Clinical Neurophysiology 130 (2019) 1502–1510

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

# Diabetes and the link between neuroplasticity and glutamate in the aging human motor cortex

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ARTICLE INFO

*Article history:*
Accepted 22 April 2019
Available online 21 June 2019

*Keywords:*
Glutamate
Cortical plasticity
Transcranial magnetic stimulation
Magnetic resonance spectroscopy
Type-2 diabetes mellitus
Impaired glucose tolerance
Insulin resistance
Aging

HIGHLIGHTS

- Cortical plasticity is impaired in older adults with either pre-diabetes or type-2 diabetes.
- Older adults with pre-diabetes show the biggest differences in cortical glutamatergic metabolite levels.
- Cortical plasticity is positively associated with glutamate concentration in type-2 diabetes.

ABSTRACT

*Objectives:* In older adults, type-2 diabetes mellitus (T2DM) impacts cognition and increases dementia risk. Prior studies suggest that impaired neuroplasticity may contribute to the cognitive decline in T2DM, but the underlying mechanisms of altered neuroplasticity are unclear. We investigated the relationship of the concentration of glutamatergic metabolites with measures of cortical plasticity in older adults across the spectrum of glucose intolerance/insulin resistance.
*Methods:* Forty adults (50–87 years: 17—T2DM, 14—pre-diabetes, 9—controls) underwent magnetic resonance spectroscopy to quantify glutamate and other key metabolites within a 2 $cm^3$ region around the hand knob of the left primary motor cortex. Thirty-six also underwent a separate transcranial magnetic stimulation (TMS) assessment of cortical *excitability* and *plasticity* using single-pulse TMS and intermittent theta-burst stimulation targeting the same brain region.
*Results:* Group differences were observed in relative concentrations of glutamine ($p = .028$), glucose ($p = .008$), total cholines ($p = .048$), and the glutamine/glutamate ratio ($p = .024$). Cortical plasticity was reduced in both T2DM and pre-diabetes groups relative to controls ($p$-values < .05). Only the T2DM group showed a significant positive association between glutamate concentration and plasticity ($r = .56$, $p = .030$).
*Conclusions:* Neuroplastic mechanisms are already impaired in pre-diabetes. In T2DM, reduced corticomotor plasticity is associated with lower cortical glutamate concentration.
*Significance:* Impaired plasticity in T2DM is associated with low glutamatergic metabolite levels. The glutamatergic neurotransmission system constitutes a potential therapeutic target for cognitive problems linked to plasticity-related deficiencies in T2DM.



## 1. Introduction

The world is facing an epidemic in type-2 diabetes mellitus (T2DM). Nearly 25% of Americans aged 65 and older have T2DM and more than half have elevated hemoglobin A1c (HbA1c) levels consistent with impaired glucose tolerance, or pre-diabetes (U.S. D.H.H.S. C.D.C.P., 2014). Diabetes can affect the central nervous

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https://doi.org/10.1016/j.clinph.2019.04.721
1388-2457/

system through neuronal toxicity of hyper- and hypoglycemia episodes, micro-vascular insults, impaired glucose and insulin transfer and resistance (Last et al., 2007). The impact of T2DM on the brain is widespread and may result in structural and functional alterations, including cortical atrophy, loss of white matter integrity, breakdown of the blood-brain barrier, disrupted functional connectivity, and altered brain metabolism (Sahin et al., 2008; Strachan, 2010; Musen et al., 2012; Cui et al., 2014; Heni et al., 2014; Marder et al., 2014; Roberts et al., 2014). Consequently, T2DM is associated with accelerated cognitive aging (Messier, 2005; Xu et al., 2010) and a two- to three-fold increase in the risk of developing dementia (Ott et al., 1999; Ravona-Springer et al., 2010). Even in the absence of subjective cognitive complaints, older adults with diabetes show deficits in verbal learning and short-term memory, visual attention, executive function, and psychomotor speed (Fontbonne et al., 2001; Knopman et al., 2001). While the relationship between diabetes-associated brain changes, cognitive deficits, and the risk of dementia is likely multifactorial, there is evidence from mouse models (Abbas et al., 2009; Grillo et al., 2015) and humans with T2DM (Fried et al., 2016) that implicates declines in the efficacy of mechanisms that support long-term potentiation (LTP)-type plasticity. Notably, glutamatergic neurotransmission plays a critical role in human cortical synaptic plasticity, learning and memory (Rowland et al., 2005; Simonyi et al., 2005; Huang et al., 2007), and may also be affected by T2DM (Trudeau et al., 2004). The goal of the present study was to further investigate the neurobiological basis for altered neuroplasticity in humans with T2DM by linking measures of brain plasticity assessed with transcranial magnetic stimulation (TMS) to the concentrations of the main glutamatergic metabolites, glutamate and glutamine, as well as other relevant brain metabolites assessed with magnetic resonance spectroscopy (MRS).

MRS is a technique to quantify in vivo the concentrations of different brain metabolites within a region-of-interest (Jissendi Tchofo and Balériaux, 2009). Several metabolites, including myo-inositol, cholines, and N-acetyl-aspartate, have been studied for their association with T2DM (Perros et al., 1997; Sahin et al., 2008). Similarly, MRS measures of glutamate, glutamine, and GABA have been used in healthy individuals to help understand neurotransmitter function underlying certain neurophysiological measures obtained with TMS, including paired-pulse TMS measures of intracortical inhibition/facilitation and repetitive TMS measures of long-term depression (LTD)-like plasticity (Stagg et al., 2009, 2011; Tremblay et al., 2013). While the link between altered synaptic plasticity and brain metabolism (including insulin signaling and energy homeostasis) has been demonstrated in mouse models of T2DM (Agrawal et al., 2014), no human studies have examined the relationship between LTP-like plasticity and cortical glutamatergic metabolites. The present study aimed to fill this gap by investigating the relationship of glutamate and other key metabolites to the mechanisms of LTP-like brain plasticity in healthy individuals as well as those along the spectrum of impaired glucose tolerance and insulin resistance. We hypothesized that impaired mechanisms of LTP-like plasticity previously observed in diabetes (Fried et al., 2016) are associated with glutamate concentrations in the motor cortex.

