

Central nervous system function in youth with type 1 diabetes 12 years after disease onset.

Running title: *Type 1 diabetes and the developing brain*

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Additional Table and Figure available in an online appendix at <http://care.diabetesjournals.org>

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Objective: This study used neurocognitive assessment and neuroimaging to examine brain function in youth with type 1 diabetes studied prospectively from diagnosis.

Research Design and Methods

Participants: Type 1 diabetes (N=106) and controls (N=75) with no significant group difference on IQ at baseline 12 years previously. *Measures:* Wechsler Abbreviated Scale Intelligence, magnetic resonance spectroscopy and imaging, metabolic control data from diagnosis.

Results: Type 1 diabetes had lower Verbal and Full Scale IQ than controls (both $p < .05$). Type 1 diabetes had lower N-acetylaspartate in frontal lobes and basal ganglia and higher myoinositol and choline in frontal, temporal lobes and basal ganglia than controls (all $p < .05$). Type 1 diabetes, relative to controls, had decreased grey matter in bilateral thalami and right parahippocampal gyrus and insular cortex. White matter was decreased in bilateral parahippocampi, left temporal lobe and middle frontal area (all $p < .0005$ uncorrected). T2 in type 1 diabetes was increased in left superior temporal gyrus and decreased in bilateral lentiform nuclei, caudate nuclei and thalami, and right insular area (all $p < .0005$ uncorrected). Early onset disease, predicted lower Performance IQ, hypoglycemia was associated with lower Verbal IQ and volume reduction in thalamus, poor metabolic control predicted elevated myoinositol and decreased T2 in thalamus and older age predicted volume loss and T2 change in basal ganglia.

Conclusions: This study documents brain effects 12 years after diagnosis in a type 1 diabetes sample whose IQ at diagnosis matched that of controls. Findings suggest several neuropathological processes including gliosis, demyelination and altered osmolarity.

Abbreviations: BGL, blood glucose level; CNS, central nervous system; FSIQ, Full Scale Intelligence Quotient; VIQ, Verbal Intelligence Quotient; PIQ, Performance Intelligence Quotient; RCH, Royal Children's Hospital, Melbourne; C, control participants; SES, socio-economic status; WASI, Wechsler Abbreviated Scale of Intelligence; FSIQ_{baseline}, FSIQ assessed at study entry 12 years previously; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NAA, total N-acetylaspartate, including N-acetylaspartylglutamate; Cr, creatine+creatine phosphate; Cho, trimethylamines or choline-containing metabolites; mI, myoinositol; Glx, glutamine+glutamate; GM, grey matter; WM, white matter; VBM, voxel-based morphometry; ROI, region of interest; Hb_{A1C}, glycosylated hemoglobin level.

The central nervous system (CNS) is a major organ system affected in type 1 diabetes as both cerebral glucose and insulin levels are frequently abnormal, even when diabetes is well-controlled (1). Intracellular calcium toxicity and excitotoxic cellular damage, triggered by the synaptic release of excessive glutamate, have been identified as two potentially important mechanisms that produce selective neuronal necrosis during severe hypoglycemia (1), but other metabolite changes may also be important. Hyperglycemia disrupts blood–brain barrier function and depresses cerebral blood flow acutely, while chronic hyperglycemia is associated with cerebrovascular disease and neuropathy (1). The impact on CNS of osmotic changes associated with constantly fluctuating glucose levels is unclear. Neurotransmitter pathways may also be affected in diabetes as insulin is involved in regulation of the amine neurotransmitters (1).

There is a growing literature documenting pathophysiological CNS changes and neurocognitive deficits in adults with type 1 diabetes (2-6), sometimes, but not universally, linked to specific illness variables such as disease duration, history of severe hypoglycemia or chronic hyperglycemia. Cognitive difficulties have also been reported in children, with deficits most evident in those with early (≤ 5 years) onset disease (7,8). Neuroimaging studies of youth have been limited to date (9,10) and understanding of the impact of type 1 diabetes on neurodevelopment is still based largely on inferences drawn from neurocognitive studies and from adult neuroimaging reports. Controlled, longitudinal studies are particularly informative in documenting illness-related changes in the CNS. The Diabetes Control and Complications Trial (DCCT) (11) found no deterioration in cognitive function in either conventionally or intensively treated patients over an 18 year

