REVIEW

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What have novel imaging techniques revealed about metabolism in the aging brain?

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ABSTRACT: Brain metabolism declines with age and do so in an accelerated manner in neurodegenerative disorders. Noninvasive neuroimaging techniques have played an important role to identify the metabolic biomarkers in aging brain. Particularly, PET with fluorine-18 (¹⁸F)-labeled 2-fluoro-2-deoxy-D-glucose tracer and proton magnetic resonance spectroscopy (MRS) have been widely used to monitor changes in brain metabolism over time, identify the risk for Alzheimer's disease (AD) and predict the conversion from mild cognitive impairment to AD. Novel techniques, including PET carbon-11 Pittsburgh compound B, carbon-13 and phosphorus-31 MRS, have also been introduced to determine Aβ plaques deposition, mitochondrial functions and brain bioenergetics in aging brain and neurodegenerative disorders. Here, we introduce the basic principle of the imaging techniques, review the findings from 2-fluoro-2-deoxy-D-glucose-PET, Pittsburgh compound B PET, proton, carbon-13 and phosphorus-31 MRS on changes in metabolism in normal aging brain, mild cognitive impairment and AD, and discuss the potential of neuroimaging to identify effective interventions and treatment efficacy for neurodegenerative disorders.

The human brain has the highest energy demands of any organ in the body, consuming more than 20% of the body's glucose and oxygen, despite comprising only 2% of total body mass [1]. The majority of this energy is used to support functional processes. Numerous studies have shown that metabolic rates of glucose and oxygen decline with age and do so in an accelerated manner in neurodegenerative disorders, such as Alzheimer's disease (AD) [2–4]. The metabolic rate reductions have been considered as major contributors to brain structural alteration (gray matter and white matter atrophy) and cognitive impairment later in life [2,5–6]. Methods that can monitor *in vivo* brain metabolic changes over time are thus critical for earlier diagnosis and longitudinal investigations of bioenergetic deficit in the aging brain and the risk for neurodegenerative disorders, as well as to assess the value of therapeutic interventions.

Noninvasive neuroimaging techniques have played a major role in identifying the metabolic biomarkers, including PET and magnetic resonance spectroscopy (MRS). Among these, PET with fluorine-18 (¹⁸F)-labeled 2-fluoro-2-deoxy-D-glucose (¹⁸FDG) tracer and proton (¹H) MRS are the well-established methods to quantify cerebral metabolic rate of glucose (CMR_{glc}) and neural metabolites, respectively. Recently, PET-based tracers, such as carbon-11 (¹¹C) Pittsburgh compound B (PIB), have been introduced to determine Aβ plaque deposition in neurodegenerative disorders. Other novel techniques, including carbon-13 (¹³C) and phosphorus-31 (³¹P) MRS have also developed to determine brain bioenergetics in aging brain. Here, we review the basic principle

KEYWORDS

• Aβ plaques • aging
• Alzheimer's disease
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• mild cognitive
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of the imaging techniques and their utilization to monitor brain metabolism changes over time, identify the risk for AD and predict the conversion from mild cognitive impairment (MCI) to AD. We will also introduce the potential interplay of neuroimaging methods and interventions that can slow down brain aging and prevent neurodegenerative disorders.

***In vivo* neuroimaging for brain metabolism measurement**

• PET

PET is currently the most common approach to study brain metabolism. It uses radioactive tracers to produce a 3D image of functional processes (e.g., metabolism) in the body. The system detects pairs of γ -rays emitted indirectly by a positron-emitting tracer, which is introduced into the body on a biologically active molecule. 3D images of tracer concentration are then constructed by computer analysis. PET has several useful attributes for human diagnostic or metabolic studies [7]: the positron-emitting radioisotopes of choice are short lived, rather simplistic, safe within the radioactivity ranges permitted; they require only very low amounts of tracer on the order of 10^{-12} – 10^{-9} M, a tolerable risk for organs; and, dynamic tissue uptake and washout of the tracer are measurable in real time (more technical details can be found in [8]).

Glucose metabolism

^{18}F FDG is the tracer for studying CMR_{glc} . It simulates a combination of both glucose transport and subsequent phosphorylation [9]. FDG is transported into tissues, including the brain, at almost the same rate as glucose itself. Like glucose, FDG can be phosphorylated by the first glycolytic enzyme (hexokinase). Unlike glucose, FDG cannot be further metabolized to fructose-6-phosphate by glucose-phosphate-isomerase, so FDG remains trapped in the tissue as FDG 6-phosphate. Hence, FDG uptake represents glucose uptake but without subsequent metabolism toward CO_2 [8].

