BENG 485: Fundamentals of Neuroimaging
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By submitting this essay, I attest that it is my own work, completed in accordance with university regulations. –Ludivine Brunissen

Neurometabolic Biomarkers for the Early Detection of Alzheimer's Disease

by Ludivine Brunissen

Introduction to Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disease, which accounts for roughly two-thirds of late-life dementias, now estimated to affect 10% of people aged 65 and older (Alzheimer's Association, 2018). As of 2018, 5.7 million Americans are living with Alzheimer's disease, accounting for $277 billion dollars in healthcare costs and lost caregiver productivity, and this number is expected to rise to 14 million by 2050 according to the Alzheimer's Association's 2018 report. The first signs of Alzheimer's disease manifest as mild short-term memory impairments, which are often difficult to distinguish from typical signs of aging (Khachaturian et al., 1985). The disease rapidly progresses to moderate and late stages, characterized by forgetting familiar words, disorientation, mood swings, agitation and eventually an inability to control autonomic functions, leading to death three to nine years after the initial diagnosis (Burns et al., 2009). Despite extensive funding for the disease's research, the cause of AD is still poorly understood. It is estimated that around 70% of the risk is genetic, with the ApoE gene, responsible for the production of apolipoprotein E involved in the formation of lipoproteins, showing the most consistently reported involvement in the disease (Ballard et al., 2011). A single copy of the ApoE4 variant of the gene has been found to increase the risk of AD two to fourfold, while homozygotes of the ApoE4 allele have a tenfold increased risk (Linsday et al., 2011). Apolipoproteins usually promote the breakdown of beta amyloid but the ApoE4 isoform has been shown to be less effective, leading to amyloid plaque buildup (Polvikoski et al., 1995). Other risk factors include cardiovascular disease, microvascular abnormalities due to hypertension, head injuries and metabolic diseases such as diabetes (Burns et al., 2009).

No treatments currently exist to reverse the progression of the disease or prevent its onset. Based on the finding that irreversible loss of cholinergic neurons that innervate the cortex and hippocampus is the cause of degeneration and loss of grey matter, acetyl-cholinesterase I inhibition is a common treatment approach aiming to restore cholinergic function and was the basis of some of the first AD drugs such as tacrine (Cognex), donezipil (Aricept), rivastigmine (Exelon) and galantamine (Razadyne) (Francis et al., 1999). However, despite the clear involvement of cholinergic neurons in the disease, cholinesterase inhibitors only offer some relief from early cognitive decline symptoms and do not alter the course of AD. The second commonly prescribed drug class are non-competitive NMDA receptor antagonists such as Memantine, which have been found to slow the clinical deterioration in moderate to severe stages of the disease (Reinsberg et al., 2003). This drug is based on findings of chronic glutamate toxicity in neurodegenerative diseases due to excessive glutamate, leading to the elevation of intracellular Ca^{2+} and excitotoxicity. Recent pharmacological advances currently still in clinical trials have focused on beta-amyloid plaques and neurofibrillary tangles, the two neuropathological hallmarks of Alzheimer's. Tau proteins are typically involved in the stabilization of microtubules but their abnormal hyperphosphorylation leads to the aggregation of neurofibrillary tangles (Buée et al., 2000). The drug AADvac1, a "tau vaccine", induces an immune response against pathological tau proteins (Ondrus et al., 2016). However, a pharmacological cure for AD would require 1) the termination of degeneration, 2) the ability to create new neurons and 3) enabling correct rewiring of these neurons; all three of which still seem like distant goals. Immediate research goals are concerned with retarding the rate of decline and preventing or retarding the rate of onset of the disease, both of which are contingent upon being able to diagnose the disease before neurodegeneration becomes irreversible.

Diagnosis of AD is currently largely based on cognitive testing, with a definite diagnosis performed at autopsy based on the observation of deposits of beta-amyloid plaques and

neurofibrillary tangles. Neuropsychological tests as the mini-mental state examination (MMSE) (Tombaugh et al., 1992), the Dementia Rating Scale (DRS) (Mattis, 1988), the Wechsler Adult Intelligence Scale–Revised (WAIS-R) full-scale IQ (Wechsler, 2014), Wechsler Memory Scale–Revised (WMS-R) logical memory II, and visual reproduction II (Wechsler, 1987) offer good reliability and validity in the diagnosis of AD. However, in early stages of the disease where only mild memory impairments are present, neuropsychological tests are largely unable to differentiate between AD and other types of dementia or normal aging. It has been estimated that an accurate early diagnosis could save up to $7.9 trillion in healthcare costs and significantly improve the management of symptoms (Alzheimer's Association, 2018). Once effective drug therapies are developed to slow the degenerative process, it is critical to apply them as early as possible to halt neurodegeneration.

There has been a recent push for the inclusion of alterations in specific biomarkers, detectable using neuroimaging, in the diagnostic criteria for clinical brain disorders, to enable both earlier detection and a more standardized diagnostic criteria than neuropsychological and behavioral evaluations can provide. The *Global Consortium for Biomarker Standardization* has recently expanded its focus to cerebrospinal fluid biomarkers such as beta-amyloid and phosphorylated tau, both of which have been associated with pre-clinical dementia stages (Carrillo et al., 2013). Furthermore, a growing body of evidence has noted a range of defects in energy metabolism in Alzheimer's patients including decreases in parietal and temporal glucose metabolism preceding cognitive deficits (Duara et al., 1986) and abnormal mitochondrial glucose metabolism (Sims et al., 1987). Neurometabolic biomarkers have the potential to improve the accuracy of preclinical AD detection and enable more effective early intervention before the manifestation of any cognitive deficits. [^{18}F]-fluorodeoxyglucose Positron Emission Tomography, or ^{18}F-FDG PET, and ^{1}H Magnetic Resonance Spectroscopy are two potential neuroimaging techniques which can help identify metabolic changes associated with AD.

