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A Preliminary Study of Brain Macrovascular Reactivity in Impaired Glucose Tolerance and Type-2 Diabetes: Quantitative Internal Carotid Artery Blood Flow Using Magnetic Resonance Phase Contrast Angiography

D Selvarajah MBChB, PhD[1], T Hughes MBChB[3], J Reeves MBChB[3], E Boland PhD, MBChB[3], J Marques PhD[2], R Gandhi MD[2], PD Griffiths, PhD[3], S Tesfaye MD[2], I.D. Wilkinson PhD[3]

1. Department of Human Metabolism, University of Sheffield, Sheffield, UK

2. Academic Department of Diabetes and Endocrinology, Royal Hallamshire Hospital, Sheffield, UK

3. Academic Unit of Radiology, University of Sheffield, Sheffield, UK

Corresponding Author

Dr Dinesh Selvarajah,

Department of Human Metabolism,

University of Sheffield,

Glossop Road,

Sheffield, S10 2JF,

United Kingdom.

Tel: 00 (44) 114 271 2204. Fax: 00 (44) 114 271 3708.

Email: d.selvarajah@sheffield.ac.uk

Novelty statement: This study sought to examine cerebrovascular reactivity in subjects with impaired glucose tolerance (IGT) and type 2 diabetes using magnetic resonance phase contrast angiography. We demonstrated impaired cerebrovascular reactivity in both cohorts in comparison with healthy volunteers. This is the first report in IGT of impaired cerebrovascular reactivity, which has previously been linked to increased risk of stroke. Furthermore, our report highlights the presence of significant cerebrovascular disease in this cohort of asymptomatic individuals.

OBJECTIVE

The aims of this study were 1) to examine cerebrovascular autoregulation in subjects with impaired glucose tolerance (IGT) and type 2 diabetes (T2DM) and 2) to clarify is cardiovascular autonomic nerve function is associated with abnormal cerebrovascular autoregulation.

RESEARCH DESIGN AND METHODS 46 subjects were recruited [12=IGT, 17=T2DM and 17=Healthy Volunteers (HV)]. Arterial blood flow (f_{ICA}) was assessed within the internal carotid artery (ICA) at baseline and 20 minutes after intravenous pharmacological stress (1g acetazolamide), using quantitative magnetic resonance phase-contrast angiography. ICA vascular reactivity [VR_{ICA}(%)] and pulsatility index (PI) was determined. All subjects underwent baroreceptor reflex sensitivity assessment.

RESULTS Subjects with IGT and T2DM had significantly lower VR_{ICA} [40.2%(19.8) and 41.5%(18.7)], respectively] compared with HV [57.0%(14.2); ANOVA p=0.02]. There was no significant difference in VR_{ICA} between T2DM and IGT groups (p=0.84). There was a significant positive correlation between baroreceptor reflex sensitivity (LF:HF) with CRV (ρ = 0.47, p=0.04). and PI (ρ = 0.46, p=0.04).

CONCLUSIONS We have demonstrated significant cerebrovascular haemodynamic abnormalities in subjects with T2DM and IGT. This was associated with greater sympathovagal imbalance. This may provide an important mechanistic explanation for increased risk of cerebrovascular disease in diabetes. It also highlights that these abnormalities may already be present in prediabetes.

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Abbreviations:

f_{ICA} Internal Carotid Artery Blood Flow

ICA Internal Carotid Artery

IGT Impaired Glucose Tolerance

MRI Magnetic Resonance Imaging

PI Pulsitility Index

T2DM Type 2 Diabetes Mellitus

VR_{ICA} Cerebrovascular Reactivity

Type 2 diabetes mellitus (T2DM) and impaired glucose tolerance increases the risk of ischaemic stoke (1-4). Although, epidemiological studies have linked increased duration of diabetes and poor metabolic control to stroke risk, paradoxically, large randomised studies have failed conclusively to show that intensively lowering glucose reduces cardiovascular event rates (including stroke) (5,6). Hence, the cause of an increased stroke risk in diabetes remains unclear.

Cerebral blood flow is carefully regulated to exceed the metabolic demands of the brain (7). Metabolic coupling mechanisms ensure that blood flow is increased in regions that are metabolically active (8) and autoregulation maintains blood flow during changes in perfusion pressure (9). Abnormal cerebrovascular autoregulation is associated with an increased risk of stroke as it impairs the ability to compensate in response to a fall in perfusion pressure (10-14). This has been widely reported in patients with diabetes as impaired cerebrovascular reactivity to a variety of haemodynamic challenges [e.g. hypercapnia, acetazolamide (ACZ) and blood pressure changes (15-18)]. Abnormal cerebrovascular autoregulation has also been demonstrated in diabetic subjects with severe cardiac autonomic neuropathy (19).

