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# Mitochondrial dysfunction, oxidative stress, neuroinflammation, and metabolic alterations in the progression of Alzheimer's disease: A meta-analysis of *in vivo* magnetic resonance spectroscopy studies

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## Abstract

Accumulating evidence demonstrates that metabolic changes in the brain associated with neuroinflammation, oxidative stress, and mitochondrial dysfunction play an important role in the pathophysiology of mild cognitive impairment (MCI) and Alzheimer's disease (AD). However, the neural signatures associated with these metabolic alterations and underlying molecular mechanisms are still elusive. Accordingly, we reviewed the literature on *in vivo* human brain $^{1}H$ and $^{31}P$-MRS studies and use meta-analyses to identify patterns of brain metabolic alterations in MCI and AD. 40 and 39 studies on MCI and AD, respectively, were classified according to brain regions. Our results indicate decreased N-acetyl aspartate and creatine but increased myo-inositol levels in both MCI and AD, decreased glutathione level in MCI as well as disrupted energy metabolism in AD. In addition, the hippocampus shows the strongest alterations in most of these metabolites. This meta-analysis also illustrates progressive metabolite alterations from MCI to AD. Taken together, it suggests that 1) neuroinflammation and oxidative stress may occur in the early stages of AD, and likely precede neuron loss in its progression; 2) the

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Author contributions

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Declaration of interest

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hippocampus is a sensitive region of interest for early diagnosis and monitoring the response of interventions; 3) targeting bioenergetics associated with neuroinflammation/oxidative stress is a promising approach for treating AD.

**Keywords**

Mild cognitive impairment; Alzheimer's disease; Neuroinflammation; Oxidative stress; Mitochondrial dysfunction; Magnetic resonance spectroscopy

## 1. Introduction

Alzheimer's disease (AD) is the most common cause of age-related cognitive decline, accounting for 60–70% of total dementia cases (WHO, 2019). It is estimated that 6.2 million Americans, or 10% of the population aged 65 and older, are currently living with Alzheimer's dementia (Alzheimer's Association, 2021; Hebert et al., 2013). In addition, more than 11 million family members and other caregivers provided around 15.3 billion hours of care to patients with Alzheimer's and other dementias in 2020, which is equivalent to $256.7 billion in unpaid labor (Alzheimer's Association, 2021). Thus, AD is a substantial and growing public health and economic burden in the US.

Unfortunately, AD is often clinically diagnosed at a stage in which the underlying pathology has reached an advanced and possibly irreversible state. Therefore, one of the major challenges in AD research is to identify targets for early intervention, which in turn could substantially reduce the morbidity, mortality, and cost of care related to AD. In this vein, mild cognitive impairment (MCI) represents an earlier clinical syndrome with less functional impairment, on the path towards AD and other dementias. An estimated 15 to 20 percent of people age 65 or older have MCI (Roberts and Knopman, 2013), and conversion rates of MCI to AD have been estimated at 15% per year and almost 45% over 5 years (Gauthier et al., 2006). Therefore, MCI may be a critical stage of illness where intervention could stop or delay the progression towards dementia (Tumati et al., 2013). However, modifying the course of disease at this early stage first requires identifying treatment targets that are reliably linked to AD pathophysiology and neuronal progression.

To date, the majority of clinical trials investigating such disease-modifying therapies have sought to halt or reverse the deposition of specific proteins in the brain, namely amyloid beta (Aβ) and microtubule-associated protein tau. These trials have been anchored in the amyloid cascade hypothesis, which proposes that accumulation of Aβ plaques in the brain is a critical early driver of AD pathogenesis. Over the past two decades, FDA approved two classes of therapies, cholinesterase inhibitors and memantine, that target cholinergic and glutamatergic neurotransmission to slow decline in the symptoms of cognition and daily functioning. Only one anti-amyloid drug (aducanumab) has very recently received FDA approval, though with significant controversy. The failure rate of anti-amyloid trials has raised questions about whether the amyloid hypothesis is complete (Kuehn, 2020; Sery et al., 2013). The paucity of available disease-modifying therapies and discouraging track record of anti-amyloid trials – in conjunction with the escalating public health and economic crises associated with AD – make clear the imperative to identify alternative treatment targets.

Toward that end, accumulating evidence indicates that enhancing brain bioenergetic metabolism, with a resulting reduction in oxidative stress and/or neuroinflammation, could be a promising treatment avenue for AD. Mitochondrial dysfunction and cellular energy deficits are increasingly thought to play a critical role in aging and AD pathophysiology (Bonkowski and Sinclair, 2016; Imai and Guarente, 2014; Sonntag et al., 2017; Sorrentino et al., 2017). For example, studies have shown hypometabolism in brain regions affected by AD (Murray et al., 2014) where the mitochondrial structure is altered (Moreira et al., 2007; Hirai et al., 2001); reduced expression and activity of mitochondrial enzymes important for energy metabolism (Maurer et al., 2000); and reduced membrane potential, increased permeability, and excess production of reactive oxygen species (ROS) in mitochondria from AD brains (Onyango et al., 2016). Alterations in mitochondrial function and metabolism might also be antecedent to AD pathology, including Aβ plaques and neurofibrillary tangles (Gibson and Shi, 2010; Onyango, 2018).

Oxidative stress (OS), which occurs when there is an imbalance between oxidant and antioxidant levels in the cell resulting in increased reactive oxygen species (ROS) production, is another important metabolic facet of AD pathology. Specifically, increased levels of ROS cause damage to macromolecules within the cell, and it is this damage of lipids, proteins, and nucleic acids that give rise to pathological consequences (Bermejo et al., 2008). In the brain, ROS are eliminated by the free radical scavenger glutathione (GSH) through a chemical reaction that converts GSH to its oxidized state (GSSG) (Cabungcal et al., 2006; Dringen, 2000). As such, higher intracellular GSH levels protect cells from ROS-mediated insults. Given that neurons are particularly sensitive to oxidative damage due to the brain's substantial metabolic requirements, alterations in GSH function can have profound effects on brain and cognitive function. Not surprisingly, research now suggests that GSH deficiencies could play a key role in the pathogenesis of various age-related neurodegenerative disorders, including AD (Bains and Shaw, 1997; Pocernich and Butterfield, 2012; Schulz et al., 2000).

As with OS, microglia-mediated neuroinflammation adversely affects brain function and is now considered a hallmark feature of various neurodegenerative diseases, including AD (Calabrese et al., 2014; Webers et al., 2020). Evidence from brain imaging supports the role of neuroinflammation in the progression of dementia. For instance, a recent meta-analysis of positron emission topography (PET) studies found evidence of increased neuroinflammation during the progression of MCI and AD (Bradburn et al., 2019). Notably, pro-inflammatory mediator expression is modulated by mitochondrial dynamics in microglial cells (Park et al., 2013). Furthermore, neuroinflammation and OS are linked closely, as neuroinflammation leads to increased OS which, in turn, causes further neuroinflammation (Fischer and Maier, 2015).

Taken together, the above findings illustrate the importance of brain bioenergetic and metabolic dysfunction in MCI and AD and, in turn, the utility of investigative methods for elucidating these pathological processes. Magnetic resonance spectroscopy (MRS) is a functional neuroimaging technique that provides one of the most direct windows into these processes. *In vivo* MRS is a non-invasive tool for characterizing alterations in metabolite concentration and, by extension, bioenergetic and metabolic dysfunction associated with

neurodegenerative disease progression (Duarte et al., 2012). Several such metabolites in the brain are present at sufficient concentrations to be detected by $^{1}$H-MRS, including N-Acetyl Aspartate (NAA), choline-containing compounds (Cho), creatine (Cr), myo-inositol (mI), and by $^{31}$P-MRS including adenosine triphosphate (ATP) and phosphocreatine (PCr). However, unlike these metabolites which could produce well-defined peaks, GSH, γ-aminobutyric acid, glucose, lactate, etc. have small peaks with overlapping resonances resulting in more measurement variability. To date, numerous MRS studies focused on MCI and AD have shown abnormal metabolite profiles. For instance, meta-analyses of MCI (Tumati et al., 2013) and AD (Wang et al., 2015) indicated decreased NAA and increased mI levels associated with MCI and AD, respectively. However, these two meta-analyses only focused on one, either MCI or AD, and $^{1}$H-MRS studies only. These analyses lack information from both MCI and AD as well as from bioenergetics associated with OS and neuroinflammation. Therefore, it is unclear whether specific patterns of the metabolic changes exist in MCI and AD, and whether these patterns could help understand disease transition from MCI to AD or give insights on the underlying molecular mechanisms. We therefore performed a meta-analysis of *in vivo* $^{1}$H- and $^{31}$P-MRS studies. The central goal of this study was to investigate the alterations in most common metabolites, including antioxidant GSH and energy-related phosphates, and to further validate the model of oxidative stress and neuroinflammation associated with mitochondrial dysfunction in MCI and AD patients (Butterfield and Halliwell, 2019; Ikawa et al., 2020; Simpson and Oliver, 2020).