A β plaques

A β plaques are one of the hallmarks of AD. Several PET tracers have been developed and introduced into clinical practice recently for A β plaques imaging. The best known tracers are ^{11}C -PIB (also known as *N*-methyl- ^{11}C]2-[4'-methylaminophenyl]-6-hydroxybenzothiazole) [10], 4-*N*-(^{11}C -methyl)amino-4'-hydroxystilbene [11] and

2-(1-[6-([2- ^{18}F -fluoroethyl][methyl]amino)-2-naph] ethylidene) malono nitrile [12]. Among these tracers, PIB is the most widely utilized and best characterized in terms of tracer kinetics, modeling and analytic methods. PIB binds to fibrillar A β plaques with high affinity.

Figure 1 shows representative images of FDG-PET and PIB-PET in an AD patient and an age-matched control [13].

• Limitations

PET scans require short-lived radiopharmaceuticals, which are produced by cyclotrons and synthesized at specially adapted on-site chemical laboratories. High costs of cyclotrons and of maintaining the extensive radiochemistry infrastructure have been the major limitations for PET to be used widespread. This limitation restricts clinical PET primarily to the use of tracers labeled with ^{18}F . ^{18}F has a 109.4-min half-life, so that a single large production should permit multiple scans to be performed at multiple PET sites, as is currently the case with ^{18}F -FDG imaging. By contrast, ^{11}C has a half-life of 20.4 min. Each patient dose requires an on-site cyclotron run and radiosynthesis of ^{11}C -PIB immediately before the scan. In addition, PET is limited by its lack of anatomic detail. This usually needs to be complemented by other imaging modalities, such as computed tomography or MRI.

• Magnetic resonance spectroscopy

An MRS spectrum is a plot (intensity vs frequency) of the number of nuclei in different local magnetic field environments. The local magnetic field environment results from an intrinsic chemical shift based on the electronic structure of the molecule in which the nuclei resides and the effects of any interactions with other nuclei. The signal recorded from the object volume of interest (**Figure 2A**) is the sum of all the component signals at the different frequencies. After the spectra acquisition, Fourier transform is used to convert the measured time-varying (time-domain) signal into a list of component frequency (frequency-domain) 'peaks'. Each peak is associated with a specific proton, carbon, or phosphorous position of a biochemical compound such as glucose or glutamate.

^1H - MRS studies have become popular due to the high natural abundance of protons. However, other nuclei, such as ^{13}C and ^{31}P , have also been introduced to study the dynamic

properties of brain metabolism. These MRS techniques have become very useful *in vivo* investigative tools for driving knowledge about the physiological processes of normal aging and the pathophysiological progression of neurodegenerative disorders.

¹H-MRS: neurochemical profile

¹H-MRS permits visualization of a variety of markers of cellular integrity and function, including those of living neurons (*N*-acetyl aspartate [NAA]), glia (myo-Inositol [mI]), high-energy metabolic products (creatine), cell membrane synthesis or degradation (choline), and some less well resolved amino acids, such as glutamate and glutamine (Figure 2B). Characterization of NAA, mI, Creatine and choline may provide a diagnostic tool, a monitor of disease progression and insight into mechanism of treatment response.

¹³C-MRS: neuroenergetics & neurotransmitter cycling

¹H[¹³C] or proton-observed carbon-edited MRS can provide noninvasive measurements of neuroenergetics and neurotransmitter cycling in the human brain [14,15]. With the infusion of ¹³C labeled glucose (or acetate), the mitochondrial glucose oxidation rates in neurons and glia can be measured *in vivo*. Figure 3 shows a diagram of neuronal and astrocyte (a type of glial cell) cell metabolism, and the interplay of neuronal and astrocyte metabolism via the glutamate/glutamine cycle. Both neurons and astrocytes can take up glucose and oxidize it in their mitochondria via the tricarboxylic acid (TCA) cycle. Excitatory glutamatergic neurons, which account for over 80% of the neurons and synapses in the cerebral cortex, release glutamate as a neurotransmitter, most of which is taken up by astrocytes [19,20] and converted to glutamine or oxidized [21,22]. Neurons lack the enzymes required for the *de novo* synthesis of glutamate, and therefore depend on astrocytes to provide substrates for the synthesis of glutamate lost during neurotransmission [23]. The neuron then converts glutamine to glutamate via phosphate-activated glutaminase. The complete series of steps from neuronal glutamate release to the resynthesis of glutamate from glutamine is called the 'glutamate/glutamine cycle'. During the process, the rates of neuronal glucose oxidation ($CMR_{glc(ox), N}$; $\mu\text{mol/g/min}$), astrocytic glucose oxidation ($CMR_{glc(ox), A}$; $\mu\text{mol/g/min}$) and glutamate/glutamine (neurotransmitter)

Figure 1. Fluorine-18-labeled 2-fluoro-2-deoxy-D-glucose-PET and Pittsburgh compound B PET in Alzheimer's disease and normal control individuals. Compared with the controls, FDG (represents brain glucose metabolism) dramatically reduced, while PIB (represents A β deposition) significantly increased in AD patients.

