 msec) can differentiate the overlapping signals from lipids/macromolecules, which is required for exact evaluation of changes in lactate (or other metabolites) at low SNR.[2,8]

Signal-to-Noise Ratio

In imaging, the SNR is a measure of genuine signal (i.e., representing actual anatomy) to noise (e.g., random quantum mottle).[131,132] The SNR is routinely evaluated in MRI by measuring the signal intensity difference between the region of interest (ROI) and the background.[133]

When MRI is used instead of 2D imaging, volume acquisition can increase the SNR. However, imaging time for spin-echo sequences is longer than GRE sequences.[134] The use of surface coils increases the SNR, and increasing the number of excitations reduces the TE while increasing the TR.[126,135]

The main challenge of formulating a proper fMRS protocol with long TE is to reduce SNR while reaching a good SNR for measuring lactate. On the other hand, SNR can be reduced by decreasing bandwidth (BW) or increasing averaging. In addition, correcting the uniformity of the field automatically or manually could enhance SNR. Placing the MRS voxel in the correct position could also decrease noise (not including air and water inside the voxels and selecting voxels with dexterity, especially where the anatomical tissue changes are large, e.g., the base of the skull). Correctly placing the suppression bands around the voxel can also be effective. Increasing the TR also helps to some extent, although the amount of TR should not be less than 2000 msec.[8,111]

fMRS Examination

The fMRI technique measures brain activity and identifies minor changes in blood flow, showing stimuli or actions (most frequently BOLD imaging). This modality relies on minute changes in a low SNR environment, making it technically challenging.[123,125]

The individual's activity or stimulus is referred to as a paradigm, and each one is meant to extract a specific cerebral response. Many paradigms with varying levels of complexity have been developed. Four paradigms were assigned to different clinical responses (visual, motor, speech, memory, and clinical practice). It can look at the brain's functional architecture to see which regions oversee specific functions. It can be used to assess the impact of stroke, trauma, and degenerative diseases such as AD on brain function.[114,116]

Repeated paradigms are separated by blocks of inactivity or alternate action in a block design. In clinical fMRI, this is by far the most common design. Individual events, rather than blocks, are used in the event-related design, and they can be dispersed arbitrarily across the research. During an fMRI examination, tapping fingers or toes, pursing lips, moving tongue, reading, seeing images, listening to speech, and playing simple word games are possible ways to show the brain's activity.[111,112]

The fMRI is now a cornerstone of neuroimaging in clinical and fundamental brain research. However, fMRI would be an ideal diagnostic tool because of its noninvasive nature and significant spatial resolution while limited in temporal resolution. On the other hand, human studies were shown to cause a consistently detectable BOLD response, with even modest lactate alterations, creating plasma concentrations equivalent to moderate muscular activity.[124,125,136] Similarly, the lactate level was significantly higher in the rodent somatosensory barrel cortex (S1bf) during stimulation or on the early visual cortex in animal experiments with the BOLD response.[137,138] Nonetheless, fMRI shows functional activity, but brain metabolism is missed. There are some techniques such as autoradiography, positron emission tomography (PET), two-photon fluorescent confocal microscopy (TPEF or 2PEF), and MRS to analyze brain metabolism.[139–141] Both autoradiography and PET are noninvasive methods and have low spatial resolution images.[142] In addition, MRS reveals the number of metabolites but not the brain activity.[135] As a result, functional MRS (fMRS) is a noninvasive and nonradioactive technique and may investigate any brain region.[143]

The peak of macromolecule is unknown; variations in their quantities have been linked to stroke, multiple sclerosis, and malignancies. Several peaks are at 0.9, 2.05, and 3.0 ppm.[144] Acetate and macromolecular proteins are among the lipids with multiple vast peaks between 0.8 and 1.3 ppm. Lipid levels, lactate, and alanine increase in various cancers.[145] The methine proton is J-coupled to the 3 methyl group protons in lactate through the C-C connection. In addition, ±3.47 Hz (J=6.93 Hz) of precession frequency shift is caused by coupling between 2 groups of protons.[146]

As proton MR spectroscopy shows, anaerobic glycolysis ends with the lactate resonance. Stroke, high-grade malignancies, and abscesses are examples of diseases compatible with our understanding of the biochemical processes taking place in the body. Long-echo proton MRS investigations use J-coupling to distinguish lactate methyl and methine proton peaks (which occur at 7 Hertz).[10]

