

Metformin attenuates white matter microstructural changes in Alzheimer's disease

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Abstract

Objectives: A body of evidence suggests that individuals with diabetes face an elevated susceptibility to developing Alzheimer's disease when compared to the general populace. Prior research has indicated the potential of metformin to confer a protective influence in postponing dementia onset among diabetic patients. Nevertheless, data are scarce regarding the impact of metformin on microstructural alterations. The primary objective of this study is to explore the influence of metformin on white matter microstructural change in non-demented individuals with diabetes.

Methods: We entered 113 non-demented diabetic subjects including 77 mild cognitive impairment (MCI), and 36 cognitively healthy individuals from Alzheimer's disease Neuroimaging Initiative (ADNI) which were then categorized as metformin users and non-users. We used the ANCOVA model to measure the association between metformin use and DTI values.

Results: Results of the univariate model indicate that metformin users had a higher FA value left hippocampal cingulum ($p = 0.003$) and right internal capsule ($p = 0.004$). Moreover, the MD value of the right inferior frontal-occipital fasciculus was lower in those who used metformin compared to those not use it ($p = 0.027$).

Conclusion: Our results revealed that metformin has protective effects on brain microstructural changes in elderly individuals with diabetes who do not exhibit signs of dementia. A comparison of the groups yielded compelling evidence of reduced neurodegeneration among those utilizing metformin.

Keywords: Alzheimer's disease, metformin, diabetes, white matter

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Introduction

Due to the demographic shift towards an aging population, there is an anticipated increase in the incidence of neurodegenerative diseases associated with aging, such as

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Alzheimer's disease (AD) (1). AD is the most prevalent cause of dementia, accounting for approximately 50% to 75% of all dementia cases, with its incidence continually rising (2). Epidemiological and clinical studies have consistently demonstrated that diabetic patients are at a heightened risk of developing AD compared to the general population (3). The pathophysiological mechanisms underlying this association are complex and multifactorial. Insulin plays a crucial role in brain functions, including the neuroinflammatory response, synaptic activity, glial cell function, and neuronal metabolism. Consequently, there is growing interest in the relationship between insulin resistance and AD (4). Additionally, numerous studies have indicated that insulin resistance may contribute to the deposition of Amyloid Beta ($A\beta$) and the phosphorylation of tau, both of which are key pathophysiological features of AD (5, 6).

The white matter of the central nervous system is particularly susceptible to ischemia (7). Pathological investigations have shown that patients with vascular dementia exhibit significantly lower myelin density in the white matter compared to age-matched controls, a condition associated with cerebral hypoperfusion (8). White matter hyperintensities (WMH), which are the characteristic neuroimaging features of cerebral small vessel diseases (CSVD), are regarded as direct manifestations of chronic cerebral hypoperfusion (9). Patients with reduced cerebral blood flow (CBF) tend to have a higher burden of WMH, with CBF being lower in regions of WMH compared to normal-appearing white matter (10). The progression of WMH correlates with cognitive decline in the elderly and is proposed as a predictor of mild cognitive impairment (11). The impact of white matter lesions on cognitive function may stem from demyelination-induced dysfunction in neural circuitry and synaptic activity (12).

Metformin is the most extensively utilized oral hypoglycemic agent for managing type 2 diabetes and metabolic syndrome (13). Type 2 diabetes is associated with an accelerated cognitive decline and an elevated risk of dementia. Among diabetic individuals, long-term metformin therapy has been shown to mitigate the risk of cognitive decline and dementia (14). Recent studies have partially attributed the cognitive benefits of prolonged metformin use to the reduction of cerebral small vessel disease (CSVD) burden (15). Emerging evidence suggests that metformin exerts a protective effect on white matter integrity. Specifically, metformin has been demonstrated to reduce demyelination and promote remyelination in models of lysophosphatidylcholine-induced demyelination in mice (16). Furthermore, metformin can accelerate myelin recovery and ameliorate behavioral deficits in animal models of multiple sclerosis (17). Additionally, metformin treatment has been shown to effectively prevent ischemia-induced brain injury by alleviating inflammation (18) and to enhance motor and cognitive performance following neonatal hypoxia-ischemia (19). These findings have generated interest in investigating whether metformin can restore white matter lesions and cognitive impairment associated with vascular contributions to cognitive impairment and dementia (VCID).