This paper will focus on identifying potential biomarkers for a pre-symptomatic diagnosis of Alzheimer's disease, namely neuroenergetic biomarkers such as decreases in glucose metabolism and cytochrome oxidase activity, as well as changes in the concentrations of neurometabolites such as N-acetylaspartate and myoinositol. First, ^{18}F-FDG-PET imaging of decreases in glucose metabolism in the posterior cingulate, temporal and parietal cortices of early AD patients, ApoE4 carriers and mild cognitive impairment (MIC) patients will be outlined. Next, ^{1}H-MRS determination of N-acetylaspartate and myoinositol changes in prodromal and clinical AD stages will be reviewed. Lastly, decreases in the activity of a key energetic enzyme, cytochrome oxidase, will be discussed as an early marker of the illness implicated in mitochondrial dysfunction. Each proposed biomarker will be reviewed for its diagnostic and prognostic potential and the current limitations and future steps of each neuroimaging method will also be addressed.

i) *^{18}F-FDG-PET imaging of glucose metabolism*

First, in a study by Minoshima et al. (1997), energy metabolic abnormalities in the posterior cingulate cortex of AD patients in very early stages were investigated using ^{18}F-FDG PET, an imaging technique widely used in cancer patients, following the consistent reports of parietotemporal metabolic reduction in AD. Unlike other neuroimaging techniques such as MRI, PET can differentiate Alzheimer's disease from normal aging as well as other dementias, making it especially clinically relevant (Minoshima et al., 1997). ^{18}F-FDG PET uses an ^{18}F analog of the 2-[^{14}C]-deoxyglucose method as a radioactively labelled glucose-6-phosphate tracer, allowing for measures of glucose utilization in local brain regions (Sokoloff, 2004). The positron-emitting radionuclide fluorine-18 is substituted for the hydroxyl group of the glucose molecule and the ^{18}F glucose analog is injected intravenously to track glucose uptake by various tissues. An operational equation is used to compute local rates of glucose metabolism (CMR_{glc}) from the kinetic analyses of arterial glucose and ^{18}F glucose concentrations in local tissues (Sokoloff, 2004).

PET can be used to detect the radioactive tracer and color-coded quantitative images of local CMR_{glc} rates are generated to visualize local glucose utilization in the brain with a spatial resolution of 100-200 m (Sokoloff, 2004). Since glucose is the main energy source of the brain, glucose utilization can be used as a correlate for neural activity and is coupled to cerebral blood flow (CBF).

In this study by Minoshima et al. (1997), 66 patients with probable Alzheimer's disease of varying severity levels (Clinical Dementia Ratings of 0.5 (questionable) in 5 patients, 1 (mild) in 39 patients, 2 (moderate) in 14 patients and 3 (severe) in 8 patients) were subjected to ^{18}F-FDG PET imaging. Cerebral glucose metabolism was measured in the posterior cingulate cortex of subjects, and linear regression analysis was performed from metabolic activities and MMSE scores on each brain pixel to predict the metabolic patterns of even earlier disease stages where behavioral and cognitive changes are absent, defined as an MMSE score of 30 (Figure 1). MMSE scores of 24-30 points, out of 30 possible points, are interpreted to indicate normal cognition, while MMSE scores between 19-23, 10-18 and 0-9 points reflect mild, moderate and severe cognitive impairments, respectively (Mungas et al., 1991). This predicted metabolic pattern at early stages was then compared with the scans of very early AD patients whose diagnosis was later confirmed (Figure 2). Measures of cortical metabolic activity were extracted on a pixel-by-pixel basis and normalized to the activity of the pons, which is preserved in Alzheimer's disease (Minoshima et al., 1997). The authors found metabolic reductions of 21-22% in the posterior cingulate cortex and cinguloparietal transitional area of early AD subjects compared to normal controls (Figure 2). As shown in Figure 1, CMR_{glc} measures in the posterior cingulate cortex and cinguloparietal transitional area decreased markedly from the control group (N) to subjects with an MMSE score of 30, extrapolated from linear regression analysis as a very early stage of AD. Further reductions in metabolic activity were noted in the MMSE 20, 10 and 0 groups, with the MMSE 0 group showing the most impaired glucose metabolism. Despite widespread decreases

in the cortical metabolic activity of the MMSE 0 group, metabolism was relatively preserved in the primary sensorimotor cortex, occipital cortex and cerebellum of all subjects in the AD group, which makes sense since these are some of the last structures to be affected by the disease. When comparing the CMR_{glc} patterns predicted from linear regression in the MMSE 30 group to the actual CMR_{glc} measurements of very early AD patients with little to no cognitive decline, whose diagnosis was later confirmed, we find that the same significant metabolic reductions are seen in the posterior cingulate and cinguloparietal area of the very early AD patients as was observed in the predicted MMSE 30 group. This study provides especially strong evidence for the involvement of the posterior cingulate cortex and cinguloparietal transitional area's glucose metabolism in the very first stages of AD, by confirming their predicted CMR_{glc} measures with that of early AD patients. Furthermore, it testifies to the ability of linear regression analysis to extrapolate data from patients at various stages in order to identify regions involved in the earliest stages of the disease where patients do not show any behavioral alterations and therefore cannot easily be recruited for imaging studies. Previous PET studies of glucose metabolism have shown consistently reduced glucose metabolism in the parietal and temporal cortices, consistent with reductions in the "Early AD" group (Minoshima et al., 1995).