period. However, this study did not enrol participants at diagnosis and recruitment was limited to those over 13 years of age. Thus, the DCCT was unable to document any illness-related effects that may have occurred prior to recruitment, in particular, the impact of diabetes on a developing CNS. Children have high cerebral energy needs associated with brain growth and "neural pruning" and may be more sensitive than adults to glucose fluctuations (1,7). It is important to document the specific neuropathological correlates of type 1 diabetes in younger populations as better understanding of the impact of childhood-onset disease on CNS development will facilitate evidence-based paediatric management regimens.

We have previously identified neurocognitive deficits 6 years after disease onset in youth studied prospectively from diagnosis (12). The current study re-evaluated this cohort 12 years after study inception.

RESEARCH DESIGN AND METHODS

Participants: Consecutive admissions to the Royal Children's Hospital, Melbourne (RCH) with newly diagnosed type 1 diabetes between 1990 and 1992 (N= 133), together with healthy controls (C, N=126), stratified for age and gender. A history of CNS disease or trauma was an exclusion criterion. Twelve years later, all participants who could be located (125 type 1 diabetes and 93 C, 94% and 74% respectively) using the study database, RCH and adult diabetes clinics, private endocrinologists and the Health Insurance Commission, were invited to take part in the current study. Of those located, 106 type 1 diabetes and 75 C agreed to participate (rates of 85% and 81% respectively).

Procedure: All participants underwent neurocognitive testing. Participants were consecutively invited to undergo neuroimaging until available funding was

exhausted. Blood glucose levels (BGL) of diabetes participants were determined prior to assessment by capillary sample to ensure that subjects had a reading above 4mmol/l. This study was approved by the Human Ethics Research Committee of the Victorian Government Department of Human Services.

Measures—Neurocognitive

assessment: Wechsler Abbreviated Scale of General Intelligence (WASI) (13) is a standardised, brief measure of intelligence providing Full Scale (FSIQ), Verbal (VIQ) and Performance (PIQ) IQ scores (Mean 100, SD 15).

Magnetic Resonance Spectroscopy (MRS) and Imaging (MRI): Imaging was carried out on a 3 tesla scanner (GE Healthcare, Milwaukee, USA).

MRS: Bilateral single voxel spectra were acquired using a standard PRESS sequence to provide a metabolite profile from three brain regions of interest (ROI), basal ganglia, frontal and temporal lobes. The ROI were positioned using T1-weighted scout images. Basal ganglia ROI were positioned over the lentiform nuclei, temporal lobe ROI were positioned to include hippocampus and mesial temporal structures and frontal lobe ROI were placed above the third ventricle sufficiently posterior to avoid interference from curvature of the skull. Acquisition parameters were TE/TR=30/3000ms. For frontal and temporal spectra, isotropic 2cm voxels were used and for basal ganglia voxel size was 2x2x1.5cm. Data were analysed using software packages, SAGE/IDL (GE Healthcare, Milwaukee, USA/RSI Inc., Boulder, USA) and LCModel(14), with a basis set of 15 metabolites acquired on the same spectrometer. Data were included in analyses if the Cramer-Rao lower bound was < 30% as determined by the LCModel. Results are presented in institutional units approximating mmol/L. *In vivo* proton MRS of brain provides information on the tissue content of total N-acetylaspartate (NAA, including N-

acetylaspartylglutamate), creatine+creatine phosphate (Cr), myoinositol (mI), trimethylamines or choline-containing metabolites (Cho), and glutamine+glutamate (Glx).