Diffusion tensor imaging (DTI) is an *in vivo* neuroimaging technique extensively employed to investigate the microstructure of white matter (WM) (20). By measuring the diffusion of water molecules within tissues, DTI offers quantitative insights into alterations in WM integrity, such as axonal and myelin damage. Although a limited number of studies have explored the impact of statins on WM microstructure using DTI (21). These investigations have been observational and may be confounded by the significantly elevated cardiovascular disease risk factors present in statin-exposed individuals, which can also influence WM microstructure. Consequently, the causative effects of statins on WM microstructure remain to be elucidated.

Although previous studies suggest the potential benefits of metformin in patients with AD and mild cognitive impairment (MCI), further research is necessary to investigate the therapeutic effects of metformin in these conditions or its potential role in delaying disease onset. Additionally, data on metformin's impact on brain health are limited (22-24). Thus, further research is warranted to determine whether there is an association between metformin use and positive WM microstructural changes in elderly individuals. This study aims to investigate the effect of metformin on WM microstructural changes in elderly individuals.

Materials and Methods

Data Acquisition

Participant information was sourced from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). Initiated in 2003 under the leadership of principal investigator Michael W. Weiner (MD), ADNI is a public-private partnership. The primary objective of ADNI is to determine whether serial MRI, positron emission tomography (PET), biological marker assessments, and clinical/neuropsychological evaluations can be combined to track the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). For this study, we enrolled 113 non-demented diabetic subjects including 77 MCI, and 36 cognitively healthy (HC) individuals. Participants were classified as metformin users if they had been on the medication for at least two years; otherwise, they were categorized as metformin non-users. Inclusion criteria required that subjects had available data on diagnostic status, Mini-Mental State Examination (MMSE) scores, and apolipoprotein E (APOE) genotyping results within the ADNI database. All MCI subjects met the criteria for amnesic MCI, which included MMSE scores between 24 and 30, a self-reported memory complaint, objective memory impairment as indicated by education-adjusted scores on the Wechsler Memory Scale Logical Memory II, a Clinical Dementia Rating (CDR) of 0.5, preserved activities of daily living, and the absence of dementia.

DTI Processing and Image Analysis

The results of the DTI regions of interest (ROI) analysis were obtained from the ADNI cohort. The DTI scans were

Table 1. Clinical and demographic characteristics of participants

Characteristic	Group	Metformin users (n=68)	Metformin non-users (n=45)	P value
Number	HC	25	11	-
	MCI	43	34	-
Age, mean (SD), years	HC	71.2 (6.6)	72.2 (6.9)	0.426
	MCI	75.5 (7.8)	74.3 (7.4)	0.141
Female sex, No.	HC	14	7	0.575
	MCI	31	18	0.784
Education, mean (SD), years	HC	16.8 (2.4)	16.4 (2.5)	0.514
	MCI	16.2 (2.8)	16.3 (2.3)	0.545

Abbreviations: HC, healthy controls; MCI, mild cognitive impairment

normalized using the Montreal Neurological Institute and Hospital (MNI) nu_correct tool (www.bic.mni.mcgill.ca/software/). Non-brain tissues were removed using the Brain Extraction Tool (BET) from FSL (25).

The T1-weighted image was aligned to a modified version of the Colin27 brain template¹⁷ using FSL's FLIRT.¹⁸ This Colin27 brain was zero-padded to achieve a cubic isotropic image size (220 x 220 x 220 mm³) and subsequently down-sampled (110 x 110 x 110 mm³) to approximate the resolution of the diffusion-weighted imaging (DWI). A single diffusion tensor was modeled at each voxel in the brain. Scalar anisotropy and diffusivity maps were generated from the resultant diffusion tensor eigenvalues ($\lambda_1, \lambda_2, \lambda_3$). Fractional anisotropy (FA), which indicates the directional dependence of the diffusion process, and mean diffusivity (MD), radial diffusivity (RD), and axial diffusivity (AxD), which reflect the extent of diffusion, were calculated. Lower FA and higher RD, AxD, and MD values are indicative of demyelination and degeneration in white matter.

