Hypothalamic Glutamine-Glutamate Metabolites and Peripheral Pro-Inflammatory Cytokines in Participants with Depression: A Case-Control Study

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Abstract

**Background:** In recent years, there has been increasing evidence of an inflammatory component due to overstimulation of the hypothalamic-pituitary-adrenal axis (HPAA) in depression. The glutamate metabolites (glutamate and glutamine) are important in this stimulation. The aim of this study was to determine the concentrations of hypothalamic glutamate metabolites in depression and to investigate their relationship to peripheral inflammation.

**Methods:** Participants with diagnosed depression (DE; \( n = 24 \)) and control subjects without depression (HC; \( n = 25 \)) were investigated. Hypothalamic glutamate metabolites were recorded using in vivo magnetic resonance spectroscopy. Peripheral cytokines (IL-6, TNF-\( \alpha \), IFN-\( \gamma \) and IL-1\( \beta \)) were assessed using Enzyme-Linked Immunosorbent Assay. For statistical analysis, generalized mixed models were computed using Poisson distributions and a log link function.

**Results:** The results show overall higher hypothalamic glutamate metabolites in DE compared to HC. High TNF-\( \alpha \) and IL-1\( \beta \) concentrations are associated with high hypothalamic glutamate metabolites in DE.

**Conclusions:** These results provide initial evidence that, in depression, increased HPAA activity is associated with peripheral inflammation favored by hypothalamic glutamate metabolites.

1. Introduction

The hypothalamus is involved in the regulation of many physical and psychological processes, including human homeostasis (1, 2) along the hypothalamic-pituitary-adrenal axis (HPAA). The HPAA regulates not only hormonal and immune functions (3) but also stress and emotional responses (4, 5).

Recently, it has been emphasized the importance of the HPAA on various mood disorders such as depression (6–8). Models that support this influence of the HPAA on depression postulate chronically dysregulated overactivation of the HPAA (9, 10), which is also associated with systemic inflammation (8, 11–13). There are animal and human studies, for instance, that showed correlations between systemic inflammation and depression (14–22). However, studies correlating hypothalamic metabolism with systemic inflammation are needed to confirm this overactivation hypothesis of the HPAA in depression.

In recent years there have been many proposals related to imaging and metabolic research for depression. One of them is in vivo proton magnetic resonance spectroscopy (\(^1\)H-MRS) (23, 24). \(^1\)H-MRS enables the non-invasive detection of the concentrations of various brain metabolites using strong magnetic fields (25). The concentrations of metabolites or neurotransmitters can be determined using \(^1\)H-MRS (26), including glutamine metabolites such as glutamate (Glu) and glutamine (Gln) (27) or the metabolite Glx, which is the sum of Gln and Glu (28). These glutamate metabolites are mainly associated with central nervous system (CNS) excitatory activity (27, 28). It is known that glutamic activity in the CNS plays a fundamental role in brain metabolic activity, glucose uptake, and excitatory electrical activity in the CNS (23).

Regarding \(^1\)H-MRS research, the number of publications on glutamate metabolites in depression has increased recently. The brain regions targeted related mainly to cognition and emotion (29), such as the hippocampus (30, 31), the anterior cingulate gyrus (32, 33) and the prefrontal cortex (34). Despite a significant number of studies on \(^1\)H-MRS in depression, few studies have involved the hypothalamus or the HPAA. Only one study found differences between Glx levels in depression (35). However, this study included multiple sclerosis (MS) patients with depression, but not depressed participants without comorbid illness. Other than this study, there is no study that evaluates hypothalamic activity by using \(^1\)H-MRS in depressed patients without other comorbid conditions.

To understand the relationship of the HPAA in depression, the present study focuses on two goals. The first goal is to determine the differences in the concentration of \(^1\)H-MRS hypothalamic glutamine metabolites (Glx, Glu, Gln) in participants with depression and community healthy controls. The second goal involves the relationship between pro-inflammatory cytokine concentrations in peripheral plasma and concentrations of hypothalamic glutamine metabolites (Glx, Glu, Gln) in participants with depression.

