Brain atrophy in ageing: estimating effects of blood glucose levels vs other type 2 diabetes effects

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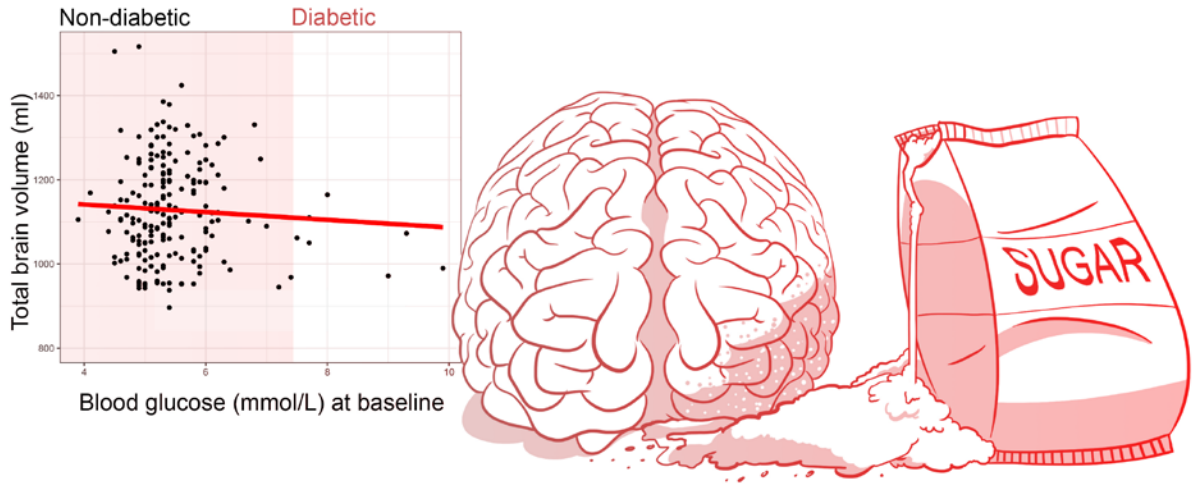

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Introduction

It is now well documented that ageing is associated with brain shrinkage, particularly in late adulthood[1, 2]. From age 60 onwards, the average adult brain atrophies ≈0.5%/yr for the whole brain, ≈0.33%/yr for grey matter and ≈0.62-0.68%/yr for white matter [3-5]. This may seem small, but cumulatively adds up to substantial volume losses over decades: between the ages of 60 and 70, approximately 54ml (5%) of total brain volume is lost, a substantial amount when coupled with cumulative atrophy across the lifespan. Type two diabetes mellitus (T2D) is associated with the development of structural brain abnormalities, including increased cerebral atrophy over time [2]. Individuals with T2D have a significantly lower total brain volume (0.1-1.5%), grey matter volume (~1%), and white matter volume (<1%) than those with normal fasting glucose (NFG), corresponding to between one and three years of age-associated atrophy[1]. There is mounting evidence that variation of blood glucose in the normal fasting glucose (NFG) range may also impact on brain structure and be associated with cognitive impairment [1, 2, 6].

Blood glucose levels and T2D are typically related, but it does not follow that an individual with T2D necessarily has high blood glucose levels. Successful glycaemic control following diagnosis can return blood glucose levels to normal. However, glycaemic control is not synonymous with addressing T2D comorbidities, such as obesity or pre-existing vascular damage, which mechanistically link T2D to cerebral atrophy. For example, while blood glucose is associated with both grey and white matter atrophy, obesity has differential effects on grey and white matter in otherwise healthy overweight and obese individuals [7, 8]. The current study examined the overlap in the association between blood glucose levels (across the whole range, and in NFG only), T2D and longitudinal brain volumes (total, white matter, and grey matter) in a healthy, community-living ageing population.

Materials and Methods

Study population

Participants were sampled from the Personality and Total Health (PATH) Through Life study, a large longitudinal study investigating ageing, health, cognition and other individual characteristics across the lifespan [9]. This study focusses on a randomly selected subsample of the oldest of the PATH cohorts (aged 60-64 years at baseline) with MRI scans taken on four occasions four years apart (*M*=4.36, *SD*=1.58), and fasting blood glucose measurements on three occasions. After exclusions due to missing data and history of neurological disorders, there were *n*=279 participants. They did not differ from the wider PATH sample except for a slightly higher education level (*M*=14.46 vs *M*=13.71; *t*(294)=4.284, p=<0.01). See supplementary material for additional information on sample selection and inclusion criterion. This study was approved by the Australian National University Ethics Committee. All participants provided written, informed consent.

Plasma glucose and brain measures

Venous blood was collected following an overnight fast of at least 10 hours. Plasma glucose was measured on a Beckman LX20 Analyzer by an oxygen rate method (Fullerton, California). Diabetic grouping was defined by non-overlapping categories of T2D (self-reported, or two or more fasting blood glucose measures >7mmol/L), IFG (not T2D and two or more blood glucose measures >=5.6mmol/L), or NFG (not T2D or IFG, two or more blood glucose measures <5.6), following American Diabetes Association guidelines [10]. T1-weighted brain scans processed with Freesurfer 5.3 to extract total brain volume (TBV), grey matter (GM) and white matter (WM) volume.