## 2. Methods

### 2.1. Human participants

The study was conducted on 40 adults (aged 50–87 years, 18 females) participants at the Beth Israel Deaconess Medical Center. All participants provided written informed consent prior to enrollment in the study which had been approved by the Institutional Review Board and was conducted in accordance with the Declaration of Helsinki.

Seventeen (17) participants had been diagnosed as having T2DM based on criteria from the National Institute of Diabetes and Digestive and Kidney Diseases (U.S. D.H.H.S. N.I.D.D.K., 2018). A maximum HbA1c cutoff of 10% was used to exclude participants whose diabetes was poorly controlled. Of the 23 non-diabetic participants, a subgroup of 14 was shown by fasting blood draw to have an HbA1c level of 5.7–6.4% consistent with pre-diabetes (U.S. D.H.H.S. N.I.D.D.K., 2018). The remaining subgroup of nine healthy controls (HC) had an HbA1c $\leq$ 5.6%. We thus defined three groups of participants: diabetes (T2DM), pre-diabetes, controls.

All participants received a brief cognitive evaluation by a neuropsychometrist, including a 30-item MMSE to assess cognitive integrity, and a 50-item Wechsler Test of Adult Reading (W-TAR; age-normed) to provide a measure of premorbid intelligence. All participants were cognitively intact (mini-mental status exam; MMSE $\geq$ 27), with a negative history of neurological disease or psychiatric illness, and without contraindications to MRI or TMS.

Following consent and screening, all participants underwent magnetic resonance imaging (MRI) and MRS, and a TMS assessment of cortical excitability and plasticity. The MRI/MRS and TMS procedures occurred on separate visits with a median time between MRS and TMS of 22 days (range 6–73). The time between MRS and TMS visits did not differ across participants' groups ($p$ = .338).

### 2.2. Magnetic resonance imaging/spectroscopy

A T1-weighted anatomical MRI scan was obtained in all participants on a 3 T scanner (GE Healthcare, Ltd., Chicago, IL, USA) using a 3D spoiled gradient echo sequence: 162 axial-oriented slices for whole-brain coverage; 240-mm isotropic field-of-view; 0.937-mm $\times$ 0.937-mm $\times$ 1-mm native resolution; flip angle = 15°; TE/TR = 2.9/6.9 ms; duration = 432 s. Cortical reconstruction and automatic segmentation were performed with FSL (version 5.9, www.fmrib.ox.ac.uk).

A 5-min PRESS (Point RESolved Spectroscopy) proton MRS sequence was acquired in all participants from a 2-cm$^3$ voxel placed around the hand knob of the left primary motor cortex (Fig. 1). First, automatic shimming was performed on the voxel, followed by manual optimization of water suppression. Then, a PRESS MRS sequence was acquired with TE/TR = 35/2000 ms, 5 kHz spectral width, 4096 complex points, 128 averages, scan time 5 min. For each water-suppressed acquisition of brain metabolites, a non-suppressed water spectrum was acquired with 2 averages for use as an internal reference for absolute quantification of metabolite concentrations. PRESS spectra were analyzed using the LCModel software (v6.3-1L) (Provencher, 1993, 2001), which performs automatic quantitation of proton MR spectra in vivo as linear combinations of model metabolite spectra. The model basis set used for analysis was composed of 17 metabolite spectra that were simulated in house using the Vespa (Soher et al., 2007) software graphical interface to the GAMMA magnetic resonance simulation library (Smith et al., 1994). In order to perform absolute quantitation of metabolites, the metabolite signal was scaled to the water signal from the same voxel using LCModel's water scaling method and estimated the water concentration in each voxel by using the voxel partial volume fractions of gray matter, white matter, and cerebrospinal fluid (Ernst et al., 1993) obtained by segmentation of the T1-weighted structural image.

Absolute concentrations (mmol/kg) were derived for eight metabolites of interest (Fig. 1): glutamate (Glu), the main excitatory neurotransmitter; glutamine (Gln), an amino acid precursor and conversion product of glutamate involved in the glutamine/glutamate cycle occurring during glutamatergic neurotransmission; glucose