AD: Alzheimer's disease; FDG: Fluorine-18-labeled 2-fluoro-2-deoxy-D-glucose; PIB: Pittsburgh compound B.

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cycling (V_{cyc} ; $\mu\text{mol/g/min}$) can be detected (for a recent review see [18]). In addition to the glutamate/glutamine cycle ¹³C and ¹H[¹³C] MRS can be used to measure GABA synthesis and GABA neurotransmitter cycling [18]. However because of the lower concentration of GABA these measurements need to be performed in larger voxel sizes or at higher magnetic fields.

³¹P-MRS: energy production & redox state

³¹P-MRS enables the noninvasive evaluation of relation concentrations of different intracellular phosphorus metabolites. Substrates of energy metabolism, such as ATP, anorganic phosphate, as well as metabolites of the cell membrane metabolism like phosphomonoesters (PME) and phosphodiesteres (PDE), pH level can be estimated [24]. In addition to static measurements, dynamic property, such as ATP production rate, can also be measured using ³¹P-MRS to perform a method known as saturation transfer [25].

Recently, researchers have extended the ³¹P-MRS method to determine the redox

Figure 2. ^1H magnetic resonance spectroscopy study design and spectrum. (A) A mid-sagittal MRI view of the voxel placement for MRS experiments. (B) A representative spectrum of proton MRS. Cho: Choline; Cr: Creatine; Glx: Glutamate and glutamine; ml: Myo-Inositol; MRS: Magnetic resonance spectroscopy; NAA: N-acetyl aspartate; ppm: Parts per million.

state in the brain by measuring intracellular NAD^+/NADH ratio (Figure 4) [26,27]. The basic function of NAD for humans is to release energy from nutrients in our diet through conversion between NADH (reduced form) and NAD^+ (oxidized form) in various redox reactions to form ATP for supporting all cellular activities and functions. Besides regulating bioenergetics and maintaining mitochondrial function, accumulating evidence has suggested that NAD^+ and NADH are also involved in other biological activities, such as calcium homeostasis [28], antioxidation and oxidative stress, gene expression, immunological functions, aging [29] and cell death [30,31]. The significantly higher intracellular level of NAD under physiological conditions [32] makes NAD the dominant component in regulating intracellular redox state. Therefore, the concentration ratio of NAD^+ and NADH (defined as $\text{RX} = \text{NAD}^+/\text{NADH}$) is widely accepted as a major representation of the intracellular redox state; and its changes have been linked to alterations in energy metabolism and transcription factors under various physiopathological conditions, including aging [33], diabetes, stroke, cancer and epilepsy [34–36].

• Limitations

One of the limitations of MRS is that it provides the metabolic composition of a given voxel, which may include more than one type of tissue. In addition, the measurable metabolites have heterogeneous distribution in the brain [37]. Therefore, results would be different if the

volume of interest is placed in different brain regions. Collectively, the variation can be found between tissue types, as well as within a tissue type at different locations.

There are other limitations specific to ^{13}C MRS. One is the heating that results from the applied radiofrequency (RF) energy to the protons bound to ^{13}C . Although advances in RF coil design have allowed human brain ^{13}C studies to be performed safely even up to 4 Tesla [38,39], concerns remain about RF heating of the eyes, which may be more vulnerable than the brain because of areas of restricted circulation. Another limitation is the requirement for a continuous infusion of the isotopically labeled substrate with venous sampling for fractional enrichment determination. This infusion must occur over a time period (typically 2 h) to capture sufficient kinetic information from spectral time courses for absolute rate estimation using metabolic modeling [40,41]. However, several studies have shown that it is possible to obtain considerable information on metabolism using simplified infusion schemes or oral ingestion [42,43]. Given that the majority of information on absolute and relative rates is derived from the early and steady-state portions of the time course, a significant reduction in the time required for a subject to be in the scanner may be possible [41].