2. Materials And Methods

2.1. Study design and selection criteria

Participants between the ages of 18 and 65 were recruited between June and September 2019. They were included in the case-control study if they met the diagnostic criteria of the International Statistical Classification of Diseases and Related Health Problems, 10th version (ICD-10) for depression (DE group, \( n = 25 \)) or as community healthy controls (HC group, \( n = 24 \)). The groups matched according to age and gender. A complete description is shown in Table 1.
Table 1 – General sample description, baseline clinical and laboratory characteristics, and hypothalamic glutamate metabolites

| Status | Age | BMI | IL-6 | TNF-α | IL-1β | IFN-γ | BDI-FS | Gln/tCr | Glu/tCr | Glx/tCr |
|--------|-----|-----|------|-------|-------|-------|--------|--------|--------|--------|
| N      |     |     |      |       |       |       |        |        |        |        |
| HC     | 25  | 25  | 25   | 25    | 25    | 25    | 25     | 25     | 25     | 25     |
| DE     | 24  | 24  | 24   | 24    | 24    | 24    | 24     | 24     | 24     | 24     |
| Mean   |     |     |      |       |       |       |        |        |        |        |
| HC     | 26.80 | 24.90 | 1.72 | 9.12  | 1.19  | 15.90 | 1.80   | 2.95   | 2.13   | 5.13   |
| DE     | 31.90 | 24.10 | 2.76 | 8.82  | 1.02  | 17.40 | 7.25   | 7.19   | 3.45   | 10.40  |
| SD     |     |     |      |       |       |       |        |        |        |        |
| HC     | 8.73  | 5.86  | 1.34 | 1.17  | 0.98  | 3.11  | 2.16   | 7.66   | 2.46   | 9.56   |
| DE     | 12.80 | 4.17  | 2.09 | 1.40  | 0.68  | 10.60 | 5.27   | 16.50  | 6.23   | 18.20  |
| Skewness |    |       |      |       |       |       |        |        |        |        |
| HC     | 3.42  | 1.92  | 1.94 | 0.34  | 3.53  | 0.60  | 4.28   | 1.75   | 4.10   |        |
| DE     | 1.30  | 0.66  | 1.00 | 1.55  | 3.74  | 4.41  | 0.53   | 4.13   | 3.34   | 3.20   |
| Q1     |     |     |      |       |       |       |        |        |        |        |
| HC     | 22.00 | 21.50 | 1.03 | 8.23  | 0.68  | 14.20 | 0.00   | < 0.01 | < 0.01 | 1.77   |
| DE     | 22.00 | 21.30 | 1.20 | 8.06  | 0.77  | 13.70 | 3.00   | 0.90   | < 0.01 | 2.13   |
| Median |     |     |      |       |       |       |        |        |        |        |
| HC     | 25.00 | 22.30 | 1.38 | 8.95  | 0.97  | 15.40 | 1.00   | 0.88   | 1.78   | 2.80   |
| DE     | 26.50 | 23.40 | 2.25 | 8.59  | 0.87  | 15.20 | 7.00   | 2.02   | 0.94   | 4.70   |
| Q3     |     |     |      |       |       |       |        |        |        |        |
| HC     | 29.00 | 25.20 | 1.81 | 9.84  | 1.11  | 17.50 | 3.00   | 1.87   | 3.37   | 3.89   |
| DE     | 36.30 | 27.40 | 3.43 | 9.48  | 1.06  | 16.70 | 10.00  | 4.82   | 4.67   | 8.66   |
| IQR    |     |     |      |       |       |       |        |        |        |        |
| HC     | 7.00  | 3.70  | 0.78 | 1.61  | 0.43  | 3.30  | 3.00   | 1.87   | 3.37   | 2.12   |
| DE     | 14.30 | 6.10  | 2.23 | 1.42  | 0.29  | 3.00  | 7.00   | 3.92   | 4.67   | 6.53   |

In the DE group, participants with comorbid psychotic disorders were not included. Participants with other psychiatric disorders (bipolar disorder, personality disorder, adaptive syndrome, or post-traumatic stress disorder) were included as long as a depressive episode prevailed in the past 6 months. Exclusion criteria for both groups were insufficient knowledge of German language and somatic restrictions that did not allow participation, especially impaired vision or hearing. With the exception of depression or depressive episode among participants in the DE group, any acute or chronic medical disease was an exclusion criterion for both groups. Participants were not included in the study if contraindications for MRI studies were identified (e.g. dental implants, pacemakers, etc.).

