

# Increased GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning

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## Abstract

Type 2 diabetes mellitus is associated with accelerated cognitive decline. The underlying pathophysiological mechanisms still remain to be elucidated although it is known that insulin signaling modulates neurotransmitter activity, including inhibitory  $\gamma$ -aminobutyric acid (GABA) and excitatory glutamate (Glu) receptors. Therefore, we examined whether levels of GABA and Glu are related to diabetes status and cognitive performance.

Forty-one participants with type 2 diabetes and 39 participants without type 2 diabetes underwent detailed cognitive assessments and 3-Tesla proton MR spectroscopy. The associations of neurotransmitters with type 2 diabetes and cognitive performance were examined using multivariate regression analyses controlling for age, sex, education, BMI, and percentage gray/white matter ratio in spectroscopic voxel.

Analysis revealed higher GABA+ levels in participants with type 2 diabetes, in participants with higher fasting blood glucose levels and in participants with higher HbA<sub>1c</sub> levels, and higher GABA+ levels in participants with both high HbA<sub>1c</sub> levels and less cognitive performance.

To conclude, participants with type 2 diabetes have alterations in the GABAergic neurotransmitter system, which are related to lower cognitive functioning, and hint at the involvement of an underlying metabolic mechanism.

**Abbreviations:** <sup>1</sup>H-MRS = proton magnetic resonance spectroscopy, CNS = central nervous system, Cr = creatine, CSF = cerebrospinal fluid, GABA =  $\gamma$ -aminobutyric acid, Gln = glutamine, Glu = glutamate, Glx = combined Glu and Gln signals, GM = gray matter, GPC = glycerophosphocholine, MEGA-PRESS = Mescher-Garwood-point resolved spectroscopy sequence, ml = myo-inositol, MMSE = mini-mental state examination, NAA = *n*-acetyl aspartate, NAAG = *n*-acetylaspartylglutamate, PCh = phosphocholine, tCho = total choline, tCr = total creatine, tNAA = total *n*-acetyl aspartate, WM = white matter.

**Keywords:**  $\gamma$ -aminobutyric acid, cognition, glutamate, magnetic resonance spectroscopy, type 2 diabetes mellitus

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## 1. Introduction

Type 2 diabetes mellitus is an endocrine disorder characterized by attenuated insulin signaling and decreased cellular responsiveness to insulin. Since systemic insulin resistance is accompanied by central insulin resistance, the complications of diabetes not only involve peripheral tissues, but also the central nervous system (CNS).<sup>[1,2]</sup> Indeed, type 2 diabetes is associated with cognitive deficits,<sup>[3]</sup> accelerated cognitive decline,<sup>[4]</sup> and an increased risk for developing dementia and Alzheimer disease.<sup>[4–7]</sup> Insulin signaling plays an important role in synaptic plasticity by modulating neurotransmitter channel activity, including excitatory, and inhibitory receptors such as  $\gamma$ -aminobutyric acid (GABA) and glutamate (Glu) receptors.<sup>[8,9]</sup> Therefore, defects in brain insulin signaling may give rise to neuronal dysfunction and impaired cognitive performance.<sup>[10,11]</sup>

Proton MR spectroscopy (<sup>1</sup>H-MRS) provides the unique opportunity to assess noninvasively the concentrations of neuro-metabolites including neurotransmitters GABA and Glu *in vivo*, through the identification and quantification of spectral peaks. GABA is the major inhibitory neurotransmitter, whereas Glu is the major excitatory neurotransmitter. At clinical field strengths ( $\leq 3.0$ T), GABA and Glu are difficult to quantify due to the spectral overlap with the signals of other metabolites, including *n*-acetyl aspartate (NAA), total creatine (tCr), and glutamine (Gln). However, advanced spectral editing methods have been developed that enable the detection of metabolites with strong spectral overlap. For example, the MEscher-GARwood-point resolved

spectroscopy sequence (MEGA-PRESS) edited  $^1\text{H}$ -MRS method can be used for the quantification of GABA.<sup>[12]</sup>