Brain metabolism in normal aging

Cross-sectional FDG-PET studies have repeatedly shown that brain glucose metabolism

Figure 3. The glutamate/glutamine cycle and ^{13}C spectra. (A) The diagram shows the metabolic pathways within glutamatergic neurons and surrounding astroglial cells. Glucose and lactate enter both the glial (V_{TCAa}) and neuronal (V_{TCA_n}) TCA cycles via pyruvate dehydrogenase (V_{PDH}), β -HB is directly incorporated into the neuronal and astroglial TCA cycles, and acetate is near-exclusively incorporated into the glial TCA cycle. Neuronal Glu that is released via neurotransmission is taken up by astroglial cells and converted by Gln synthetase to Gln at a rate proportional to the Glu/Gln cycle. The synthesis of Gln is believed to be exclusively within astroglia and other glial cells. In addition to neurotransmitter cycling, Gln may be synthesized *de novo* starting with the pyruvate carboxylase reaction (V_{PC}). Gln synthesized via pyruvate carboxylase can replace neurotransmitter Glu oxidized in the astrocyte or elsewhere (and be recycled back to the neuron) or leave the brain (V_{efflux}) to remove ammonia and maintain the nitrogen balance. To measure the rates of these pathways, ^{13}C -labeled substrates are used and the flow of ^{13}C isotope into Glu and Gln is measured using ^{13}C MRS. For detailed descriptions of how these pathways are tracked using ^{13}C -MRS, and how isotopically labeled substrates and rates are calculated by metabolic modeling, see [16,17]. **(B)** A ^{13}C spectrum with of [1- ^{13}C] glucose infusion.

α -KG: α -ketoglutarate; β -HB: β -hydroxybutyrate; AcCoA: Acetyl-CoA; Asp: Aspartate; Gln: Glutamine; Glu: Glutamate; Glut1: Glucose transporter 1; Lac: Lactate; MCT1: Monocarboxylate transporter 1; OAA: Oxaloacetate; ppm: Parts per million; Pyr: Pyruvate; TCA: Tricarboxylic acid; V_{efflux} : Efflux rate; V_{TCAa} : Tricarboxylic acid flux rate of astrocytes; V_{TCA_n} : Tricarboxylic acid flux rate of neurons.

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Figure 4. Representative surface-coil localized *in vivo* ^{31}P magnetic resonance spectra of normal cat brains at 16.4 T. Full spectrum is shown at the bottom. Enlarged spectral region (chemical shift from -9 to -11.5 ppm) with phase and baseline correction is shown in the bottom inset (gray lines), superimposed by the model predicted spectrum (red line). The model decomposed individual signals of NAD^+ (black line), NADH (green line) and $\alpha\text{-ATP}$ (blue line) are shown in the middle inset, and the residue of model fittings to the original ^{31}P spectra is plotted in the top of inset.

PCr: Phosphocreatine; PDE: Phosphodiester; Pi: Inorganic phosphate;
PME: Phosphomonoester; ppm: Parts per million.

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decreases with age in healthy subjects, with a differential effect across brain regions. The most frequently reported areas are frontal cortex, anterior cingulate cortex, ventral and dorsal lateral prefrontal/inferior frontal cortex, medial prefrontal areas and precentral and perisylvian areas. [44–47]. These regional changes are paralleled by global CMR_{glc} reductions of approximately 6% per decade [48], although work by Rappaport and colleagues suggest that if vascular factors such as hypertension are controlled for there is little drop before the age of 70 [49]. Interestingly, this rate of CMR_{glc} decrease is consistent with decreased brain structural integrity and the estimated rate of decrease in synaptic density measured post-mortem in healthy elderly brains [50,51]. This

correspondence further suggests that FDG-PET measures and synaptic density are strongly related.

Reduced synaptic density is associated with cognitive impairment [52]. In line with this, longitudinal FDG-PET studies that monitored changes in cognitive performance in healthy aging subjects showed that CMR_{glc} changes in the parieto-temporal cortex correlate with cognitive decline over time. Studies show that CMR_{glc} in memory and learning-related brain regions among normal aging subjects, including hippocampus and entorhinal cortex, accurately predict future decline from normal cognition to mild cognitive impairment (MCI) and AD [53,54]. With a 6–14 years of follow-up scans, the rate of hippocampal CMR_{glc} reduction in healthy elderly individuals was less than 1% per year, whereas patients who decline to MCI and AD had significantly higher rates of CMR_{glc} reductions of 2.4–4.4% per year, respectively.