Each participant, or its legal authorized representative, was fully informed of this study and gave their written consent to participate. This study was approved by the ethics committee from the Faculty of Medicine of the Justus Liebig University (JLU) and carried out in accordance with the Helsinki Declaration and the ethical standards of the APA.

2.2. Data collection

2.2.1. Blood sampling

3 mL fasting venous blood samples were collected between 8:00 am and 12:00 pm with Potassium Ethylenediaminetetraacetate (EDTA) tubes (2.7 mL K3E tube with 1.6 mg EDTA/mL) and then centrifuged at 4 °C with 1100 x g for 15 minutes. After centrifugation, 1.5 mL plasma was collected and immediately stored at -20 °C. Every 4 weeks, the blood samples were collectively delivered to a university research facility, which was about 30 km away, and stored at -80 °C for further use.

Levels of interleukin 6 (IL-6), interleukin 1 beta (IL-1β), TNF-α and interferon-gamma (IFN-γ) were measured using ELISA kits (Quantikine ELISA kits, R&D-Systems Inc., Minneapolis, Minnesota, United States of America) with the minimal detection dose of: IL-6 = 0.70 pg/mL, IL-1β = 1.00 pg/mL, TNF-α = 4.00 pg/mL and IFN-γ = 8.00 pg/mL. Intra- and inter-precision values were < 10%.

Values below the ELISA detection limit were considered as 0 pg/mL and included in the analysis. Maximum plasma cytokine values (indicated by the software as > Max) were excluded from the analysis.

Cytokine concentrations were calculated using the Tecan Reader and Magellan Reader Software (Tecan Group Ltd., Männedorf, Switzerland). For the parameter calculation, the Marquardt's 4-parameter estimation method was used.

2.2.2. Depression
DE were defined according to the ICD-10 criteria (36). Clinical diagnosis of DE was made by clinical experts of the psychiatry department from the University Hospital Giessen and Marburg (location - Giessen).

The German version of the BDI-FS (37) was used to assess the severity of depressive symptoms. The BDI-FS delivers values in the range between 0 and 21. The highest value indicates a higher depressiveness. This instrument, validated in Germany with a representative sample ($n = 2467$), showed good internal consistency (Cronbach’s $\alpha = 0.84$) and convergent validity with the Patient-Health-Questionnaire-9 ($r = 0.67$). This inventory defines different categories for DE: minimal (0 to 3 points), mild (4 to 8 points), moderate (9 to 12 points) and severe (13 to 21 points).

2.2.3. MR data acquisition

All participants underwent a $^1$H-MRS imaging protocol using a 3.0 T MRI scanner (SIEMENS MAGNETROM Prisma) at the Bender Institute for Neuroimaging (BION) of the JLU. The measurements were carried out with a 64-channel head coil. The $^1$H-MRS imaging protocol included a T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) image in sagittal position, a $^1$H-MRS localizer protocol for chemical shift imaging (CSI), and a CSI with spin echo sequence (SE) $^1$H-MRS. Figure 1 shows the region of interest (ROI) used for this study, with two different orientations: sagittal and diagonal.

MPRAGE parameters were set to TR/TE = 1580 ms/2.3 ms, 176 slices, slice thickness = 0.94 mm, field of view (FoV) = 240 mm x 240 mm, matrix size = 256 x 256 voxels, duration = 4 minutes and 29 seconds. For running the $^1$H-MRS localizer, images from the T1-weighted MPRAGE were used to locate the slice containing the hypothalamus (reference structures: thalamus, mammillary bodies, inferior and superior colliculi, pituitary gland and fornix). The parameters were set to TR/TE = 6000 ms/115 ms, 1 slice group 1, 11 slices, FoV = 220 mm x 220 mm, slice thickness = 5 mm, distortion factor = 0%, duration = 1 minute and 14 seconds. The parameters for the CSI with SE $^1$H-MRS protocol were set to TR/TE = 1700 ms/30 ms, FoV = 160 mm x 160 mm, voxel size = 10 mm x 10 mm x 15 mm, volume of interest (VOI) = 80 mm x 80 mm, number of signal averaging = 3, duration = 6 minutes and 53 seconds.