## 2. Materials and Methods

### 2.1 Data extraction and inclusion/exclusion criteria

We performed meta-analyses on the metabolite ratios (e.g. NAA/Cr) and absolute concentrations (quantified by the internal reference of Cr and external reference of water signal, respectively) of NAA, Cho, mI, and Cr categorized by brain location because these were the most common measures reported in MCI and AD studies. In addition, due to the small number of GSH and $^{31}$P-MRS studies, we summarized the GSH result of each study and performed meta-analyses for the averaged levels of metabolites of phosphorous compounds, e.g. PCr and ATP, regardless of brain locations. PubMed, EMBASE, Cochrane databases were searched to identify journal articles published between 1 January 1989 and 30 January 2021, using the search terms: MRS or magnetic resonance spectroscopy and (1) MCI or (2) AD or (3) brain metabolites or (4) brain phosphate metabolism. In addition, we limited the search to English language studies only. The procedure of the literature searching along with the inclusion/exclusion criteria as well as the data extraction are plotted in Fig.1 (details, see Supplementary Materials).

### 2.2 Meta-analysis

The current meta-analyses were conducted using RevMan (version 5.3, Cochrane Collaboration) with a random-effects model and following the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher et al., 2009). Differences in metabolite ratios and concentrations of MCI/AD relative to healthy controls (HCs) were calculated for each study. After excluding outliers (identified by the ROUT

method) (Motulsky and Brown, 2006), the weighted averages of relative changes were calculated using the weight factors provided by RevMan software (version 5.3, Cochrane Collaboration). Hedges' g was used as the effect size to analyze the differences in the means of two groups, which were divided by the pooled standard deviation (Cheung, 2015). To address potential heterogeneity, a subgroup meta-analysis was performed according to brain region, and further leave-one-out analysis (excluding the study which contributes the highest heterogeneity) was performed if the heterogeneity ($I^2$) was larger than 50%. Meta-analyses were conducted only if the number of studies was larger than three. Otherwise, we presented summarized results from each study. Details of analyses on heterogeneity and publication biases are provided in the Supplementary Materials. Lastly, Pearson correlation was performed to determine the relationship between Hedge's g effect sizes for metabolite concentrations and Mini-Mental State Exam (MMSE) scores in MCI/AD patients.

## 3. Results

### 3.1 Description of studies

The studies were classified according to the region of interest, as well as the reported metabolite ratios or concentrations. For *in vivo* $^{1}$H-MRS data, 40 MCI (1473 HCs and 1238 patients) and 39 AD (1682 HCs and 1248 patients) studies were included. Of the 40 studies that included MCI patients, 25 included data from the posterior cingulate cortex (PCC), 15 from the hippocampus or medial temporal lobe (MTL), and 7 from parietal white matter areas (PWM). Of the 39 studies that included AD patients, 23 included data from the PCC, 15 from the hippocampus or MTL, 7 from PWM, and 6 from parietal gray matter areas (PGM). Regarding $^{31}$P-MRS data, a total of 123 patients and 119 HC subjects from eight AD studies and one MCI study were included (Bottomley et al., 1992; Brown et al., 1989; Forlenza et al., 2005; Gonzalez et al., 1996; Mecheri et al., 1997; Pettegrew et al., 1994; Rijpma et al., 2018; Smith et al., 1995). Summaries of all studies included in the current analysis are provided in Table 1.

### 3.2 $^{1}$H-MRS studies in MCI and AD

The specific Hedges' $g$ values, heterogeneities, number of studies, and $p$ values for each metabolite in each region of interest, as well as the overall effect are shown in Table 2. Forest plots for each metabolite were also provided in the Supplementary Materials (Fig. S1–S14). Compared to HCs, our overall analysis (Fig. 2) indicated similar alterations in metabolite concentration in both MCI and AD patients, albeit more pronounced in the latter. Specifically, we observed the following in MCI and AD patients, respectively: NAA was decreased by 9.6% and 12.9%; mI was increased by 6.3% and 7.4%; Cho was decreased by 4.3% and 5.4%; and Cr was decreased by 6.4% and 6.9%. Regarding metabolite ratios, our overall analysis showed that NAA/Cr was decreased by 6.4% and 10.8%, and mI/Cr was increased by 16.2% and 19.4%, in MCI and AD subjects, respectively. Cho/Cr did not significantly differ in either MCI or AD versus HC subjects. Regarding region-specific findings, the largest metabolite alterations were most often observed in the hippocampus in both clinical groups (Fig. 2C and Fig. 2D). Moreover, most of the observed metabolite changes were greater in AD than MCI across brain regions, such as hippocampus, PCC, and

PWM, except for Cho alterations, which were only observed in the hippocampus in MCI and AD.

After excluding studies that contributed to the highest heterogeneity (see forest plots in Supplementary Materials), the results were not changed significantly apart from the following: Increased mI concentration in the PCC in MCI patients ($p$ value changed from 0.06 to 0.002 after excluding Pilatus et al., 2009), decreased overall Cho concentrations in AD ($p$ value changed from 0.93 to 0.04 after excluding Marjanska et al., 2019), and decreased Cr concentrations in the PCC of AD patients ($p$ value changed to from 0.13 to 0.002 after excluding Marjanska et al., 2019).

To date, four studies have measured GSH levels in MCI and AD subjects respectively, and this precluded region-specific meta-analysis of those data. Instead, we performed an overall meta-analysis. Relative to HC subjects, lower GSH concentrations were reported in MCI and AD patients by (Mandal et al., 2015) and (Shukla et al., 2020), respectively, while a separate study reported a non-significant difference in AD (Marjanska et al., 2019). The GSH/Cr ratio was reported as either increased or no significant difference in MCI and AD patients (Duffy et al., 2014; Mullins et al., 2018; Oeltzschner et al., 2019). The overall effect sizes of GSH/Cr ratio and GSH concentrations are shown in Table 2.

Pearson correlation was performed to determine the association of alterations in metabolite concentrations and the decreases in MMSE scores in MCI/AD patients compared to HCs. A strong negative correlation (r = −0.86, p = 0.006) was observed between Hedge's g effect size for MMSE scores and mI concentrations in the hippocampus of AD patients. A positively trending correlation (r = 0.62, p = 0.08) was also observed between Hedge's g for NAA and MMSE scores in the hippocampus (Fig. 3). No significant correlation of metabolite concentrations with MMSE scores was observed in PCC and PWM. All of the Hedge's g for metabolite and MMSE scores were extracted from the forest plots in the Supplementary Materials.

### 3.3 $^{31}$P-MRS studies in MCI and AD

Due to the small number of $^{31}$P-MRS studies, we were unable to analyze differential effects as a function of specific brain region. Moreover, only one $^{31}$P-MRS study to date has examined MCI subjects (Mandal et al., 2012), and it did not show significant differences in phosphodiester (PDE), inorganic phosphate (Pi), PCr, ATP, or phosphomonoester (PME) in the hippocampus compared to HC subjects. Regarding AD studies, a significant decrease was observed in PDE level (Effect size in hedge's g $(ES)$ = −1.15 [−2.23, −0.06], $p$ = 0.04, $I^2$ = 88%), whereas differences in PME, Pi, PCr, PCr/Pi, ATP, and pH were not significant compared to HC subjects. After accounting for heterogeneity via leave-one-out analyses, we observed significantly increased PME ($ES$ = 0.52 [0.14, 0.90], $p$ = 0.007, $I^2$ =16%) and Pi ($ES$ = 0.34 [0.01, 0.67], $p$ = 0.050, $I^2$ = 28%), and significantly decreased PCr/Pi ($ES$ = −0.82 [−1.31, −0.32], $p$ = 0.001, $I^2$ = 0%) in AD patients. However, PDE was not significantly different ($ES$ = −0.66 [−1.51, 0.19], $p$ = 0.13, $I^2$ = 80%). Forest plots were shown in the Supplementary Materials (Fig. S15).