As  $^1\text{H}$ -MRS facilitates the assessment of neurotransmitters GABA and Glu, it is a suitable technique to explore whether an altered neurotransmitter metabolism underlies cognitive problems in type 2 diabetes. A previous  $^1\text{H}$ -MRS study with respect to neurotransmitters in depressed type 2 diabetes has reported decreased Glu levels<sup>[13]</sup> compared with healthy controls. Moreover, lower GABA levels were observed in patients with diabetic neuropathy.<sup>[14]</sup>

To our knowledge, no studies explored the neurotransmitter metabolism of impaired cognitive performance in type 2 diabetes. Therefore, the current study was designed to assess whether neurotransmitters (GABA and Glu) are related to type 2 diabetes status, cognitive performance, and the potential interaction between type 2 diabetes and cognitive performance.

## 2. Materials and methods

### 2.1. Study population

Forty-seven participants with type 2 diabetes and 41 participants without type 2 diabetes were recruited from the first 866 participants of the Maastricht Study for additional brain MRI measurements. The Maastricht Study is an ongoing observational, prospective, population-based cohort study that focuses on the etiology, pathophysiology, complications, and comorbidities of type 2 diabetes. Participants are between 40 and 75 years of age and live in the southern part of the Netherlands.<sup>[15]</sup> Participants are considered to have diabetes according to the WHO 2006 criteria if they use diabetes medication or if they have a fasting blood glucose  $\geq 7.0$  mmol/L or a 2-hours blood glucose  $\geq 11.1$  mmol/L. Participants without type 2 diabetes are characterized by fasting blood glucose  $< 6.1$  mmol/L and a 2-hours blood glucose  $< 7.8$  mmol/L. At baseline inclusion, participants underwent an extensive battery of measurements, including cognitive performance tasks, blood pressure measurements, and blood sampling. A detailed overview is provided in Schram et al.<sup>[15]</sup> After their baseline measurements of the Maastricht Study, participants were invited to participate in this MRI study.

Participants with the highest and lowest cognitive scores were selected from the first 866 participants to increase the probability of finding MRI differences associated with cognitive decrements (Table 1). A detailed selection procedure is provided in van Bussel et al.<sup>[16]</sup> In brief, the division of participants in a low and high cognition group was based on a cumulative score of three neuropsychological tests covering the domains of verbal memory, attention and flexibility, and executive functioning (Table 1). For matching, the scores for each neuropsychological test were adjusted for age, sex, and education level by linear regression and the cumulative cognition score was calculated by adding the corresponding z-scores (standardized residuals) of the three neuropsychological tests. Exclusion criteria for participants were: a known history of stroke or neurological disease, if the time span between enrollment in the Maastricht Study and MRI was  $> 1.5$  years, incomplete cognitive assessments, type 1 diabetes mellitus, mild cognitive impairment, participants with the metabolic syndrome, participants with color blindness, participants with an unknown diabetes status, and for participants without type 2 diabetes, an impaired fasting blood glucose level. The low and high cognition groups were matched on age, sex, and education level, and display a similar distribution of participants with and without type 2 diabetes (Table 1).

**Table 1**

**Characteristics of the two cognition groups\*.**

|                            | Low cognitive function (n=39) | High cognitive function (n=41) | P value            |
|----------------------------|-------------------------------|--------------------------------|--------------------|
| Type 2 diabetes (% , n)    | 53.8 (n=21)                   | 48.8 (n=20)                    | 0.650 <sup>†</sup> |
| Age, y                     | 61.1 $\pm$ 9.7                | 62.7 $\pm$ 6.6                 | 0.369              |
| Sex (male, % , n)          | 56.4 (n=22)                   | 56.1 (n=23)                    | 0.978 <sup>†</sup> |
| Education                  |                               |                                | 0.889 <sup>†</sup> |
| Low (% , n)                | 15.4 (n=6)                    | 19.5 (n=8)                     |                    |
| Middle (% , n)             | 46.2 (n=18)                   | 43.9 (n=18)                    |                    |
| High (% , n)               | 38.5 (n=15)                   | 36.6 (n=15)                    |                    |
| Verbal memory              | 37.1 $\pm$ 10.1               | 49.6 $\pm$ 9.1                 | $< 0.001$          |
| Executive function, s      | 63.3 $\pm$ 35.6               | 34.7 $\pm$ 12.6                | $< 0.001$          |
| Verbal fluency             | 20.3 $\pm$ 4.9                | 27.6 $\pm$ 5.7                 | $< 0.001$          |
| Cumulative cognition score | -2.31 $\pm$ 2.20              | 2.12 $\pm$ 1.28                | $< 0.001$          |