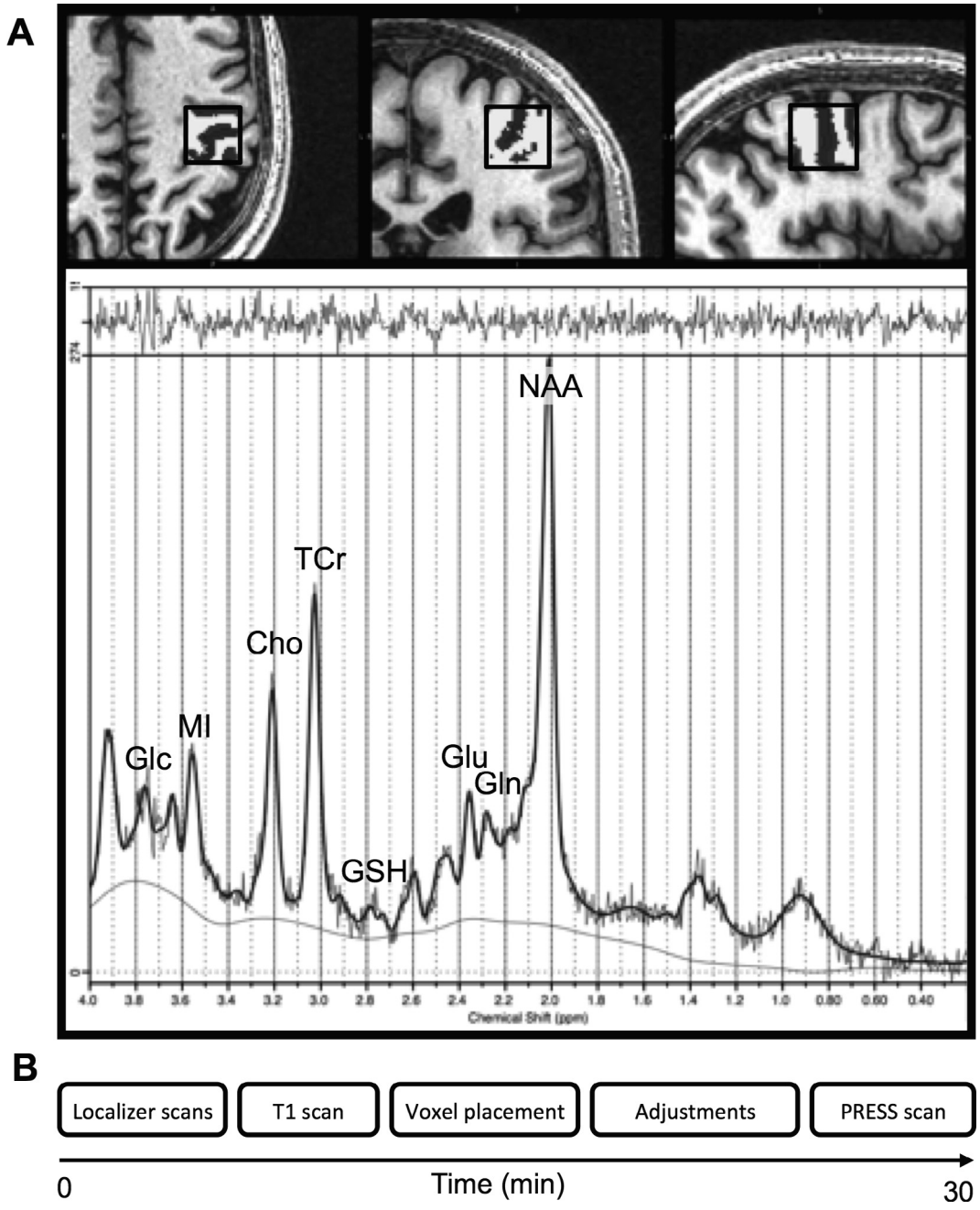


**Fig. 1.** *Location of tissue-segmented voxel centered around the left primary motor cortex "hand knob" (top) and resulting PRESS (Point RESolved Spectroscopy) spectrum analyzed using LCModel (bottom).* Metabolites of interest included: glucose (Glc); myo-inositol (MI); total cholines (Cho; including glycero-phosphocholine and phosphocholine); total creatines (TCr; including creatine and phosphocreatine); glutathione (GSH); glutamate (Glu); glutamine (Gln); N-acetylaspartate (NAA). Adjustments during MRS acquisition included automatic transmitter frequency and shim (field homogeneity within the voxel) adjustments and manual adjustment of the water suppression.

(Glc), the main energetic substrate for the brain; myo-inositol (MI), an osymolyte that is the precursor and degradation product of membrane phospholipids and cytosolic second messenger inositol-phosphates (e.g. IP3); N-acetyl-aspartate (NAA), a marker of neuronal viability that is synthesized in neuronal mitochondria; glutathione (GSH), an important antioxidant in the brain; total cholines (Cho; including glycerol-phosphocholine and phosphocholine), the precursors and degradation products of membrane phospholipids; and total creatine (TCr; including creatine and phosphocreatine), the energetic buffer system that regenerates ATP after expense in order to maintain energy potential. The combined concentration of glutamate and glutamine (Glx) was also derived. After confirming, via one-way ANOVA (with age as a covariate), that absolute TCr concentrations did not differ by group ($F = .24$, $p = .784$), all other metabolites were expressed as the ratio to TCr. Thus the reported relative metabolite concentrations reflect absolute concentrations normalized by the TCr energetic buffer levels in each individual. This method of calculating the metabolite concentrations relative to absolute TCr concentrations differs from those that directly report metabolite to TCr ratios without prior evaluation of TCr absolute concentrations and statistical comparison of TCr group levels. In the latter case, the relative metabolite concentrations reported are blind to any potential

individual or group differences in TCr, which could bias the results. In addition, the ratio of glutamine to glutamate (Gln/Glu) was calculated to assess glutamatergic activity and provide an index of glutamatergic neurotransmission (Hall et al., 2015).

### 2.3. Transcranial magnetic stimulation

TMS was performed in 36 subjects (7–HC, 14–PreDM, 15–T2DM). All parameters used in the study conformed to current recommended guidelines for the safe application of TMS endorsed by the International Federation of Clinical Neurophysiology (IFCN) (Rossi et al., 2009). Motor evoked potential (MEPs) elicited by suprathreshold single-pulse TMS were recorded from the right first dorsal interosseous muscle using surface electromyography. Following IFCN guidelines (Rossini et al., 2015), each individual's resting motor threshold (RMT) and active motor threshold (AMT) were measured with Nexstim (Nexstim Plc, Finland) and Magventure (MagVenture A/S, Denmark) biphasic figure-of-8 coils, respectively, and used to set the intensity of subsequent stimulation. A Navigated Brain Stimulation system (Nexstim) was used to identify the hand region of the primary motor cortex and ensure consistent targeting throughout the experimental session (Fig. 2).

The parameters of single-pulse and intermittent theta-burst (iTBS) TMS used in the present study have been reported in detail elsewhere (Fried et al., 2016). Briefly, cortical *excitability* was defined as the average amplitude of MEPs elicited with single TMS pulses delivered at 120% of RMT. Three blocks of 30–35 pulses (with a random 5000–6000 ms interval) were acquired before iTBS (Baseline) and after iTBS (at 5, 10 and 20 min). The first 20 min following iTBS corresponds to the peak effect in normal individuals (Wischnewski and Schutter, 2015) and is when the greatest difference between diabetes patients and controls was previously observed (Fried et al., 2016). A Shapiro-Wilk test revealed a positive skew in raw MEP amplitudes ($W = .84$, $p < .001$), so all μV values were $Log_{10}$ transformed to meet the assumption of normality ($W = .99$, $p = .992$). The transformed data were then averaged across trials to yield two measures, Baseline and Post-iTBS, and back-transformed into geometric means. Cortical *plasticity* was defined as the percent change in cortical excitability (MEP%Δ) induced by iTBS.

### 2.4. Statistical analysis

Statistical analyses were performed in JMP Pro (version 13.0, The SAS Institute, Cary, NC) using a normal distribution and a two-tailed 95% confidence interval ($\alpha = .05$).