## 4. Discussion

The current study aimed to characterize and quantify oxidative stress and neuroinflammation associated with bioenergetic and metabolic abnormalities in MCI and AD via meta-analysis of prior research that has utilized *in vivo* $^{1}$H-MRS and $^{31}$P-MRS. The results from our meta-analyses revealed several noteworthy patterns, which we interpret in greater detail below: (1) Concentrations of NAA and Cr were significantly lower in MCI and AD patients compared to HC subjects, while mI was significantly higher, and these patterns were preserved across different brain regions; (2) alterations in Cho were observed only in the hippocampus in both clinical groups; (3) significantly reduced overall GSH concentration was found in MCI but not AD patients; (4) compared to other brain regions, the hippocampus showed the most significant alterations for most of these metabolites in both MCI and AD patients (Fig. 2C and Fig. 2D), which aligns with extensive research highlighting the centrality of this region in AD disease pathophysiology and progression; and (5) after performing the leave-one-out analyses to account for heterogeneity, we noted significantly increased PME and Pi and decreased PCr/Pi in AD, which could be associated with mitochondrial dysfunction and oxidative stress.

### 4.1 Significance of metabolic alterations in MCI and AD

Our meta-analyses showed consistently decreased NAA/Cr and NAA concentrations in both patient groups in almost all regions assessed, which broadly aligns with previous research in MCI/AD (Wong et al., 2020) and other neuropsychiatric disorders, such as schizophrenia (Whitehurst et al., 2020). This is noteworthy given that NAA exists exclusively within neurons and plays crucial and diverse roles in the nervous system. The generation of NAA is correlated with mitochondrial function and is thought to play a role in neuroenergetics (Moffett et al., 2007). Therefore, the observed reductions in NAA and NAA/Cr in MCI/AD may reflect neuronal loss in addition to nonstructural and physiological changes associated with impaired mitochondrial activity (Bornstein et al., 2020).

The observed increase in mI concentration in MCI/AD is important given that mI has been proposed as a glial marker, is a constituent of the lipid component of biomembranes, and plays an important role in the phosphatidylinositol second messenger system (Brand et al., 1993; Kim et al., 2005; Rae, 2014). Altered cerebral mI concentrations are implicated in many neuropsychiatric disorders (Sekar et al., 2019). For example, elevated mI levels have been observed in AD, gliomatosis cerebri, diabetes melitus, systemic lupus erythematosus, multiple sclerosis, etc (Chhetri, 2019). As an indicator of the glial activation, mI is considered the most likely candidate MRS-marker of inflammation in AD (Chaney et al., 2019). Decreased NAA and increased mI have been generally reported and associated with each other in the same regions (for instance, PCC, hippocampus, and PWM, as illustrated in Fig.2) in AD, which suggests a link between increased neuroinflammation and decreased neuron viability (Chaney et al., 2019). It has been reported that reduced NAA and increased mI are associated with increased cerebrospinal fluid tau, and mI is negatively correlated with cerebrospinal fluid Aβ−42, reduction of which indicates brain Aβ amyloidosis (Piersson et al., 2020). In addition, mI levels have been shown to predict the progression to AD with a 70% sensitivity and 85% specificity (Targosz-Gajniak et al., 2013). Thus, accumulating

evidence suggests that increases in mI concentration may precede decreases in NAA in AD, with increased mI potentially reflecting neuroinflammation in AD (Chaney et al., 2019; Kantarci et al., 2008a), though further investigation is needed to confirm this dynamic.

Interestingly, the current study observed variable correlations between NAA and mI levels and cognitive performance on the MMSE. This finding is not only consistent with previous research in MCI and AD (Ackl et al., 2005; Foy et al., 2011), but also in stroke, hereditary ataxias, and alcohol dependence (Krahe et al., 2020; Morley et al., 2020; Wang et al., 2017). Changes in NAA concentration are closely related to MMSE and the cognitive part of the AD Assessment Scale scores (Jessen et al., 2000). Moreover, NAA and mI are also relevant to performance in verbal memory testing (Auditory Verbal Learning Test) and general cognition (Dementia Rating Scale) (Kantarci et al., 2002). Thus, NAA and mI may be the most sensitive MRS markers to monitor AD progression and treatment response, as indicated by Fig. 2.

As with NAA, we observed reductions in Cr overall and in almost all regions in both MCI and AD patients. Cr (including PCr) is a well-known energy shuttle which also serves as an intracellular buffer for ATP by providing a ready supply of high energy phosphate through the creatine kinase reaction. Findings from rodent research suggested that Cr exerts a neuroprotective effect by buffering ATP levels (Beal, 2011). Similarly, beneficial effects of Cr supplementation have been shown in neurodegenerative and neurological diseases linked with mitochondrial dysfunction (Adhihetty and Beal, 2008; Andres et al., 2008; Marques and Wyse, 2019; Smith et al., 2014). Interestingly, the observed alterations in mI/Cr in the current study were faster/larger than that of NAA/Cr (Fig. 2A). This is noteworthy given that Cr is often used as an internal reference for *in vivo* MRS quantification based on the assumption that Cr is constant and stable in the altered pathophysiological conditions (Foy et al., 2011; Shukla et al., 2020). However, this notion needs should be taken with caution in light of the current meta-analysis showing decreases in Cr concentrations in both MCI and AD patients relative to healthy controls. As a consequence, the metabolite ratio values involving Cr may be differentially affected across clinical and healthy comparison groups, potentially complicating the interpretation of these ratios. Therefore, we further analyzed the absolute concentrations such as NAA, mI, and Cho (Fig. 2B) and found that NAA concentrations were largely altered more than that of mI, which is the opposite of what we observed in the NAA/Cr and mI/Cr metabolite ratios. The absolute Cho concentration was also shown to be decreased, while the Cho/Cr ratio remained unchanged (Fig. 2A, 2B). These differential results call into question the practice of normalizing metabolite levels by using Cr as an internal reference, and this should be carefully considered in future research.

The current analysis found reductions in Cho concentration in MCI/AD, though only in the hippocampus. Cho is considered as a cell membrane marker which indicates cellular proliferation (Duarte et al., 2012). Changes in Cho reflect non-steady-state alterations in membrane turnover in pathological states, such as AD, tumors, inflammation, infections, and schizophrenia (Rae, 2014). Cortical acetylcholine (ACh) is critical for the cognitive processes and ACh deficit has been reported to be a primary event in the early stage of AD and related to the cognitive symptoms of dementia (Muir, 1997). Previous research has also found that increasing ACh can enhance cholinergic functioning and subsequently

improve AD patient outcomes (Akincioğlu and Gülçin, 2020; Saxena and Dubey, 2019), partially through the suppression of inflammation by administration of acetylcholinesterase inhibitors (Pollak et al., 2005). In addition, being a byproduct of ACh hydrolysis, free choline is an endogenous and selective α7 agonist for nicotinic ACh receptors (nAChR) (Alkondon et al., 1997). Targeting an α7 nAChR has been suggested as a possible strategy to reduce microglial activation (and neuroinflammation), thereby enhancing the clearance of misfolded and aggregated proteins that typify AD (Hernandez et al., 2010; Medeiros et al., 2014). High Cho dietary intake has also been associated with reduced pro-inflammatory markers in serum (Detopoulou et al., 2008). Regretfully, ACh level cannot be directly quantified by *in vivo* $^{1}$H-MRS, although one recent study suggested that Cho MRS had the potential to serve as a proxy of ACh function in human brains (Lindner et al., 2017). $^{1}$H-MRS detectable Cho signal are mostly from glycerophosphocholine and phosphocholine, which provide free choline for synthesizing ACh (Rae, 2014; Wang et al., 2008). Thus, our finding of decreased Cho levels in both MCI and AD may indicate increased inflammation and cell membrane changes, particularly in hippocampus. However, it is worth noting that increased Cho levels have also been reported as a non-specific marker of neuroinflammation in patients with HIV (Zahr et al., 2014). Therefore, the exact interpretation of changes in Cho signal is complicated due to the multiple metabolic pathways involved.

GSH is an important antioxidant that displays remarkable metabolic and regulatory versatility (Dwivedi et al., 2020). In the context of relatively few GSH studies in MCI/AD, the current meta-analysis observed overall reductions in GSH levels in MCI but not AD, raising the possibility that GSH reductions may play an important role in the early stages of AD before later increasing as the disease progresses. This seemingly paradoxical trajectory may have some indirect empirical support from rodent models of other neurodegenerative diseases. For example, higher brain GSH levels were found in R6/2 Huntington's Disease mice due to GSH overproduction induced by oxidative stress (Tkac et al., 2007). Similarly, McLaughlin et al. found frontal cortex GSH increases in GT-tg bi-genic mice after short-term Tat protein exposure and induced oxidative stress (McLaughlin et al., 2017). Thus, GSH may remain near normal, or even increase slightly as indicated in the early stages of some illnesses, but then is reduced as the disease progresses. To test this hypothesis, more evidence is needed. Of note, studies measuring GSH in the brain by *in vivo* MRS have been controversial, with no consistent pattern reported in the limited publications that exist. These discrepancies may reflect the challenge of measuring GSH by *in vivo* MRS due to the limited sensitivity and spectral resolution of MRI scanners, low concentration of GSH (~ 1mM), and partially overlapped resonances with other metabolites.