Statistical analyses

To contrast the effects of blood glucose and diabetes diagnosis, a multilevel model was fitted with diabetic group (NFG, IFG, or T2D) as a predictor of repeated blood glucose measures, which were nested by individual (random intercept model). Overlap was explored via coefficient direction, significance, and a multilevel extension of the Variance Inflation Factor (VIF) from the RMS package (v 4.5).

Exploration of the association between blood glucose and brain volumes proceeded in three modelling steps: (1) blood glucose as key predictor (2) blood glucose and diabetes diagnosis as key predictors, and (3) blood glucose as key predictor in individuals with NFG only.

To investigate the association between blood glucose and brain volumes, models (1), (2) and (3) were fit for each of TBV, GM and WM, with blood glucose (mmol/L) as a fixed-effect predictor of volume (ml). To investigate the longitudinal between-subjects association between blood glucose and change in brain volumes over time, models (1), (2) and (3) were fit for each brain volume, with the interaction between blood glucose (mmol/L) and time (age, centered on 60) as a fixed-effect predictor of volume (ml). All models controlled for time (age centered on 60), gender, years of education, and intracranial volume, hypertension, BMI, smoking status, and Goldberg depression score (see supplementary materials and [6, 7, 9]).

Alpha was set at 0.05.

Results

Overlap between blood glucose and diabetes diagnosis

Multilevel modelling of diabetic status throughout the study as a predictor of repeated blood glucose measures showed individuals in the NFG group had an average blood glucose level of 5.11mmol/L (95%CI[5.022,5.192]). Individuals in IFG and T2D groups had significantly higher blood glucose levels (*b*=0.76 mmol/L, 95%CI[0.58,0.94] and *b*=1.74 mmol/L, 95%CI[1.55,1.94]). There was moderate but not strong correlation between blood glucose and diabetes group (VIF for glucose=1.04, IFG=1.05, T2D=1.08). Further multilevel models indicated that blood glucose and diabetes group did not significantly interact to predict TBV (*b*=-3.08ml, 95%CI[-10.15,3.98]), GM (*b*=-4.08 ml, 95%CI [-8.52, 0.35]) or WM (*b*=0.94ml, 95%CI [-4.38,6.25]).

Blood glucose, diabetes group and baseline brain volumes

Blood glucose was not significantly associated with TBV or WMV (Table 1). Diabetes group (NFG, IFG, and T2D) was significantly associated with brain volumes; compared to those in the NFG group, participants with T2D had significantly lower total brain volume (*b*=-30.64ml, 95%CI[-44.62, -16.67], p<0.001); lower grey matter volume (*b*=-9.62ml, 95%CI[-18.01,-1.24], p<0.05); and lower white matter volume (*b*=-19.59ml, 95%CI[-29.63,-9.54]).

Blood glucose, T2D and longitudinal change in brain volumes

Interactions between blood glucose and time (Table 1) indicated blood glucose was associated with a significant decrease in TBV each year beyond age 60 (≈0.43 ml per 1mmol/L per year) and grey matter volume (≈1.45ml per 1mmol/L per year). Interactions between time and diabetic group indicated T2D was significantly associated with GM atrophy only (≈0.75 ml per 1mmol/L per year; see supplementary materials).

Discussion

This study clarifies the synergetic but not completely overlapping contribution of higher blood glucose levels and T2D on the ageing brain. The main findings were that while a T2D diagnosis was more strongly associated with brain structure than variability in plasma glucose levels, the latter also played a significant role beyond T2D. Indeed, while T2D was associated with total brain, grey matter and white matter volume, when the sample was limited to individuals without diabetes or impaired fasting glucose, an individual with a subtly higher blood glucose in the normal range (e.g. 5.5mmol/L versus 5mmol/L) were predicted to have an ≈0.06% comparatively greater decrease in total brain volume each year. In combination with typical age-associated changes in brain volume, additional atrophy of this magnitude may be associated with an increased risk of mild cognitive impairment and Alzheimer's disease.

The current study reaffirmed an association between T2D and blood glucose and brain volumes, and extended previous findings of indicating an association between high blood glucose in the normal range and change in brain volume over time [1, 2]. Our results emphasize that the association between blood glucose and brain atrophy is important even in healthy individuals. Although baseline brain volumes and atrophy rates were broadly commensurate with the broader literature, total brain and white matter atrophy in the current sample was markedly (approximately 1-2ml per year) less than has been previously suggested [3]. Further, average blood glucose levels within individuals with T2D were lower than the diagnostic cutoff for T2D (6.85mmol/L is less than 7mmol/L), indicating some degree of post-diagnosis glycaemic control was taking place. Significant findings within this neurologically healthy (due to stringent exclusion criterion) and highly educated[9] sample further highlight the importance of blood glucose as a factor in healthy brain ageing.

We also demonstrated that blood glucose levels and T2D are neither synonymous with one another nor their associations with brain volumes and atrophy. Although high blood glucose is a prerequisite for diagnosis, successful glycaemic control can return blood glucose levels to NFG levels without necessarily altering T2D comorbidities, such as obesity or inflammation [8]. While separating individuals with NFG and T2D into distinct groups for study is a valid approach, the current study highlights the need to carefully consider the relationship between covariates and T2D, and urges caution in synthesising conclusions relating to blood glucose across studies conducted in only NFG or only T2D.

