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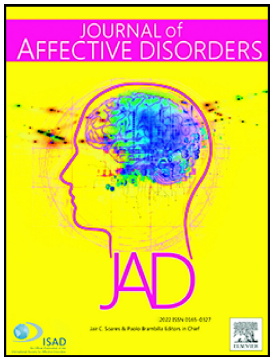

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Serum levels of carnosine may be associated with the duration of MDD episodes

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ABSTRACT

Background: Major depressive disorder (MDD) is a recurrent disorder that incurs a high societal burden. However, the etiology of MDD remains unclear. The functioning of several systems associated with the etiopathogenesis of MDD, such as inflammatory and stress systems, is partially modulated by the dipeptide carnosine.

Methods: The study comprised 99 MDD patients and 253 non-depressed controls aged 20–71 years. Fasting serum samples were analyzed using ultra-performance liquid chromatography coupled to mass spectrometry to determine the serum levels of carnosine and its constituent, histidine. We compared these metabolites in three different settings: 1) MDD patients vs. non-depressed controls and 2) remitted vs. non-remitted MDD patients, as well as 3) changes in the metabolite levels during the follow-up period within a) the remitted group and b) the non-remitted group. In addition, we assessed the possible effect of medications on the measured metabolites.

Results: We observed higher serum levels of carnosine in the MDD group compared to the control group at baseline (OR=1.895, 95%CI=1.223–2.937, p=0.004). Elevated serum levels of carnosine were also associated with a longer duration of the depressive episode (Z=0.406, p=0.001). However, the use of any antipsychotic medication (n=36) was associated with lowered carnosine levels (p=0.010 for use vs. non-use). At the follow-up, remitted and non-remitted participants displayed no significant differences in their carnosine levels (Z=-0.14, p=0.891) or histidine (Z = -1.39 p=0.164)

Conclusions: An increase in circulating carnosine may characterize depressive episodes and may represent a protective homeostatic reaction against MDD-related oxidative stress and inflammation.

Introduction

Major depressive disorder (MDD) is a recurrent psychiatric disorder with an extensive societal impact. Depressive disorders are the leading cause of disability globally, and altogether 322 million individuals have been estimated to suffer from depression (World Health Organization, 2017). The etiology of the disorder is multifactorial, and MDD is accompanied by several changes in the central nervous system (CNS) and peripheral physiology. Peripheral physiological changes linked to MDD include chronic low-grade inflammation (Tolmunen et al., 2015), a high level of oxidative stress (Maes et al., 2011; Moylan et al., 2014), and altered function of the hypothalamic-pituitary-adrenal (HPA) axis (Zorn et al., 2017). Individuals with MDD are also at increased risk of cardiovascular morbidity and mortality (Penninx, 2016), but the specific mechanisms contributing to this risk remain unclear.

Carnosine (beta-alanyl-L-histidine) is a dipeptide composed of two amino acids, beta-alanine and histidine. It is highly concentrated in muscle and brain tissues. Furthermore, carnosine has properties that are deemed beneficial for cardiovascular and neural health. It participates in the modulation of vascular tone (Miller and O'Dowd, 2000; Nagai et al., 2012; O'Dowd and Miller, 1998; Ririe et al., 2000) and protects against ischemic damage (Dursun et al., 2011). Carnosine has also been suggested to modulate HPA axis functions (Tsoi et al., 2011) and to act as a neurotransmitter or neuromodulator in glutaminergic sensory neurotransmission (Baslow, 2010). In addition, it has anti-oxidative (Aldini et al., 2011; Aydin et al., 2010; Bonfanti et al., 1999; Decker et al., 2000; Ma et al., 2010), anti-glycating (Ghodsi and Kheirouri, 2018), and anti-inflammatory properties in murine cells and humans (Caruso et al., 2017; Houjeghani et al., 2018). For a more detailed review of the biochemical properties and history of research regarding carnosine, please see Kwiatkowski et al. (2018) and Boldyrev et al. (2013).