Reduced CMR_{glc} also suggests mitochondrial dysfunction in aging brain, since normally there is tight coupling between brain glucose uptake and glucose oxidation by the mitochondria. Mitochondrial oxidative phosphorylation of glucose is the predominant mode of energy generation (ATP production) in mammalian species. The decreased functionality of mitochondria has been implicated in the loss of brain function in normal aging and neurodegenerative disease [55]. Using combined infusions of $[1\text{-}^{13}\text{C}]$ glucose and $[2\text{-}^{13}\text{C}]$ acetate with ^{13}C MRS, Boumezbeur *et al.* identified the difference of *in vivo* mitochondrial function between a healthy group of elderly subjects and a group of young adult controls [56]. They found that, compared with young subjects, neuronal mitochondrial metabolism (TCA cycle) and glutamate/glutamine cycle flux was 24–28% lower in elderly subjects. The reduction in individual subjects correlated strongly with reductions in NAA (a neuronal biomarker) and glutamate concentrations consistent with chronic reductions in brain mitochondrial function. In the elderly, glial mitochondrial metabolism increased 30% compared with that of young subjects, indicating age-related changes in glial mitochondrial metabolism [57]. The metabolic changes could not be explained by changes in brain volume/tissue composition and primarily reflected changes at a cellular level.

Redox state is highly associated with mitochondrial function. In a preliminary study using ^{31}P -MRS, Zhu *et al.* found a profound reduction

in the NAD⁺/NADH redox in the healthy aging human brains. They also found that the total NAD level slowly declined in older individuals' brain [27]. It is suggested that the age-related NAD and redox changes are likely the indications of increased oxidative stress, deteriorating cellular metabolic activities and functions occur during the aging process [29].

Brain metabolism in neurodegenerative disorders

• Mild cognitive impairment

MCI is a transitional state between normal aging and dementia. MCI is a diagnosis given to individuals who experience memory problems greater than normally expected with typical aging, but who do not show other symptoms of dementia, such as impaired judgment or reasoning. A person with MCI is at an increased risk of developing AD, which is characterized by brain accumulation of senile A β plaques and hyperphosphorylated tau (neurofibrillary tangles) in the medial temporal lobe (MTL) and cortical areas of the brain [58].

Metabolic changes have been found in MCI using ¹H-MRS. Elevated mI was detected in MCI and quantitative metabolite measures correlate with degrees of cognitive impairment in AD; NAA/mI ratio reduced in the MTL in MCI patients. Moreover, the level of NAA/mI reduction was correlated with the performance on visual recognition memory. Clinical follow-up data suggest that patients with impaired visual recognition memory are more likely to develop probable AD and MCI converters were found to have metabolic changes in the MTL at baseline in comparison to controls [59].

Instead of showing further reduction in glucose metabolism (CMR_{glc}; determined by FDG-PET) compared with the normal aging, Cohen *et al.* has recently found that patients with MCI had a transient increase in CMR_{glc} [60]. They also found that the local elevations in glucose metabolism were found to be associated with A β plaque accumulation (determined by PIB-PET) at the MCI stage of AD. Specifically, CMR_{glc} in anterior cingulate showed positive correlation with PIB in most brain areas in MCI, and CMR_{glc} and PIB retention were positively correlated locally in the precuneus/parietal cortex. They suggested that, in MCI, higher basal metabolism could either be exacerbating A β deposition or increasing the level of A β necessary for cognitive impairment sufficient of the clinical diagnosis of AD. Only

after extensive A β deposition has been present for longer period of time does A β become the driving force for decreased metabolism in clinical AD.

When the severity of hypometabolism of the parieto-temporal cortex and posterior cingulate cortex (PCC) is associated with that of MTL, FDG-PET is able to differentiate MCI patients from normal aging individuals with a 85% sensitivity and 71% specificity, and from AD patients with a 100% sensitivity and 77% specificity [61]. FDG-PET is also able to predict the conversion rate of MCI to AD. In a recent study, Landau *et al.* followed-up patients with amnesic MCI (n = 85) for 1.9 $\pm$ 0.4 years and evaluated the predictors of subsequent conversion to AD and cognitive decline (Alzheimer's Disease Assessment Scale-Cognitive Subscale). They found that patients with MCI converted to AD at an annual rate of 17.2%. Subjects with MCI who had abnormal results on both FDG-PET and episodic memory were 11.7-times more likely to convert to AD than subjects who had normal results on both measures (p $\leq$ 0.02) [62]. Taken together, these results showed that FDG-PET can predict longitudinal cognitive decline, and baseline FDG-PET and episodic memory predict conversion to AD.

• Alzheimer's disease

AD is the most common cause of dementia among the elderly and the seventh leading cause of death in the USA. In addition to the accumulation of senile A β plaques and hyperphosphorylated tau (neurofibrillary tangles), AD is a neurodegenerative disorder characterized by progressive memory deficits, cognitive impairments, and personality changes. Support for an early mitochondrial dysfunction that precedes the histopathological hallmarks described above in AD continues to increase [63–66]. Perturbations of mitochondrial function in terms of altered morphology, compromised electron transfer complexes, and TCA cycle deficiencies have been long identified in post-mortem tissues of Alzheimer's patients [67,68].