The key strength of the current study was the combination of tight age range and longitudinal follow up from age 60 which would be expected to minimise cohort effects and might provide some support for possible causative links. Although, as in all correlational studies, causation cannot be demonstrated. Moreover, reverse causality cannot be excluded (e.g. poorer cognitive functioning due to decreased brain volumes leading to lifestyle and dietary changes that increase blood glucose, or other risk factors such as obesity). The nature of the relationship between T2D, blood glucose and brain atrophy should therefore be clarified by future experimental work.

Conclusions

In conclusion, this study showed that the impact of blood glucose on the brain is not exclusive to T2D, and that blood glucose levels even in the normal range can have a significant impact on total brain and grey matter atrophy. These results emphasize the need to consider the role of higher normal blood glucose as a risk factor for brain health.

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Statistical analysis: conducted by Erin Walsh, Australian National University.

Author contributions:

Dr. Walsh contributed to the design of the study, conducted all statistical analyses, and managed all aspects of manuscript preparation and submission.

Prof. Sachdev contributed to the design of the study, and provided methodological input and theoretical expertise.

Assoc. Prof Cherbuin, Dr. Shaw and Prof. Anstey contributed to the design of the study, provided methodological input and theoretical expertise, advised on statistical analyses, and contributed to writing and editing of the manuscript.

Disclosures

Dr. Walsh reports no disclosures

Dr. Shaw reports no disclosures

Prof. Sachdev reports no disclosures

Prof. Anstey reports no disclosures

Assoc. Prof. Cherbuin reports no disclosures

Conflicts of interest: none.

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Table 1. Multilevel model coefficients for the association between blood glucose, diabetes group, and brain volumes

	Total brain volume			Grey matter volume			White matter volume		
	[1]	[2]	[3]	[1]	[2]	[3]	[1]	[2]	[3]
Fixed									
Glucose	-0.81	0.16	2.58	-1.56*	-1.23	1.85	0.55	1.26	0.73
	[-3.09, 1.47]	[-2.18, 2.50]	[-4.02, 9.17]	[-2.99, -0.14]	[-2.70, 0.25]	[-2.25, 5.95]	[-1.15, 2.25]	[-0.50, 3.02]	[-4.36, 5.81]
Time	-4.26***	-4.29***	-4.12***	-2.87***	-2.88***	-2.74***	-1.15***	-1.17***	-1.15***
	[-4.65, -3.87]	[-4.68, -3.90]	[-4.61, -3.63]	[-3.12, -2.63]	[-3.13, -2.64]	[-3.04, -2.43]	[-1.44, -0.85]	[-1.46, -0.88]	[-1.53, -0.77]
IFG		7.25			4.28			2.97	
		[-5.35, 19.86]			[-3.26, 11.81]			[-6.06, 12.00]	
T2D		-30.64***			-9.62*			-19.59***	
		[-44.62, -16.67]			[-18.01, -1.24]			[-29.63, -9.54]	
Intercept	370.38***	352.84***	357.46***	248.01***	240.76***	229.84***	112.16***	101.39***	114.65**
	[300.68, 440.08]	[284.73, 420.95]	[264.50, 450.42]	[207.09, 288.94]	[199.78, 281.73]	[173.71, 285.96]	[62.30, 162.02]	[52.30, 150.49]	[45.85, 183.44]
Random effects (variance)									
Intercept	1504.85 [1227.91,1777.83]	1384.84 [1119.17, 1625.05]	1383.79 [1069.14, 1692.91]	495.22 [400.92, 587.54]	485.13 [389.57, 571.12]	489.31 [376.58, 599.51]	745.92 [605.38, 883.82]	698.23 [560.78, 822]	726.9 [555.74, 894.38]
Residual	302.55 [253.92, 353.03]	302.1 [253.59, 352.32]	296.65 [238.6, 357.24]	122.18 [102.53, 142.81]	121.74[102.17, 142.21]	116.02[93.38,139.77]	172.74[144.96, 201.83]	173.36[145.46, 202.53]	178.91[143.68,216.3]
Model fit									
AIC.	5509.68	5479.36	3617.63	4954.22	4940.9	3258.86	5162.59	5139.64	3411.04
Fixed effects									
Glucose	3.82	5.16*	14.02*	0.90	1.39	6.49	2.53	3.50*	6.78
	[-0.36, 8.00]	[0.93, 9.38]	[2.14, 25.90]	[-1.75, 3.54]	[-1.29, 4.08]	[-0.96, 13.93]	[-0.63, 5.69]	[0.29, 6.71]	[-2.44, 16.01]