Data are mean  $\pm$  standard deviation.

\* Only participants who were included in the final analysis; independent samples *t* test.

<sup>†</sup> Pearson  $\chi^2$  test.

After taking into account those who declined the invitation and exclusion of participants with MRI contra-indications, a total of 47 and 41 participants with and without type 2 diabetes were included, respectively.

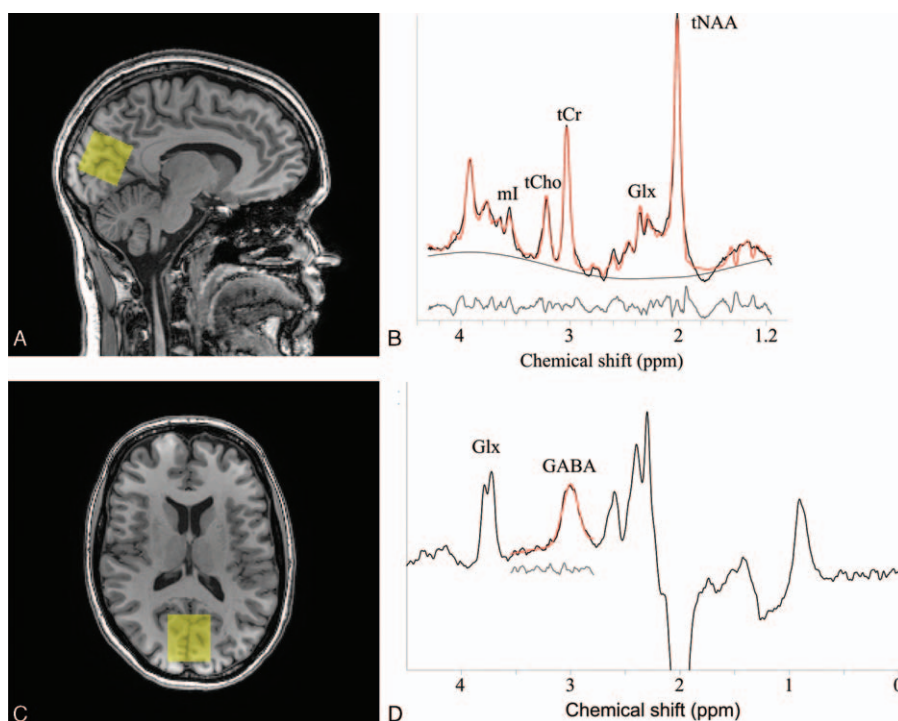
Prior to MRI, all participants underwent a general cognitive function test (Mini-Mental State Examination, MMSE<sup>[17]</sup>) to assess clinically significant differences in cognitive performance compared with the baseline cognitive tests at enrollment in the Maastricht Study. None of the participants were excluded based on a MMSE score of  $\leq 24$ . Structural brain and MR spectroscopy scans were obtained from all participants. This study was approved by the Medical Ethics Committee of the Maastricht University Medical Center (MUMC+), the Netherlands, and all participants gave written informed consent. The study is registered at <http://www.clinicaltrials.gov> with identifier NCT01705210.