Even though carnosine participates in the modulation of several physiological systems relevant to depression and its connection to cardiovascular conditions, there have been relatively few studies on possible changes in carnosine metabolism in psychiatric diseases. Nevertheless, carnosine supplementation has been reported to have a positive effect on cognition in schizophrenia patients (Chengappa et al., 2012). As an adjuvant to risperidone, carnosine has been reported to reduce the negative symptoms of schizophrenia (Ghajar et al., 2018). Low circulating carnosine levels have also previously been linked with autism spectrum disorders (Bala et al., 2016). Furthermore, carnosine has been reported to display antidepressive effects in rats (Tomonaga et al., 2008). Supplementation with carnosine has also been found to ameliorate autistic symptoms in children with autism spectrum disorders (Chez et al., 2002), increase cognitive functioning and physical capacity in the elderly (Szcześniak et al., 2014), and reduce symptoms of obsessive–compulsive disorder as adjuvant to fluvoxamine (Arabzadeh et al., 2017). Hipkiss (2015) suggested that dietary supplementation with carnosine could be beneficial in the treatment of depression. In addition, Araminia et al. (2020) investigated the effect of carnosine as an adjuvant treatment for depressive symptoms. They demonstrated a greater decrease in symptoms in a group receiving both antidepressive medication and carnosine compared with a group on antidepressive medication only (Araminia et al., 2020). However, to the best of our knowledge, no study has investigated the serum levels of carnosine in MDD patients compared with non-depressed controls in a naturalistic setting.

In this study, we sought to explore the possible role of carnosine and its constituent, histidine, in MDD. We hypothesized that 1) serum carnosine levels are decreased during MDD, 2) remitted MDD patients display similar serum carnosine levels to a healthy control group, and 3) antidepressant or antipsychotic drugs may affect the serum levels of carnosine. We analyzed the serum levels of carnosine and histidine in a longitudinal sample of MDD patients and a non-

depressed control group. These metabolites were compared 1) between MDD patients and non-depressed controls and 2) within the MDD group, between remitted and non-remitted MDD patients. We also examined changes in the metabolite levels of interest during the follow-up period within a) the remitted MDD patient group and b) the non-remitted MDD patient group. Moreover, we repeated our analyses after excluding users of any medication that was associated with differences in the measured metabolites.

METHODS

Study samples

Two samples were utilized in the present study: 1) a naturalistic follow-up study sample of patients with MDD and 2) a general population-based sample of non-depressed individuals. The participants were aged 20-71 years. The Research Ethics Committee of the Northern Savo Hospital District approved both studies, and the participants provided written informed consent before entering the study. The study samples geographically represent the same population.

The patient sample consisted of 99 outpatients with diagnosed MDD. The patients were recruited from the Department of Psychiatry at Kuopio University Hospital. The collection of samples was ceased when the determined group size was acquired. The diagnosis of MDD was confirmed at baseline by using the Structured Clinical Interview for DSM-IV (SCID) (DSM-IV; American Psychiatric Association 1994). In the follow-up study (mean follow-up time 8 months; range 5–13 months), 78 of the initial 99 patients participated in the re-assessments. All participants gave venous blood samples at baseline and again on follow-up. The exclusion criteria were a history of epilepsy, bipolar disorder, psychotic disorders, mental symptomology due to substance abuse, and current somatic conditions preventing participation in the study. Individuals with a history of a partial

response to antidepressants were not excluded.

The non-depressed control sample was from a population-based follow-up study of 480 individuals living in the municipality area of Lapinlahti, Finland. The sample included in this study was collected as part of the 5-year follow-up of the study in 2010. Altogether, 257 non-depressed controls were derived from the population-based Lapinlahti study sample, with exclusion criteria as follows: an elevated level of depressive symptoms, i.e., BDI scores ≥ 10 (Beck et al., 1961), at the Lapinlahti Study baseline or the 5-year follow-up, or the reported use of antidepressive medication. Participants completed a background questionnaire and underwent a complete health examination, including anthropometric measurements (Savolainen et al., 2014).

Background data

The following variables were formed based on questionnaires completed by the participants in both study samples: the frequency of weekly physical exercise (≥ 1 times vs. < 1 time), regular smoking (yes vs. no), weekly alcohol use (0–5 portions vs. ≥ 6 portions; 1 portion corresponds to 1 bottle of beer, 1 glass of wine, or 4 cl of spirits), marital status (married or living with a partner vs. living alone) and education level (university, polytechnic or college education vs. lower than university, polytechnic or college education). Depressive symptoms were evaluated with the 21-item BDI (Beck et al., 1961). The use of prescription and over-the-counter medications was recorded with a questionnaire and double-checked from the prescription documents the patients provided at the study visit.