For $^{31}$P studies, we couldn't meta-analyze relevant MCI data due to the paucity of prior research but decreased PDE and PCr/Pi levels and increased PME and Pi levels were observed in the AD studies. The increased Pi and decreased PCr/Pi are consistent with the notion of abnormal energy metabolism associated with mitochondrial dysfunction and oxidative stress in AD. However, most of these studies involved small sample sizes, were performed at 1.5T, and were conducted decades ago (Forlenza et al., 2005; Gonzalez et al., 1996; Mecheri et al., 1997; Pettegrew et al., 1994; Smith et al., 1995). Thus, further research with larger samples and using more advanced techniques are needed to better characterize and understand potential alterations in $^{31}$P metabolite concentration in AD.

### 4.2 Hippocampus-specific metabolic alterations

The current meta-analysis found the largest and most consistent alterations in metabolite concentration in the hippocampus (Fig. 2C and Fig. 2D). This is not surprising given that the hippocampus is both critical for learning/memory and anatomically central to AD neuropathology progression. Longitudinal fMRI studies reveal that early hippocampal hyperactivation is a predictor of cognitive decline in patients with MCI and early AD (O'Brien et al., 2010). Different hippocampal sub-regions, namely the CA1 and dentate gyrus, appear particularly vulnerable to early neuritic plaque aggregation associated with altered synaptic density within the perforant pathway (Hyman et al., 1986), possibly reflecting the sensitivity of CA1 to microglial mediated neuroinflammation in response to neuritic plaque pathology (Moodley and Chan, 2014). A previous study also demonstrated hippocampal atrophy is the most robust structural MRI biomarker of AD at the prodromal stage (Risacher et al., 2009).

Of note, the current study also found a significant correlation between mI concentrations and MMSE scores in AD patients, as well as a positively trending correlation between NAA concentrations and MMSE scores, both of which were observed in the hippocampus but not in the other brain regions (Fig. 3). This finding suggests that increases in mI concentration and decreases in NAA concentration in the hippocampus are associated with declines in cognitive function. Taken together, it seems that hippocampus would be the most sensitive among all the brain regions during the progression of AD and treatment response.

### 4.3 Molecular mechanisms of AD suggested by metabolites' changes

AD is a complex and multi-factorial disorder involving mitochondrial dysfunction, OS, neuroinflammation, Aβ and tau burden, etc (Fig. 4). Over 90% of all cases are first diagnosed after age 65. Earlier ages of onset are rare and are usually associated with a dominant genetic mutation of amyloid precursor protein and presenilin (PS1 and PS2), which represents only 1–5% of AD cases. However, most of the molecular AD research in cells and mice has used these mutations in the search for the cause of AD (Behl, 2017), thus, the models based on Aβ might be not adequately modeling human AD. In addition, Aβ alone appears to be insufficient for the development of AD, and changes in tau are nonspecific to AD and are seen in other neurodegenerative disorders as well as in nondemented elderly individuals (Behl, 2017). Even though Aβ proves to be correlated with AD, the results of amyloid-targeting therapies for AD have been controversial in clinical trials (Herrup, 2015; Kuehn, 2020). Aducanumab becomes the first FDA-approved anti-amyloid drug in June 2021. Aβ burden as well as tau burden relevant to the consequences of other pathological mechanisms of AD need further studies. For instance, it has been found that both OS and mitochondrial dysfunction induce aggregation of misfolded proteins, such as Aβ and tau (Lévy et al., 2019). Aβ plaques could be detected and engulfed by microglia through TAM (Tyro3, Axl, and Mer) receptors (Huang et al., 2021). Besides, microglia are capable of binding to soluble Aβ oligomers via cell surface receptors including cluster of differentiation CD36, CD14, and Toll-like receptors (TLR4, 6 and 9), etc. The binding of Aβ to CD36, TLR4 or TLR6 results in activation of microglia and further leads to the production of proinflammatory cytokines and chemokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α which may directly impair neuronal function (El

Khoury et al., 2003; Ye et al., 2013). These findings suggest a close connection between amyloidogenesis and neuroinflammation (Webers et al., 2020).

Although the nature of AD pathogenesis is complex, it is known that OS plays a key role starting in early AD stages (Chang et al., 2014). Under physiological conditions, there is a balance between oxidant and antioxidant species. OS occurs when the level of oxidants present exceeds the levels of antioxidants present (Dalle-Donne et al., 2006). States of intensive energy metabolism and inflammation lead to the production of ROS, which can have toxic effects that damage proteins, lipids, and DNA, as well as result in neuron death (Bermejo et al., 2008; Kuka et al., 2013). ROS are eliminated by the free radical scavenger GSH in the brain (Cabungcal et al., 2006; Dringen, 2000), thus alteration in GSH metabolism may have profound effects on neurons.

OS, neuroinflammation, and mitochondrial dysfunction are intertwined in the pathology of AD and could act as a consequence of each other (Fig. 4). Thus, it's hard to tell which is the primary upstream cause in the progression of AD. The mitochondrial cascade hypothesis indicates that mitochondrial dysfunction is considered a primary and early event in the pathological cascade of AD (Swerdlow, 2020; Swerdlow et al., 2010). Mitochondrial dysfunction has also been observed prior to amyloid plaque deposition (Gillardon et al., 2007). Evidence suggests that mitochondrial dysfunction could result in overproduction of ROS/reactive nitrogen species leading to OS (Bhat et al., 2015; Calabrese et al., 2005). In AD, mitochondrial abnormalities are considered the main source of OS (Perry et al., 2002). Besides mitochondrial dysfunction, there are other possible causes of oxidative stress in the brain, e.g., neuroinflammation, protein aggregation, and decreased antioxidant defenses (Simpson and Oliver, 2020). Previous PET studies also indicated that mitochondrial dysfunction has shown in the early stage of AD, and this mitochondrial-related energy failure may precede glycolysis-related hypometabolism in regions with pathologically confirmed early neurodegeneration in AD, e.g. hippocampus (Terada et al., 2020). On the other hand, mitochondrial dysfunction in microglial cells has been found to inhibit portions of the IL-4-induced alternative response, which is associated with a reduction of inflammation (Ferger et al., 2010). Notably, besides the Aβ pathway, microglia activated by OS also releases proinflammatory cytokines such as IL-1β, IL-6, IL-8, and TNFα, and up-regulates the expression of chemokines such as C-C motif ligand (CCL)-2, chemokine receptors CCR3 and CCR5, resulting in local inflammatory responses, causing the death of neural cells (Heneka et al., 2015; Xia et al., 1998). Taken together, a **schematic framework** of hypothesized mechanism of mitochondrial dysfunction, oxidative stress, and neuroinflammation with the altered metabolites in AD was proposed in Fig. 4, which illustrated that the ROS formation, enhanced activation of microglia and other immune cells, and expression of cytokines are associated with neuroinflammation in AD (Kinney et al., 2018). OS and neuroinflammation are inextricably linked, neuroinflammation leads to increased OS which in turn causes subsequent inflammation (Fischer and Maier, 2015), and antioxidants protect neurons by reducing OS and chronic inflammation in AD (Prasad, 2017). In that sense, early intervention of AD could target mitochondrial dysfunction, OS, and neuroinflammation. And enhancing brain bioenergetics and antioxidant supplementation could be potential ways to treat AD at an early stage (Perez Ortiz and Swerdlow, 2019;

Petrovic et al., 2020; Viña et al., 2011). For instance, raising brain nicotinamide adenine dinucleotide (NAD) levels has recently aroused intense scholarly attention as a potential treatment intervention (Hou et al., 2018; Hikosaka et al., 2021; Wang et al., 2021; Rajman et al., 2018; Trammell et al., 2016), which can also reduce neuroinflammation in a mouse model of AD (Hou et al., 2021).