Unless otherwise specified, each dependent variable was tested against the null hypothesis of no difference between groups using

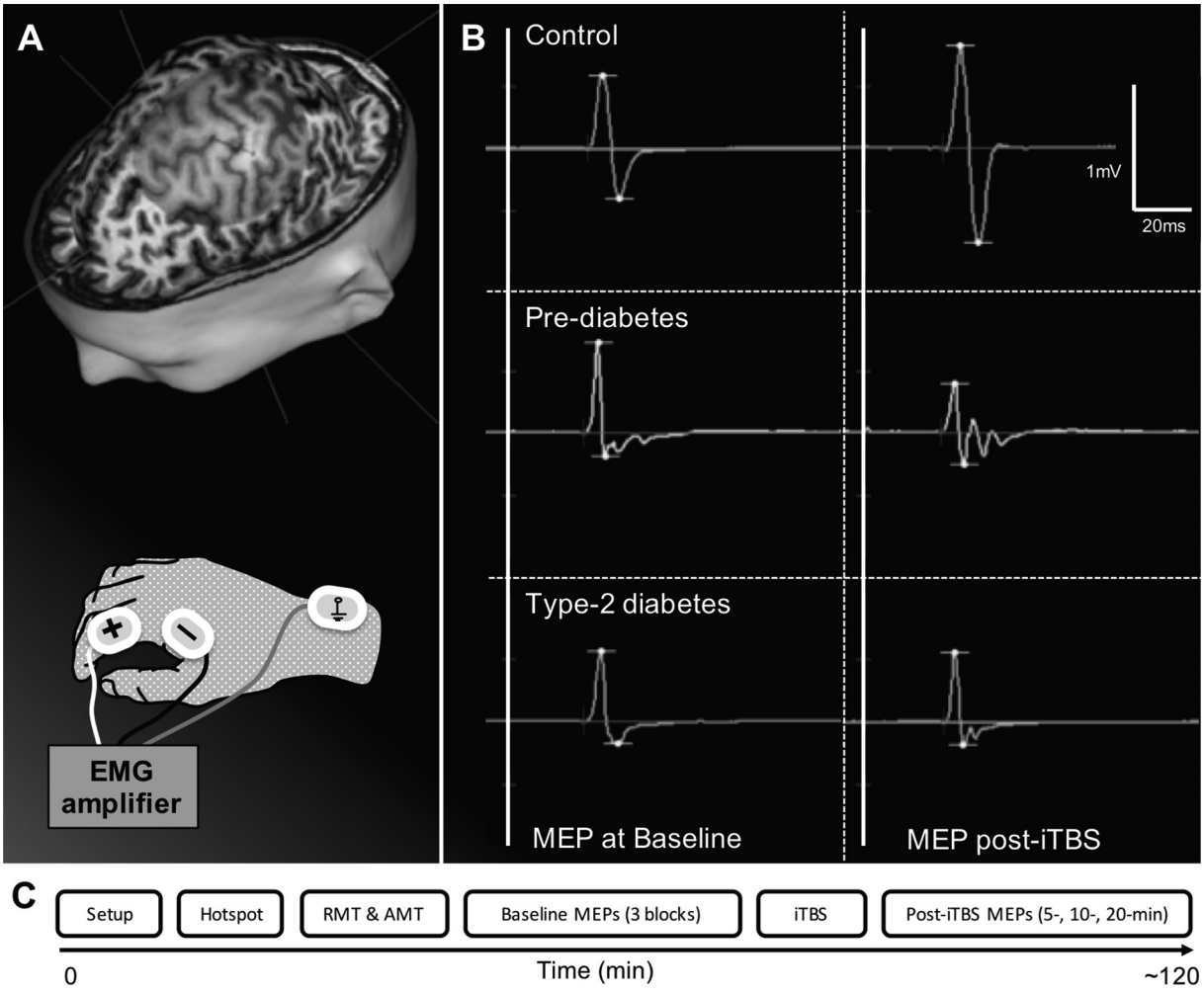


**Fig. 2.** *Transcranial magnetic stimulation (TMS) assessments of cortical excitability—average amplitude of motor evoked potentials (MEPs) elicited by single-pulse TMS—and cortical plasticity—the change in excitability induced by intermittent theta-burst stimulation (iTBS).* (A) Magnetic Resonance (MR)-guided TMS was applied to the left primary motor cortex "hand knob" and resulting MEPs were recorded from the right first dorsal interosseous muscle by surface electromyography (EMG). (B) Example MEP traces from control, pre-diabetes, and type-2 diabetes subjects before and after iTBS.

Fisher's exact tests for categorical variables and one-way analysis of variance (ANOVA) for continuous variables. Tukey's honestly significant difference (HSD) tests were used for all post-hoc pairwise comparisons. Participant age differed significantly (see Section 3) and was thus added as a covariate to the ANOVAs for metabolite concentrations, neuropsychological measures, and TMS-based neurophysiological assessments.

To examine group differences in relative metabolite concentrations, all metabolites of interest (Glu/TCr, Gln/TCr, Glx/TCr, Gln/Glu, Glc/TCr, MI/TCr, NAA/TCr, GSH/TCr, Cho/TCr) were first entered into a multivariate ANOVA (MANOVA) with the main factor of *group* and *age* as a covariate. Follow-up one-way ANOVAs were performed on each metabolite with *group* and *age*. Individual p-values were subjected to a 5% false-discovery rate (FDR) threshold using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

To investigate group differences in cortico-motor reactivity and plasticity, the variables of interest (RMT, AMT, Baseline MEPs, MEP%Δ) were entered into separate one-way ANOVAs with *group* and *age*. Individual p-values were subjected to a 5% FDR threshold.

To investigate the impact of iTBS on MEP amplitude within each group, MEP amplitudes (μV) were entered into separate random-effects linear regression analyses with a main effect of *time* (Baseline, 5-min, 10-min, 20-min).

The relationship between cortical plasticity and relative glutamate concentration was first assessed by multiple linear regression using a full-factorial model, with *age* and the *time between MRS and TMS visits* as covariates, to investigate if there was an overall relationship between Glu/TCr and MEP%Δ and if that relationship was different between groups. This was followed by separate linear regression analyses between Glu/TCr and MEP%Δ, with *age* and the *time between MRS and TMS visits* as covariates, to highlight the relationship in each group.