Laboratory analyses

The participants in both study populations were instructed to fast for 12 hours before venipuncture. Samples were stored at -70 °C until analyzed in one batch. The blood samples were used to quantify the concentrations of a batch of metabolites related to different aspects of the studies conducted in the patient sample and non-depressed control sample described here. All samples were analyzed as singletons.

Metabolites were extracted from the serum samples using acetonitrile (1:4, sample:solvent) and then analyzed using an ACQUITY UPLC-MS/MS system (Waters Corporation, Milford, MA, USA). A detailed protocol and instrument conditions have been described elsewhere (Nandania et al., 2018).

Serum high-density lipoprotein cholesterol (HDL-C; mmol/l) and glycated hemoglobin (Hb1Ac; %) were analyzed according to the routine protocol in the accredited medical laboratory of Kuopio University Hospital. The exact analytical protocols have been described in detail elsewhere (Chang et al., 1998; Siekmann et al., 1976).

Statistical methods

The chi-squared test and Fisher's exact test were used to analyze the differences between groups for categorical variables. The normality of the distribution for the continuous variables was examined using the Kolmogorov-Smirnov test. Due to the non-normal distribution of the continuous variables, the Mann-Whitney U-test was used. We also checked the metabolites for potential outliers: There were no outliers for carnosine (i.e., no values exceeding 3rd quartile + 1.5*interquartile range or no values below 1st quartile – 1.5*interquartile range). For histidine there

were 2 cases that exceeded the 3rd quartile + 1.5*interquartile range and 1 case which was below 1st quartile–1.5*interquartile range; We checked these values for potential errors and found the values to be physiological. Therefore, we removed no cases based on possible outliers in values of histidine. The changes in metabolite levels between baseline and follow-up assessments within each group were analyzed using the Wilcoxon signed-rank test. Correlations between the measured metabolites and the duration of the depressive episode, HbA1c, BDI, HDL, a diagnosis of high blood pressure or diabetes were examined using the Spearman correlation coefficient.

Logistic regression analysis (method: enter) was conducted. In baseline analyses, we constructed four models: the basic model (Model 1) was adjusted for age and sex. In the lifestyle model (Model 2), three factors (regular smoking, physical exercise, and alcohol use) were added to Model 1 to investigate the potential confounding effects of lifestyles factors. In the socioeconomic model (Model 3), education and marital status were added to Model 1. In the hyperglycemia model (Model 4), serum levels of glycated hemoglobin (HbA1C) were added to Model 3. We also utilized a cholesterol model (Model 5), in which HDL-C was added to Model 3. The potential confounders were chosen based on 1) a known influence of the examined variables (HbA1C and HDL-C; Peng et al., 2015; Zieliński and Kusy, 2015) on carnosine or histidine, or 2) an observed difference between the MDD and control groups (regular smoking; physical exercise; alcohol use; see Table 1).

In the follow-up setting, a smaller number of participants was available for the analyses. Thus, no larger models were utilized in the multivariate analysis to remain with the recommended 10% limit for the number of covariates utilized and to avoid overfitting of the models (Babyak, 2004). Thus, five separate models with fewer confounders than in baseline models were used. Several models were compared by assessing the Goodness of fit (Cox and Snell R-square). Significant alcohol

consumption, education and cohabitation were not added to the final models' duo to the poor Goodness of fit. Model 1 was adjusted for age and sex, and in Model 2 regular smoking was added to the Model 1. In Model 3 regular exercise was added to the Model 1. In Model 4 HbA1C was added to the Model 1. Finally, in Model 5 HDL was added to Model 1.

To further examine the possible effect of HbA1c on the correlation between serum level of carnosine and depression in this sample, moderation analysis was conducted. Depression was coded in the following manner: depressed = 1, not depressed = 0. To provide clinically directly interpretable findings carnosine and HbA1c were used in analyses in their original form, not as z-scores. Moderation analyses were performed with the conditional modeling program PROCESS macro v.3.3 for SPSS utilizing the Johnson-Neyman technique, Model 1 (Hayes, 2018), which utilizes an ordinary least-squares regression. If the upper and lower bounds of 95% confidence intervals did not contain zero, the indirect effect was considered significant.