MRI: Volumetry: A fast spoiled gradient recalled echo at steady state (FSPGR) sequence was used (TR/TE/TI 13.8/2.7/500 ms, voxel size: 0.48×0.48×2 mm). **T2 relaxometry:** A modified, optimised Carr-Purcell-Meiboom-Gill (CPMG) multi-echo sequence (14) was used (8 echoes, TE=28.9-231 ms, TR=6.24 sec, 24 slices, 5 mm slice thickness, in-plane voxel size: 0.94×1.88 mm). The slice plane was perpendicular to the long axis of the hippocampus. T2 maps were generated by fitting to a mono-exponential model with the inclusion of a baseline that minimizes the contribution of long T2 components (mainly CSF) to the fit. **Analyses:** Images were warped to standard space in which they could be compared, and smoothed (with a 10 mm kernel). All analyses were performed using SPM2 (<http://www.fil.ion.ucl.ac.uk/spm2>). Separate grey matter (GM) and white matter (WM) volumetry analyses were performed using optimized voxel-based morphometry (VBM) (15). Voxel-wise T2 changes were assessed using the approach of voxel-based relaxometry (VBR)(16).

Biomedical measures: Participants diagnosed at 5 years or younger were classified as having early onset disease. Participants reported any episodes from diagnosis of hypoglycemia associated with seizure/coma and these were corroborated through scrutiny of medical records. The sample was dichotomized into those who reported no events and those with a history of ≥1 event. Hb_{A1C} measurements from diagnosis were obtained for each patient (range 9-57, median 37) from hospital and clinic databases. The percentage of total time from diagnosis that Hb_{A1C} was ≥9.0% was

calculated to estimate overall metabolic control.

Statistical analyses: SPSS version 14 (SPSS, Chicago IL) was used for statistical analyses of demographic, IQ and MRS data.

IQ: Group differences were examined using analysis of co-variance (ANCOVA), covarying for SES, FSIQ_{baseline} and time between baseline and current assessment.

MRS: Mean bilateral metabolite concentrations in each ROI were used in analyses. Group differences were examined using ANCOVA with age as a co-variate.

MRI: ANCOVA was used to examine group differences in volume and T2, covarying for age. Volume analyses also included total brain volume and gender as co-variables.

Multiple linear regression was used to predict IQ from illness variables (age of disease onset, hypoglycemia, metabolic control), SES, FSIQ_{baseline} and time between baseline and current assessment. Age of disease onset was highly correlated with age; hence age was used as a predictor for regression analyses of MRS/MRI data, together with hypoglycemia and metabolic control. MRI regression analyses were restricted to anatomical areas shown to differ in the initial type 1 diabetes/C comparisons.

RESULTS

Sample characteristics of participants presented in Table 1.

Type 1 diabetes and C did not differ on FSIQ_{baseline}, age, gender ratio, SES or psychiatric symptoms. Time between baseline and current assessment was shorter in type 1 diabetes (95% CI: 0.37–1.0 years). Type 1 diabetes and C who underwent neuroimaging did not differ significantly on age or gender ratio, and there were no differences between type 1 diabetes who had neuroimaging and those who did not on age of disease onset, hypoglycemia or metabolic control. Group

mean scores \pm SD on IQ, MRS and MRI variables are presented in Tables 2 & 3.

Group (type 1 diabetes, C) differences on IQ and MRS/MRI variables—IQ: Type 1 diabetes had lower VIQ ($p=.03$) and FSIQ ($p=.05$) than C.

MRS: The largest mean differences were in mI and NAA, with mI higher and NAA lower in type 1 diabetes. Smaller statistically significant mean differences (type 1 diabetes higher) were found in Cho in all locations.

MRI: Type 1 diabetes, relative to C, had decreased GM volume in bilateral thalami, right parahippocampal gyrus, and right insular cortex (see Figure A1 in the online appendix available at <http://care.diabetesjournals.org>). Mean WM volume was decreased in bilateral mesial temporal lobes (parahippocampal region), other areas of the left temporal lobe, and in the left middle frontal area. T2 relaxation times in type 1 diabetes were increased in the left superior temporal gyrus and decreased in bilateral lentiform nuclei, caudate nuclei and thalami, and the right insular area (all $p<.0005$ uncorrected).

Illness-related predictors of CNS outcome in type 1 diabetes—Regression analyses of illness related predictors of IQ, MRS and MRI findings are presented in Table A1 of the online appendix.