2.2.4. Imaging processing

Two blinded researchers (neuroimaging scientists) evaluated the data obtained from the $^1$H-MRS. The data analysis was carried out on the selected layer with TARQUIN 4.3.1. This software was selected based on positive reports in the literature (38, 39). For the current study, three glutamine metabolites from the water-suppressed CSI were quantified using SE $^1$H-MRS data, namely Glu, Gln and Glx. Total creatinine (tCr) values were also calculated and used as a reference signal for the analysis.

The $^1$H-MRS data obtained for the hypothalamic region could contain a different number of voxels for each participant. To solve this problem, the voxels with a signal-to-noise ratio (SNR) for the glutamate metabolites and a reference signal of more than 3 were selected. Hypothalamic voxels with an SNR < 3 for these metabolites were discarded. The glutamate metabolite concentrations (Glx, Glu, Gln) were calculated for these selected voxels and divided by a reference metabolite (tCr). The methodological considerations for $^1$H-MRS data analysis, in particular for determining the metabolite concentrations, have been published elsewhere in the literature (40). Finally, the voxel with the highest concentration was selected for each participant and for each of the metabolites (Glx/tCr, Glu/tCr, Gln/tCr) and fed into the statistical analysis.

2.3. Statistical Analysis

The data set in this study consists of the metabolic data of the hypothalamus (Glx/tCr, Glu/tCr, Gln/tCr) and the plasma peripheral pro-inflammatory cytokines (IL-6, IL-1β, TNF-α, IFN-γ). Both showed positively skewed, non-Gaussian distributions with a few large values. The TARQUIN software expresses the hypothalamic metabolic data (Glx/tCr, Glu/tCr, Gln/tCr) in ppm (parts per million), which corresponds to count data with a discrete distribution and is best represented by a Poisson distribution. Accordingly, generalized mixed models were calculated using the Poisson distribution with log link-function for Glx/tCr, Glu/tCr and Gln/tCr as dependent variables.

Differences in hypothalamic metabolites between DE and HC were investigated with the fixed effects metabolite (Glx/tCr, Glu/tCr, Gln/tCr) and group (DE, HC). For analyzing differences in hypothalamic metabolites with respect to the pro-inflammatory cytokines, the data from the pro-inflammatory cytokines was dichotomized using the median split method. These dichotomized variables were individually introduced into the model as fixed effects together with the group status (DE, HC). A model was calculated for each combination of a hypothalamic metabolite and a cytokine.

For all models, the variables age, BMI and gender were included as covariates to control the possible effects of these variables. Smoking behavior and medication intake explained the variance of the models only minimally, so that they were not included in the models.

Models were computed using the R software v. 3.6 (41) and the toolbox GAMLj (42). This toolbox also is implemented in the software jamovi 1.2.5.0 (43).

3. Results

3.1. General sample description

General sample description, including baseline clinical and laboratory characteristics of the 49 participants included in the study are listed in Table 1. Descriptive data regarding hypothalamic metabolites are also represented in Table 1.

3.2. $^1$H-MRS hypothalamic glutamate metabolites: group differences.

The first goal was whether there was a difference between the groups in the hypothalamic metabolites. The main effect of group indicated group differences ($\chi^2 (df = 1) = 24.26, p < 0.001, OR = 2.24, CI95 [1.62; 3.08]$). There was no interaction metabolite x group (DE, HC) in the model ($\chi^2 (df = 2) = 2.79, p = 0.25$), which
indicates an overall effect of the group on the metabolites. Confounding variables showed effects for age ($\chi^2 (df = 2) = 4.20, p = 0.04$), for BMI ($\chi^2 (df = 2) = 7.23, p = 0.007$), and a negligible effect for gender ($\chi^2 (df = 2) = 2.98, p = 0.08$). The results of this group comparison are shown in Fig. 1.