As an exploratory analysis to examine the link between cortical plasticity and cognitive function in our sample, we related our measures of cognitive status (MMSE) and premorbid IQ (W-TAR) to the TMS measures of cortical plasticity first applying a multiple linear regression analysis to look for an overall relationship, and then by computing individual Pearson correlation coefficients to assess the relationship within each group of participants.

## 3. Results

Complete data and full statistical details are reported in Table 1.

### 3.1. Demographics, health, and neuropsychological function

Fisher's exact tests yielded no difference in gender ($p = .919$) or handedness proportions across the three groups ($p = .170$). The one-way ANOVA yielded significant *group* variance in participant age ($p = .014$). Specifically, controls were younger as a group than pre-diabetic or diabetic participants ($p$-values < .05). Importantly,

**Table 1**
Study data and statistical details.

| | Healthy Controls | Pre-diabetes | Type-2 Diabetes | One-way ANOVA | | | | Post-hoc | Relationship to age | |
|---|---|---|---|---|---|---|---|---|---|---|
| | (HC; n = 9) | (PreDM; n = 14) | (T2DM; n = 17) | *df* | *F* | *P* | $\eta_p^2$ | Tukey's HSD | $R_p$ | *P* |
| *Demographics* | | | | | | | | | | |
| # Female (%) | 4 (44%) | 7 (50%) | 7 (41%) | N = 40, df = 2, 2-tailed p = 0.919; Fisher's Exact Test | | | | | – | – |
| # Right handed (%) | 8 (89%) | 14 (100%) | 13 (76%) | N = 40, df = 2, 2-tailed p = 0.170; Fisher's Exact Test | | | | | – | – |
| Age (y) | 54.89 ± 7.1 | 65.71 ± 10.7 | 64.47 ± 7.5 | 2,37 | 4.84 | 0.014 | 0.21 | **HC < T2DM, PreDM** | – | – |
| Time between visits (d) | 22.00 ± 15.6 | 26.86 ± 16.5 | 34.13 ± 22.1 | 2,33 | 1.12 | 0.338 | 0.06 | *n.s.* | – | – |
| *Health indices* | | | | | | | | | | |
| Body Mass Index (kg/m$^2$)[a] | 26.72 ± 6.3 | 26.78 ± 5.3 | 28.58 ± 4.0 | 2,28 | 0.52 | 0.601 | 0.04 | *n.s.* | −0.09 | 0.622 |
| Hemoglobin A1c (%)[b] | 5.36 ± 0.2 | 5.87 ± 0.2 | 7.51 ± 1.2 | 2,34 | 25.72 | <0.001 | 0.60 | **HC, PreDM < T2DM** | 0.02 | 0.896 |
| Fasting Glucose (mg/dL)[c] | 88.44 ± 8.1 | 91.31 ± 4.7 | 146.80 ± 38.9 | 2,33 | 21.21 | <0.001 | 0.56 | **HC, PreDM < T2DM** | −0.07 | 0.707 |
| *Neuropsychological Status* | | | | | | | | | | |
| MMSE (#/30) | 29.22 ± 0.7 | 29.57 ± 0.8 | 29.13 ± 1.1 | 2,35 | 0.85 | 0.437 | 0.05 | *n.s.* | 0.11 | 0.504 |
| W-TAR (age-normed) | 109.89 ± 10.6 | 118.64 ± 9.7 | 110.69 ± 14.4 | 2,35 | 1.63 | 0.210 | 0.09 | *n.s.* | **0.45** | **0.006** |
| *Relative Metabolite Concentration*[†] | | | | | | | | | | |
| Glutamate | 1.51 ± 0.2 | 1.38 ± 0.1 | 1.42 ± 0.2 | 2,36 | 0.45 | 0.644 | 0.02 | *n.s.* | −0.28 | 0.094 |
| Glutamine | 0.35 ± 0.2 | 0.22 ± 0.1 | 0.30 ± 0.1 | 2,36 | 3.97 | 0.028 | 0.18 | **HC > PreDM** | 0.00 | 0.978 |
| Total Glutamate + Glutamine | 1.86 ± 0.1 | 1.60 ± 0.0 | 1.72 ± 0.1 | 2,36 | 1.88 | 0.168 | 0.09 | *n.s.* | −0.20 | 0.218 |
| Glutamine/Glutamate | 0.22 ± 0.0 | 0.02 ± 0.1 | 0.21 ± 0.1 | 2,36 | 4.13 | 0.024 | 0.19 | **HC > PreDM** | 0.12 | 0.490 |
| Glucose[d] | 0.16 ± 0.0 | 0.12 ± 0.0 | 0.24 ± 0.1 | 2,17 | 6.54 | 0.008 | 0.43 | **T2DM > PreDM** | **0.68** | **0.001** |
| Myo-inositol | 0.75 ± 0.2 | 0.71 ± 0.1 | 0.73 ± 0.2 | 2,36 | 0.03 | 0.769 | 0.00 | *n.s.* | **−0.41** | **0.010** |
| N-acetyl-aspartate | 2.12 ± 0.4 | 2.15 ± 0.2 | 2.05 ± 0.3 | 2,36 | 0.54 | 0.588 | 0.03 | *n.s.* | −0.19 | 0.256 |
| Glutathione | 0.27 ± 0.0 | 0.22 ± 0.0 | 0.23 ± 0.0 | 2,36 | 1.01 | 0.376 | 0.05 | *n.s.* | 0.08 | 0.639 |
| Total Cholines | 0.15 ± 0.0 | 0.17 ± 0.0 | 0.15 ± 0.0 | 2,36 | 3.31 | 0.048 | 0.16 | *n.s.* | −0.16 | 0.337 |
| *Cortico-motor Reactivity and Plasticity*[e] | | | | | | | | | | |
| Resting Motor Threshold (% MSO) | 50.57 ± 6.2 | 43.79 ± 9.5 | 47.13 ± 13.2 | 2,32 | 1.51 | 0.236 | 0.09 | *n.s.* | 0.20 | 0.254 |
| Active Motor Threshold (% MSO) | 48.86 ± 8.1 | 45.57 ± 7.9 | 48.53 ± 13.1 | 2,32 | 0.57 | 0.573 | 0.03 | *n.s.* | 0.14 | 0.426 |
| Baseline MEPs (mV) | 1.25 ± 0.5 | 1.34 ± 0.3 | 0.73 ± 0.1 | 2,32 | 1.66 | 0.206 | 0.09 | *n.s.* | 0.05 | 0.788 |
| % Δ in MEPs 5–20 min post-iTBS[††] | 50.43 ± 24.6 | −14.29 ± 11.3 | 0.20 ± 7.5 | 2,32 | 4.83 | 0.015 | 0.23 | **HC > T2DM, PreDM** | 0.06 | 0.743 |