Multiple levels of analyses indicate a dysfunction of glucose metabolism and mitochondrial bioenergetics as antecedents to the development of Alzheimer's pathology [69–72]. A decline in brain glucose uptake (and metabolism) can appear decades prior to the onset of histopathological changes inherent in AD [73–75]. A growing evidence has highlighted the importance of FDG-PET as a tool to distinguish AD

from other dementias, predict and track decline from normal cognition to AD, and to identify individuals at risk for AD before the onset of cognitive decline (Figure 1). The FDG-PET results have shown that AD patients show consistent CMR_{glc} deficits in the parietotemporal cortex and PCC and MTL [73,76–77]. As the disease progresses, frontal association cortices become involved, while cerebellum, basal ganglia, striatum, primary visual and sensorimotor cortices remain preserved [73,76]. The *in vivo* pattern of hypometabolism is found in the vast majority of clinically diagnosed AD patients, and in over 85% pathologically confirmed AD cases [13,76].

CMR_{glc} is highly correlated with clinical disabilities in dementia [78]. Clinical AD symptoms always occur accompanied with CMR_{glc} reduction. Furthermore, the characteristic AD-pattern of CMR_{glc} decreases yield high sensitivity in distinguishing AD from normal controls and from other types of dementia, including Lewy body, frontotemporal and cerebrovascular dementia [75]. These findings suggest that FDG-PET is a powerful tool in differential diagnosis of the major neurodegenerative disorders.

MRS has also been used to differentiate AD from normal controls in the context of metabolic abnormality. Characteristic and consistent abnormalities in AD are decreased NAA and elevated mI in the medial temporal and parieto-occipital cortex. These are thought to represent neuronal loss/dysfunction and gliosis, in anatomic distributions that reflect early pathological involvement and atrophy patterns in AD. Less consistent disturbances of glutamine and glutamate, and Cho have also been reported. NAA/mI ratios of the PCC discriminate reliably between AD subjects and normal individuals, and provides a useful clinical test, as an adjunct to structural imaging [79].

In a pioneering study, Lin *et al.* infused three patients with [^{13}C] glucose and found a reduction of neuronal glucose oxidation and glutamate/glutamine neurotransmission rate in AD patients compared with age-matched controls, consistent with TCA impairment. They also reported that the ^{13}C -MRS measures were significantly correlated with measures of neuronal integrity: reductions in NAA concentration, suggesting that impairment of TCA and neurotransmission may be a contributing factor in the cognitive impairment characteristic of AD [80].

Using a novel multivoxel ^{31}P -MRS imaging experimental scheme and an advanced ^{31}P signal

processing technique, Mandal *et al.* determined the pH and neurochemicals from both hippocampal areas in young healthy individuals and patients with AD. They found that a significant decrease of PME and increase of PDE levels in the left and right hippocampus of AD patients was found compared with the control subjects. The pH in AD was found to be negatively correlated with phosphorus creatine level in AD subjects. In the left hippocampus, the increase in pH to alkaline range (in normal aging, pH is decreased to acidic range), significant increments of phosphorus creatine, γ -ATP and PDE, as well as a decrease of PME in AD subjects, provide additional crucial clinical information. They conclude that measures by ^{31}P -MRS can be used as biomarkers for AD and potentially aid in the diagnosis [81].

Although not as direct a measure of metabolism as ^{13}C and ^{31}P MRS, ^1H MRS has the potential of identifying alterations in aging. The ^1H nucleus is the most sensitive for performing MRS studies and spectroscopic images of NAA and glutamate have been obtained with a spatial resolution as good as 5 mm in plane [82]. ^1H MRS studies have found reductions in NAA levels (reflect neuronal status) and increases in mI (reflect glial status) with healthy aging as well as in MCI, and AD [83–86].

In addition to significantly reduced brain metabolism, AD patients showed dramatic A β plaque deposition determined by PIB-PET (Figure 1). Several studies demonstrated significant PIB retention in AD patients as compared with normal controls and MCI, mostly in the frontal cortex, parietotemporal, PCC/precuneus, occipital lobes, thalamus and striatum [87–91], consistent with the known pattern of A β plaques deposition observed at post-mortem. However, the correlation between PIB retention and cognition does not appear to be as strong as with FDG-PET (i.e., glucose metabolism) [92–94].

Despite the fact that in AD A β load and hypometabolism seem to spatially coincide in multiple brain regions, the lack of a direct correlation between the two measures is consistent with the finding that while FDG correlates reliably with the measures of cognitive decline, PIB does not [87,93–96]. These reports are in line with longitudinal data on A β accumulation obtained with PIB, suggesting that A β levels remain relatively stable as cognitive functions deteriorate. There is a growing consensus in the field that once AD pathology is fully present, A β is no longer a

driving factor of the continued cognitive decline [97–99]. This is consistent with the notion that A β plaque distribution does not necessarily correlate with clinical symptoms in AD [100]. As a result, neuroimaging related to metabolic profile, for example, FDG-PET and MRS, have advantages in clinical diagnosis in AD over PIB-PET.