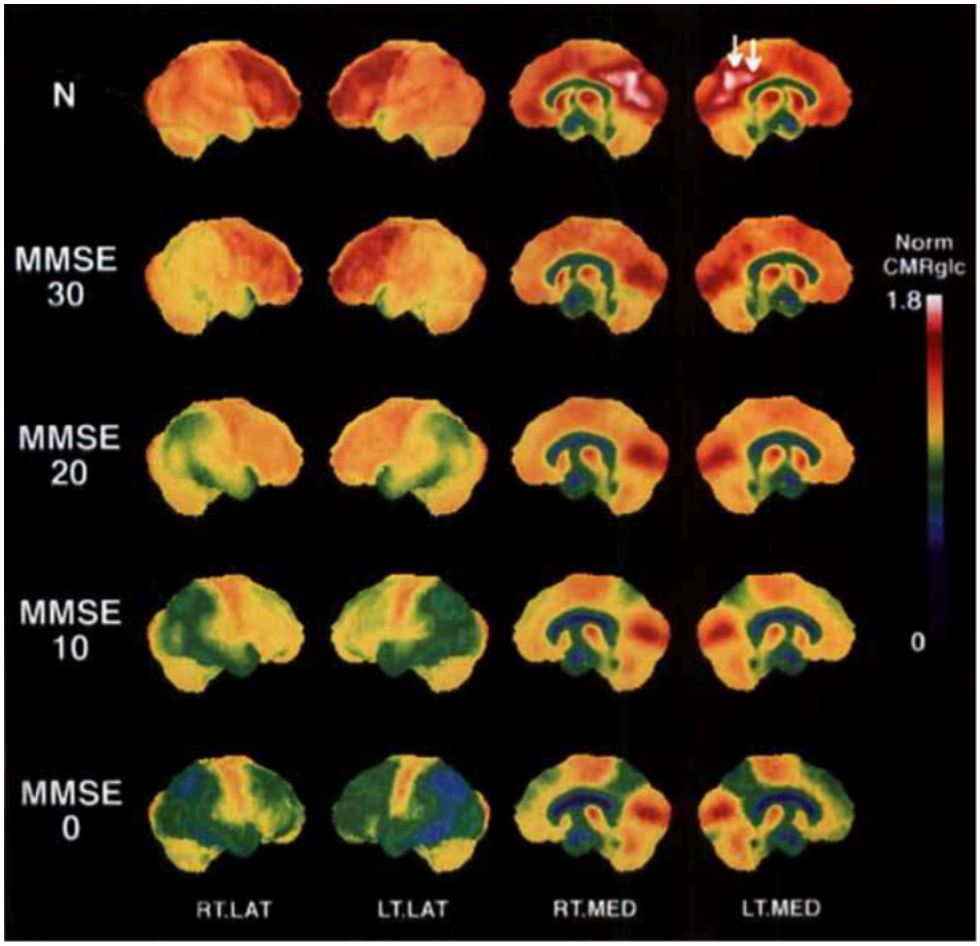

Figure 1: Cerebral glucose metabolic activity (CMR_{glc}) in Alzheimer's disease subjects with MMSE scores of 20, 10 and 0 and predicted cerebral glucose metabolic activity for subjects with an MMSE score of 30, compared to age-similar control subjects (N). The predicted metabolic activity of patients with MMSE scores of 30 is extrapolated from linear regression analysis of CMR_{glc} measures from subjects with MMSE scores of 0 to 23. Cerebral metabolic activity is normalized to pontine activity. RT.LAT: right lateral, LT. LAT: left lateral, RT. MED: right medial, LF. MED: left medial. Metabolic activity in the posterior cingulate cortex and cinguloparietal transitional area is indicated by the two white arrows in the left medial view of the N group. Reprinted from Minoshima et al. (1997).