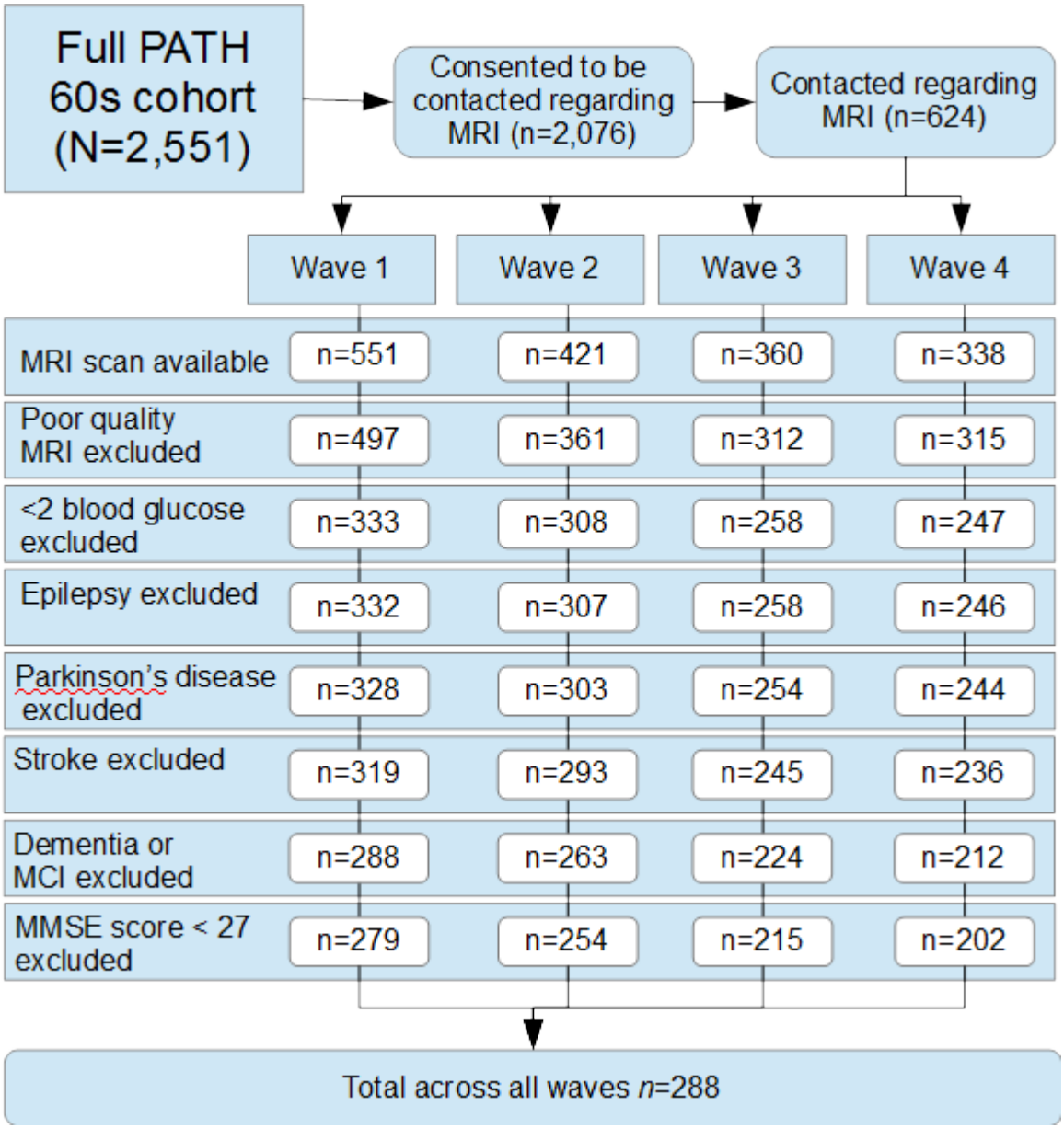
Time	-1.61	-1.43	2.87	-1.45*	-1.38*	0.10	-0.01	0.11	2.56
	[-3.68, 0.46]	[-3.49, 0.64]	[-3.21, 8.96]	[-2.77, -0.14]	[-2.69, -0.07]	[-3.72, 3.92]	[-1.58, 1.56]	[-1.46, 1.68]	[-2.18, 7.29]
Glucose x time	-0.48*	-0.52**	-1.37*	-0.26*	-0.27*	-0.55	-0.21	-0.23	-0.72
	[-0.85, -0.11]	[-0.88, -0.15]	[-2.55, -0.18]	[-0.49, -0.02]	[-0.50, -0.04]	[-1.30, 0.19]	[-0.48, 0.07]	[-0.51, 0.05]	[-1.65, 0.20]
IFG			7.09		4.19			2.90	
			[-5.53, 19.71]		[-3.35, 11.72]			[-6.15, 11.94]	
T2D			-31.61***		-10.14*			-20.00***	
			[-45.60, -17.62]		[-18.53, -1.75]			[-30.07, -9.93]	
Intercept	343.87***	324.27***	300.97***	233.85***	225.65***	206.87***	100.81***	88.56***	84.65*
	[271.24, 416.51]	[253.20, 395.34]	[196.06, 405.89]	[190.97, 276.74]	[182.71, 268.59]	[142.87, 270.87]	[48.58, 153.04]	[37.04, 140.08]	[6.01, 163.30]
Random effects (variance)									
Intercept	1504.85 [1227.91, 1777.83]	1384.84 [1119.17, 1625.05]	1383.79 [1069.14, 1692.91]	495.22[400.92, 587.54]	485.13[389.57, 571.12]	489.31[376.58, 599.51]	745.92[605.38, 883.82]	698.23[560.78, 822]	726.9[555.74 ,894.38]
Residual	302.55 [253.92, 353.03]	302.1 [253.59, 352.32]	296.65 [238.6, 357.24]	122.18 [102.53, 142.81]	121.74 [102.17, 142.21]	116.02 [93.38, 139.77]	172.74 [144.96, 201.83]	173.36 [145.46, 202.53]	178.91 [143.68, 216.3]
Model fit									
AIC.	5,506.74	5,475.33	3,613.74	4,954.03	4,940.14	3,258.84	5,164.58	5,141.06	3,410.34