As brain metabolism is critical to sustain brain functions and glucose metabolism is severely impaired in AD, alternative fuel substrate may be useful to maintain the energy balance and thus retard AD progression. In addition to glucose, brain cells use ketone bodies as energy sources (see [2]). As a result, ketone bodies are the main replacement fuel for the brain when glucose availability is insufficient. Clinical studies have shown that the affected brain regions in AD are at least partially viable and that cognition can improve when exogenous supply of ketone bodies to the brain is increased [101,102]. This suggests that although glucose metabolism (glycolysis) may be impaired in AD, other metabolic pathways (e.g., ketone metabolism) may be relatively normal in AD. This also implies that if brain hypometabolism in AD can be retarded or prevented, the clinical fallout may be reduced. The long-term metabolic and cognitive benefits of ketogenic therapies in AD may be further investigated using *in vivo* neuroimaging techniques, such as ^{13}C MRS with 2,4-(13)C(2)-D- β -hydroxybutyrate infusion [103].

Evaluation of risk & treatment efficacy for AD

The e4 allele of the *APOE4* gene is a major genetic risk factor for AD [104]. Individuals that possess one or two *APOE4* alleles have a four- to eight-fold increased risk of developing AD, and the age of onset of AD is reduced by 7–15 years in a dose-dependent manner, compared with the noncarriers [105]. Using FDG-PET, Reimen and colleagues have shown that young *APOE4* carriers (20–39 years old) had abnormally low CMR_{glc} bilaterally in the posterior cingulate, parietal, temporal and prefrontal cortex, regardless of their sex, age, educational level and clinical ratings of neuropsychological test scores. The study suggested that carriers of a common Alzheimer's susceptibility gene have functional brain abnormalities in young adulthood, several decades before the possible onset of dementia [106].

Similar observations were also found in late to middle-age carriers. They had abnormally low rates of glucose metabolism in the same

brain regions as patients with probable AD. Reimen and colleagues further characterized longitudinal CMR_{glc} declines in those individuals and estimated the power of PET to test the efficacy of treatments to attenuate these declines in 2 years, and considered how this paradigm could be used to efficiently test the potential of candidate therapies for the prevention of AD. They studied *APOE4* carriers and noncarriers at 50–63 years of age with a reported family history of Alzheimer's dementia before and after an interval of approximately 2 years. The *APOE4* heterozygotes had significant CMR_{glc} declines in the vicinity of temporal, posterior cingulate, and prefrontal cortex, basal forebrain, parahippocampal gyrus, and thalamus, and these declines were significantly greater than those in the *APOE4* noncarriers. In testing candidate primary prevention therapies, they detected a 25% attenuation in these CMR_{glc} declines with 80% power and p-value of 0.005 in 2 years with subject numbers between 50 and 115 cognitively normal *APOE4* heterozygotes per active and placebo treatment group [5].

PIB-FDG has also been shown to be valuable for the early detection of amyloid pathology in elderly individuals with a known risk factor for AD, including *APOE4* carriers and those with a maternal history of AD. Increased PIB retention in AD-vulnerable brain regions was found in cognitively normal (CN) *APOE4* carriers compared with the noncarriers [107], and in CN individuals with a maternal history of AD relative to those with a paternal history and negative family histories [108]. Although the predictive value of PIB abnormalities in the asymptomatic *APOE4* carriers and adult children of AD-mothers is unknown, PIB measures may be useful to discriminate the risks (high vs low) for AD in CN individuals [107,108].

Conclusion

Brain metabolism is an important biomarker for aging and neurodegenerative disorders. FDG-PET and ^1H -MRS have been widely used to provide important biomarkers to evaluate brain metabolic changes in normal aging and neurological disorders. Newly developed neuroimaging techniques, including PIB-PET, ^{13}C - and ^{31}P -MRS, will enhance the range of metabolic biomarkers and has the promise of both increasing diagnostic power and the ability to monitor treatment, as well as understanding the etiology

of disease. Combining with other imaging techniques (e.g., MRI), genetic effects, interventions and treatment efficacy that can retard brain aging and the onset of neurodegenerative disorders can be effectively evaluated in living human brain.