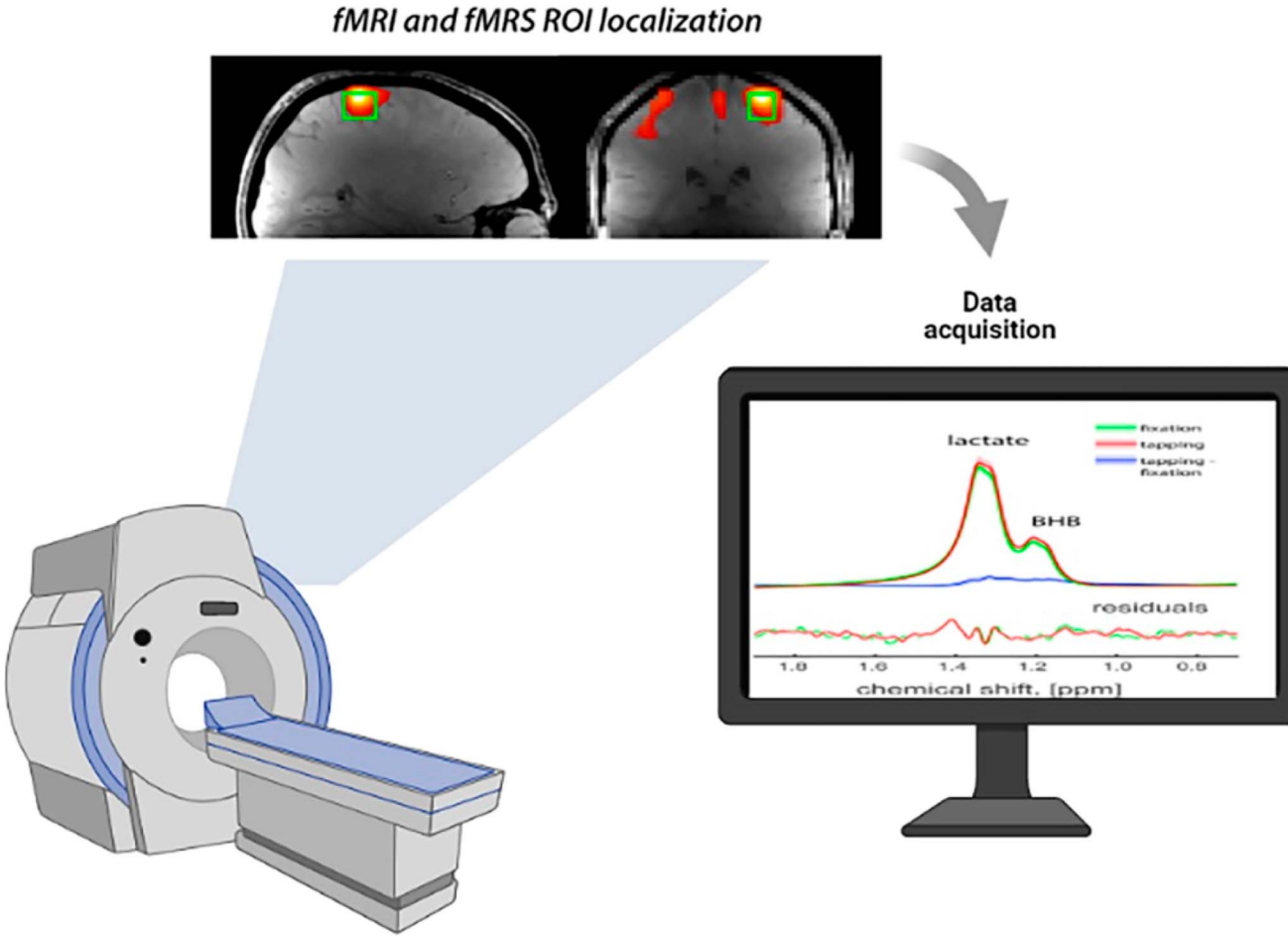

FIGURE 2. fMRS experimental design and results. Note that the figure was created by using the website BioRender.