### 2.2. Magnetic resonance imaging

MRI data were acquired on a 3T scanner (Achieva TX, Philips Healthcare, Best, The Netherlands) using a 32-element head coil for parallel imaging. The MRI protocol consisted of structural scans for neuroradiological evaluation (including T1-, T2-, T2\*-weighted, and fluid attenuated inversion recovery (FLAIR) sequences) and  $^1\text{H}$ -MRS scans. A 3-dimensional T1-weighted (T1) fast field echo sequence (TR/TE 8.1/3.7 ms, 1.00 mm isotropic voxel size, 170 continuous slices, matrix size of 240  $\times$  240, and 7:56 minutes acquisition time) was acquired and used for the positioning of the spectroscopic voxel and voxel segmentation.  $^1\text{H}$ -MRS were acquired from a 3  $\times$  3  $\times$  3 cm<sup>3</sup> voxel located in the occipital lobe (Fig. 1A and B) due to its favorable signal to noise profile using a single voxel PRESS sequence (TR/TE 2000/38 ms, 128 averages, MOIST water suppression, and 4:52 minutes acquisition time). Additionally, a spectrum (16 averages) was recorded of unsuppressed water. For GABA, a MEGA-PRESS sequence (TR/TE=2000/68 ms, 320 averages, editing pulses at 1.9 (ON), and 7.46 ppm (OFF) interleaved in 40 blocks, MOIST water suppression, and 10:40 minutes acquisition time) was acquired.<sup>[12]</sup>

### 2.3. Data analysis

The metabolite concentrations within the PRESS voxel were analyzed using the LCModel (linear combination of model



**Figure 1.** (A) Sagittal and (B) axial view of T1-weighted image of participant without diabetes indicating the  $^1\text{H}$ -MRS voxel (yellow) in the occipital lobe. (C) Representative PRESS spectrum (black line) and LCMoDel fit (red line). The smooth black line shows the estimated baselines by LCMoDel and the gray line shows the residuals between the raw data (black line) and the fit (red line). The peaks in the spectrum represent mI, tCho, tCr, Glx, and tNAA metabolites. (D) Representative MEGA-PRESS spectrum (black line) and the Gannet fit (red line), yielding GABA+ concentration. The gray line shows the residuals between the raw data and the fit.

spectra) software package (version 6.3-1B), which analyzes the *in vivo* MR spectra as a linear combination of the spectra of the individual metabolites.<sup>[18]</sup> LCMoDel performs water-scaling automatically and uses a simulated basis set. The simulated basis set was provided by Dr Provencher and included the following 16 metabolites: alanine, aspartate, creatine (Cr), GABA, glucose, Gln, Glu, glycerophosphocholine (GPC), phosphocholine (PCh), myo-inositol (mI), lactate, NAA, *n*-acetylaspartylglutamate (NAAG), scyllo-inositol, taurine, and guanine. Metabolite estimates were excluded from analysis if the Cramér-Rao lower bound, an estimate of the error in metabolite quantification and used as reliability indicator, was greater than 15% range. Glu satisfies the requirements of the Cramér-Rao lower bound <15%. Post hoc analyses were performed for all other metabolites which fulfilled the Cramér-Rao criteria: Glx (the combined signal of Glu and Gln), mI, total choline (tCho; sum of GPC and PCh), total NAA (tNAA; sum of NAA and NAAG), and tCr (sum of Cr and phosphocreatine). Figure 1C shows a typical spectrum and its best-fit model. In this manuscript, metabolic concentrations are reported relative to water<sup>[19]</sup> and expressed in institutional units (i.u.). To this end, after the *in vivo* measurement, the signal from unsuppressed tissue water was recorded from the same voxel, which served as an endogenous concentration reference. No corrections for relaxation were performed.

The GABA concentration (quantified in i.u. relative to the unsuppressed water signal from the same volume) was estimated by analyzing the MEGA-PRESS spectrum using the Gannet 2.0 toolkit, a Matlab-based quantification batch analysis tool for analyzing GABA MEGA-PRESS spectra.<sup>[12]</sup> A detailed overview is provided in Edden et al.<sup>[12]</sup> As the GABA concentration

estimation likely contains contribution from macromolecules and homocarnosine, it will therefore be referred to as GABA+. Figure 1D shows the fitted GABA+ signal. From here on, the GABA+/H<sub>2</sub>O ratio is referred to as GABA+ level.