## 5. Limitations

Several limitations of our meta-analysis should be mentioned. Firstly, the heterogeneity we observed between studies may relate to factors such as age, illness duration, diagnosis, symptom severity, and medication exposure, which may vary between cohorts. Secondly, data quality is another concern. Cramér–Rao Lower Bounds values, which are used to estimate metabolite goodness of fit, were not provided for most of the studies. Therefore, we cannot fully evaluate how MRS data quality affected the results of our meta-analyses. Thirdly, the number of GSH and $^{31}$P studies were too small to permit region-specific analyses. Fourthly, the etiology of MCI patients and the disease stage amongst AD patients were also not considered in this review due to insufficient information provided in the published MRS studies with MCI and AD patients. Lastly, MRI platforms, MRS sequences, and quantification methods (e.g., correcting partial volume effect of gray/white matter and cerebrospinal fluid) differed between studies and could not be corrected due to insufficient details provided.

## 6. Conclusion

In conclusion, the present meta-analysis indicates a similar pattern of metabolic alterations in MCI and AD, albeit with increased severity in AD. These results (particularly in Fig. 2) support the hypothesis (Fig.3) that neuroinflammation and oxidative stress associated with mitochondrial dysfunction play a critical role in AD etiopathogenesis and pathophysiology (Chaney et al., 2019; Gadhave et al., 2020; Tobore, 2019), and eventually lead to decreased neuronal function, which is implied by the changes in *in vivo* MRS markers of increased mI and decreased NAA, GSH, and PCr/Pi. Among all the MRS markers, NAA and mI seem most sensitive in detecting the progression of AD. The hippocampus is the most sensitive during AD progression, suggesting it could be an important brain region for early diagnosis and prevention. Lastly, targeting abnormal bioenergetic processes associated with neuroinflammation/oxidative stress could be a promising approach for earlier intervention in AD.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Abbreviations:

| | |
|---|---|
| **Aβ** | amyloid beta |
| **ACC** | anterior cingulate cortex |
| **ACh** | acetylcholine |
| **AD** | Alzheimer's disease |
| **ATP** | adenosine triphosphate |
| **Cho** | choline-containing compounds |
| **Cr** | creatine |
| **ETC** | electron transport chain |
| **fMRI** | function magnetic resonance imaging |
| **GSH** | glutathione |
| **GSSG** | oxidized glutathione |
| **HC** | healthy control |
| **IL** | interleukin |
| **MCI** | mild cognitive impairment |
| **mI** | myo-inositol |
| **MMSE** | Mini-Mental State Exam |
| **MRS** | magnetic resonance spectroscopy |
| **MTL** | medial temporal lobe |
| **NAA** | N-acetyl aspartate |
| **nAChR** | nicotinic ACh receptors |
| **NAD** | nicotinamide adenine dinucleotide |
| **PCC** | posterior cingulate cortex |
| **PCr** | phosphocreatine |
| **PDE** | phosphodiester |
| **PET** | positron emission topography |
| **PGM** | parietal gray matter |
| **Pi** | inorganic phosphate |
| **PME** | phosphomonoester |

| | |
|---|---|
| **PWM** | parietal white matter |
| **OS** | oxidative stress |
| **ROS** | reactive oxygen species |
| **TCA** | tricarboxylic acid |
| **TLR** | Toll-like receptors |
| **TNF** | tumor necrosis factor |

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Zimny A, Szewczyk P, Trypka E, Wojtynska R, Noga L, Leszek J, Sasiadek M, 2011. Multimodal imaging in diagnosis of Alzheimer's disease and amnestic mild cognitive impairment: value of magnetic resonance spectroscopy, perfusion, and diffusion tensor imaging of the posterior cingulate region. J. Alzheimers Dis. 27, 591–601. 10.3233/JAD-2011-110254. [PubMed: 21841260]

**Highlights:**

- Neuroinflammation and oxidative stress (OS) may occur in the early stages of AD;
- Neuroinflammation and OS likely precede neuronal loss in AD;
- The hippocampus is the most vulnerable brain region in the progression of AD.