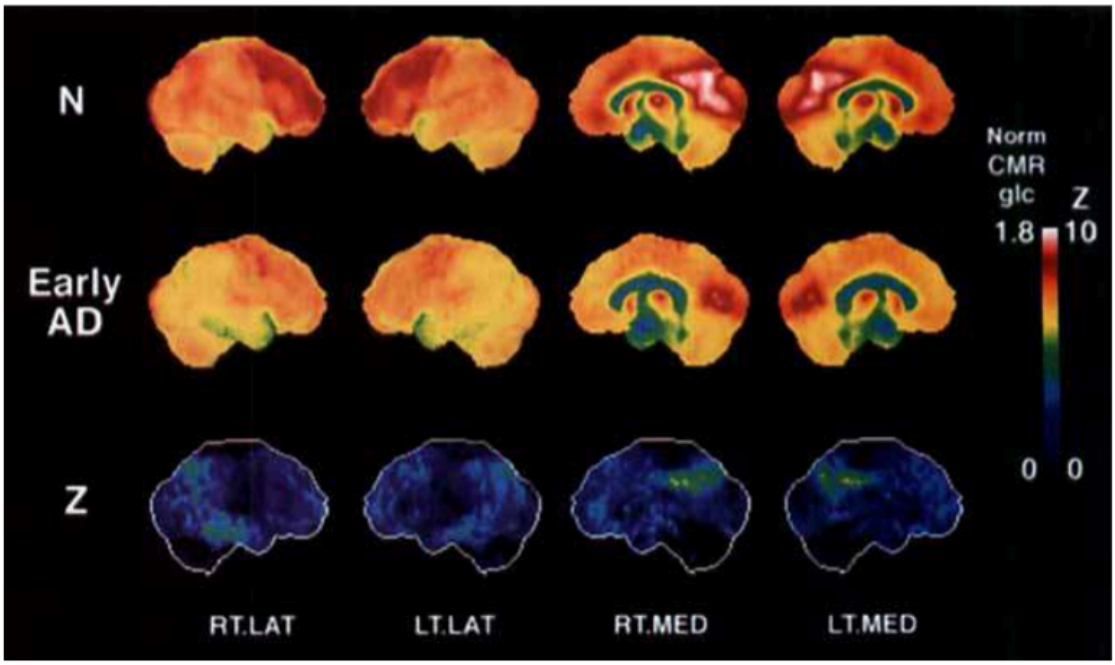

Figure 2: Cerebral metabolic activity in patients with very early AD (Early AD) compared to normal controls (N). Group-averaged images were compared and converted to Z score images (Z). RT.LAT: right lateral, LT. LAT: left lateral, RT. MED: right medial, LF. MED: left medial. Significant metabolic reductions can be seen in the posterior cingulate cortex and cinguloparietal area of the Early AD group compared to normal controls. Milder metabolic reductions can be seen in the parietal and temporal cortices. Reprinted from Minoshima et al. (1997).

A discordance between these findings of early metabolic reductions and the absence of pathological signs in the posterior cingulate has been noted by the authors. Indeed, no increase in neurofibrillary tangles of amyloid plaques was found in the posterior cingulate of AD patients (Minoshima et al., 1997). This raises the possibility of functional alterations in the posterior cingulate without structural changes in very early stages of AD. It is plausible to think that functional alterations may precede or even disagree with any structural abnormalities, which would make metabolic changes measured via ^{18}F-FDG PET an even earlier biomarker than neurofibrillary tangles and amyloid plaques. The authors have hypothesized that this early metabolic reduction in the posterior cingulate cortex may be due to a "global derangement of cortical glutamatergic neurons which interconnect the posterior cingulate to other cortical structures" (Minoshima et al., 1997). Thus, while the earliest affected structure in AD was thought to be the entorhinal cortex based on the distribution of neurofibrillary tangles, ^{18}F-FDG

PET imaging of metabolic reductions was able to implicate the posterior cingulate cortex in very early stages of the disease and is readily able to differentiate between pre-clinical AD and other dementias. This suggests that reductions in glucose metabolism in the posterior cingulate and cinguloparietal area could potentially constitute some of the earliest biomarkers of the disease detectable through imaging.

Next, a similar ^{18}F-FDG PET study by Small et al. (2000) looked at cerebral glucose metabolism in nondemented individuals at genetic risk of Alzheimer's disease with normal memory performance and ApoE4 carriers, and compared it to that of controls and subjects with a clinical AD diagnosis. Combining genetic risk and functional imaging measures is another promising alternative to Minoshima et al's (1997) previously described method for the detection of preclinical AD. Lower parietal, temporal and posterior cingulate cerebral glucose metabolism has been reported in patients with clinical AD (Small et al., 2000). The authors found the lowest metabolic rates in the AD group, intermediate rates in the ApoE4 carriers and the highest rates in the control group, as expected. Significantly lower glucose metabolism was found in the inferior parietal and temporal cortices of the asymptomatic ApoE4 carriers, two areas which show early deposition of plaques and neurofibrillary tangles, as well as the posterior cingulate, which showed a 5% reduction in metabolic rate (Small et al., 2000). Furthermore, following a 2-year follow-up the ApoE4 carriers with the lowest baseline metabolism showed the most severe cognitive decline.

Another ^{18}FDG-PET study by Drzezga et al (2003), measuring relative glucose cerebral metabolic rates ($rCMR_{glc}$) in patients with mild cognitive impairment (MCI), considered a transitional stage to clinical dementia, similarly found that prognostic subgroups can accurately be predicted in subjects with comparable neuropsychological status from their initial pattern of cortical metabolism. Indeed, MCI subjects who went on to develop clinical AD a year later showed the lowest $rCMR_{glc}$ at the time of imaging. Interestingly, in addition to replicating the

decreases in metabolic activity in the parietal and posterior cingulate cortices observed by Minoshima et al. (1997) and Small et al. (2000), they also observed newly emerging metabolic declines in the bilateral prefrontal cortical regions; regions which have rarely been implicated in early AD. According to Fabrigoule et al.'s (1998) hypothesis, a prefrontal metabolic decrease may lead to an early functional deterioration of control processes, responsible for widespread connectivity disruptions later in the disease course.

In conclusion, Small et al. (2000) and Drzezga et al.'s (2003) observations that metabolic deficits predict cognitive decline in pre-symptomatic individuals further supports the use of glucose metabolism as a marker for the onset and progression of AD and indicates that AD pathogenesis occurs well before the first apparition of symptoms. This finding is consistent with the knowledge that the brain "compensates for regional neuronal dysfunction, leading to normal clinical neuropsychological performance, although biological degeneration is occurring." (Minoshima et al., 1997). Both studies underscore the relevance of ^{18}FDG-PET as an objective measure of functional declines in the earliest stages of the disease as well as a prognostic measure. This ability to detect pre-symptomatic metabolic changes testifies to PET's sensitivity and applicability to the detection of bioenergetic biomarkers. All three studies discussed reported well replicated metabolic declines in the posterior cingulate, temporal and parietal cortices in three different subject populations corresponding to pre-symptomatic stages of AD (early AD, ApoE4 carriers and MCI), testifying to these findings' reliability. It would therefore appear that declines in glucose metabolism can be used as reliable early biomarkers in asymptomatic individuals to help develop more sensitive diagnostic schemes and prognoses for patients.