Given the increased risk of stroke in prediabetes, we evaluated whether IGT is associated with abnormal cerebrovascular autoregulation by examining vascular reactivity of the internal carotid artery in response to pharmacological vasodilatory challenge using quantitative magnetic resonance (MR) angiography. We also examined the relationship between cerebrovascular autoregulation and cardiovascular autonomic function. Therefore, the aims of this study were 1) to examine the cerebrovascular autoregulation in patients with IGT and 2) to examine the relationship between cerebrovascular autoregulation and autonomic function assessed by baroreceptor gain using established spectral techniques.

RESEARCH DESIGN AND METHODS

Forty six subjects [IGT, n=12; T2DM, n=17 and 17 healthy volunteers (HV)] were studied. Impaired glucose tolerance was defined as previously described (20). We excluded any patients with uncontrolled diabetes, a history of cerebrovascular disease or the presence of MRI findings compatible with stroke/ischaemic change or had evidence of significant carotid artery stenosis; known symptomatic heart disease that can alter cardiac output; peripheral vascular disease; neurological diseases; uncontrolled hypertension; insulin treatment; renal impairment; current smokers or ex-smokers (<12months) and medications (e.g. β-blockers) or illnesses (e.g. anaemia or polycythemia) that can alter cerebrovascular reactivity, central chemosensitivity or cerebral blood flow. Standard MR exclusion criteria were adopted. If present, the degree of diabetic retinopathy (DR) was quantified using digital retinal photography from the annual eye screening database. All subjects gave written, informed consent before participation in the study, which had prior approval by the Regional Ethics Committee.

Baroreceptor Reflex Sensitivity

Spectral measurements of baroreflex gain were performed in the morning, in the supine position using the Portapres Device (Finapres Medical Systems, Amsterdam, The Netherlands). Portapres self-adjustment was performed for 15 minutes to ensure patient and signal stabilisation. Following self-adjustment, an 8-min recording of ECG and blood pressure signals was performed.

Cardiac and systolic arterial pressure time series were computed using dedicated software provided by the manufacturer. Computation of spectral components in the low frequency (LF) band (from 0.04 to 0.15 Hz) and in the high frequency (HF) band

(from 0.15 to 0.45 Hz) was performed. The square root of the ratio between cardiac and systolic arterial pressure spectral components in both bands provided the alpha-LF and alpha-HF indices respectively. The ratio between LF and HF was also calculated.

Magnetic Resonance Imaging Protocol

All data were acquired at 3.0 T (Achieva3.0T, Philips Healthcare, Best, Holland). Standard T2-weighted and Fluid Attenuated Inversion Recovery (FLAIR) images were acquired in the axial plane using 2-dimensional, fast-spin-echo techniques. Finger-prick blood glucose testing was performed during the MR imaging session to ensure it remained within 4-12 mmol/l. Magnetic Resonance Angiography (MRA): Standard time-of-flight MRA was performed over the right carotid artery bifurcation to aid subsequent angiographic slice placement. Quantitative phase-contrast angiography (PCA) data (TE=4.4ms; TR=8.0ms; FA=10^{O}; velocity encoding factor [VENC]=120cm/s) were acquired from a single 4mm thick slice with in-plane pixel dimensions of 1.72 x 1.59 cm^2 . The PCA-acquisition slice was placed approximately 3cm distal to the carotid bifurcation, perpendicular to the axis of flow within the right ICA, in order to maximise the extent of laminar flow-detection. Centrally acquired vector ECG data were used to retrospectively gate 40 time points within the cardiac cycle allowing blood flow to be assessed during each phase of the cardiac cycle. Following initial PCA, 1g ACZ (Diamox Sodium parenteral, Wyeth laboratories, Maidenhead) was administered intravenously over 10 minutes through a cannulated antecubital vein whilst the subject remained in the scanner. An identical PCA acquisition was repeated 20 minutes post-ACZ administration.