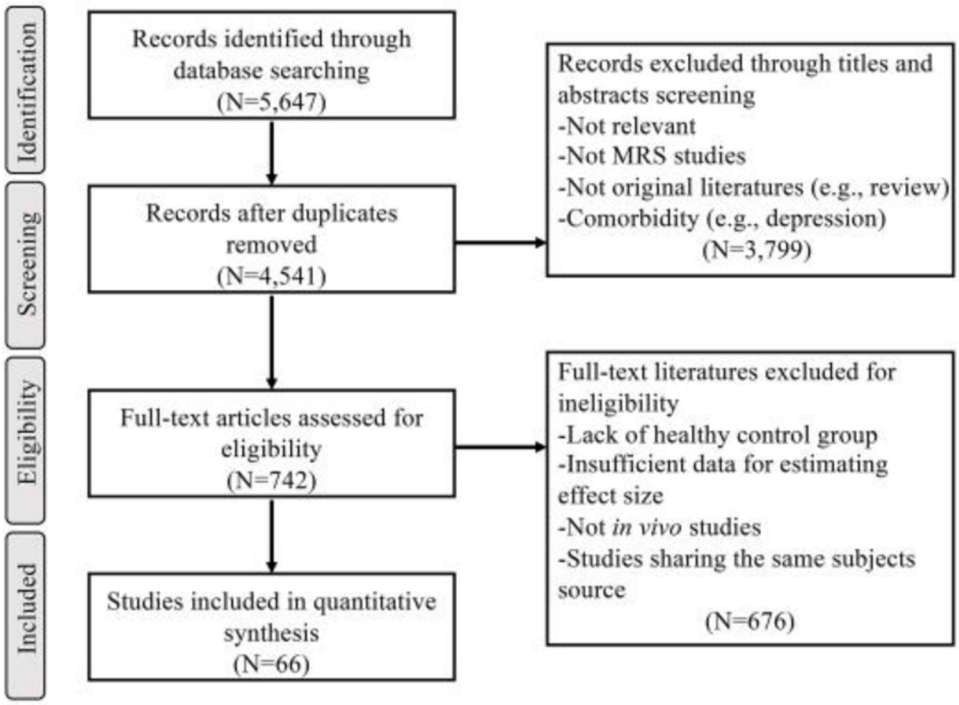


**Figure 1.**
Flowchart of studies screening, and inclusion/exclusion criteria.

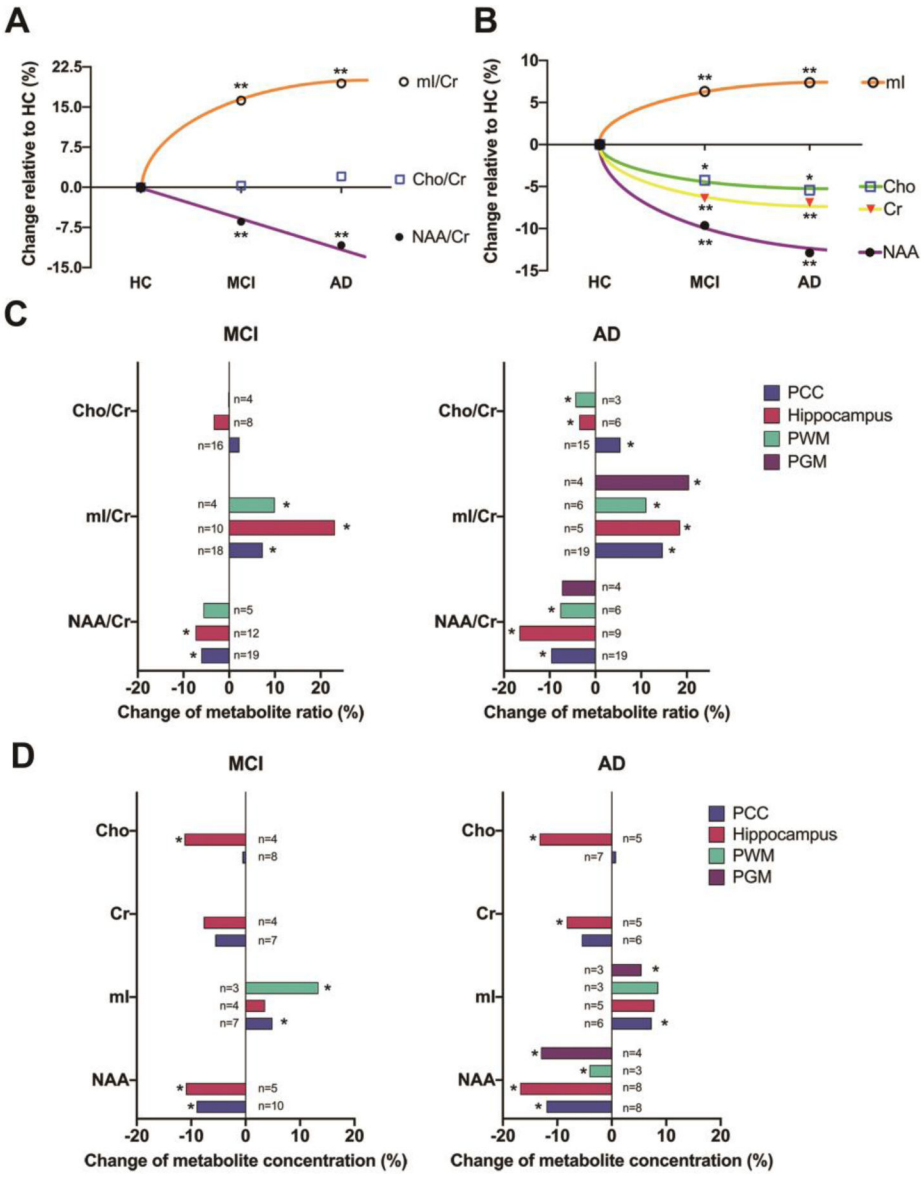


**Figure 2. Semi-quantifications of metabolite ratios and concentrations.**

Panel (A) and Panel (B) respectively depicts the overall metabolite ratios and concentrations alterations in mild cognitive impairment (MCI) and Alzheimer's disease (AD) compared to healthy control (HC). Panel (C) and Panel (D) depicts the metabolite ratios and concentrations, respectively, with the focus on the alterations in specific brain regions in MCI and AD compared to HC. *, $p < 0.05$; **, $p < 0.01$ (compared to HC) after leave-one-out analysis; The other abbreviations have the same meanings as noted in the main manuscript.

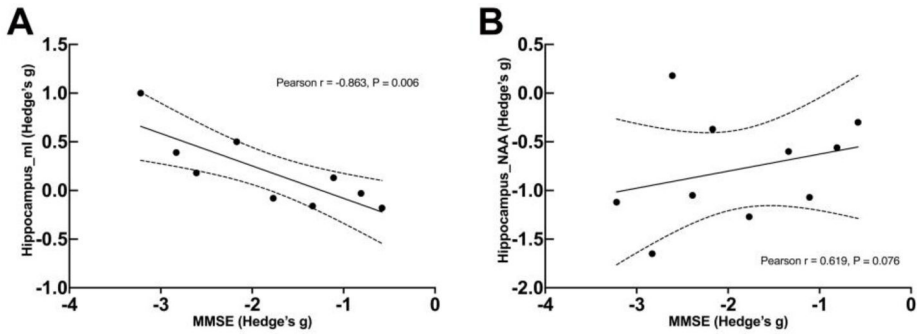


**Figure 3. Correlations between Hedge's g effect sizes for metabolite concentrations and Mini-Mental State Exam (MMSE) scores in the hippocampus of MCI/AD patients.**

Panel (A) depicts the correlations between Hedge's g for myo-inositol (mI) and MMSE scores. Panel (B) depicts the correlations between Hedge's g for N-acetyl aspartate (NAA) and MMSE scores.

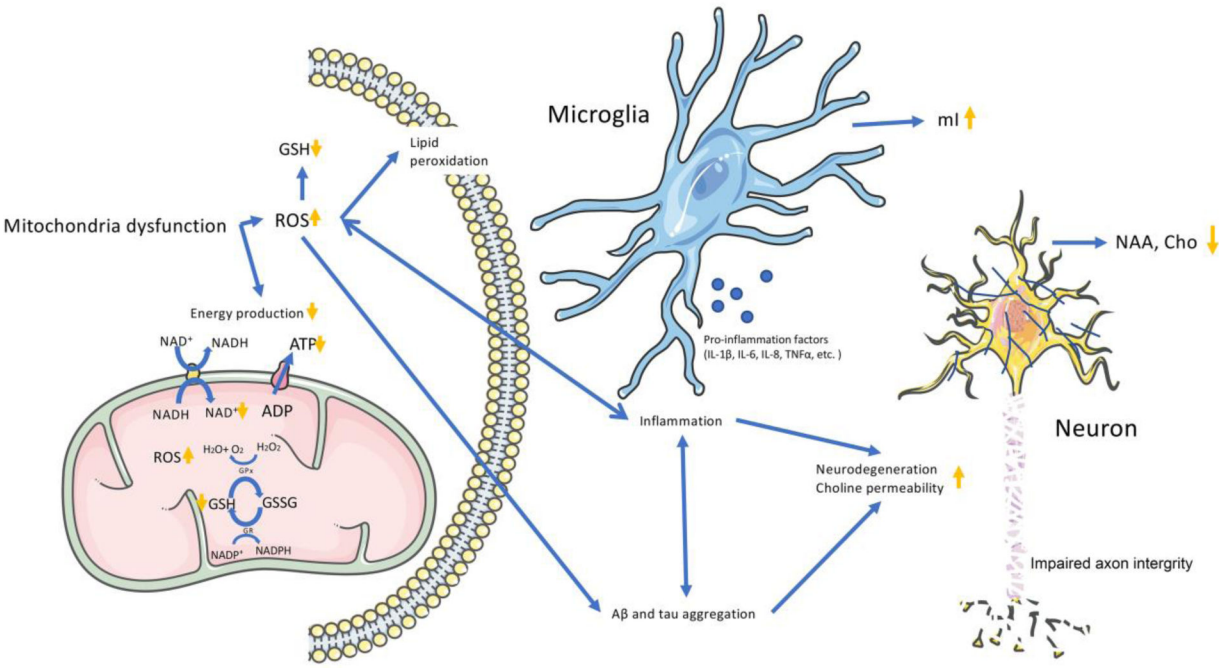


**Figure 4. A schematic view of mitochondrial dysfunction, oxidative stress, neuroinflammation, and metabolic response in the progression of AD.**

Mitochondrial dysfunction results in decreased phosphocreatine (PCr) and adenosine triphosphate (ATP), overproduction of reactive oxygen species (ROS), and occurrence of oxidative stress manifested in decreased nicotinamide adenine dinucleotide ($NAD^+$) and glutathione (GSH) levels (Bhat et al., 2015). The overproduction of ROS would further lead to lipid peroxidation, neuroinflammation and Aβ/tau aggregation (Ikawa et al., 2020). Neuroinflammation is induced by microglia and astrocytes activation, accompanying with the increased level of mI. The neuroinflammation and/or Aβ/tau aggregation ultimately result in neural dysfunction with increased choline (Cho) permeability, then lead to decreased NAA and Cho levels, and finally aggravate the progression to AD.

**Table 1.**

List of included MCI and AD studies.