The possible confounding role of medication was assessed in two steps. Firstly, possible differences in the levels of carnosine and histidine were examined using the Mann-Whitney U-test between MDD patients using and not using 1) any antidepressive medication, 2) SSRIs, 3) duloxetine, 4) venlafaxine, 5) mirtazapine, 6) any antipsychotic or 7) quetiapine. Due to small group sizes, we did not examine the effects of bupropion and trazodone specifically (bupropion, n = 6; trazodone, n = 1). Secondly, the possible confounding role of medications was further assessed by repeating the baseline multivariate Model 2 after the exclusion of individuals with any medication having significant metabolite differences between medication users vs. non-users. Models 3-5 were not applied to avoid over-adjusting the sample due to the smaller sample size following the exclusions. Moreover, Model 2 had the best Goodness of fit (Cox and Snell R-square), compared to other possible combinations of used confounders.

An online calculator was used for the post hoc power analyses (https://sample-size.net/logistic-regression-sample-size/). The final logistic regression model (i.e., Model 5) was chosen over other models because it provided the second-best goodness of fit after Model 4, adjusted for HbA1c, which was considered biased because HbA1c was significantly modified by quetiapine usage, and therefore in this sample acted as a proxy for quetiapine use, rather than a true factor impacting the association between MDD and carnosine.

All other analyses were performed with SPSS 25.0 for Windows statistical software (SPSS Inc., Chicago, IL). Simple moderation analyses were performed using PROCESS macro v.3.1 for SPSS (Hayes, 2018). Two-tailed p-values below 0.05 were considered to indicate statistical significance.

RESULTS

MDD patients vs. non-depressed controls at baseline

Participants with MDD were younger than the non-depressed controls. Furthermore, they were more likely to smoke and less likely to exercise regularly (Table 1). In unadjusted analyses, no significant differences in measured metabolites were observed between the MDD group and the non-depressed control group (Table 2). No differences were observed in age ($p = 0.152$), sex ($p = 0.663$), marital status ($p = 0.575$), alcohol use ($p = 0.324$), smoking ($p = 0.964$), regular exercise ($p = 0.964$), or Beck Depression Inventory (BDI) scores ($p = 0.493$) between the depressed individuals who participated in the follow-up re-assessments and those who did not.

In adjusted logistic regression models, the MDD group showed significantly lower levels of

carnosine compared to non-depressed controls in all models except the model adjusted for age and sex (Model 1, Table 3) and a model where HbA1C was added as a covariate (Model 4, Table 3). However, when we excluded patients using antipsychotic medication from Model 2, the MDD group had a significantly higher mean carnosine level than the non-depressed group (odds ratio, OR = 1.895, CI (95%) = 1.223 – 2.937, p = 0.004).

Metabolites associated with MDD remission status at the follow-up

At the time of the follow-up, 57.7% of patients still fulfilled the diagnostic criteria for MDD. Non-remitted participants had higher baseline BDI scores and were less likely to exercise regularly at baseline compared with remitters (Table 3). At the follow-up, remitted and non-remitted participants displayed no significant differences with regards to the investigated metabolites (Tables 2 and 4). Furthermore, there were no significant differences in serum levels of carnosine (z = -1.618, p = 0.106) or histidine (z = -1.155, p = 0.248) between those who participated in the follow-up assessment and those who did not.

Within-group changes in metabolite levels during the follow-up period

During the follow-up period, neither carnosine nor histidine did not show significant changes from baseline to the follow-up assessment within either the remitted or the non-remitted group (Table 5).

Correlations among measured metabolites and other variables

Within the MDD group, higher carnosine level correlated with longer duration of the current depressive episode (p = 0.003; Supplementary Table 1). This correlation persisted when we

excluded the patients using any antipsychotic medication (n = 36) (Z = 0.406, p = 0.001; Supplementary Table 2.). In the MDD group, the increased levels of HbA1C correlated with decreased levels of carnosine (p = 0.024, Supplementary Table 1). In addition, in the MDD group, those diagnosed with high blood pressure (n = 32) had lower levels of histidine (p = 0.047, Z = −1.990) compared to those without this diagnosis (n = 67). There were no significant differences in measured metabolites between those with diagnosis vs. no diagnosis of diabetes. Moreover, HDL showed no correlation with the measured metabolites (Supplementary Table 2).