Quantitative velocity and flow - encoded information were extracted from the MR-PCA datasets obtained from each subject via user-defined region of interest, post-acquisition, proprietary processing software (Q-flow, Philips Healthcare, Best, Holland). Regions of interest were drawn on the resultant phase contrast images corresponding to the time-point of maximum arterial flow. The following established markers of ICA haemodynamics and autoregulation were used as outcome variables (21,22):

1. Arterial blood flow (f_{ICA}, ml/s), refers to the average volume of blood per unit time
2. ICA vascular reactivity (VR_{ICA}, %) = [(f_{ICA}POST - f_{ICA}PRE) / f_{ICA}PRE] x 100

Where f_{ICA}PRE = mean blood flow through the ICA pre ACZ administration and f_{ICA}POST = mean blood flow through the ICA flow post ACZ administration.

3. Pulsatility index (PI) = [f_{ICA}MAX- f_{ICA}MIN] / f_{ICA}MEAN

Where f_{ICA}MAX = maximum flow during the cardiac cycle, f_{ICA}MIN = minimum flow during the cardiac cycle and f_{ICA}MEAN = mean flow throughout the cardiac cycle. Severely impaired VR_{ICA} was defined as <10% increase as described previously (23).

Statistical Analysis

All analyses were performed using standard statistical techniques (SPSS 21.0, IBM Inc.). Baseline characteristics were described as means and standard deviation for normally distributed variables. The appropriate tests for normality were conducted to guide subsequent analysis. Demographic and outcome variables were compared between groups using one-way ANOVA, with Fisher's post-hoc test. Paired sample t-

tests were used to compare mean f_{ICA} determined pre- and post-ACZ. Finally, ANCOVA (age as covariate) was used to examine mean PI and RI of HV, IGT and T2DM subject groups divided into those with (T2DM-DR; n=7) and without (T2DM-NoDR; n=7) retinopathy. The linear association between the baroreceptor sensitivity and MR measures of ICA haemodynamics and autoregulation was assessed by Spearman correlation analysis in IGT and T2DM subgroups.

RESULTS

There were no significant group differences in age or gender distribution (Table 1). IGT and T2DM subjects had significantly greater body mass index and waist circumference compared with HV (Table 1). Mean HbA1c in the T2DM cohort was 9.5(1.9)% (75.0mmol/mol). There were no significant differences in pre-ACZ mean f_{ICA} between groups [ml/s; HV (4.36(0.7), IGT 4.88(0.9), T2DM 4.34(1.0); ANOVA p=0.19; Figure 1B]. In all groups there was a significant increase in subject f_{ICA} post-ACZ [ml/s; HV (6.85(1.2), IGT 6.75(1.0), T2DM 5.37(2.4); p<0.001; Figure 1B].

VR_{ICA} (%) was significantly greater in HV [57.0(14.2)] compared to IGT [40.2(19.8)] and T2DM [41.5(18.7), ANOVA p=0.02; Figure 1C, post-hoc tests Table 1]. After correction for BMI and waist circumference mean VR_{ICA} remained significantly greater in HV (ANCOVA p=0.04). There was no significant difference in VR_{ICA} (%) between IGT and T2DM subjects (p=0.84). One subject each from T2DM (5.9%) and IGT (8.3%) cohorts had severely impaired $VR_{ICA.}$ Subjects with T2DM [1.14(0.3)] had the highest mean PI compared to HV [1.01(0.2)] and IGT [1.04(0.2); ANOVA p=0.24, Table 1]. T2DM-DR subjects were older compared to T2DM-NoDR subjects [years, 60.6(15.8) vs. 51.0(9.5)]. After correction for age, mean PI were

significantly higher in T2DM-DR when compared with the T2DM-NoDR subgroup, IGT and HV groups [ANCOVA, p=0.02; Figure 1D, post-hoc tests Table 1].

There was a significant positive correlation between baroreceptor reflex sensitivity measure LF:HF with CRV (ρ = 0.47, p=0.04). and PI (ρ = 0.46, p=0.04). Only HF and not LF demonstrated a significant correlation with CRV (ρ =-0.53, p=0.02) and PI (ρ=-0.46, p=0.04).

DISCUSSION

In this preliminary study, we have demonstrated impaired cerebrovascular reactivity not only in T2DM subjects but also in those with IGT. This suggests poor cerebral blood flow reserve and abnormal cerebral autoregulation. Therefore, during an acute ischaemic insult collateral vessels may be unable to re-perfuse the ischaemic penumbra to maintain its viability. To our knowledge, this is the first report of impaired cerebrovascular reactivity in subjects with IGT. Endothelial cell mediated vasodilatation is thought to play an important role in the intracerebral response to ACZ (24) and could be responsible for impaired cerebrovascular reactivity in IGT and T2DM.