Note. IFG = Impaired Fasting Glucose, T2D = Type 2 Diabetes. All models control for age, gender, years of education, ICV, hypertension, BMI, smoking status, and Goldberg depression score (coefficients not shown). Values in square brackets are 95% confidence intervals. “Time” is the age variable centred on age at baseline (60).

Supplementary information on sample selection

This study focusses on the oldest of the PATH cohorts, aged 60-64 years at baseline (*n*=2551). Of these participants, 2076 consented to be contacted regarding an MRI scan, and a random subsample of 622 participants were invited to participate in the MRI sub-study. This included an MRI scan taken on three occasions at least four years apart (M=4.36, SD=1.58, range=3.24 to 12.7 years between scans), and fasting blood glucose measurements collection on four occasions. Once taken, scans were excluded on the basis of poor quality or failed processing (as described in the MRI acquisition and image analyses section). Participants were excluded on the basis of having fewer than two blood glucose measures, a history of self-reported epilepsy, Parkinson's disease, stroke, or mild cognitive disorder; and mild cognitive impairment or dementia indicated by International Consensus Criterion, the clinical dementia rating scale. In order to avoid confounding neurobiological changes specific to dementia pathology, MMSE was used alongside self-report to detect possible dementia as in Shaw (2016)[5], with the high cut-off based on a validation study using MMSE to establish probable dementia in population studies [11]. This resulted wave 1 *n*=279, wave 2 *n*=254, wave 3 *n*=215, and wave 4 n=202.

Figure e-1. Exclusion criterion and sample size



Note. MCI = mild cognitive impairment. MMSE = mini–mental state examination. Numbers for each row indicate *n* after exclusion criterion have been applied.

Table e-1. Included vs excluded demographics at baseline

	Included			Excluded		
Demographics	n	M (%)	SD	n (b)	M (%)	SD
Age	279	63.09	1.44	2271	62.99	1.47
Years of education	261	14.35	2.46	2162	13.71	2.88
BMI	260	26.28	4.2	2059	26.82	4.87
Blood glucose	197	5.46	0.81	76	5.89	1.93
Hypertension (yes)	171	(62%)		1429	(64%)	
Gender (Female)	135	(47%)		1099	(48%)	
Smoking (Ever, yes)	115	(41%)		1139	(50%)	
Diabetes (IFG)	52	(19%)		33	(0.1%)	
Diabetes (T2D)	44	(16%)		351	(15%)	
Volumes (ml)	n	M	SD	n (b)	M	SD
Total Brain	279	1120.76	115.84	230	1121.86	112.05
Grey Matter	279	597.88	56.55	230	578.38	63.49
White Matter	279	492.81	64.37	230	514.79	93.93
Lateral ventricles	279	21.42	11.83	230	23.69	22.13

Note. Demographics and brain volumes reported at baseline. Demographic % expresses percentage of sample with the given trait. Atrophy % expresses slope (*b*) as percentage of intercept, calculated from hierarchical model with age as sole predictor of volume.

Table e-2. Demographic characteristics at baseline

	All			NFG only			T2D only		
Demographics	n	M (%)	SD	n (b)	M (%)	SD	n	M (%)	SD
Age	279	63.09	1.44	183	63.16	1.46	44	62.92	1.4
Years of education	261	14.35	2.46	176	14.54	2.39	38	13.5	2.82
BMI	260	26.28	4.2	172	25.31	3.87	41	29.57	4.45
Blood glucose	197	5.46	0.81	133	5.12	0.39	32	6.44	1.31
Hypertension (yes)	171	(62%)		104	(56%)		31	(70%)	
Gender (Female)	135	(47%)		94	(51%)		20	(45%)	
Smoking (Ever, yes)	115	(41%)		67	(36%)		21	(47%)	
Volumes (ml)	n	M	SD	n (b)	M	SD	n	M	SD
Total Brain	279	1120.76	115.84	183	1126.17	116.76	44	1106.18	124.9
Grey Matter	279	597.88	56.55	183	599.7	56.6	44	593.29	67.48
White Matter	279	492.81	64.37	183	496.48	65.8	44	483.58	60.28
Atrophy (ml/yr)	b	(%)		b	(%)		b	(%)	
Total Brain volume	-4.11	(-0.36)		-3.99	(-0.34)		-5.14	(-0.45)	
Grey Matter	-3.00	(-0.50)		-2.92	(-0.10)		-3.65	(-0.60)	
Cortical White Matter	-0.79	(-0.15)		-0.81	(-0.16)		-1.16	(-0.23)	