The T1 images were used for the  $^1\text{H}$ -MRS voxel segmentation (Fig. 1A and B) to account for differences in tissue composition which may influence the metabolite concentrations. The  $^1\text{H}$ -MRS voxel was coregistered to the T1 image and automatically segmented as white matter (WM), gray matter (GM), or cerebrospinal fluid (CSF) using FSL FAST (FMRIB's Automated Segmentation Tool, Oxford University, Oxford, UK).<sup>[20]</sup> Then, percentages of WM, GM, and CSF in the  $^1\text{H}$ -MRS voxel were calculated and the metabolite concentrations were corrected for CSF content. Furthermore, the ratio of GM to WM in the voxel was used as a covariate in linear regression analyses.<sup>[21]</sup>

After careful analyses, data from 41 type 2 diabetes participants and 39 participants without type 2 diabetes remained suitable for final analysis, as data from 8 participants were excluded due to claustrophobia ( $n=2$ ), impaired fasting blood glucose levels ( $n=2$ ), parkinsonism ( $n=1$ ), brain injury due to an accident ( $n=1$ ), an incidental finding (i.e., tumor,  $n=1$ ), and unreliable data ( $n=1$ ). In addition, 1 included participant had a missing GABA measurement.

## 2.4. Statistical analysis

Descriptive participant characteristics are reported as mean  $\pm$  standard deviation. Group characteristics were tested using independent samples *t* tests and Pearson  $\chi^2$  tests using SPSS (Statistical Package for Social Sciences, version 20, IBM Corp, Armonk, NY), with  $\alpha=0.05$ .

**Table 2**

**Clinical characteristics of participants with and without type 2 diabetes.**

|                                   | Participants with type 2 diabetes (n=41) | Participants without type 2 diabetes (n=39) | P value |
|-----------------------------------|--|---|---------|
| Type 2 diabetes-related variables |  |   |         |
| Duration of diabetes, y           | 9.6 ± 6.7                                | —   |         |
| Fasting blood glucose, mmol/L     | 7.7 ± 1.6                                | 5.1 ± 0.3                                   | <0.001  |
| HbA <sub>1c</sub> , %             | 6.8 ± 0.5                                | 5.6 ± 0.4                                   | <0.001  |
| HbA <sub>1c</sub> , mmol/mol      | 50.7 ± 5.6                               | 38.0 ± 4.5                                  | <0.001  |
| Type 2 diabetes medication        |  |   |         |
| None, %                           | 12.2                                     | 100   | <0.001* |
| Insulin, %                        | 2.4                                      | —   |         |
| Oral medication, %                | 75.6                                     | —   |         |
| Insulin and oral medication, %    | 9.8                                      | —   |         |
| Clinical variables                |  |   |         |
| BMI, kg/m <sup>2</sup>            | 29.2 ± 3.5                               | 24.7 ± 2.8                                  | <0.001  |
| SBP, mm Hg                        | 152 ± 18                                 | 131 ± 18                                    | <0.001  |
| DBP, mm Hg                        | 83 ± 10                                  | 76 ± 13                                     | 0.012   |
| Cognitive Score                   |  |   |         |
| Baseline MMSE total score         | 28.6 ± 1.4                               | 29.4 ± 0.8                                  | 0.006   |
| Repeated MMSE total score         | 28.7 ± 1.2                               | 29.1 ± 0.9                                  | 0.169   |

Data are mean ± standard deviation.

(Baseline/repeated) MMSE = (the Maastricht Study/before MRI) Mini-Mental State Examination. BMI = body mass index, DBP = diastolic blood pressure, HbA<sub>1c</sub> = glycated hemoglobin, SBP = systolic blood pressure. Independent samples *t* test.

\* Pearson  $\chi^2$  test.