A shared information-based elastic registration algorithm, as previously described, was employed to align the FA image from the Johns Hopkins University (JHU) DTI atlas²⁰ to each subject. Nearest-neighbor interpolation was used to apply the deformation to the stereotaxic JHU "Eve" white matter atlas labels

(http://cmrm.med.jhmi.edu/cmrm/atlas/human_data/file/AtlasExplanation2.htm) to prevent label intermixing. This ensured that the atlas ROIs were placed in the same coordinate space as the DTI maps. The average FA and MD values were then calculated within the boundaries of each ROI mask for each subject.

Additionally, tensor-based spatial statistics (26) were performed, extracting the mean FA in the ROIs along with the skeleton. Tract-based spatial statistics (TBSS), an automated and observer-independent method for assessing FA in major white matter tracts on a voxel-wise basis across groups of subjects, was

executed following the ENIGMA-DTI group's protocols (http://enigma.loni.ucla.edu/wpcontent/uploads/2012/06/ENIGMA_TBSS_protocol.pdf). In summary, all subjects were registered to the ENIGMA-DTI template in International Consortium for Brain Mapping (ICBM) space, a stereotaxic probabilistic white matter atlas. Standard TBSS steps were undertaken to project individual FA maps onto the skeletonized ENIGMA-DTI template. ROI extraction was also conducted to determine the mean FA in ROIs along with the skeleton, as detailed in the ENIGMA ROI protocol (http://enigma.loni.ucla.edu/wpcontent/uploads/2012/06/ENIGMA_ROI_protocol.pdf).

Statistical Analysis

Statistical analyses were conducted using SPSS Statistics version 16 (IBM Corp., Armonk, NY). Clinical and demographic comparisons between groups stratified by metformin exposure were performed using the t-test for continuous variables and the chi-square test for categorical variables (27). Neuroimaging variables were log-transformed to meet normal distribution criteria before statistical analyses. The association between metformin exposure and neuroimaging parameters was evaluated using an ANCOVA model adjusted for age, sex, APOE $\epsilon 4$ genotype, and MMSE score. To address type I errors due to multiple comparisons, the Benjamini-Hochberg correction was applied.

Results

Patient demographic

This study included 157 subjects with a mean age of 73.3 (± 7.2) years, of whom 68 individuals were classified as metformin users. The clinical and demographic characteristics of the subjects are detailed in Table 1. There were no significant differences between metformin users and non-users in terms of clinical and demographical characteristics (Table 1).