Note. Demographics and brain volumes reported at baseline. Atrophy (ml/yr) drawn from fixed effects in an unadjusted multilevel model with age as a predictor of the volume of interest, nested by participant. Demographic % expresses percentage of sample with the given trait. Atrophy % expresses slope (*b*) as percentage of intercept, calculated from hierarchical model with age as sole predictor of volume. For context in the literature, from age 60 annual volume loss is estimated for the whole brain (1100-1200ml) at 5.4ml (≈0.5%/yr), for grey matter volume (620-720ml) at 2.4ml-4ml (≈0.33%/yr), and for white matter volume (450-500ml) at 3.1ml (≈0.62-0.68%/yr) [3, 4, 12, 13].

Supplementary information on Socio-demographic and health measures

Total years of education, history of stroke epilepsy and diabetes was assessed by self-report. Participants were screened as potentially having mild cognitive impairment or dementia based on self-report, and diagnoses confirmed by clinical interview including Mini-Mental State Examination[14]. Body mass index (BMI) was computed based on self-reports of weight and height with the formula weight (kg)/height $(m)^2$. Hypertension was assessed by self-reported use of antihypertensive medication, or two blood pressure measures taken three minutes apart while participants were seated (systolic > 140, diastolic > 90). Diabetic group was defined by non-overlapping categories of T2D (self-reported, or two or more fasting blood glucose measures >7mmol/L), IFG (not T2D and two or more blood glucose measures >=5.6mmol/L), or NFG (not T2D or IFG, two or more blood glucose measures <5.6), following American Diabetes Association guidelines [10]. APOEε4 genotype was obtained at baseline as in Jorm et al (2007)[15], briefly cheek swab samples were obtained using Qiagen DNA Blood kits, and two SNPs (rs429358 and rs7412) were assayed via TaqMan Assays (Applied Biosystems Inc., Foster City, CA, USA). Participants were classified as APO*E4 carriers if they possessed one or two ε4 alleles, or otherwise as non-carriers.

Supplementary information on MRI image processing

Acquisition parameters for MRI scans are described in detail elsewhere [16]. Briefly, scans were three-dimensional structural fast-field echo sequence T1-weighted MRI, analyzed on a Linux workstation using FreeSurfer v5.3[17]. For reasons beyond the researcher's control, scanners differed at some waves but participants were scanned on the same scanner at each wave. Wave 1 scans were acquired on a Philips Gyroscan ACS-NT scanner (Philips Medical Systems, Best, the Netherlands) at a coronal orientation (1.5mm slices, repetition time 28.05ms, echo time 2.64ms, flip angle 300, matrix size 256x256). Wave 2 scans were acquired on a Philips Gyroscan ACS-NT scanner at a coronal orientation (1.5mm slices, repetition time 8.93ms, echo time 3.57ms, flip angle 80, and matrix size 256x256). Wave 3 scans were acquired on a Siemens 1.5T Avanto scanner (Siemens Medical Solutions) at a sagittal orientation (1mm slices, repetition time 1160ms, echo time 4.17ms, flip angle 150, and matrix size 512W512). Wave 4 scans were acquired on a Siemens 1.5T Espree scanner (Siemens Medical Solutions) at a sagittal orientation (1mm slices, repetition time 1160ms, echo time 4.24ms, flip angle 150, and matrix size 512x512).

MRI image processing was undertaken in FreeSurfer v5.3[17]. The longitudinal pipeline of this automated software constructs a within-person template from multiple-wave data, and uses it to extract reliable volume estimates[18]. This workflow began with the default cross-sectional process. All scans were aligned to unbiased median common space via the software's normalized MRI template. They underwent intensity normalization via B1 bias field correction, had the skulls stripped by excluding voxels that are non-brain across the majority of scans, and neck removed via reference to the template. They then underwent Talairach registration, and further normalization based on the Global Cellular Automata Model. The longitudinal element of analysis then began. Following motion correction (minor discrepancies across scans being

replaced by the average value across those discrepancies), a new within-subject template was established from cross-sectional scans. Scans were then processed as in the cross-sectional stream, but in reference to the within-subjects template (rather than the more generic MRI template). At this point, scans are resampled to match the template (here, 1.0mm isotropic due to the lower native space voxels of the Siemens scanner). By using this longitudinal pipeline, differences between scanner strengths and manufacturers are minimized by an image processing procedure that focusses on within-subjects registration.

Intracranial volume was estimated from the within-subject template, following the assumption that head size should not change over time. Total brain, grey matter, white matter, and ventricle volume were estimated from each scan using the FreeSurfer segmentation and parcellation procedure, which has been shown to have good reliability across field strengths and scanner manufacturers[19]. At this point, an in-house script was uses to identified scans with outlier values for gray and white matter volumes. These scans were visually checked and removed from further analysis if FreeSurfer processing had failed.