| Literatures | Region of interest | Magnetic Field (Tesla) | Pulse sequence | Subjects number (MCI/AD/HC) | Metabolites quantification |
|---|---|---|---|---|---|
| (Jessen et al., 2000) | Medial temporal lobe, central cortical region | 1.5 | PRESS | 38 (-/20/18) | NAA/Cr, Cho/Cr |
| (Stoppe et al., 2000) | PGM, PWM | 2.0 | - | 42 (-/30/22) | NAA/Cr, Cho/Cr, mI/Cr, NAA, Cr, Cho, mI |
| (Catani et al., 2001) | PWM | 1.5 | PRESS | 36 (11/14/11) | NAA/Cr, Cho/Cr, mI/Cr |
| (Block et al., 2002) | Hippocampus, lateral temporal lobe, occipital lobe | 1.5 | - | 56 (-/34/22) | NAA/tCr, Cho/tCr |
| (Chantal et al., 2002) | MTL (Hippocampus), Frontal cortex, parietotemporal cortex | 1.5 | PRESS | 28 (-/14/14) | NAA, Cho, Cr, mI |
| (Hattori et al., 2002) | PCC, Parietooccipital WM | 3.0 | PRESS | 21 (-/9/12) | NAA/Cr, Glx/Cr, Cho/Cr, mI/Cr |
| (Schuff et al., 2002) | Bilateral hippocampus, temporal lobe, parietal lobe, frontal lobe | 1.5 | PRESS | 110 (-/56/54) | NAA |
| (Herminghaus et al., 2003) | PGM, PWM, Frontal GM, Frontal WM | 1.5 | STEAM | 43 (-/28/15) | NAA/Cr, mI/Cr, Glx/Cr, TMA/Cr |
| (Kantarci et al., 2004) | left PCC | 1.5 | PRESS | 327 (-/121/206) | NAA/Cr, Cho/Cr, mI/Cr |
| (Ackl et al., 2005) | Hippocampus, Parietal GM, Parietal WM | 1.5 | PRESS | 59 (19/18/22) | NAA/Cr, mI/Cr, mI/NAA |
| (Chao et al., 2005) | Hippocampal region, medial temporal lobe | 1.5 | PRESS | 48 (-/24/24) | NAA/Cr, NAA |
| (Metastasio et al., 2006) | PWM | 1.5 | PRESS | 54 (25/-/29) | NAA/Cr, Cho/Cr, mI/Cr |
| (Zhu et al., 2006) | PGM, PWM, Frontal GM, Frontal WM | 1.5 | - | 36 (-/14/22) | NAA/Cr, mI/Cr, NAA/mI, NAA, mI |
| (Franczak et al., 2007) | Hippocampus | 0.5 | PRESS | 10 (5/-/5) | mI/Cr, Cho/Cr, Glx/Cr, NAA/Cr, Glx/NAA, mI/NAA, NAA, mI, Cho, Cr, Glx |
| (Griffith et al., 2007) | PCC | 3.0 | PRESS | 34 (-/15/19) | NAA/Cr, Cho/Cr |
| (Kantarci et al., 2007) | PCC | 1.5 | PRESS | 194 (49/60/85) | NAA/Cr, Cho/Cr, mI/Cr |
| (Rami et al., 2007) | PCC, Temporal, Temporalparietal | 1.5 | PRESS | 89 (27/35/27) | NAA/Cr, Cho/Cr, mI/Cr, NAA, Cho, Cr, mI |
| (Kantarci et al., 2008b) | Hippocampus | 1.5 | PRESS | 243 (143/-/100) | NAA/Cr, Cho/Cr, mI/Cr |
| (Ding et al., 2008) | PCC | 1.5 | PRESS | 40 (-/20/20) | NAA/Cr, Cho/Cr, mI/Cr |
| (Olson et al., 2008) | PCC | 1.5 | STEAM | 71 (47/-/24) | NAA/Cr, NAA/Cho, NAA/mI, Cho/Cr, mI/Cr, Glx/Cr, NAA, Cho, mI, Glx, Cr |
| (Garcia Santos et al., 2008) | PCC | 1.5 | PRESS | 44 (10/-/34) | NAA/Cr, Cho/Cr, NAA/Cho, mI/Cr, NAA/mI |
| (Watanabe et al., 2008) | PCC, Hippocampus, Occipital lobe | 1.5 | PRESS | 56 (-/30/26) | NAA/Cr, mI/Cr, Cho/Cr, NAA/mI, NAA, mI, Cho, Cr |
| (Jessen et al., 2009) | MTL (Hipp) | 1.5 | PRESS | 279 (136/98/45) | NAA/Cr, mI/NAA, NAA, Cr, Cho, mI |
| (Pilatus et al., 2009) | PCC, PWM | 1.5 | PRESS | 27 (15/-/12) | NAA, Cho, mI, Cr, Glx |
| (Siger et al., 2009) | Frontal lobe G/W, PWM, PGM | 1.5 | - | 47 (14/17/16) | mI, NAA |

| Literatures | Region of interest | Magnetic Field (Tesla) | Pulse sequence | Subjects number (MCI/AD/HC) | Metabolites quantification |
|---|---|---|---|---|---|
| (Wang et al., 2009) | PCC, Hippocampus | 3.0 | PRESS | 48 (16/16/16) | NAA/Cr, mI/Cr, Cho/Cr, mI/NAA |
| (Zhang et al., 2009) | Hippocampus, Temporalparietal | 1.5 | - | 40 (14/13/13) | NAA/Cr, mI/Cr |
| (Chao et al., 2010) | PCC | 1.5 | STEAM | 31 (13/-/18) | NAA/Cr, mI/Cr, NAA/mI |
| (Griffith et al., 2010) | PCC | 3.0 | PRESS | 71 (29/-/42) | NAA/Cr, Cho/Cr, mI/Cr |
| (Watanabe et al., 2010) | PCC, Hippocampus, Occipital, ApPoDeepWM | 1.5 | PRESS | 169 (47/70/52) | NAA, mI, Cho, Cr |
| (Foy et al., 2011) | Hippocampus | 1.5 | PRESS | 98 (21/38/39) | NAA, mI, Cho, Cr |
| (Modrego et al., 2011) | PCC | 1.5 | PRESS | 106 (71/-/35) | NAA/Cr, Cho/Cr, mI/Cr, NAA/mI, NAA |
| (Silveira de Souza et al., 2011) | PCC | 1.5 | PRESS | 68 (10/25/33) | NAA/Cr, Cho/Cr, mI/Cr, mI/NAA |
| (Zimny et al., 2011) | PCC | 1.5 | PRESS | 68 (23/30/15) | NAA/Cr, Cho/Cr, mI/Cr, mI/NAA, mI/Cho |
| (Gordon et al., 2012) | PCC | 3.0 | PRESS | 39 (-/11/28) | NAA/Cr, Cho/Cr, mI/Cr, NAA/Cho, NAA/mI, NAA, Cho, mI, Cr |
| (Lim et al., 2012) | PCC | 3.0 | STEAM | 78 (19/36/23) | NAA/Cr, mI/Cr |
| (Seo et al., 2012) | PCC, Hippocampus, ERC, Occipital WM | 3.0 | PRESS | 24 (13/-/11) | NAA/Cr, Cho/Cr |
| (Shiino et al., 2012) | PCC, bilateral hippocampus | 1.5 | PRESS | 144 (-/99/45) | NAA/Cr, Cho/Cr, mI/Cr, Glx/Cr, mI/NAA, NAA, Cr, Cho, mI, Glx |
| (Wang et al., 2012) | PCC, Hippocampus | 3.0 | PRESS | 135 (32/47/56) | NAA/Cr, Cho/Cr, mI/Cr, NAA/mI |
| (Yang et al., 2012) | PCC, PWM, Dorsal Thalamus, Lentiform nucleus | 1.5 | PRESS | 29 (14/-/15) | NAA/Cr, Cho/Cr, mI/Cr, NAA/mI, NAA, mI, Cho, Cr |
| (Targosz-Gajniak et al., 2013) | PCC, Hippocampus, Parietal lobe | 1.5 | PRESS | 76 (41/-/35) | NAA/Cr, Cho/Cr, mI/Cr, Glx/Cr, NAA/Cho |
| (Duffy et al., 2014) | Anterior and posterior cingulate | 3.0 | PRESS | 95 (54/-/41) | GSH/Cr |
| (Fayed et al., 2014) | PCC | 1.5 | PRESS | 295 (66/36/193) | Glu, Glu/Cr, Glx, Glx/Cr, mI, mI/Cr, NAA, NAA/Cr, Cho, Cho/Cr |
| (Graff-Radford et al., 2014) | PCC, frontal lobe, occipital lobe | 1.5 | PRESS | 183 (-/35/148) | NAA/Cr, Cho/Cr, mI/Cr |
| (Suriyajakryuththana et al., 2014) | Frontal and paiertal white matter | 3.0 | - | 20 (7/10/3) | NAA/Cr, Cho/Cr, mI/Cr |
| (Mandal et al., 2015) | Frontal cortex, Hippocampus | 3.0 | MEGA-PRESS | 64 (22//21/21) 66 (28/19/19) | GSH |
| (Riese et al., 2015) | PCC | 3.0 | MEGA-PRESS | 39 (15/-/21) | GABA (AU), Glx (AU), NAA (AU) |
| (Yin et al., 2015) | Hippocampus | 3.0 | PRESS | 27 (11/-/16) | NAA/Cr, mI/Cr, mI/NAA |
| (Zhu et al., 2015) | Hippocampus, Basal ganglia, Frontal lobe | 3.0 | PRESS | 86 (52/-/34) | NAA/Cr, Cho/Cr, mI/Cr |
| (Chen et al., 2016) | PCC, Hippocampus, Frontal lobe WM, PAWM | 3.0 | PRESS | 78 (38/-/40) | NAA/Cr, mI/Cr, Glu/Cr, Cho/Cr |