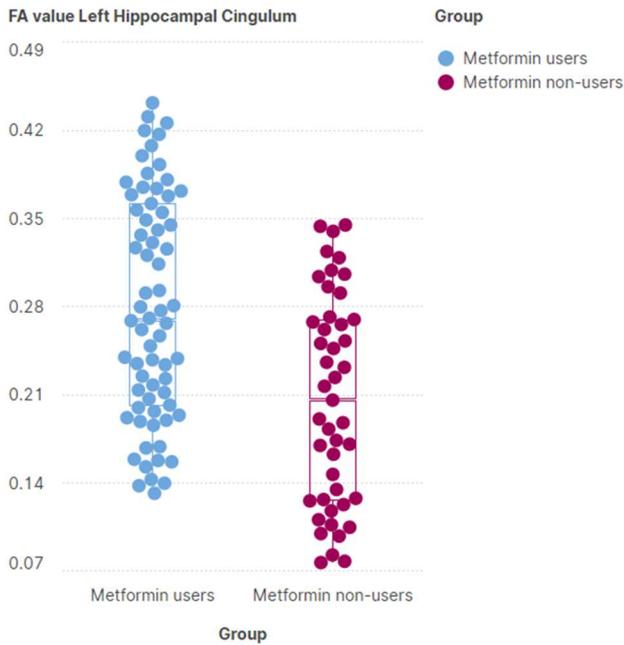


Figure1. Box plot of the FA in the left hippocampal cingulum

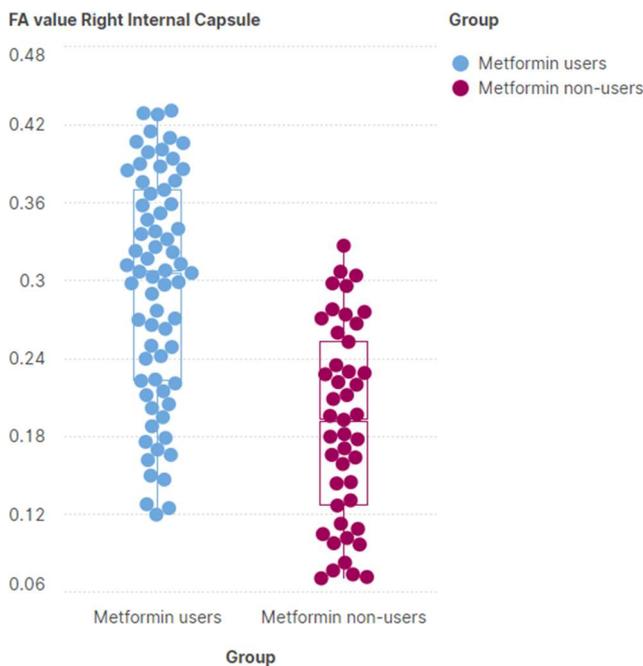


Figure2. Box plot of the FA in the right internal capsule

Metformin and DTI

A univariate linear model was employed to compare hippocampal and cortical volumes between metformin users

and non-users, with adjustments made for age, sex, and APOE $\epsilon 4$ genotype.

The univariate model results indicate that metformin users exhibited significantly higher FA values in the left hippocampal cingulum ($p = 0.003$) (Figure 1) and the right internal capsule ($p = 0.004$) (Figure 2). Additionally, the MD value of the right inferior frontal-occipital fasciculus was significantly lower in metformin users compared to non-users ($p = 0.027$) (Figure 3).

Discussion

In this study, we examined the impact of metformin on brain structure by analyzing FA and MD values in non-demented diabetic patients. Our univariate model results revealed that metformin users had significantly higher FA values in the left hippocampal cingulum ($p = 0.003$) and the right internal capsule ($p = 0.004$), indicating enhanced white matter integrity in these regions. Additionally, metformin users exhibited significantly lower MD values in the right inferior fronto-occipital fasciculus compared to non-users ($p = 0.027$), suggesting reduced microstructural damage or increased cellularity in this area.

Recent evidence has established type 2 diabetes mellitus as a major risk factor for neurodegenerative diseases, including AD (28, 29). This association has spurred numerous investigations into the therapeutic effects of anti-diabetic agents on AD progression (30, 31). Insulin resistance, impaired glucose metabolism, and mitochondrial dysfunction have been proposed as mechanisms linking AD and type 2 diabetes (32, 33). Notably, increased insulin resistance has been observed in brain regions like the hippocampus and cerebellum in AD patients (28, 34).

Metformin, a small, water-soluble compound of the biguanides class, is a first-line treatment for type 2 diabetes mellitus in patients with a glomerular filtration rate (GFR) above 30 ml/min/1.73m² (35). It regulates blood glucose by reducing hepatic glucose production, enhancing peripheral glucose uptake, and improving insulin sensitivity (36). However, the literature presents conflicting results regarding metformin's impact on neurodegenerative diseases. Some studies indicate that metformin has neuroprotective effects, potentially improving cognition or delaying cognitive decline in elderly diabetic patients (37), while other research associates long-term, high-dose metformin use with an increased risk of AD development (38). Our findings suggest that metformin use is associated with higher FA values and lower MD values, indicating a potential protective effect on brain microstructure in non-demented diabetic individuals.

Magnetic resonance imaging (MRI), computed tomography (CT), and positron emission tomography (PET) are key tools for studying AD progression (39). MRI, in particular, can quantify brain atrophy in AD's early stages and predict future clinical manifestations. Jack et al. used serial MRI to compare brain atrophy levels across different groups, concluding that MRI combined with clinical measures is a precise marker for AD progression (40).