*Abbreviations*: MMSE = mini-mental status exam; W-TAR = Weschler Test of Adult Reading; MSO = maximum stimulator output. Values represent mean ± standard deviation. Analyses reflect the results of a one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) tests. Partial correlation and significance of age as covariate shown when appropriate. [†]Individual metabolites were measured in mmol of brain tissue (gray and white matter) and expressed as the ratio of total creatine.
[††]Post-iTBS measures calculated as percent change from baseline

[a] Body mass index was not obtained from 2 pre-diabetic and 6 diabetic subjects.
[b] Hemoglobin A1c was not obtained from 1 control and 2 diabetic subjects.
[c] Fasting glucose was not obtained from 1 control, 1 pre-diabetic, and 2 diabetic subjects.
[d] Glucose could not be reliably detected by MRS in 4 controls, 9 pre-diabetic, and 6 diabetic subjects.
[e] TMS data were not obtained in 1 control and 2 diabetic subjects.

the time between MRS and TMS visits did not differ across groups ($p = .338$). As expected, there were significant group differences in HbA1c ($p < .001$) and fasting glucose levels ($p < .001$), with diabetes participants having higher values than controls or pre-diabetic participants, indicative of insulin resistance. Controlling for *age*, the one-way ANOVA demonstrated no variance by *group* in MMSE ($p = .437$) or W-TAR scores ($p = .210$), indicating equivalent cognitive status and premorbid intelligence.

### 3.2. Cortical metabolites

Glu, Glx, TCr, MI, NAA, GSH and Cho concentrations were reliably determined over all subjects as indicated by mean (±SD) Cramer-Rao lower bounds (CRLB) of 9 (±2)%, 8 (±2)%, 3 (±1)%, 8 (±3)%, 4 (±1)%, 15 (±7)% and 7 (±4)% respectively. Due to its lower concentrations, Gln was less reliably determined with a mean (±SD) CRLB of 39 (±6)% (range 25–51%). We included all Gln measurements even with high CRLBs (up to the highest: 51%) in our analyses in order to avoid bias by preferential selection of higher concentrations (Kreis, 2016; Provencher, 2018). In healthy non-diabetic subjects at the scanner field strength of 3 Tesla, brain Glc cannot always be reliably determined due to low concentrations, a complex spectral pattern, and spectral overlap with other metabolites. In subjects with diabetes, brain glucose concentrations are higher and their measurement is typically more reliable. Consequently, absolute Glc levels could not be reliably detected in 4 control, 9 pre-diabetic, and 6 diabetic subjects. Therefore, the significant group differences in Glc/TCr should be interpreted with caution as they reflect differences between group levels that are barely detectable and only moderately reliably measured.

Controlling for *age*, the MANOVA yielded a significant main effect of *group* (Wilks' $\lambda = 0.08$, $F_{18,18} = 2.64$, $p = .023$), indicating an overall difference in metabolite concentrations across the three groups. Follow-up one-way ANOVAs demonstrated this effect was driven mainly by group differences in Gln/TCr ($p = .028$), Gln/Glu ($p = .024$), Glc/TCr ($p = .008$), and Cho/TCr ($p = .048$), with Glc/TCr surviving the 5%FDR cutoff. By comparison, Glu/TCr, Glx/TCr, MI/TCr, NAA/TCr, and GSH/TCr levels were equivalent across groups at the .05–level. Tukey's HSD tests revealed Gln/TCr and Gln/Glu were significantly lower in pre-diabetes than controls, while Glc/TCr levels were significantly higher in the diabetes group than in pre-diabetes ($p$-values < .05) (Fig. 3A).

### 3.3. Cortical excitability and plasticity

The random-effects linear model revealed that for HC there was significant variation in MEP amplitude by *Time* ($F_{3,18} = 3.47$, $p = .038$, $\eta_p^2 = .37$). Post-hoc Tukey's HSD tests indicated this was driven by a significant increase in MEP amplitudes from baseline to 10-min post-iTBS ($p < .05$). By comparison, neither PreDM, nor T2DM showed a significant modulation of MEPs by iTBS ($F$-ratios < 1.19, $p$-values > .324).

Controlling for *age*, the one-way ANOVA revealed no significant variance by *group* in RMT, AMT, or baseline MEP amplitude ($p$-values > .206) suggesting that cortical excitability, and by extension, the integrity of the cortico-spinal pathway was equivalent in diabetic, pre-diabetic and control groups. By contrast, the primary variable of interest, MEP%Δ, was significantly different across groups ($p = .015$) and survived the 5%FDR cutoff. Tukey's HSD tests indicated that iTBS-induced facilitation of MEPs was significantly lower in both pre-diabetes and diabetes than controls ($p$-values < .05), indicating reduced LTP-like plasticity in these subjects (Fig. 3B).