### 3.3. Association between the peripheral pro-inflammatory cytokines and ¹H-MRS hypothalamic glutamate metabolites

The second goal was to determine whether there were significant associations between the measured pro-inflammatory peripheral cytokines and the glutamate hypothalamic metabolites. Pro-inflammatory cytokine concentrations were classified as low or high by using the median split method. The focus was on the main effects of the cytokine (low, high) and the interaction effects of the cytokine x group. Corresponding odds ratio, confidence intervals and p-values are listed in Table 2 and are shown in Fig. 2.

**Table 2**

| Cytokine (low vs. high) | Group (DE vs. HC) x Cytokine (low vs. high) |
|------------------------|---------------------------------------------|
|                        | OR, CI95                                    | p    | OR, CI95                                    | p    |
| Glu/tCr                | 1.72, [1.00; 2.95]                          | 0.05*| 3.73, [1.35; 10.32]                         | 0.01*|
| IL-6                   | 1.53, [0.83; 2.83]                          | 0.18 | 1.07, [0.38; 3.04]                          | 0.90 |
| IL-1β                  | 1.72, [0.91; 3.26]                          | 0.09 | 3.34, [1.26; 8.82]                          | 0.02*|
| IFN-γ                  | 1.86, [1.15; 3.01]                          | 0.01*| 1.70, [0.50; 5.70]                          | 0.39 |
| Gln/tCr                | 1.58, [0.82; 3.03]                          | 0.17 | 3.93, [0.96; 16.18]                         | 0.06 |
| IL-6                   | 1.36, [0.63; 2.93]                          | 0.43 | 1.38, [0.35; 5.40]                          | 0.65 |
| IL-1β                  | 1.16, [0.56; 2.42]                          | 0.69 | 5.91, [1.61; 21.72]                         | 0.007*|
| IFN-γ                  | 3.54, [1.70; 7.37]                          | <0.001*| 0.94, [0.20; 4.45]                         | 0.94 |
| Glu/tCr                | 2.61, [1.17; 5.84]                          | 0.02*| 2.73, [0.82; 9.13]                          | 0.10 |
| IL-6                   | 1.77, [0.81; 3.88]                          | 0.16 | 0.54, [0.16; 1.88]                          | 0.33 |
| IL-1β                  | 4.65, [1.99; 10.84]                         | <0.001*| 1.98, [0.53; 7.34]                         | 0.31 |
| IFN-γ                  | 0.79, [0.44; 1.44]                          | 0.45 | 2.44, [0.56; 10.64]                         | 0.23 |

### 4. Discussion

**First goal - Group differences with regard to ¹H-MRS hypothalamic glutamate metabolites**

The analysis revealed a group effect, but no interaction effect (metabolite x group). This means that participants with depression overall had higher hypothalamic glutamate metabolites than healthy controls.

This study is the first to examine the concentration of hypothalamic glutamine metabolites (Glx, Gln and Glu) in depression with no medical comorbid condition. A study by Kantorová et al studied Glx levels in MS patients and healthy subjects (35) and showed that Glx levels in MS patients correlated positively with indicators of depression. Similarly, the results of this study show that participants with primary depression had higher levels of hypothalamic glutamate metabolites than healthy controls.

While this study is, as far as we know, the first one to report elevated levels of hypothalamic glutamate metabolites in patients with depression, there are several ¹H-MRS-based studies in depression reporting changes in glutamate metabolites for other CNS regions. For example, the meta-analysis of Moriguchi et al, which included 49 studies, showed reduced Glx concentrations in depressed patients only in the medial prefrontal cortex (mPFC) (34). However, the relevant literature remains ambiguous, suggesting that depression may exhibit different metabolic activation patterns and excitatory activities. On the one hand, there is the study of Walter et al, which found that the Gln levels in the pregenital anterior cingulate cortex (pgACC) of patients with depression were significantly reduced compared to a group of healthy controls (44). Similar results have been reported by Block et al who found a significant reduction in the Gln concentration in the hippocampus in depression (45). On the other hand, the study by Bhagwagar et al reported increased Glu and Gln concentrations in the occipital cortex in depression (46, 47). However, none of the mentioned ¹H-MRS studies with depression has considered the hypothalamic region.