IQ: In each regression, SES and FSIQ_{baseline} contributed significantly to the model. In addition, hypoglycemia predicted lower VIQ (R^2 change=.032, $p=.01$), and early disease onset predicted lower PIQ (R^2 change=.135, $p<.001$) and FSIQ (R^2 change=.064, $p<.001$). Verbal IQ of the hypoglycemia subgroup was nearly one third of a standard deviation below type 1 diabetes with no hypoglycemia (adjusted $M=93.8$, $SE 1.3$ vs $M=98.2$, $SE 1.1$). Early onset participants had a PIQ more than half a standard deviation, and a FSIQ one third of a standard deviation below later onset type 1

diabetes (adjusted $M=101.6$, $SE\ 2.5$ vs $M=109.2$, $SE\ 1.7$ and $M=97.8$, $SE\ 2.1$ vs $M=103.4$, $SE\ 1.4$ respectively).

MRS: Poor metabolic control predicted higher levels of mI in basal ganglia ($R^2\text{change}=.055$, $p=.04$) and older age predicted lower levels of Glx in frontal lobes ($R^2\text{change}=.116$, $p<.01$) and NAA in temporal lobes ($R^2\text{change}=.133$, $p<.01$).

MRI: Regression analyses of MRI data in areas implicated in the previous group comparisons revealed the dominating influence of age in explaining variation in volume and $T2$: $R^2\text{change}=.247$, $p<.001$ (GM volume lentiform), $R^2\text{change}=.060$, $p<.02$ (GM volume thalamus), $R^2\text{change}=.578$, $p<.001$ ($T2$ lentiform), $R^2\text{change}=.418$, $p<.001$ ($T2$ thalamus). Hypoglycemia predicted reduced GM volume in the thalamus ($R^2\text{change}=.045$, $p<.03$) and poor metabolic control was associated with decreased $T2$ in the thalamus ($R^2\text{change}=.021$, $p<.05$).

In view of the strong association between older age, and volume and $T2$ reduction in type 1 diabetes, we investigated how group and age predicted each of the MRI variables; the models included an interaction term of group by age which was statistically significant in all analyses. For volume, the models predicted small positive changes with age for C and relatively larger negative changes for type 1 diabetes (lentiform, interaction $p=0.002$, age slopes: T1DM= -0.0038 , C= 0.0001 ; thalamus, interaction $p=0.046$, age slopes: T1DM= -0.0025 , C= 0.0012). For $T2$, the models predicted relatively larger negative changes for T1DM than for C (lentiform, interaction $p=0.013$, age slopes: T1DM= -5.11 , C= -3.17 ; thalamus, interaction $p=0.017$, age slopes: T1DM= -5.25 , C= -2.85).

BGL at time of testing: Correlations between BGL prior to neuropsychological assessment and IQ scores, and between BGL prior to scanning and metabolite profiles were

calculated and ranged from $r=.001$ to $r=-.242$ (all $p>.05$). To further examine the possible confound of intercurrent hyperglycemia on study findings, data from type 1 diabetes participants with $BGL >15\text{mmol/l}$ at time of testing ($n=34$) were removed and the data re-analysed. Group differences on VIQ and FSIQ became trends only ($p<.06$ and $P<.07$ respectively). The significance of the group difference on basal ganglia NAA was also reduced ($p<.08$) after removal of type 1 diabetes participants with $BGL > 15$ ($n=19$) at the time of scanning. All other group differences and illness related predictors of IQ and metabolites were unchanged.

CONCLUSIONS

This study is the first to document CNS effects 12 years after diagnosis in youth with type 1 diabetes whose neurocognitive profile at disease onset was not different from that of C. Metabolites and IQ results were largely unchanged after removal of data from type 1 diabetes participants with high BGL at the time of assessment, suggesting that study findings reflect stable changes in CNS function. Group differences on IQ were marginal and appear to reflect the selective impact of specific illness risk factors. Lower VIQ was associated with a positive history of hypoglycemia, while early onset disease predicted lower PIQ and FSIQ. The association between early onset disease and lower IQ, particularly PIQ, is a consistent finding (7,8). Hypoglycemia-related effects on VIQ have also been reported previously in the paediatric literature (8,12) but were not found in the DCCT (11), suggesting that the developing CNS may be especially vulnerable to hypoglycemia. It is important to note that illness-related effects on IQ, while statistically significant, are small and individuals with type 1 diabetes function well within the average range. However, even mild decrements in ability may have functional significance during childhood when new

knowledge is being acquired. Of participants who had reached school leaving age at follow-up (type 1 diabetes N= 76, C n=55), 17% fewer type 1 diabetes than C (68% versus 85%, $p < .01$) completed Year 12, the pre-tertiary year of education in Australia.