Previous research has shown that metformin activates AMP-activated protein kinase (AMPK), significantly increasing the generation of amyloid-beta ($A\beta$) species (34). It also upregulates

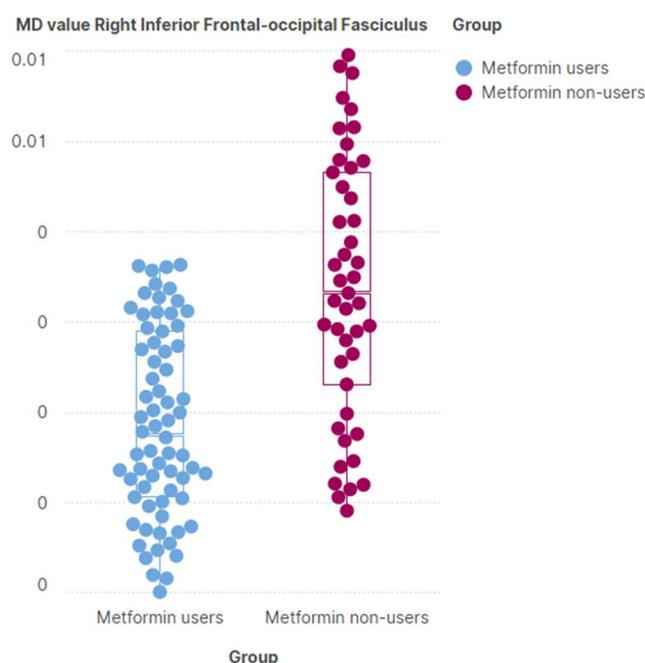


Figure 3. Box plot of the MD in the right inferior frontal-occipital fasciculus

β -secretase (BACE1), enhancing protein levels and enzymatic activity, and induces mitochondrial dysfunction (41). These neuropathological mechanisms contribute to neurodegeneration and cognitive decline. While metformin has been associated with a lower risk of dementia, some studies suggest it may have harmful effects when used as monotherapy in elderly AD patients with diabetes (41). In contrast, treatments like insulin and rosiglitazone have shown cognitive improvements in mild to moderate AD patients, and glucagon-like peptide-1 receptor agonists have reduced AD risk in diabetic patients (22).

In our study, non-metformin users had smaller volumes in the cingulate, parietal, and frontal lobes. Although cortical changes in AD progression are well-documented, subcortical changes are less understood (24). Metformin can cross the blood-brain barrier, offering neuroprotection through anti-inflammatory mechanisms and improved brain energy metabolism (18). However, it can also increase $A\beta$ production, leading to amyloid plaque formation, neuronal toxicity, and brain atrophy. Emerging evidence suggests that COVID-19 and other viral infections are associated with white matter brain changes, including microstructural abnormalities that may contribute to neurological and cognitive complications observed in affected individuals (42, 43). Pathological alterations in white matter integrity have been associated with autism spectrum disorder, indicating that these changes may contribute to the atypical neural connectivity and cognitive characteristics observed in individuals with the condition (44).

Conclusion

Our results indicate that metformin has protective effects on brain volumes in a combined sample of MCI and CH diabetic

individuals, suggesting lower neurodegenerative WM changes in metformin users. Given the conflicting evidence on metformin's role in AD development, further studies are needed to elucidate its pharmacological mechanisms on brain metabolism. Our study's limitations include a lack of longitudinal design and a small sample size. Future research should involve larger, longitudinal studies to comprehensively examine metformin's impact on microstructural changes in MCI and AD.

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Declarations

Funding

We do not have any financial support for this study.

Conflict of interest

The authors have no conflicts of interest to disclose.

Availability of data

The datasets analyzed during the current study are available upon request with no restriction.

Code availability

Not applicable

Ethical approval

The data in this paper were obtained from the ADNI database (adni.loni.usc.edu). It does not include any examination of human or animal subjects.

Consent for publication

This manuscript has been approved for publication by all authors.

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