EXECUTIVE SUMMARY

In vivo neuroimaging for brain metabolism measurement

- PET and fluorine-18 (^{18}F)-labeled 2-fluoro-2-deoxy-D-glucose (^{18}F FDG) tracer have been widely used to measure cerebral metabolic rate of glucose (CMR_{glc}).
- PET-based carbon-11 Pittsburgh compound B tracer has been recently introduced to determine A β plaque deposition *in vivo*.
- Proton magnetic resonance spectroscopy (MRS) has been used to quantify neural metabolites.
- Carbon-13 MRS has been developed to measure *in vivo* mitochondrial glucose oxidative rates in neurons and glia, and glutamate/glutamine neurotransmission rate.
- Phosphorus-31 MRS has been developed to determine intracellular phosphorus metabolites. Newly developed method enables the redox state (an indicator of oxidative stress) *in vivo* by measuring the NAD $^{+}$ /NADH ratio.

Brain metabolism in normal aging

- CMR_{glc} declines with age, particularly in regions of the frontal cortex, anterior cingulate cortex, ventral and dorsal lateral prefrontal/inferior frontal cortex, medial prefrontal areas and precentral and perisylvian areas.
- A carbon-13 MRS study showed that the neuronal mitochondrial metabolism and glutamate/glutamine neurotransmission rate declined 24–28%, but glial mitochondrial metabolism increased 30%, in elderly individuals. The result indicates that brain metabolism is altered in aging brain.
- A phosphorus-31 MRS study showed altered redox state in aging brain; elderly individuals had lower total NAD levels and NAD $^{+}$ /NADH ratio, compared with young healthy controls.

Brain metabolism in neurodegenerative disorders

- Compared with normal aging, CMR_{glc} has a transitioning increase in patients with mild cognitive impairment (MCI).
- The increase of CMR_{glc} (measured by FDG-PET) is correlated with A β plaques deposition (determined by Pittsburgh compound B PET) in MCI.
- FDG-PET is able to differentiate MCI from normal aging and Alzheimer's disease (AD) patients.
- AD patients have significantly impaired glucose uptake, mitochondrial function and energy production in the brain.
- In AD patients, CMR_{glc} correlates better with the measures of cognitive declines than A β accumulation.
- Ketone bodies may be alternative fuels to glucose and thus a potential treatment for AD patients.

Evaluation of risk & treatment efficacy for AD

- Individuals with e4 allele of the *APOE4* gene have high risk for AD. They show dramatic reduction in CMR_{glc} decades before the onset of AD.
- FDG-PET is able to evaluate treatment efficacy in late to middle-age APOE4 carriers.

Conclusion

- Brain metabolism is an important biomarker for aging and neurodegenerative disorders. Multimetric neuroimaging techniques can provide complementary yet multiple levels of metabolic biomarkers to monitor the changes of aging brain and to diagnose the risk for neurodegenerative disorders early.

Future perspective

- Genetic effects, interventions or treatment efficacy (e.g., diet, exercise and pharmaceutical approach) that can retard aging and neurodegenerative disorders can be effectively evaluated in living human brain with the multimodal neuroimaging techniques.

Future perspective

In vivo neuroimaging methods have shown to be promising to detect changes in brain metabolism with age and to early diagnose the risk for neurodegenerative disorders. Imaging methods have been shown to be useful for identifying genetic effects [5,55], effective interventions and treatment efficacy [109,110] that could slow down brain aging and prevent AD. For example, in a recent study, we examined the effects of rapamycin, a pharmaceutical intervention to extended longevity, on brain metabolic and vascular functions in mice modeling AD (hAPP mice) using multimetric neuroimaging methods (PET, MRI and MR angiography) [109]. We demonstrated that chronic rapamycin treatment started after disease onset restored cerebral blood flow and brain vascular density, reduced cerebral amyloid angiopathy and microhemorrhages, decreased amyloid burden, and improved cognitive function in symptomatic hAPP mice. The results from the study may have tremendous translation potential because rapamycin is US FDA approved, and the treatment efficacy of rapamycin in humans can be monitored over time using the same noninvasive neuroimaging methods used in the animal study.

In a preliminary study, we showed that chronic caloric restriction could enhance brain

mitochondrial and metabolic functions in aging rats using ^{13}C -MRS [111]. Taken together, interventions that have positive impacts on brain function (e.g., diet and exercise) can be evaluated by neuroimaging both in preclinical research (e.g., animal models) and in clinical trials.

In addition to metabolic measurements, neuroimaging has also provided other valuable biomarkers to evaluate brain aging and neurodegenerative disorders. MRI has been extensively used to measure brain structural and hemodynamic integrity in normal aging, MCI and AD (see [112]). The multimetric neuroimaging techniques will provide complementary yet multiple levels of biomarkers to monitor the changes of aging brain and to early diagnose the risk for neurodegenerative disorders.

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