The overall higher concentrations of hypothalamic glutamate metabolites found in the present study show that there are no negative changes in neuronal activity such as apoptosis. In various diseases such as hepatic encephalopathy, a higher and sustained increase of Glu is observed in the CNS (48). In such a case, increased Glu levels cause apoptosis of the neurons, which leads to cognitive and consciousness disorders (48). The overall effect on the three hypothalamic glutamate metabolites could also suggest that overexpressed astrocyte activity in the hypothalamus may be associated with depression.

Astrocytes are part of the glutamate metabolism in the CNS by capturing the Glu produced by the neurons and converting it to Gln (49). In addition to the intervention of astrocytes in the glutamate-glutamine metabolism, astrocytes support neurons also with energy and metabolism. In this case, Gln is an
important source of energy for neurons (50, 51). The energy consumption associated with depolarization and excitation in the neurons (Glu) promotes an immediate Gln metabolism and, like glucose, supports the neuronal energy requirement (50, 51). This effect could explain the overall effect on the hypothalamic glutamate metabolites in depression, suggesting that the hypothalamus of depressed people has a higher level of energy, metabolism, and nerve activity. This means that an overreaction of astrocytes could correspond to an overactivation of some neurons, which at the same time would correspond to a stronger excitation (Glu, neurons) and a stronger cell metabolism (Gln, astrocytes) in the hypothalamus of depressed patients. Further studies in this direction are still missing to confirm such a proposal.

Second goal - Correlations of the MRS glutamate metabolites and the peripheral plasma pro-inflammatory cytokine concentrations between DE and HC

The results of this study showed that participants with depression had positive correlations between high TNF-α concentrations and Glx/tCr, between high IL-1β concentrations and Gln/tCr, and between high IL-1β concentrations and Glx/tCr. Here, the word "high" refers with respect to the median value, after the median splitting (see Chap. 3.3). The effects of possible confounding factors such as age, BMI, and gender were considered.

The positive relationship between hypothalamic glutamine metabolites and high peripheral pro-inflammatory cytokine levels in participants with depression but without additional disorders is particularly interesting because few published studies are available to support this correlation. Gonçalves-de-Rezende et al reported correlations between the cortisol awakening response and Glx concentrations (33) in patients with postpartum depression, indicating a positive relationship between hypothalamic activity and pro-inflammatory metabolites (e.g., cortisol). Another study on the relationship between glutamate brain metabolites and peripheral inflammation is the work of Haroon et al (52). The hypothalamus was not specifically examined in this study, but rather changes in cortical and subcortical glutamate metabolites and their relationship to pro-inflammatory markers and depression symptoms during interferon treatment in patients with hepatitis C. The results showed a relationship between inflammation markers increased glutamate concentrations and reduced motivation (52). Slavich et al showed stronger associations between activity in the dorsal anterior cingulate cortex (dACC) and in the anterior insula with an increase in the TNF-α-soluble receptor 2 (53). These brain regions were also associated with negative affect and distress. The differences between the study by Slavich et al and the present study concern the sample (healthy young adults), the imaging methods and the examined areas of the brain (dACC and anterior insula).