This study found lower mean NAA and higher mean mI and Cho in type 1 diabetes than C. The magnitudes of these differences were in the range of 10% to 15% and are similar to those reported for diseases such as dementia, epilepsy and Parkinson's disease (17,18). There are also similarities between current findings and previous reports in diabetes (4,5,10), although direct comparison is difficult because methodology has varied, with different brain regions assessed and some samples including both type 1 and type 2 participants. With one exception (10), study populations have been older than the current sample. NAA is a marker of neuronal density or viability, with lower levels indicative of neuronal death and/or decreased neuronal metabolism (17). Lower mean NAA is consistent with animal models of diabetes suggesting that chronic hyperglycemia reduces neuronal number and impedes myelination (19). While we were not able to relate NAA directly to metabolic control in the current study, NAA levels correlated inversely with lifetime glycemic exposure in another report (5).

Myoinositol is a marker of changes in osmolarity and increased levels are associated with both gliosis and demyelination (17). In the current study, poorer metabolic control was associated with higher levels of mI in basal ganglia which, together with other reports of elevated mI in patients recovering from DKA (20), suggests that higher levels may represent a homeostatic response of the brain to prolonged hyperglycemia. Cho levels are increased with demyelination and other forms of cell membrane breakdown, including gliosis (17). In diabetes, increases in both mI and Cho are consistent with glial proliferation

due to tissue hypoxia in the context of chronically elevated blood glucose levels (21). As with a previous report (10), metabolite profiles were not related to history of hypoglycemia.

Volumetric measurements can identify regionally specific macroscopic atrophy associated with neuronal degeneration. Musen et al. (2) using VBM, reported reduced GM densities in temporal brain regions as well as left thalamus. Our finding of decreased mean GM in thalamus is similar, but, in addition, we found reduced mean GM volume in insular cortex and frontal precentral regions and reduction in mean WM volume in mesial temporal areas. Previous reports have linked VBM changes to a previous history of either chronic hyperglycemia (2,3,8) and/or severe hypoglycaemia (2,8). In the current study, hypoglycemia was associated with volume reduction in the thalamus, poor control predicted decreased T2 in the thalamus and there were strong associations between older age and reduced volume and T2 in anterior and temporal brain regions as well as thalamus, caudate and lentiform nuclei.

The volume group differences found in this study reach levels of approximately 10%, comparable to changes in temporal lobe epilepsy (TLE) where volume reduction is approximately 10-15% (22). T2 group differences of 3% in the current study are smaller than those of 5-20% reported in other CNS diseases (23) and may in part reflect an osmotic influence of acutely elevated glucose on tissue water distribution as well as, or instead of, any longer term impact of the illness. In the current study, older age was the strongest predictor of volume and T2 decrease in type 1 diabetes. Older age is a surrogate for later disease onset in this cohort as duration of illness was controlled, hence these findings are counter-intuitive given the consistent association between early disease onset and neurocognitive deficits reported previously (7,8,11). It is now recognised though, that

CNS maturation continues into the third decade of life (24) and it is possible that findings reflect an interaction between type 1 diabetes and the final stages of neurodevelopment, with the early onset subgroup yet to experience this disruption. The suggestion by Biessels et al. (25) that diabetes is a model for “accelerated (CNS) aging” provides an alternative explanation for our findings. That is, rather than reflecting disrupted *neurodevelopment*, the greater volume loss in our older, and later-onset participants, may represent the earliest stages of cerebral microvascular disease or some other *neurodegenerative* process.