| Literatures | Region of interest | Magnetic Field (Tesla) | Pulse sequence | Subjects number (MCI/AD/HC) | Metabolites quantification |
|---|---|---|---|---|---|
| (Guo et al., 2016) | ACC, PCC | 3.0 | PRESS | 44 (13/15/16) | NAA/mI, NAA/Cr, Cho/Cr, mI/Cr |
| (Waragai et al., 2017) | PCC | 1.5 | PRESS | 274 (53/21/200) | NAA/Cr, mI/Cr, NAA/mI |
| (Zeydan et al., 2017) | PCC | 3.0 | sLASER | 46 (14/-/32) | Cr, mI, Cho, Glu, NAA, Glu/mI |
| (Mullins et al., 2018) | PCC | 3.0 | PRESS | 52 (-/25/27) | Glucose, Asc, Lac, NAA, Glu, Gln, sI, PCr, mI, GSH, Ala, NAAG, GABA |
| (Marjanska et al., 2019) | PCC, OCC | 7.0 | STEAM | 49 (-/16/33) | Asc, Asp, GABA, Gln, Glu, GSH, mI, NAA, NAAG, PE, sI, Tau, Cho, Cr |
| (Oeltzschner et al., 2019) | ACC | 7.0 | STEAM | 26 (13/-/13) | GABA/tCr, Glu/tCr, GSH/tCr, NAA/tCr, NAAG/tCr, mI/tCr |
| (Shukla et al., 2020) | ACC, PCC and Cingulate | 3.0 | MEGA-PRESS | 64 (19/18/27) | GSH, NAA (AU), Cr (AU), Cho (AU) |
| (Wong et al., 2020) | Hippocampus, PCC | 7.0 | sLASER | 35 (8/9/16) | Glu, NAA |
| (Brown et al., 1989) | Frontal, Temporoparietal regions | 1.89 | - | -/17/17 | PCr/Pi, Pi, PME/PDE, PME, PCr |
| (Bottomley et al., 1992) | Whole brain | 1.5 | - | -/11/14 | PCr, NTP, PME, Pi, PDE, PCr/NTP, Pi/NTP, PCr/Pi, PDE/NTP, PME/NTP, PDE/PME |
| (Pettegrew et al., 1994) | Dorsal prefrontal cortex | 1.5 | - | -/12/21 | PME, Pi, PDE, PCr, pH |
| (Smith et al., 1995) | Frontal lobe | 1.5 | - | -/17/8 | pH, PME, Pi, PDE, PCr, Total NP, PCr/Pi, PCr/NTP, Pi/NTP, PME/PDE |
| (Gonzalez et al., 1996) | Whole brain | 1.5 | - | -/16/8 | β-NTP, PCr, PME, PDE, Pi, PCr/Pi, NTP/Pi, PME/PDE, PDE/NTP, PME/NTP |
| (Mecheri et al., 1997) | Hippocampus | 1.5 | - | -/24/11 | PME, Pi, PDE, PCr, γ-ATP, α-ATP, β-ATP |
| (Forlenza et al., 2005) | Left prefrpntal cortex | 1.5 | ISIS | -/18/16 | PME, PDE, PME/PDE, PCr, Pi, γ-ATP, α-ATP, β-ATP, total ATP |
| (Rijpma et al., 2018) | ACC, Hippocampus, retrosplenial cortex | 3 | MRSI | -/31/31 | PCr, Pi, PCr/Pi, total ATP, NAD(H), PEth, PCh, GPEth, GPCh, pH |

"-", not available.

**Table 2.**

Hedges'g for MCI and AD studies in each region of interest before leave-one-out analysis.

| Metabolite | Region of interest | MCI | | | | AD | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | k | Hedges'g [95% CI] | $I^2$ | p value | k | Hedges'g [95% CI] | $I^2$ | p value |
| NAA/Cr | PCC | 19 | −0.53 [−0.78, −0.28] | 80% | **<0.001** | 19 | −0.96 [−1.22, −0.69] | 82% | **<0.001** |
| | Hippocampus | 12 | −0.37 [−0.59, −0.14] | 61% | **0.001** | 9 | −0.85 [−1.20, −0.50] | 84% | **<0.001** |
| | PWM | 5 | −0.34 [−0.85, 0.17] | 71% | 0.19 | 6 | −0.85 [−1.43, −0.26] | 84% | **0.004** |
| | PGM | - | - | - | - | 4 | −0.78 [−1.71, 0.14] | 92% | 0.1 |
| | overall | 36 | −0.46 [−0.62, −0.30] | 76% | **<0.001** | 38 | −0.90 [−1.10, −0.71] | 84% | **<0.001** |
| mI/Cr | PCC | 18 | 0.36 [0.12, 0.65] | 83% | **0.003** | 19 | 0.85 [0.52, 1.18] | 89% | **<0.001** |
| | Hippocampus | 10 | 0.70 [0.37, 1.03] | 79% | **<0.001** | 6 | 0.90 [0.60, 1.19] | 62% | **<0.001** |
| | PWM | 5 | 0.69 [0.10, 1.29] | 78% | **0.02** | 6 | 0.72 [0.23, 1.21] | 77% | **0.004** |
| | PGM | - | - | - | - | 4 | 0.93 [0.54, 1.32] | 57% | **<0.001** |
| | overall | 33 | 0.52 [0.33, 0.71] | 80% | **<0.001** | 35 | 0.85 [0.65, 1.06] | 85% | **<0.001** |
| Cho/Cr | PCC | 16 | 0.14 [−0.08, 0.35] | 70% | 0.15 | 15 | 0.39 [0.20, 0.58] | 62% | **<0.001** |
| | Hippocampus | 8 | −0.02 [−0.38, 0.34] | 80% | 0.92 | 6 | −0.24 [−0.45, −0.03] | 41% | **0.02** |
| | PWM | 4 | −0.04 [−0.31, 0.23] | 0% | 0.76 | 3 | −0.26 [−0.51, −0.02] | 0% | **0.04** |
| | overall | 28 | 0.08 [−0.08, 0.24] | 69% | 0.35 | 24 | 0.15 [−0.04, 0.34] | 78% | 0.13 |
| GSH/Cr | overall | 2 | 0.16 [−0.76, 1.08] | 89% | 0.73 | 1 | 0.49 [−0.06, 1.04] | - | - |
| NAA | PCC | 10 | −0.77 [−1.07, −0.48] | 70% | **<0.001** | 8 | −1.13 [−1.38, −0.88] | 44% | **<0.001** |
| | Hippocampus | 5 | −0.79 [−1.22, −0.37] | 71% | **<0.001** | 8 | −0.96 [−1.27, −0.65] | 81% | **<0.001** |
| | PWM | - | - | - | - | 3 | −0.28 [−0.51, −0.06] | 0% | **0.01** |
| | PGM | - | - | - | - | 4 | −0.74 [−1.17, −0.31] | 72% | **<0.001** |
| | overall | 15 | −0.78 [−1.01, −0.55] | 68% | **<0.001** | 23 | −0.90 [−1.09, −0.70] | 78% | **<0.001** |
| mI | PCC | 7 | 0.26 [−0.01, 0.53] | 56% | 0.06 | 6 | 1.00 [0.29, 1.72] | 93% | **0.006** |
| | Hippocampus | 4 | 0.16 [−0.25, 0.58] | 68% | 0.44 | 5 | 0.35 [−0.10, 0.79] | 87% | 0.13 |
| | PWM | 3 | 0.68 [0.36, 1.01] | 0% | **<0.001** | 3 | 0.45 [−0.01, 0.91] | 66% | 0.06 |
| | PGM | - | - | - | - | 3 | 0.25 [0.01, 0.49] | 0% | **0.04** |
| | overall | 14 | 0.31 [0.10, 0.52] | 62% | **0.004** | 17 | 0.51 [0.25, 0.78] | 86% | **<0.001** |
| Cho | PCC | 8 | −0.06 [−0.33, 0.20] | 56% | 0.64 | 7 | 0.60 [−0.06, 1.26] | 92% | 0.08 |
| | Hippocampus | 4 | −0.53 [−0.84, −0.22] | 49% | **<0.001** | 5 | −0.60 [−0.75, −0.45] | 0% | **<0.001** |
| | overall | 12 | −0.24 [−0.47, −0.01] | 67% | **0.04** | 12 | 0.02 [−0.39, 0.42] | 92% | 0.93 |
| Cr | PCC | 7 | −0.57 [−1.02, −0.12] | 79% | **0.01** | 6 | −0.37 [−0.85, 0.11] | 82% | 0.13 |
| | Hippocampus | 4 | −0.46 [−0.66, −0.27] | 0% | **<0.001** | 5 | −0.41 [−0.58, −0.24] | 22% | **<0.001** |
| | overall | 11 | −0.50 [−0.76, −0.24] | 68% | **<0.001** | 11 | −0.40 [−0.63, −0.18] | 69% | **<0.001** |
| GSH | overall | 2 | −1.01 [−1.27, −0.76] | 0% | **<0.001** | 3 | −0.40 [−2.37, 1.58] | 98% | 0.69 |

k, number of studies; $I^2$, heterogeneity. "-", not available. The threshold for significance is $p < 0.05$ showed as bold.