Linear regression analyses, adjusted for age, sex, education level, BMI,<sup>[22]</sup> and percentage GM/WM ratio were performed to assess the association of the neurotransmitter concentrations (GABA+ and Glu) with type 2 diabetes status and cognition status. Furthermore, the interaction term between type 2 diabetes and cognition status (lower vs higher cognitive performance) was added to the linear regression model to investigate the combined effect of type 2 diabetes and less cognitive performance on metabolite concentrations.

All analyses were repeated replacing the dichotomous type 2 diabetes status by either fasting blood glucose levels or glycated hemoglobin (HbA<sub>1c</sub>), a measure for long-term blood glucose control. In addition, post hoc analyses were also performed for other metabolite concentrations (Glx, mI, tCho, tNAA, and tCr).

### 3. Results

#### 3.1. Characteristics

Table 1 shows the baseline characteristics of the low and high cognition groups, as participants were selected based on cognitive performance. The groups were matched on age, sex, education, and type 2 diabetes status, but score different on cognition. Table 2 shows the clinical characteristics of participants based on diabetes status. As expected, participants with type 2 diabetes had higher fasting blood glucose levels, higher HbA<sub>1c</sub> levels, higher BMI, and both higher systolic and diastolic blood pressure (Table 2). Participants with type 2 diabetes scored significantly worse on baseline MMSE score (*P*=0.006). Baseline and repeated MMSE did not differ for all participants (*P*=0.280).

#### 3.2. <sup>1</sup>H-MRS

Linear regression (Table 3) revealed higher GABA+ levels in participants with type 2 diabetes (*P*=0.041), but no differences in Glu concentrations. Linear regression analyses that included the interaction term (diabetes status × cognition status) revealed no significant interaction.

Additional linear regression analyses replacing type 2 diabetes status by fasting blood glucose levels or HbA<sub>1c</sub> showed similar results (Table 3): higher GABA+ levels in participants with higher fasting blood glucose levels and in participants with higher HbA<sub>1c</sub> levels. Furthermore, linear regression analyses that included the interaction term (HbA<sub>1c</sub> levels times cognition status) revealed higher GABA+ levels in participants with both higher HbA<sub>1c</sub> levels and less cognitive performance.

#### 3.3. Post hoc analyses

Post hoc analyses (data not shown) revealed lower tNAA levels in participants who scored less on cognitive performance ( $\beta$ =0.504, *P*=0.030). Other metabolite concentrations did not show a significant analyses with type 2 diabetes or cognitive status (*P*>0.267). In addition, higher tCho ( $\beta$ =0.518, *P*=0.013) levels were observed in participants with both higher HbA<sub>1c</sub> levels and less cognitive performance (interaction analyses).

### 4. Discussion

The current study examined whether neurotransmitter levels are related to type 2 diabetes, cognitive performance, and the

**Table 3**

**Relationship between the neurotransmitters GABA+ and Glu with type 2 diabetes (either dichotomous status, fasting blood glucose levels, or HbA<sub>1c</sub>) and cognitive performance and their corresponding interaction term.**

| Metabolites | Concentration (i.u.) |              | Model 1                |          |                  |          | Model 2     |          |                       |              | Model 3     |          |                   |              |             |          |
|-------------|----------------------|--------------|------------------------|----------|------------------|----------|-------------|----------|-----------------------|--------------|-------------|----------|-------------------|--------------|-------------|----------|
|             |                      |              | Type 2 diabetes status |          | Cognition status |          | Interaction |          | Fasting blood glucose |              | Interaction |          | HbA <sub>1c</sub> |              | Interaction |          |
|             | Diabetes             | Nondiabetes  | $\beta$                | <i>P</i> | $\beta$          | <i>P</i> | $\beta$     | <i>P</i> | $\beta$               | <i>P</i>     | $\beta$     | <i>P</i> | $\beta$           | <i>P</i>     | $\beta$     | <i>P</i> |
| GABA+*      | 1.68 ± 0.45          | 1.66 ± 0.39  | 0.626                  | 0.041    | 0.057            | 0.800    | -0.661      | 0.159    | 0.338                 | <b>0.016</b> | -0.381      | 0.104    | 0.316             | <b>0.026</b> | 0.464       | <0.05    |
| Glu         | 11.39 ± 1.15         | 11.31 ± 1.06 | 0.311                  | 0.323    | -0.133           | 0.572    | -0.077      | 0.874    | -0.022                | 0.880        | 0.220       | 0.379    | -0.013            | 0.928        | 0.141       | 0.575    |