Despite these promising results, some limitations still exist before ^{18}FDG-PET imaging of neuroenegetic biomarkers can become the standard for early AD diagnosis. A review study by Smailagic et al. (2015) analyzed the "diagnostic accuracy of the ^{18}F-FDG PET index test for detecting people with MCI at baseline who would clinically convert to Alzheimer's disease at

follow-up." They noted a sensitivity for conversion from MCI to AD between 25% and 100% based on various studies, which is a wide range of prognostic accuracy (Smailagic et al., 2015). This may be due to clinical heterogeneity in subjects across studies, so future ^{18}FDG-PET should seek to adopt more standardized clinical criteria for their AD, MCI and/or early AD groups. Numerous PET studies have also ignored the global baseline neuronal activity signal in their analysis, which is a key factor in avoiding the misinterpretation of glucose metabolism changes (Mortensen et al., 2018). Furthermore, longitudinal ^{18}F-FDG PET studies like Drzezga et al (2003) and Minoshima et al. (1997) should also make sure to obtain longitudinal scans from healthy controls in case declines in cerebral glucose metabolism are also observed as a consequence of healthy aging, in order to adjust metabolic declines in the clinical group for the effects of healthy aging if need be.

Lastly, the most significant limitation of ^{18}FDG-PET studies of glucose metabolism concerns inconsistencies in the way in which CMR_{glc} is measured (relative vs absolute measures). Indeed, all three studies detailed in this paper use relative CMR_{glc} measurements, derived from the normalization of absolute CMR_{glc}. Mortensen et al. (2018) have analyzed some of the pitfalls of global mean normalization (GMN), frequently employed in PET studies, which "reveals differences from healthy individuals as fractional changes across regions relative to a global mean" (Mortensen et al., 2018). The authors compared CMR_{glc} measurements with and without GMN between healthy controls and subjects under various physiological and clinical states (congenitally blind, sedated, etc.). They found global alterations in CMR_{glc} without GMN in the subjects with altered physiological or clinical states compared to controls. Use of GMN instead showed regional and bidirectional CMR_{glc} changes and a loss of global information. Mortensen et al. (2018) advocate for the use of a quantitative approach to baseline metabolic activity without GMN, in order to both preserve global CMR_{glc} changes and allow for the detection of regional CMR_{glc}.

When comparing the normalized results from Minoshima et al. (1997), Drzezga et al. (2003) and Small et al. (2000) to ^{18}FDG-PET studies which make use of absolute measures, one can see that the glucose metabolic reductions appear much more global when observed at absolute scales than the regional differences in the posterior cingulate and temporoparietal cortices reported with normalization. An ^{18}FDG-PET study of CMR_{glc} by Borght et al. (1997) compared the patterns of metabolic reduction in subjects with Alzheimer's disease and Parkinson's disease (PD) to healthy controls. Both absolute and normalized cerebral metabolic rates were assessed using pixel-by-pixel analyses. The authors noted significant global glucose metabolic declines in both AD (5.47 ± 0.63 mg/100 g/min, $p < 0.0001$) and PD groups compared to controls (7.22 ± 0.85 mg/ 100 g/ min). Cerebral metabolic rates were then normalized, with each pixel representing a mean metabolic value averaged across all subjects in each group, by averaging across both hemisphere and using the pons as a point of reference, similarly to what was done by Minoshima et al. (1997). Normalization of CMR_{glc} led to the identification of disease specific regional CMR_{glc} alterations in AD and PD groups, with the most severe hypometabolism observed in the posterior cingulate of AD patients. Therefore, despite the fact that AD and PD patients showed similar hypometabolism, disease specific regional accentuations were confirmed, which supports ^{18}FDG-PET's use in differentiating between AD and other neurodegenerative conditions using early hypometabolism in the posterior cingulate as a biomarker. This study shows the importance of reporting both absolute and relative/normalized findings to obtain both a global and regional picture of metabolic reductions in AD.

Another study by Tohgi et at. (1998) looked at regional oxygen cerebral metabolic rates ($rCMRO_2$), closely related to the rate of glucose oxidation, using $^{15}O_2$-PET and noted similar global declines in oxygen metabolism throughout the frontal, parietal and temporal cortices of patients with senile dementia of Alzheimer's type (SDAT). While most PET studies have looked at glucose metabolic rates, cerebral blood flow (CBF) is coupled to both CMR_{glc} and $CMRO_2$ at

rest (while CBF is only coupled to CMR_{glc} during focal activity). Absolute $CMRO_2$ measures at rest displayed in Figure 3 show global declines in oxygen metabolism in the frontal, parietal and temporal cortices of the SDAT group, similarly to the global glucose hypometabolism noted by Borght et al. (1997). These global findings have also been corroborated by calibrated fMRI studies of resting oxygen metabolism in Alzheimer's patients (Lajoie et al., 2017).