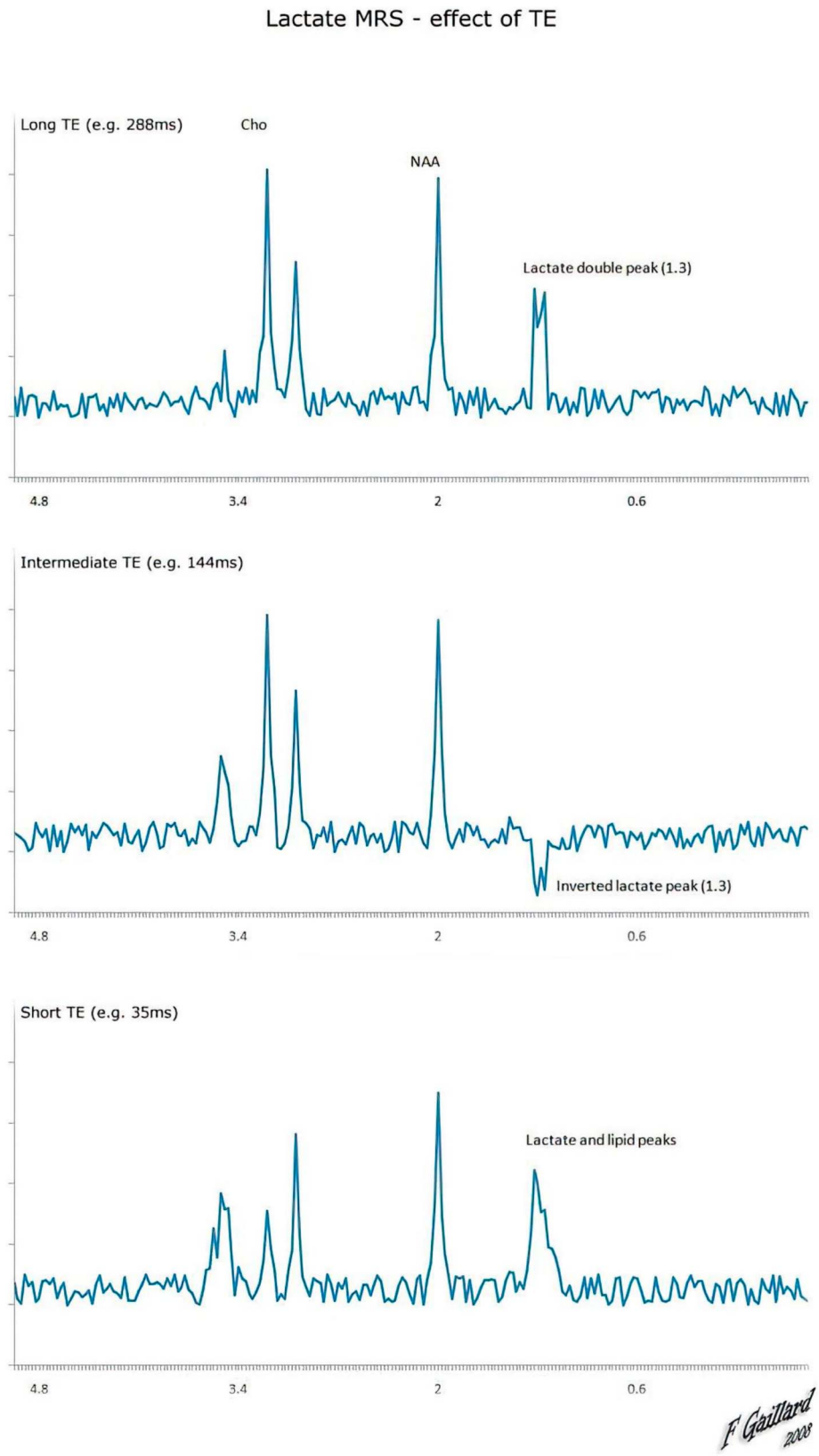

FIGURE 3. Long TE can differentiate the overlapping signals from lipids/macromolecules; the main challenge of formulating a proper fMRS protocol with long TE is to reduce SNR while reaching a good SNR for measuring lactate.