Concentrations are mean ± standard deviation. Standardized  $\beta$  (95% CI) indicates increments/decrements of the metabolites with type 2 diabetes status and cognitive status or the interaction term. The interaction term represents participants with both type 2 diabetes and less cognitive performance.

GABA+ =  $\gamma$ -aminobutyric acid, Glu = glutamate, i.u. = institutional units, n=80, \*n=79, *P*=*P* value.

Model 1 = dichotomous type 2 diabetes status, dichotomous cognition status, age, sex, education level, BMI, percentage GM/WM ratio in voxel, and interaction between diabetes and cognition status.

Model 2 = model 1 with fasting blood glucose levels instead of the dichotomous type 2 diabetes status.

Model 3 = model 1 with HbA<sub>1c</sub> levels instead of the dichotomous type 2 diabetes status.

Note: cognition status is only reported for model 1 because the results did not differ for models 2 and 3.

potential interaction between diabetes and cognitive performance. The main findings were that GABA+ is higher in diabetes, as well as in participants with higher fasting blood glucose levels and in participants with higher HbA<sub>1c</sub> levels, that GABA+ concentrations were higher in participants with both higher HbA<sub>1c</sub> levels and less cognitive performance. To our knowledge, this is the first study to investigate the role of neurotransmitters in type 2 diabetes and cognitive decrements.

Higher GABA+ levels were found in participants with type 2 diabetes, in participants with higher fasting blood glucose levels, and in participants with both higher HbA<sub>1c</sub> levels and less cognitive performance. Only one small diabetes study on GABA <sup>1</sup>H-MRS is available that reports lower GABA levels in patients with diabetic neuropathy, although translation of this result to the current type 2 diabetes population is not straightforward.<sup>[14]</sup> In addition, our study population is relatively healthy in terms of good treatment control according to glucose levels and diabetic neuropathy is not yet reported in this group. Nevertheless, increased GABA levels have been found in a type 2 diabetic rat model.<sup>[23]</sup> GABAergic inhibition is involved in the control of many behaviors, such as anxiety, psychosis, aggression, depression, mood, and cognition.<sup>[24]</sup> One possible explanation of how GABA affects cognition is by means of its inhibitory function on dopamine release in the mesocortical dopamine pathway, which partly projects to prefrontal cortex. It has previously been hypothesized that this pathway regulates cognition and executive functions.<sup>[24]</sup> Thus, GABA could downregulate the dopamine activity which might eventually lead to impaired cognitive performance. This issue cannot be resolved with the results of the current study, future studies are needed to confirm this hypothesis.

No significant changes in Glu levels were found for participants with type 2 diabetes, less cognitive performance, nor their interaction, whereas a previous <sup>1</sup>H-MRS study did report decreased subcortical Glu levels in type 2 diabetes.<sup>[13]</sup> In contrary to our participants, these patients were suffering from a major depression, which could explain the difference in Glu concentrations. In addition, an important methodological difference is that the voxel placement and field strength (1.5T) differs compared with our study. Rather than excluding a potential role for the glutamatergic neurotransmitter system in less cognitive performance and type 2 diabetes, it could be the case that our applied technique for detecting Glu is simply not sensitive enough. An alternative <sup>1</sup>H-MRS technique, making use of spectral editing, might yield better results, due to an enhanced sensitivity.<sup>[25]</sup>

The post hoc analyses revealed significant results regarding tNAA and tCho, and although this study was not designed specifically to investigate these metabolites, the results are still interesting. For participants who scored less on cognitive performance, we observed lower tNAA levels. tNAA is a surrogate marker of normal functioning neurons and these results indicate that a decline in neuronal integrity is associated with cognitive deficits. Similar results have been found in patients with mild cognitive impairment, which might suggest that tNAA could be a predictor for cognitive deficits.<sup>[26]</sup> With respect to cognition and type 2 diabetes, one <sup>1</sup>H-MRS study observed no differences in NAA metabolite concentrations and concluded that cognitive decline cannot be explained by this metabolite.<sup>[27]</sup> Nevertheless, this study was performed at lower field strength (1.5 Tesla), which could make the quantification less sensitive to NAA differences.