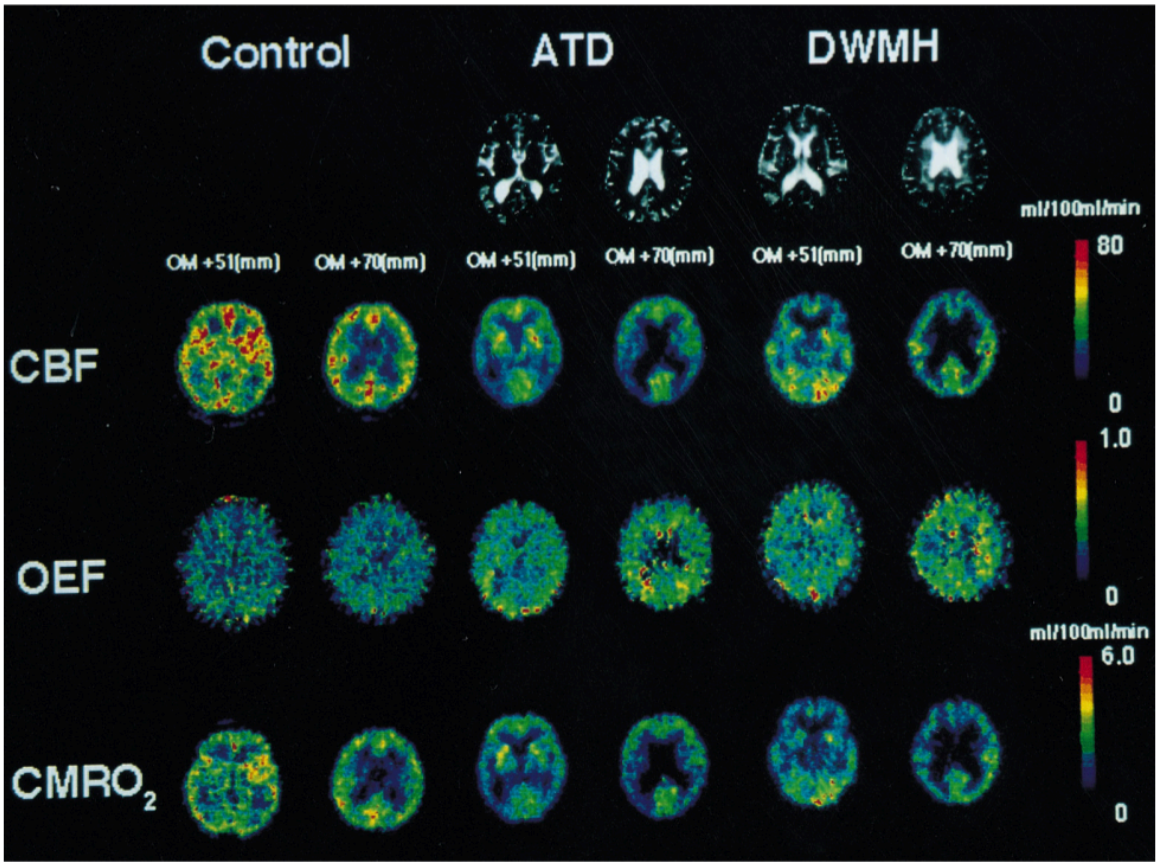

Figure 3: Absolute $CMRO_2$, oxygen extraction fraction (OEF) and cerebral blood flow (CBF) in a patient with SDAT (ATD group), a demented patient with deep white matter high signal (DWMH) and a control subject at rest. Global declines in CBF and $CMRO_2$ are observed in the SDAT and demented DWMH groups. Reprinted from Tohgi et at. (1998).

Thus, absolute CMR_{glc} and $CMRO_2$ studies of metabolism in Alzheimer's disease point to much more global changes than were observed from a relative scale, which seem to overlap with other neurodegenerative conditions such as PD or other dementias. However, regional effects specific to AD such as hypometabolism in the posterior cingulate cortex are well replicated, supporting their use as early clinical biomarkers. It is crucial however that future

studies of glucose metabolism report both absolute and relative/normalized findings, or replace global mean normalization methods, which tend to obscure global changes, with the quantitative approach proposed by Mortensen et al. (2018). Lastly, absolute CMR_{glc} measures using ^{18}FDG-PET require continuous arterial blood sampling throughout the procedure, which is inconvenient in a diagnostic clinical setting. Alternative approaches involve determining a "ratio of dose injected and body weight as a proportional index of arterial input" (Mortensen et al., 2018). Therefore, some precautions need to be taken to confirm ^{18}F-FDG PET's sensitivity and prognostic value before ^{18}F-FDG PET imaging of glucose metabolism changes can be used routinely in a clinical setting, especially since ^{18}F-FDG PET is a high-cost investigation. Absolute CMR_{glc} measurement can be adapted to clinical settings for use along with normalized measures.

ii) *^{1}H-MRS imaging of neurometabolite changes*

Secondly, another neuroimaging method, in addition to ^{18}FDG-PET, which has gained interest in recent years for the imaging of early AD biomarkers, is ^{1}H Magnetic Resonance Spectroscopy or ^{1}H MRS. MRS is based on the magnetic properties of atomic nuclei and relies on the principle that nuclei with an odd number of protons or neutrons possess an intrinsic magnetic moment under strong magnetic fields (de Graaf, 2004). Both MRI and MRS rely on the same magnetic resonance principles: first, nuclei with a net spin are subjected to a strong external magnetic field to polarize the sample. Next, the nuclei are excited by a radiofrequency pulse at the corresponding Larmor frequency, calculated from the product of the gyromagnetic ratio of that isotope and the magnetic field magnitude, B_0 (de Graaf, 2004). Relaxation back to a lower energy state, or free induction decay, is converted to a frequency signal displayed in MRS. Specific metabolites can be detected *in vivo* and displayed as frequency peaks representing their abundance. The location of a specific metabolite on the x-axis is determined by shifts in resonance frequency, based on the chemical environment of the nucleus of interest. The most

commonly used MRS isotope is ^{1}H for its high sensitivity due to its abundance in water and fat containing tissues and gyromagnetic ratio (de Graaf, 2004). Some drawbacks of the ^{1}H isotope include high water resonance and a narrow chemical shift range.