The effect of medication use in the MDD sample

Of the 99 MDD patients, 84 (84.8%) used antidepressive medication, and 36 (36.4%) used antipsychotic medication at the baseline. The antidepressive medications used were distributed as follows: 1) selective serotonin reuptake inhibitors (SSRI), n = 42 (42.4%); 2) venlafaxine, n = 21 (21.2%); 3) mirtazapine, n = 13 (13.1%); 4) duloxetine, n = 12 (12.1%); 5) moclobemide, n = 8 (8.9%); 6) bupropion, n = 6 (6.1%); and 7) trazodone, n = 1 (1.0%). The antipsychotics used were distributed as follows: 1) quetiapine, n = 28 (28.3%); 2) clozapine, n = 1 (1.0%); 3) olanzapine n = 3 (3.0%); 4) sertindole, n = 1 (1.0%); 5) perphenazine, n = 1 (1.0%); 6) levomepromazine, n = 1 (1.0%); 7) aripiprazole, n = 1 (1.0%); and 8) risperidone, n = 1 (1.0%). In the MDD group, the use of any antidepressive medication (n = 84) was associated with increased levels of histidine (p = 0.023). The use of any SSRI (n = 42), mirtazapine (n = 13), venlafaxine (n = 21), duloxetine (n = 12), bupropion (n = 6) or mirtazapine (n = 13) did not associate with alterations in the metabolite levels. For all other individual types of antidepressive medication used in the MDD group, the group sizes were too small for meaningful statistical analysis (Table 6).

Use of any antipsychotic medication (n = 36) or quetiapine (n = 28) was associated with lower

serum levels of carnosine (p = 0.010 and p = 0.004, respectively). For all other individual types of antipsychotics used in the MDD group, the group sizes were too small for meaningful statistical analysis (Supplementary Table 3).

In the follow-up setting, when comparing remitted and non-remitted MDD patients, the results remained unaltered after the exclusion of patients using a) any antidepressive medication, b) mirtazapine, or c) any antipsychotic medication (data not shown).

Moderation analyses

HbA1C moderated the effect of carnosine on depression (b = – 13.6, SE = 6.28, p = 0.03). However, the moderative effect of HbA1c for the association of carnosine with depression disappeared after the exclusion of patients using any antipsychotic medication (n = 36) (b = –5.8, SE = 7.4 , p = 0.432).

Before the exclusion of antipsychotic medications, when the levels of HbA1c were greater than or equal to 5.72%, the carnosine levels had a significant negative association with depression, meaning that higher levels of HbA1c were associated with a decreased likelihood of belonging to the depressed group. As the levels of HbA1c increased, the negative correlation between carnosine and depression got stronger, the highest levels of HbA1c being 9.7% (b = – 61.87, SE = 27.59, p = 0.02). On the contrary, in very low levels of HbA1c, serum levels of carnosine were positively associated with belonging to the depressed group; when HbA1c ≤ 2.95%, higher levels of carnosine slightly increased the likelihood of having depression. However, as stated before, these moderation effects disappeared after the exclusion of patients using antipsychotic medication.

Post hoc power analyses

Using the parameters extracted from the final logistic regression model (Model 5: OR = 1.44, relative proportions of the sample groups q_1 =0.39 and q_0 = 0.61, Variance Inflation Factor = 1.042), group sizes required to detect the investigated group differences as significant were 102 for the MDD group and 159 for the control group, or 261 for the whole sample.

DISCUSSION

The MDD group had lower serum levels of carnosine compared to the control group at baseline. This finding persisted regardless of adjustments for age, sex, alcohol use, regular smoking, physical exercise, education level and co-habitation. The use of antipsychotic medication, however, was associated with decreased levels of carnosine in the MDD group, and appeared to explain the initially observed difference between the MDD group and the control group. After the exclusion of individuals with any antipsychotic use, serum levels of carnosine and belonging to the MDD group still had a significant association, but the direction of this association was reversed: the MDD group had a higher serum level of carnosine compared to the control group. Moreover, the serum levels of carnosine positively correlated with the duration of the current depressive episode, and the most notable increase in the level of carnosine only occurred after the depressive episode had lasted 40 months. Histidine did not display any significant differences between MDD and control groups.