The results of this study showed no positive associations between high IL-6 or IFN-γ concentrations and glutamine hypothalamic metabolites in patients with depression. In addition, TNF-α did not correlate with the individual Gln or Glu concentrations, but with the combined Gln and Glu concentrations, i.e., the Glx concentration. This indicates a possible synergistic effect of Gln and Glu, which leads to an increase in the variance when the two substances are combined. TNF-α could therefore affect both substances more directly than either. For example, TNF-α could have a direct impact on the metabolites involved in neuronal metabolism and energy support (Gln), as well as excitatory transmission and toxicity (Glu). However, further evidence is needed to understand this possible effect of TNF-α on hypothalamic and brain metabolism, cell energy processes and excitatory transmission in the human CNS. Finally, in participants with depression, higher IL-1β levels were associated with both Gln and Glu levels. This indicates that the increase in IL-1β in depression depends more on hypothalamic neuron metabolism and energy support (Gln) than on excitatory neuronal activity (Glu). The IL-1 family has been shown to induce the activity of various neurotransmitters in the HPAA (54). The induction of various neurotransmitters due to the presence of cytokines from the IL-1 family is mainly related to the activation of cortisol metabolism. The latter is usually associated with depression symptoms (54). In contrast to the current data situation, the results of the present study showed that the HPAA activity in depression is not due to excitatory neural activity, but mainly to an increased cell metabolism (Gln) in the HPAA. However, further studies (i.e., the role of IL-1β on the hypothalamus in depression) are needed to confirm these results.

Limitations

The current study has shown interesting results regarding hypothalamic glutamate-glutamine metabolites, inflammatory cytokines and depression. However, some aspects should be considered when evaluating the result. As usually, a larger sample could improve the generalizability of the study results. However, the estimated power (1-β) for a total sample size of 49 participants is 0.98, which is above the accepted minimum power threshold of 1-β = 0.80. This indicates that the sample size for this study design is sufficient to achieve the study goals.

More women than men were included in the study. However, the ratio of female to male participants was the same in both groups examined and corresponds to the statistics of depression in Germany (55, 56).

Attention should be drawn to a limitation by the method for non-invasive detection of glutamate metabolites, 1H-MRS with CSI. Although all extracted voxels come from a brain slice that is selected to best contain the hypothalamus, there is a theoretical possibility that the signals attributed to the hypothalamus may also come from other neighboring structures (i.e., thalamus, corpus callosum, hippocampus, etc.). As far as we know, the method is currently the best for detecting non-invasive hypothalamic glutamate levels.

Conclusions

This study shows hypothalamic glutamate-glutamine differences between people with depression and people without depression. A positive correlation between TNF-α and Glx concentrations was found in depression. However, there was no significant correlation between TNF-α and Glu or Gln concentrations. This suggests that Gln and Glu could play a synergistic role in the relationship with TNF-α. This possible synergistic role could mean that the effects on neuronal metabolism (i.e., Gln) and on excitatory neurotransmission (i.e., Glu) are even greater, the higher the TNF-α concentration. IL-1β positively correlated with Glx and Gln levels in depression, indicating a possible effect of IL-1β on the metabolism of hypothalamic neurons. With this in mind, future studies are needed to clarify the role of metabolism and hypothalamic function in peripheral systemic anti-inflammatory.

Declarations
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Ethics approval and consent to participate

Each participant, or its legal authorized representative, was fully informed of this study and gave their written consent to participate. This study was approved by the ethics committee from the Faculty of Medicine of the Justus Liebig University (JLU) and carried out in accordance with the Helsinki Declaration and the ethical standards of the APA.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to the Data protection law of the European Union, but are available from the corresponding author on reasonable request.

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- Manuscript contribution: This is the corresponding author and BPP is responsible for everything concerning the submission process on behalf of all authors of the paper. Additionally, BPP wrote the introduction, methods, results, discussion and conclusions. Corrected the manuscript, did the data analysis and the literature search.

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- Manuscript contribution: CB contributed mainly with the methods section, especially with the technical issues of the magnetic resonance imaging (Spectroscopy). In addition, CB contributed with the data recollection for this manuscript, regarding MRS measuring techniques.

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Competing Interests

Non-financial competing interests: The authors declare that they have no competing interests.

Financial competing interests

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All other authors have no conflicts to declare.

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Additional Information

Ethical approval and consent to participate: This study was approved by the ethic committee of the JLU medical faculty (Annex 1). Additionally, this study is part of a big project to investigate inflammatory factors and fatigue in patients with depression and multiple sclerosis. The code of this project in the ethic committee is AZ 81/18. Ethical approval and the informed consents in its original language (German) are by request.

Consent for publication: Not applicable

Availability of data and materials: The data that support the findings of this study are not publicly available due to the approved law of data protection from the European Union but are available from the corresponding author on strictly grounded reasonable requests.

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