Furthermore, our study also observed higher tCho levels in participants with both higher HbA<sub>1c</sub> levels and less cognitive performance. Choline is involved in membrane turnover, which is a process of loss and replacement of cellular membrane, inflammatory processes, astrocytosis, and in the synthesis of the neurotransmitter acetylcholine and all these processes could affect cognitive performance.<sup>[24,28]</sup> Interestingly, Sahin et al.<sup>[29]</sup> found higher choline levels in participants with poor glycemic control. Similar higher choline levels have been found in patients with Alzheimer Disease characterized by poor recognition memory performance.<sup>[30]</sup> Therefore, altered membrane metabolism seems to underlie cognitive decrements both in Alzheimer Disease and type 2 diabetes, which may indicate a shared mechanism.

This study has several strengths: first, to our knowledge, it is the first study to investigate the neurotransmitter system in participants with type 2 diabetes in relationship with cognitive functioning. Second, the participants are extensively characterized. Third, the detection and quantification of the neuro-metabolites was applied at higher field strength (3T) and using editing, which results in improved spectral resolution, compared with 1.5 T in most other studies in type 2 diabetes.

A number of limitations also need to be addressed. First, the study had a cross-sectional design, so the results should be interpreted cautiously in terms of causality. Nevertheless, the first results are promising and open directions to future longitudinal studies assessing neurotransmitter metabolism in type 2 diabetes and cognitive decrements. Second, our voxel was placed in the occipital lobe, chosen for optimal spectral quality, rather than neuropsychological relevance.<sup>[4]</sup> However, the fact that we do find significant effects, despite the occipital placement of the voxel, might indicate that metabolic effects are global. Future studies could apply spectroscopic imaging to explore multiple brain regions to assess whether spatial variations could be related to specific cognitive domains. Third, the association between receptor activity and metabolite concentration is not necessarily linear but rather intricate. Fourth, as the interaction analyses only observed an effect with GABA for the continuous HbA<sub>1c</sub> levels rather than the dichotomous diabetes status, it seems that HbA<sub>1c</sub> levels are more sensitive to detect an interaction effect.

#### 4.1. Clinical perspectives

Unfortunately, our study was not able to measure cerebral insulin levels and therefore we were not able to link whether insulin causes directly the increase in GABA+ levels. Other studies already have shown the involvement of insulin on increased activity of dopamine neurons, and increased expression of the GABA receptors.<sup>[8,9,31]</sup> Thus insulin could also indirectly (independent of GABA+) be involved in the process of cognitive decrements in participants with type 2 diabetes. Therefore an important question to be addressed in future studies is whether insulin modulates directly the levels of GABA+ in the brain of participants with type 2 diabetes and causes cognitive decrements. Furthermore, our results open directions for future (longitudinal and pharmacological) studies, which are needed to unravel the underlying mechanism of cognitive decrements in type 2 diabetes. It would be interesting to investigate and validate whether induced changes in the GABAergic system or choline mechanism, for instance with drug therapies (GABAergic drugs or choline agonists), can lead to improvements in cognitive performance or prevention of cognitive deficits in participants

with type 2 diabetes. Interestingly, drug treatment with Xanomeline, a muscarinic acetylcholine receptor agonist, has already been shown to provide promising results in improving cognitive performance in Alzheimer disease, which was reflected by the normalization of cerebral choline to normal levels.<sup>[32]</sup>

In conclusion, this study revealed alterations in GABAergic neurotransmitter system in participants with type 2 diabetes, which were related to lower cognitive functioning.

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