Then, as in Shaw (2016), volumes were orthogonalised with respect to a scanner covariate in order to further adjust for differences attributable to scanner effects. This was carried out on all available MRI scans from PATH, comprising 2445 scans for participants aged 44-78 years, using MATLAB (2012b, The MathWorks, Natick, MA). For each vertex across the cortex, a general linear model was estimated to remove variance associated with a scanner covariate corresponding to the scanner for each wave (numbered 1,2,3 or 4). The impact of orthogonalisation over the total sample, and in the sample in the current study, can be seen in Figure e-2.

Following adjustment for scanner effects, the second script identified individuals with changes in volume exceeding 8% between waves. These trajectories were visually checked, with the

intention of excluding individuals on the basis of large volume changes inconsistent with the general trajectory of the individual (e.g. consistent increase from waves 1, 3, and 4 with a sudden large drop at wave 2).

Scanner used (Philips Gyroscan ACS-NT, Siemens 1.5T Avanto or Siemens 1.5T Espree) was entered as a dummy variable to ensure significant results were not spuriously due to scanner effects. Key relationships were unaffected by the inclusion of scanner as a covariate (glucose and grey matter volume in all *b*=-1.53 95%CI[-2.87, -0.17]; T2D and grey matter volume in all b=-9.62 95%CI[-18.01, -1.23]; T2D and white matter volume, b=-18.60, 95%CI[-28.76, -8.45]; T2D; glucose and total brain volume change in all -0.38*[-0.70, -0.05] and NGT-only -1.63**[-2.66, -0.60]; glucose and grey matter volume change in all -0.27*[-0.51, -0.04]).

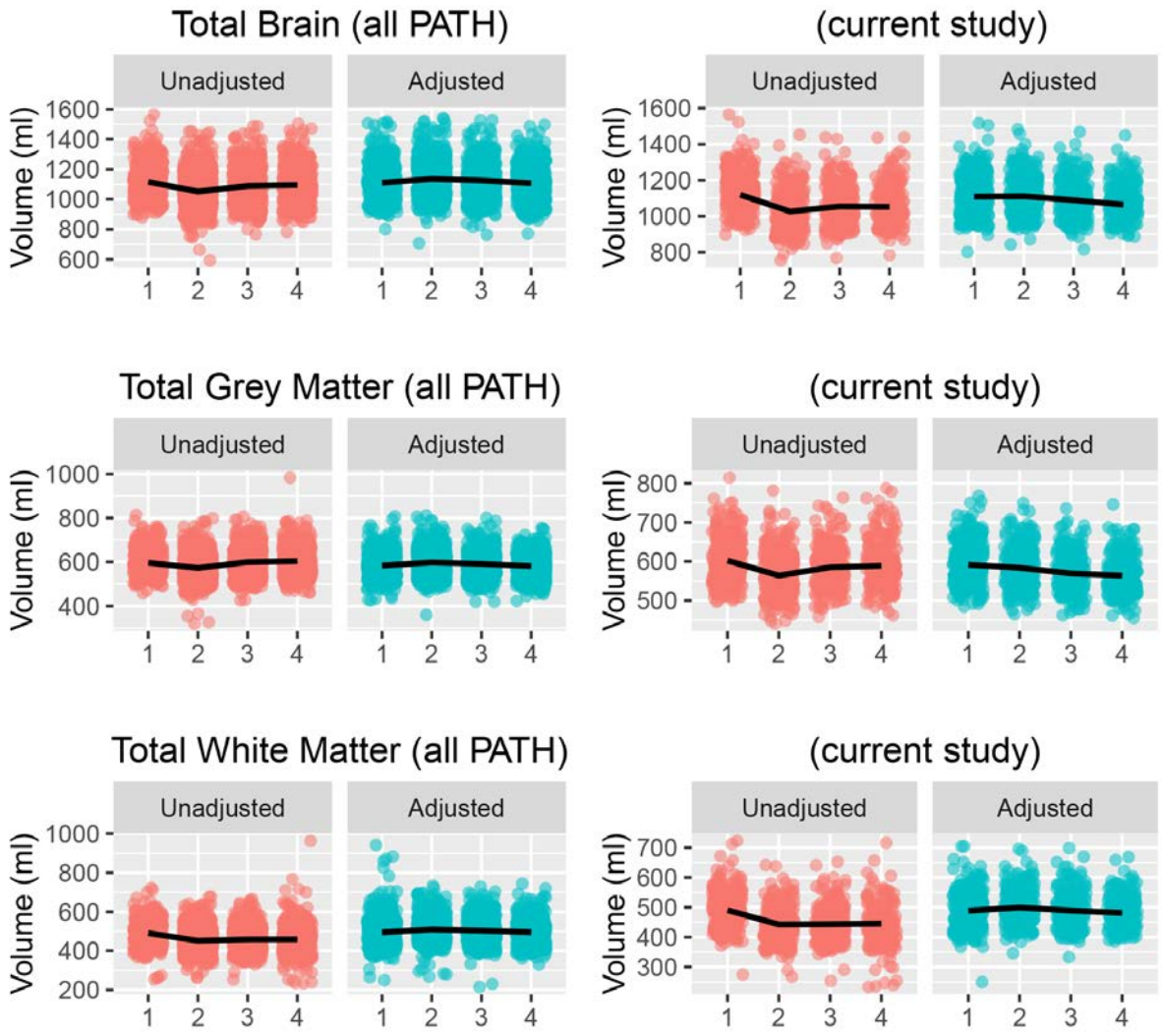


Figure e-2. Impact of scanner orthogonalisation procedure on brain volumes in total PATH study (2445 scans from participants aged 44-78 years) and current study. Points have been jittered on the x axis and made transparent for readability. Black line joins median point for each group.