The Gosling PI is a measure of vascular resistance originally performed on the brachial artery in humans (25). It has since been frequently used in studies to reflect increase in cerebrovascular resistance (26,27). In our study, ICA PI was significantly greater in T2DM subjects with microvascular disease (DR). This is consistent with the literature and thought to reflect greater cerebrovascular microangiopathy. Thus, our finding of impaired CVR but not pulsatility in IGT subjects probably reflects a relatively greater degree of endothelial vasodilatory dysfunctional than a microangiopathic pathology. This would suggest it could be

amenable to pharmacological treatment (e.g. low-dose aspirin is known to have vasoactive properties that may be through a cyclooxygenase independent pathway) or aggressive risk factor modification.

Autonomic nervous system alterations may also play a role in the cerebrovascular haemodynamic differences demonstrated. We found that changes in sympatho-vagal balance (LF:HF ratio) were moderately associated with CVR and PI. This appears to be driven by the variance in HF but not LF. Efferent vagal activity is a major contributor to the HF component of spectral analysis of baroreceptor gain as seen in clinical and experimental observations. Interpretation of the LF component is less clear, some consider it a marker of sympathetic modulation and by others as a parameter that includes both sympathetic and vagal influences. The LF:HF ratio assesses the controlled and balanced activity of the two branches of the autonomic nervous system. Although the results of this study are consistent with the hypothesis of autonomic dysfunction being involved in abnormal cerebrovascular reactivity, the cross-sectional design limits interpretation of causality. Further work is necessary to examine if abnormal sympatho-vagal balance results in worse outcomes post stroke and if pharmacological treatments can ameliorate this.

A number of different techniques have been used to assess CVR. The commonest method is the use of transcranial Doppler ultrasound measurement of middle cerebral artery velocity. Using this method several investigators have demonstrated reduced CVR in patients with type 1 and 2 diabetes (28-29). Impaired CVR was also demonstrated in diabetic subjects with established microvascular complications [retinopathy (30) and nephropathy (31)]. Transcranial Doppler ultrasound is a non-invasive, inexpensive technique that only measures blood velocity. Its main limitation is it assumes that changes in velocity are directly

proportional to changes in blood flow and therefore the arterial diameter remains constant (32). Consequently it overestimates blood flow (33). There is also significant inter-operator variability influencing its reliability and 5-15% of patients cannot undergo transcranial Doppler of the middle cerebral artery as there is an insufficient temporal bone acoustic window (34). Magnetic resonance PCA provides a reliable and reproducible method of quantifying blood flow and can be used to investigate arterial blood input to the brain, within the carotid and vertebral arteries. It also measures both blood flow and velocity and does not rely on the assumption of arterial diameter being constant. The disadvantages are that it is costly, requires longer imaging times and there are more contraindications to MRI scanning compared to ultrasound techniques. A recent study reported impaired vascular reactivity in subjects with impaired glucose tolerance and T2DM by measuring changes in retinal vessel diameter to flickering light (35). However, their exclusion of subjects with retinopathy, limits the widespread application of this method to the population of diabetes as a whole.

The main limitation of the current study is the relative small sample size. However, our goal was to assess whether abnormalities in cerebrovascular autoregulation are present in IGT. The data presented here at least preliminarily support this view. There are also limitations associated with the use of ACZ. Although it is the most applied stimulus for cerebral vasodilatation in a clinical setting, there is a degree of individual variability in response to a standardised dose of ACZ. While a single 1g dose of ACZ is often used, this does not necessarily produce a maximum CBF if it is less than 15mg/kg (36). Even with an effective supramaximal ACZ dose, CBF continues to respond to ventilation induced changes in PCO_2 and to changes in blood pressure (37), both are confounding factors that

can affect CBF. Future studies should consider using CO_2 inhalation as an alternative stimulus as its administration is non-invasive and easily terminated (38). Such studies may lead to identification of objective haemodynamic correlates of cerebrovascular disease that may serve as surrogate biological endpoints to evaluate interventions designed to prevent vascular complications of diabetes.

AUTHOR CONTRIBUTIONS

D.S, researched data and wrote manuscript, T.H, researched data and contributed to discussion, J.R, researched data and contributed to discussion, E.C, researched data and contributed to discussion, J.M, researched data and contributed to discussion, R.G, researched data and contributed to discussion, P.D.G, contributed discussion. S.T, researched data, contributed to discussion and reviewed/edited manuscript, I.D.W, researched data, contributed to discussion and reviewed/edited manuscript.

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