Kantarci et al. (2000) compared ^{1}H MRS findings in the superior temporal lobe, posterior cingulate cortex and medial occipital lobe of subjects with MCI, probable AD and normal controls. They specifically considered the following neurometabolites: N-acetylaspartate (NAA)/creatine (Cr) ratios and myoinositol (MI)/Cr ratios. Creatine is a physiologically stable metabolite, used as a reference. NAA is synthesized in neuronal mitochondria and decreases have been used as markers of neuronal loss. MI is the most abundant neurometabolite, located in glia, and known for its osmolytic properties (Kantarci et al., 2000). The authors noted "significantly lower NAA/Cr ratios in AD patients compared to both MCI and normal control subjects in the left superior temporal and the posterior cingulate volumes of interest (VOI)" (Kantarci et al., 2000) as well as significantly higher MI/Cr ratios in the posterior cingulate VOI of MCI and AD subjects compared to controls (Figure 4). By positioning the ^{1}H MRS voxels in "areas of the brain involved at different neurofibrillary stages" and considering patients at different stages of the disease (AD, MCI and controls), the authors aimed to assess the temporal course of alterations in the metabolites measured by ^{1}H MRS. Their results suggest that the first alteration is an increase in MI/Cr in the posterior cingulate, since MCI subjects showed significantly higher MI/Cr than controls (Figure 4), followed by a decrease in NAA/Cr which develops later in the disease (Kantarci et al., 2000). The biological mechanism by which AD leads to an increase in MI is still unclear, but it is plausible to think that ^{1}H MRS imaging of MI/Cr ratios in areas implicated in the earliest stages of the disease could serve as early biomarkers. A possible relationship has been suggested between elevated MI, glial activation and inflammation in the pathogenesis of Alzheimer's (Kantarci et al., 2000).

However, several limitations affect the use of ^{1}H MRS for early diagnostic purposes. First, several patients with AD take drugs which affect neurometabolite concentrations, such as donepezil hydrochloride (taken by 14 out 21 AD subjects in this study), which increases NAA/Cr ratios (Kantarci et al., 2000). This could have diminished the "magnitude by which NAA/Cr ratios in probable AD patients differed from MCI and control subjects," (Kantarci et al., 2000) leading to a false negative. Another complication of NAA/Cr detection using MRS is tissue heterogeneity. Specifically, white matter has a lower creatine content so using white matter voxels can lead to false negatives, while using volumes with substantial grey matter may overestimate the NAA/Cr ratio (de Graaf et al., 2004). Linear regression analysis should be used to compensate for tissue heterogeneity. Furthermore, the authors did not note any significant metabolite changes in ApoE4 carriers versus noncarriers, which is difficult to reconcile with Small et al's (2000) findings of significant glucose metabolism reductions in the parietal, temporal and posterior cingulate cortices of asymptomatic ApoE4 carriers. It could be that there are indeed no observable neurometabolite changes in ApoE4 carriers, which would make ^{18}F-FDG-PET a better diagnostic imaging method than ^{1}H-MRS for this at-risk group. However, it could also be that these conflicting findings are due to the echo time used in this study, or the lack of clinical heterogeneity in the patient populations used across studies which is a significant issue for both ^{18}F-FDG-PET and ^{1}H-MRS studies of AD biomarkers. The short echo time (30msec) used in this study has for instance been associated with metabolite quantization errors due to an irregular baseline, as well as an artificial elevation of NAA levels due to its overlap with the glutamine, glutamate, GABA (Glx) peak (Cinafoni et al., 2011). Lastly, the significant overlap in metabolite ratios between the three clinically defined group in this study limits the value of ^{1}H-MRS for the clinical diagnosis of early AD.

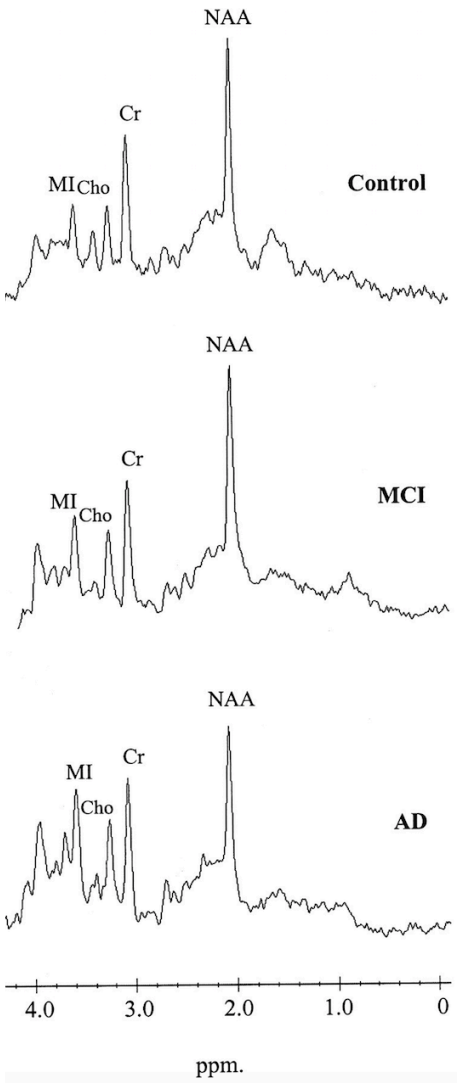

Figure 4: ^{1}H MRS spectra obtained from the posterior cingulate VOI of a control, MCI and probable AD subject. An echo time of 30 msec was used. Lower NAA/Cr is found in the AD group compared to the control and MCI group. Higher MI/Cr is found in both the AD and MCI groups compared to controls ($p<0.01$). Reprinted from Kantarci et al. (2000).