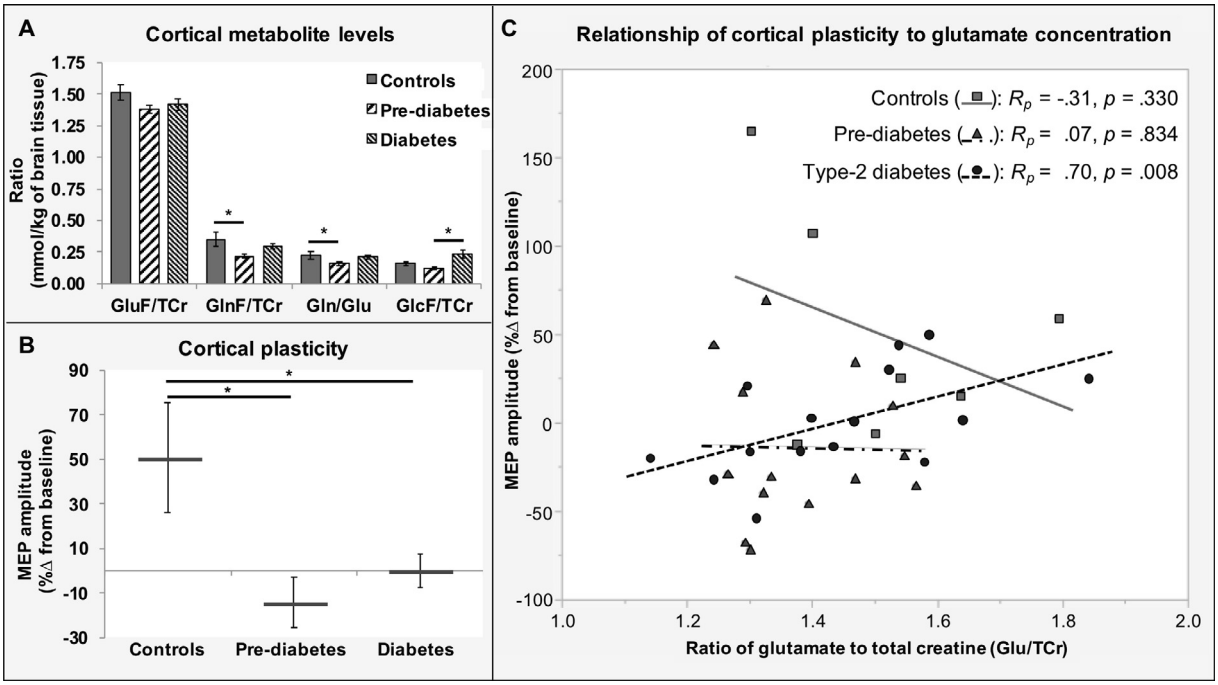


**Fig. 3.** *Measures of cortical metabolism, cortical plasticity, and their relationship in healthy controls, pre-diabetes, and type-2 diabetes.* (A) The three groups did not differ in the relative (to total creatine; TCr) concentration of glutamate (Glu), but group differences were observed for glutamine (Gln), glucose (Glc), and total cholines (not shown), as well as the glutamine/glutamate ratio (Gln/Gln). (B) Cortical plasticity, defined as the percent change (%Δ) in motor evoked potential (MEP) amplitude induced by intermittent theta-burst stimulation (iTBS), was significantly higher in controls than either pre-diabetes or type 2 diabetes groups. (C) In the diabetes group alone, there was a significant positive correlation between measures of cortical plasticity and glutamate metabolism indicating the efficacy of neuroplastic mechanisms may depend on the availability of free glutamate at the synapse. * $p < .05$.

### 3.4. Relationship of glutamate concentration to cortical plasticity

Multiple linear regression revealed a significant *group*MEP%Δ* interaction ($F_{2,29}$ = 4.57, $p$ = .019, $\eta_p^2$ = .25) indicating the relationship between glutamate concentration and iTBS-induced facilitation differed between groups controlling for *age* and the *time between MRS and TMS visits*. Neither main effect (*group*, *MEP%Δ*) was significant (*p*-values > .189). Follow-up linear regression analyses (Fig. 3C) revealed that Glu/TCr and MEP%Δ were positively correlated in diabetes (partial $R_{12}$ = .70, $p$ .008), but not pre-diabetes or controls (*p*-values > .423). These results suggest that for T2DM, the ability of iTBS to induce cortical plasticity in the motor cortex may depend on the primary motor cortical concentration of glutamate.

### 3.5. Relationship of cortical plasticity to cognitive status and premorbid IQ

For both MMSE and W-TAR, the multiple linear regression analyses revealed no significant main effects of *group* or *MEP%* Δ and no *group*MEP%Δ* interaction (*F*-ratios < 1.72, *p*-values > .199). Planned Pearson correlations revealed weak-to-moderate non-significant associations (−.39 < *R*'s < .34, *p-values* > .234) between MEP%Δ and either MMSE or W-TAR at the individual group level. These results suggest that there are no overarching relationships between cortical plasticity and broad measures of cognitive status and premorbid IQ.

## 4. Discussion

T2DM accelerates cognitive aging and increases the risk of developing Alzheimer's disease (AD) related dementia (Arvanitakis et al., 2004; Ravona-Springer et al., 2010). While the impact of diabetes on the aging brain is likely multifactorial, a previous study from our group (Fried et al., 2016) reported reduced iTBS measures of LTP-like cortical plasticity associated with lower verbal learning and memory performance in older adults with T2DM. These findings are consistent with rodent models of diabetes (Sweetnam et al., 2012; Agrawal et al., 2014) and suggest that declines in the efficacy of neuroplastic mechanisms, specifically those that support LTP-like plasticity, may contribute substantially to declines in core cognitive functions seen in some diabetic patients and may be an important link between T2DM and AD (Abbas et al., 2009). The present study confirms and extends these findings, showing the mechanisms of LTP-like plasticity are already impaired in pre-diabetes, and provides mechanistic insight into this alteration by relating measures of plasticity to glutamate concentrations in the motor cortex. As the aftereffects of iTBS are NMDAR-dependent (Huang et al., 2007), the aim of the present study was to investigate if glutamatergic metabolites in the brain are abnormal in T2DM and linked to the mechanisms of LTP-like plasticity as assessed by TMS. While no differences were observed in the relative concentration of glutamate between the three groups, there was a significant positive association between higher glutamate levels and greater facilitation from iTBS in diabetes participants. These results suggest that fluctuations in glutamate concentration play a substantial role in the lack of LTP-like plasticity observed in some diabetic participants and by extension implicate a deficiency of the glutamatergic neurotransmission system underlying plasticity in T2DM. The higher glutamate concentrations required to attain close to normal levels of plasticity in the T2DM group could be explained by abnormalities at the level of glutamatergic receptors, such as a deficiency in number, or changes in relative distribution or configuration. Such receptor changes could occur as an adaptation or compensation mechanism during prolonged periods of insulin resistance in the pre-diabetic state, which impact brain glucose, glutamate and glutamine metabolism and concentrations.