Supplementary information on sensitivity analysis

Multilevel modelling of repeated blood glucose measures at each point as predicted by diabetic diagnosis (NFG vs T2D) at each point (allowing for incidence throughout the duration of the study), or T2D at any point in the study (thus considering only diagnosed T2D) led to similar conclusions. For diabetic group at each point, T2D has 1.77 mmol/L 95%CI[1.526, 2.025] significantly higher blood glucose than NFG, VIF =1; for diagnosed diabetes only, T2D has 1.566 mmol/L 95% CI[-1.78, -1.35] significantly higher blood glucose than NFG, VIF =1. Blood glucose did not significantly interact with diabetic group at each point to predict any brain volumes, with the exception of grey matter volume (*b*=-3.69ml, 95%CI [-0.01, -0.7.37]). This is the same trend as the main findings, suggesting significance was reached due to larger cell sizes for comparison (as this grouping includes NFG and IFG in a single diabetes group).

In brain volume models, coefficient directionality and significance remained largely consistent whether all or a subset of covariates were included. Sixty six participants were identified as APOE*4. Addition of this as a predictor to all models did not affect coefficient directionality or significance in most models (data not shown). The interaction between glucose and time as a predictor of total brain volume over time lost significance in the fully adjusted model including NFG only (b=-1.03, 95%CI[-2.21, 0.15], p=0.08), which may reflect a genuine effect of APOE*4 or be due to model instability from uneven APOE*4 carriers. The addition of a dummy variable for scanner type (Philips Gyroscan ACS-NT, Siemens 1.5T Avanto or Siemens 1.5T Espree) did not affect conclusions: across all models, key relationships remained significant.

Table e-3. Multilevel model results examining whether the association between blood glucose and change in brain volumes differ as a function of diabetic status

	Total Brain Volume	Grey Matter Volume	Cortical white Matter Volume	Lateral ventricle volume
Fixed effects				
Glucose	14.80 [2.89, 26.72]*	6.91[-0.58, 14.40]	7.18[-1.92, 16.28]	-0.54 [-3.39, 2.31]
IFG	28.88[-136.13, 193.89]	-19.50 [-123.09, 84.09]	39.62 [-86.25, 165.48]	8.39 [-31.25, 48.02]
T2D	38.12 [-36.26, 112.50]	37.28 [-9.38, 83.94]	0.40 [-56.27, 57.08]	-2.09 [-20.10, 15.93]
Time	2.84 [-3.27, 8.96]	-0.07 [-3.91, 3.78]	2.75 [-1.92, 7.42]	1.13 [-0.33, 2.58]
Glucose*IFG	-5.53 [-34.37, 23.32]	2.92 [-15.19, 21.03]	-6.81 [-28.82, 15.20]	-1.17 [-8.09, 5.74]
Glucose*T2D	-12.52 [-25.81, 0.76]	-7.97 [-16.32, 0.37]	-4.16 [-14.30, 5.98]	1.47 [-1.71, 4.65]
Glucose*Time	-1.36[-2.55, -0.16]*	-0.52 [-1.27, 0.23]	-0.75 [-1.67, 0.16]	-0.07 [-0.35, 0.22]
IFG*Time	0.84 [-12.29, 13.97]	2.70 [-5.55, 10.95]	-1.05 [-11.08, 8.98]	-1.23 [-4.36, 1.91]
Diabetes*Time	-6.38 [-13.61, 0.85]	-2.97 [-7.51, 1.58]	-3.13 [-8.65, 2.40]	0.71 [-1.01, 2.43]
Glucose*IFG*Time	0.05 [-2.27, 2.36]	-0.37 [-1.83, 1.08]	0.26 [-1.50, 2.03]	0.22 [-0.33, 0.77]
Glucose*T2D*Time	1.13 [-0.19, 2.44]	0.45 [-0.37, 1.28]	0.62 [-0.39, 1.62]	-0.04 [-0.35, 0.28]
Random effects (variance)				
Intercept	1385.86[1059.83,1727.65]	491.71[373.54,615.46]	690.65[522.88,866.36]	149.41[116.27,184.8]
Residual	295.52[230.84,355.9]	117.82[91.98,142.17]	174.93[136.52,211.26]	16.17[12.58,19.43]
Model information				
Observations	567	567	567	567
-LL	-2,712.59	-2,446.43	-2,548.03	-2,002.24
AIC	-2,712.59	-2,446.43	-2,548.03	-2,002.24

Note. IFG = Impaired Fasting Glucose, T2D = Type 2 Diabetes. All models control for age, gender, years of education, ICV, hypertension, BMI, smoking status, and Goldberg depression score (coefficients not shown). Values in square brackets are 95% confidence intervals. “Time” is the age variable centred on age at baseline (60), so years in study. Models include data from all waves of data collection.

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