If lengthy echo durations (144 and 288 msec) (multiples of 1/7 Hz) are used, it can differentiate lactate signals from lipid signals in the blood. Spin or J-coupling inverts the resonance when sampling with a 144 ms echo period.[143,147] As a result, the lactate doublet peak may be distinguished from macromolecules and lipids. Still, the signal strength is reduced, particularly troublesome at low concentrations. At 288 msec, the doublet peak appears above the baseline.[8,110] The lactate peak seen at the doublet may be unbalanced due to transmission and reception problems. In addition, the signal should physically match the high-fat sign-on imaging at a time interval of 288 ms if it originates from lipids. Thus, anaerobic glycolysis is the cause of lactate development which is considered pathological. Depending on the situation, the lactate signal may potentially represent mitochondrial dysfunction.[148]

A greater B0 field demands different methods to B1-related abnormalities in J-difference MRS in vivo, although the MEGA-PRESS sequence is the most frequent lactate detection by 1H-MRS with a long TE J-difference, which naturally eliminates overlapping signals. The MEGA-PRESS is a J-difference edited MRS pulse sequence consisting of 'ON' and 'OFF' subexperiments.[149] In contrast to the 'OFF' experiment, the' ON' experiment uses frequency-selective radio frequency editing pulses to edit the lactate molecule's 1.32 ppm resonance.[8]

Lactate is separated from overlapping resonances and macromolecules using spectral editing with J-modulation, which uses the quantum mechanical characteristics of specific molecules to "edit" them out of the overall 1H-MRS spectrum.[8,111] On the other hand, J-editing is prone to subtraction mistakes caused by motion, which can hide tiny changes in the lactate by using quantum mechanical characteristics of lactate to remove it from the overall spectrum; J-edited 1H-MRS can provide direct insights into the overlapped resonances that occur from numerous molecules such as fatty acids or macromolecules.[110] Because of considerable suppression of the relatively short T2 for the lipid and macromolecule background signals, 1H-MRS has demonstrated that physiological modulations of lactate in the visual cortex may be seen even at 1.5 T using long TE.[143]

Overall, J-edited fMRS has high sensitivity and specificity for task-induced lactate modulation[8] and shows the rate of increase in lactate concentration.[2] Compared with the fixation condition, a significant increase was detected in lactate levels.[8,108,111] The rate of lactate increases significantly between 7 and 15 percent.[110,112,114] In addition, the result shows that lactate increased on activating the visual cortex but did not change on deactivating the posterior cingulate cortex[111]; however, increases in lactate during motor stimulation are less significant[2] (Figure 2, Figure 3).[150]

LIMITATIONS AND FUTURE PROSPECTS

Although the prospect of different derivatives of MRI imaging in AD is promising, several issues limit the possible beneficence of these techniques in routine clinical consideration. One problem is the low specificity of certain imaging signs and quantitative outputs (such as those of MRS) in differentiating dementia from other common clinical pathologies, such as stroke, benign senile changes, and mild to moderate mental decline.[151] Furthermore, there is no consensus on the criteria for patient selection for specific derivatives of MR imaging in dementia, and simply changing the study population could have significant effects on the diagnostic profile of the studied modality (most notably the positive and negative predictive values). Importantly, the relative importance of quantifiable findings (such as those of MRS) is not known compared with anatomical studies, which are the major yield in conventional MR imaging.[152] Note that, in contrary to Cholin and NAA, specifically NAA can demonstrate relationship to disease severity,[153–155] clinical results for this association for lactate levels are limited. A recent clinical study on AD and mild cognitively impaired adults showed that CSF lactate levels were associated with age and blood-brain barrier integrity but not with clinical severity or CSF biomarkers of AD.[156] This hypothesis should be investigated through clinical studies, and future research could focus on the possible role of lactate imaging in AD diagnosis by focusing on the combined efficacy of novel quantitative MR methods and the anatomic preciseness offered by conventional MR imaging.

CONCLUSION

This comprehensive review concludes that fMRS with J-editing is a cutting-edge technique to detect lactate in ADs. This modality during functional activation by omitting overlapping lipid and macromolecule signals provides high SNR than other modalities to measure lactate. However, this method is still not indicated for diagnostic purposes based on the clinical evidence. Still, it seems that lactate evaluation with high B0 fMRS equipment increase 3–7 Tesla provides more accurate results.

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