The current findings are suggestive of a number of possible illness-related neuropathological processes, including gliosis, demyelination, and changes in osmolarity and neural cell type/viability. Anterior and temporal brain regions have particularly high glucose demands and increased sensitivity to glucose disruption (1), thus it not surprising that these brain regions show reduced brain volumes, altered T2 relaxation times and metabolite changes. Relationships between specific illness variables and CNS effects are more difficult to disentangle. There were strong associations between age, and IQ and structural brain changes, but in the opposite direction. Early onset diabetes (and younger age) was associated with lower PIQ and FSIQ, while older age predicted lower levels of NAA, reduced brain volumes, and altered T2. Metabolite profiles are more suggestive of hyperglycemia, than of hypoglycemia-mediated neurotoxicity in the developing CNS. It is possible that specific illness variables exert different effects on the CNS but inconsistent associations may also reflect difficulties in obtaining reliable and comprehensive metabolic control histories, including documentation of diabetes complications. Further studies, involving large, multi-centre cohorts, meticulous

collection of metabolic control histories, documentation of diabetes complications and tight control over the glycemic level of participants at the time of assessment, are needed to fully understand the pathogenesis of CNS changes in childhood-onset diabetes.

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Table 1 Sample characteristics

	Type 1 diabetes	C	Mean difference (type 1 diabetes – Control)		P-value
			Estimate	95% CI	
Sample size (n)	106	75			
Age (years)	20.5 ± 4.3	21.0 ± 3.8	−0.50	−1.74, 0.74	0.4
Female gender	49%	51%	−1.6%	−16.0%, 12.9%	0.9
SES (1=high, 7=low)	4.3 ± 1.1	4.1 ± 1.1	0.25	−0.08, 0.59	0.14
Mental Health (YSR/YASR total t-score)	48.7 ± 11.5	49.5 ± 11.0	−0.75	−4.17, 2.66	0.7
FSIQ _{baseline}	108.0 ± 15.1	110.6 ± 12.2	−2.66	−6.83, 1.52	0.2
Time between baseline assessment and 12 year follow-up (years)	12.7 ± 1.1	12.0 ± 1.1	0.70	0.37, 1.03	<0.001
Neuroimaging (n)	79	51			
Age (years)	20.3 ± 4.3	20.6 ± 3.6	−0.25	−1.69, 1.18	0.7
Female gender	41%	47%	−6.6%	−2.3%, 10.5%	0.5
BGL at imaging (mmol/l)	12.6 ± 5.4				
BGL at neurocognitive testing (mmol/l)	11.7 ± 5.9				
Most recent Hb _{A1c} (%)	9.2 ± 1.8				
Age at type 1 diabetes onset (years)	7.1 ± 3.7				
Early disease onset (%)	38%				
≥ 1 episode severe hypoglycemia (%)	44%				
Mean time Hb _{A1c} >9.0% (%)	41.95 ± 26.31				

Results for type 1 diabetes and C are presented as mean ± standard deviation. Abbreviations: controls (C), confidence interval (CI), socio-economic status (SES), Youth Self Report (YSR), Young Adult Self Report (YASR), Full-scale IQ assessed at study entry 12 years previously (FSIQ_{baseline}), blood glucose level at time of assessment (BGL), mmol/l glycosylated hemoglobin level (Hb_{A1c}),

Table 2 Group (type 1 diabetes, C) differences on IQ and metabolites

Outcome variable	Means		Mean difference*		P-value
	type 1 diabetes	Control	Estimate	95% CI	
IQ					
VIQ	96.2	100.4	-3.64	-6.97, -0.30	0.03
PIQ	106.4	109.1	-2.02	-5.46, 1.43	0.25
FSIQ	101.3	105.1	-3.03	-6.07, 0.00	0.05
Metabolite					
mI basal ganglia	3.46	3.17	0.29	0.11, 0.48	0.002
mI frontal lobe	4.17	3.52	0.65	0.46, 0.84	<0.001
mI temporal lobe	4.39	3.91	0.48	0.22, 0.75	<0.001
Cr basal ganglia	6.88	6.96	-0.08	-0.32, 0.16	0.5
Cr frontal lobe	5.39	5.37	0.01	-0.12, 0.15	0.9
Cr temporal lobe	5.31	5.32	-0.02	-0.29, 0.26	>0.9
Glx basal ganglia	11.50	11.37	0.14	-0.35, 0.63	0.6
Glx frontal lobe	9.99	9.76	0.16	-0.26, 0.58	0.4
Glx temporal lobe	8.43	8.31	0.12	-0.37, 0.61	0.6
Cho basal ganglia	1.63	1.56	0.07	0.01, 0.14	0.027
Cho frontal lobe	1.61	1.48	0.13	0.07, 0.19	<0.001
Cho temporal lobe	1.72	1.64	0.09†	-0.00, 0.18†	0.04†
NAA basal ganglia	7.98	8.47	-0.49	-0.83, -0.15	0.005
NAA frontal lobe	8.27	8.67	-0.41	-0.65, -0.16	0.001
NAA temporal lobe	7.50	7.84	-0.35	-0.72, 0.03	0.07