Beyond glutamate, the present study revealed group differences in metabolite concentrations overall, which were driven by mainly by glutamine, the glutamine/glutamate ratio, and glucose. Interestingly, while the diabetes group had the highest levels of brain glucose, it was the pre-diabetes group that showed lower levels of glutamine and a lower glutamine/glutamate ratio compared to diabetes participants or controls. Glutamine plays an essential role in glutamatergic neurotransmission by providing a source for replenishment of neuronal glutamate presynaptic vesicles via recycling of synaptic glutamate. During glutamatergic neurotransmission, glutamate released into the synaptic cleft is captured by astrocytes and converted to glutamine by glutamine synthase, which is then transferred to neurons and recycled to glutamate by glutaminase to replenish the presynaptic vesicular pool (i.e., glutamate-glutamine cycling) (Magistretti and Allaman, 2015). As such, glutamine and the Gln/Glu ratio may be more closely related to levels of glutamatergic neurotransmission than glutamate alone and the Gln/Glu ratio has been proposed as an index of glutamatergic activity (Hall et al., 2015). Therefore these results suggest that even subtle glucose intolerance may be associated with a broader disruption to glutamine metabolism and glutamatergic function. The results highlight the early effects of insulin resistance on glutamatergic metabolites and support an explanation of progressive disruption of the glutamatergic neurotransmission system ultimately leading to more consequential impairment in T2DM. The pre-diabetes group also showed impaired mechanisms of LTP-like plasticity similar to those found in T2DM. The majority of pre-diabetic individuals in the current study had a reduction in MEP amplitudes following iTBS rather than facilitation—a similar impairment in the mechanisms of NMDAR-dependent LTP-like plasticity as observed in older adults with T2DM (Fried et al., 2016) and AD (Koch et al., 2012). However, unlike the diabetic participants, the failure to show iTBS-induced potentiation was not significantly related to glutamate concentration in pre-diabetes, which implies that the factors that influence the efficacy of neuroplastic mechanisms may be more complex for impaired glucose tolerance and early-stage insulin resistance. Compared to diabetes, the impact of pre-diabetes on the aging brain is not well understood, despite its prevalence. Moreover, as pre-diabetes is often undiagnosed, affected individuals are unlikely to pursue medication or diet and lifestyle changes that could slow or reverse its progression, and which may influence the response to iTBS (Gomes-Osman et al., 2017).

The results of the present study should be interpreted in the context of several limitations. The sample sizes for the different groups were fairly small and unequal. The study was initially conceived as a comparison of diabetes versus non-diabetic controls with sample sizes similar to several recent studies (Novak et al., 2014; Fried et al., 2016), but the prevalence of pre-diabetes in older adults necessitated a further subdivision. A post-hoc power analysis revealed the present sample provided 80% power to detect medium-large effect sizes ($\eta^2 \geq .23$). Information on HbA1c and fasting glucose levels, as well as medications and comorbidities was incomplete, thus preventing a fuller investigation of the role of these factors. The use of the PRESS MRS sequence and scanner field strength of 3 Tesla did not allow reliable measurements of GABA concentrations in the voxel of interest. Thus this study was not able to examine the role of the inhibitory GABAergic system in plasticity mechanisms. Future studies should attempt to replicate and extend the present findings using larger and better characterized cohorts.

## 5. Conclusions

Together, the results of the present study suggest that both glutamatergic metabolism/neurotransmission mechanisms and NMDAR-dependent LTP-like plasticity are altered from the earliest stages of insulin resistance. In T2DM, the efficacy of neuroplastic mechanisms is at least partially dependent on the concentration of glutamate in the motor cortex. The abnormalities in brain metabolism and neuroplasticity found in pre-diabetes provide further evidence that early diagnosis and management of impaired glucose tolerance are critical. While an investigation of the impact of diabetes management strategies, including the impact of medication, is beyond the scope of the current paper, future studies could use these TMS- and MRS-based measures of glutamatergic function to chart the neurophysiological and neurometabolic consequences of progression from pre-diabetes to T2DM and the response to therapeutic interventions to improve glycemic control and reduce insulin resistance.

## Acknowledgements

The authors thank E. Seligson, S. Saxena, K. McDonald, and S. Buss (Beth Israel Deaconess Medical Center) for their assistance in data collection, A. Connor and J. Macone (Beth Israel Deaconess Medical Center) for assistance with evaluation of participant health and medical history, as well as A. Cypess (National Institute of Diabetes and Digestive and Kidney diseases) and E. Horton (Joslin Diabetes Center) for their assistance with recruitment and classification of diabetic participants.

This study was primarily funded by the National Institutes of Health (NIH R21 NS082870) with support from the Harvard Catalyst | The Harvard Clinical and Translational Science Center (NCRR and the NCATS NIH, UL1 RR025758). Dr. Pascual-Leone was also supported in part by the Sidney R. Baer Jr. Foundation, the NIH (R01HD069776, R01NS073601, R21 MH099196, R21 NS085491, R21 HD07616). The content is solely the responsibility of the authors and does not necessarily represent the official views of Harvard Catalyst, Harvard University and its affiliated academic health care centers, the National Institutes of Health, or the Sidney R. Baer Jr. Foundation.

## Declaration of Competing Interest

Dr. Pascual-Leone serves on the scientific advisory boards for Starlab Neuroscience, Neuroelectrics, Neosync, NovaVision, and Cognito; and is listed as an inventor on several issued and pending patents on the real-time integration of transcranial magnetic stimulation with electroencephalography and magnetic resonance imaging. The authors declare no competing interests.

## Author contributions

A.P.-L. and N.R.B. contributed to study concept and design. P.J.F. and N.R.B. contributed to data collection. P.J.F. and N.R.B. contributed to the data analysis. P.J.F., A.P.-L., and N.R.B. contributed to interpretation of the data. P.J.F., A.P.-L., and N.R.B. contributed to drafting and revising the manuscript.

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