iii) *Cytochrome oxidase activity and early energetic changes*

Another active area of research regarding energetic biomarkers for Alzheimer's disease, beyond glucose metabolism, is concerned with the activity of a key enzyme in the oxidative phosphorylation pathway of ATP synthesis: cytochrome oxidase, following the reports of decreased activity in the platelets of Alzheimer's patients (Parker et al., 1990). Cytochrome oxidase complexes play a key role in oxidative ATP production as electron carriers in the electron transport chain, making this enzyme's catalytic activity a good marker for neuronal activity (Wong-Riley, 1989). Following the findings that defects in glucose metabolism are

implicated in the pathogenesis of AD, Mutsiya et al. (1994) examined the activities of enzymes involved in the oxidative phosphorylation pathway in the frontal, temporal, parietal and occipital cortices of postmortem Alzheimer's patients. Autopsied brain specimens of patients with clinically confirmed AD were obtained, and purified mitochondria and tissue homogenates were assayed for complexes I-V activity. A significant decrease (25-30% compared to age-matched controls) in complex IV cytochrome oxidase activity was noted across all cortices (Mutsiya et al., 1994). A defect in cytochrome oxidase activity could contribute to the stunted energy metabolism caused by decreased glucose utilization and contribute to neurodegeneration. Several hypotheses have been proposed by the authors to explain this finding. First, it could be that reduced cytochrome oxidase activity is the result and not the cause of reduced energy requirements in AD brains as a consequence of neuronal degeneration. However, findings that cytochrome oxidase levels are normal despite reduced activity and that other oxidative phosphorylation enzymes are unaffected suggest that this alteration does not merely result from degeneration (Mutsiya et al., 1994). Oxidative damage to mitochondrial DNA has been proposed as an alternative mechanism (Mecocci et al., 1994).

Cytochrome oxidase has been implicated in AD pathogenesis since sodium azide inhibition of cytochrome oxidase leads to a marker increase in amyloidogenic fragments in neurons (Gabuzda et al., 1994) and reductions in the closely maintained ATP levels can activate kinases which phosphorylate tau protein, which is one mechanism for neurofibrillary tangles formation. Therefore, it is still unclear from this study whether an impaired complex IV cytochrome oxidase activity is the primary or secondary consequence of AD, but this further implicates energy metabolism defects in the pathogenesis of AD. It could be that cytochrome oxidase's reduced activity is an independent finding which is part of a broader range of energetic impairments implicated in AD. Reduced cytochrome oxidase activity has been shown experimentally following "deafferentation due to a decrease in neuronal firing and Na/K ATPase activity," (Mutsiya et al., 1994) which could make this a useful biomarker for the first

neuronal losses in early AD. Furthermore, Lin et al. (2006) have associated cytochrome oxidase deficits with mitochondrial dysfunctions, which have been heavily implicated in AD pathogenesis. Specifically, Crouch et al. (2005) and Manczak et al. (2006) have reported an interaction between amyloid beta and mitochondria, leading to an inhibition of cytochrome oxidase and increases in the generation of free-radicals, responsible for oxidative stress and damage. Oxidative damage has been noted in very early stages of the disease, well before the onset of amyloid plaque pathology (Lin et al., 2006). Further studies are needed to see whether or not cytochrome oxidase activity can be assayed in pre-clinical AD subjects or ApoE4 carriers to serve as an alternative biomarker to glucose metabolism.

Conclusion

Throughout this paper, the main biomarkers for the early detection of Alzheimer's disease have been reviewed. Glucose metabolism deficits in the posterior cingulate, temporal and parietal cortices of pre-symptomatic Alzheimer's patients, ApoE4 carriers and MIC patients have been well replicated by ^{18}F-FDG-PET studies (Minoshima et al. (1997), Small et al. (2000), Drzezga et al. (2003)) and shown to accurately predict cognitive decline upon follow-up.
The observation of metabolic deficits in pre-symptomatic subjects supports the use of glucose metabolism as a marker for the onset and progression of AD and testifies to PET's sensitivity for the detection of bioenergetic biomarkers. Next, NAA/Cr decreases and MI/Cr increases were observed using ^{1}H-MRS, with MI increases occurring earlier in the posterior cingulate, with a possible association with inflammation, and NAA decreases developing later in the disease as a result of neuronal loss and dysfunction. Lastly, reductions in complex IV cytochrome oxidase activity have been observed as another early bioenergetic marker and may be associated with mitochondrial dysfunctions. Neurometabolic biomarkers have the potential to improve the accuracy of preclinical AD detection to enable more effective early intervention before the manifestation of any cognitive deficits and significant neuronal loss. ^{18}F-FDG-PET imaging of

glucose metabolism is perhaps the closest to use in a clinical setting compared to ^{1}H-MRS and enzyme assays. However, there is a dire need for reporting of both absolute and normalized CMR_{glc} measures or for the use of an alternative quantitative method to global mean normalization to ensure that regional effects are considered in the context of some of the more global changes in metabolism reported by Borght et al. (1997) and Tohgi et al. (1998). Findings concerning metabolic biomarkers for Alzheimer's disease have the potential to inform future therapies by emphasizing the early targeting of energy metabolism, mitochondrial processes and inflammation pathways. Similar neuroimaging approaches can also be applied to other neurodegenerative diseases such as Parkinson's disease, which also still lack early diagnostic biomarkers.

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