*Adjusted mean difference estimate (from ANCOVA model) is type 1 diabetes – control, and confidence interval (CI) is 95%.

† This analysis was based on non-parametric procedures because of an extreme value in the original data set; the estimate and approximate CI are for a difference in the location of the populations. The p-value is from the Mann-Whitney test.

Table 3 Group (type 1 diabetes,C) differences on grey matter (GM) volume, white matter(WM) volume and T2 relaxation time

Outcome variable	Cluster*	Means†		Mean difference‡		¶
		type 1 diabetes	Control	Estimate	99·9% CI§	
GM volume decrease						
Left thalamus	1718	0·531	0·568	0·038	0·005, 0·071	3·93
Right thalamus	1623	0·525	0·563	0·038	0·005, 0·071	3·93
Left frontal precentral/insular gyrus	373	0·532	0·575	0·051	0·003, 0·098	3·55
Right superior frontal gyrus	287	0·431	0·478	0·049	0·003, 0·095	3·58
Right frontal precentral gyrus	78	0·349	0·390	0·046	0·003, 0·088	3·60
Right parietal postcentral gyrus	104	0·375	0·417	0·046	0·002, 0·089	3·51
Right parahippocampal gyrus	79	0·513	0·554	0·046	0·001, 0·091	3·45
WM volume decrease						
Right parahippocampal WM	183	0·275	0·329	0·050	0·006, 0·094	3·82
Left parahippocampal WM	290	0·231	0·275	0·042	0·002, 0·082	3·55
Left middle frontal WM	207	0·149	0·201	0·049	0·007, 0·090	3·94
Left temporal WM	156	0·092	0·131	0·037	0·003, 0·071	3·69
Left insular WM	158	0·182	0·230	0·043	0·004, 0·082	3·69
Left middle temporal WM	126	0·386	0·458	0·074	0·006, 0·142	3·56
T2 decrease						
Left lentiform nucleus	299	625·5	638·0	14·3	1·0, 27·6	3·63
Right lentiform nucleus	1058	645·4	662·9	19·1	3·1, 35·1	4·01
Left caudate nucleus	1843	614·0	627·7	15·4	2·2, 28·6	3·92
Right caudate nucleus	2901	644·7	659·3	16·2	2·2, 30·3	3·88
Left thalamus	496	678·8	693·2	15·5	1·78, 29·2	3·81
Right thalamus	753	653·7	669·8	17·6	2·6, 32·63	3·95
Red nuclei (bilaterally)	225	664·1	676·4	13·7	1·0, 26·4	3·63
Right frontal WM	251	706·1	727·7	22·8	1·4, 44·18	3·59
Right insular/superior temporal gyrus	146	712·0	736·0	26·0	1·2, 50·9	3·53
Corpus callosum	24	706·9	722·0	15·7	0·7, 30·7	3·52
Right parietal WM	23	716·6	732·4	16·3	0·3, 32·3	3·44
T2 increase						
Left middle temporal GM	37	793·8	762·1	-30·0	-59·6, -0·5	-3·43

MR regions are indicated as abnormal by voxel-based analysis (type 1 diabetes vs C using 1-tailed t-tests at $p=0·0005$, non-corrected).

* The cluster size is the number of voxels in a contiguous volume of supra-threshold voxels.

† Mean values of voxel-based data are not adjusted for covariates. The units of the T2 values are in milliseconds, the units of volume change are in litres.

‡ The mean difference is adjusted for covariates.

§ The bidirectional confidence intervals are shown at 99·9% (equivalent threshold to the two 1-tailed t-tests at $p=0·0005$).

¶ Since significance is the selection criteria for these areas, the t -statistic is reported.