Our results demonstrated that the use of antipsychotics was related to lower serum levels of carnosine, whereas a depressive status itself correlated with higher levels of carnosine. Before we excluded the users of any antipsychotic medication, a group that mainly comprised users of quetiapine, HbA1C strongly affected the results of multivariate models on carnosine. Moderation

analysis demonstrated that HbA1C moderated the relationship between serum levels of carnosine and MDD.

At the follow-up assessment, contrary to our hypotheses, no significant differences in changes in the serum levels of carnosine or histidine levels were observed between remitted vs. non-remitted groups. This could be due to the relatively short duration of the follow-up period (mean 8 months).

Carnosine has been suggested to protect against increased all-cause mortality associated with MDD (Cuijpers et al., 2014), as well as several somatic conditions linked with MDD, such as diabetes (Mezuk et al., 2008), hypertension (Meng et al., 2012), stroke (Dong et al., 2012), obesity (Luppino et al., 2010), and dementia (Cherbuin et al., 2015). Moreover, several rodent studies have indicated the neuroprotective effects of carnosine (Afshin-Majd et al., 2015; Corona et al., 2011; Herculano et al., 2013) and also protection against stress-induced changes in metabolism (Tsoi et al., 2011).

We observed a decrease in the levels of serum carnosine linked to the use of quetiapine. Quetiapine, among several other antipsychotics, has adverse effects on cardiovascular health, including hyperglycemia (Jain et al., 2017). A recent meta-analysis concluded that carnosine enhances glycemic control and reduces obesity (Menon et al., 2020). A decrease in the levels of carnosine might be one of the mechanisms contributing to metabolic side effects of quetiapine and other antipsychotics, or a compensatory mechanism linked with the adverse metabolic effects of quetiapine. This, however, remains to be further investigated in future studies.

Our finding of increased carnosine levels in the MDD group after the exclusion of individuals using antipsychotic medication may represent a compensatory mechanism, and therefore be in line with earlier literature suggesting that carnosine supplementation might be beneficial during depressive

episodes (Hipkiss, 2015). It could be hypothesized that carnosine helps the system to protect itself against the inflammatory and oxidative burden linked with depression, especially in the case of longer episode durations. However, the possible mechanism for the increase of carnosine in this context remains unclear and requires further study, possibly in animal models. These findings also raise a question as to whether substitution with carnosine or other histidine-containing dipeptides could ameliorate metabolic side-effects associated with antipsychotics, posing yet another hypothesis that remains to be tested in the future.

Carnosine (beta-alanyl-L-histidine) is composed of two amino acids, beta-alanine and histidine (Kwiatkowski et al., 2018). In the present study, histidine levels showed no differences between the MDD and control groups. However, we did not measure serum levels of β-alanine, which has been shown to be a factor limiting the *in vivo* activity of carnosine synthase, an enzyme synthesizing carnosine (Kwiatkowski et al., 2018). Therefore, it is possible that serum levels of β-alanine may change during depression, even if serum levels of histidine remain stable. This, however, remains to be investigated in the future.

We observed an increase in serum levels of carnosine with longer durations of MDD. The mechanisms causing this observed increase remain undisclosed. Based on our findings and previous literature, we suggest a hypothesis that carnosine could be beneficial during MDD by protecting various systems from damage often associated with MDD. Carnosine has multiple functions, several of which could theoretically provide protection against depression.

Depression has been strongly associated with an increased state of oxidative and nitrosative stress (Maes et al., 2011; Moylan et al., 2014), and the possible beneficial effects of carnosine may thus be explained by its antioxidative qualities (Aldini et al., 2011; Aydin et al., 2010; Bonfanti et al., 1999;

Decker et al., 2000; Ma et al., 2010). Carnosine acts as a free radical scavenger (Dursun et al., 2011; Ma et al., 2012) and increases the activity and levels of several antioxidative enzymes such as glutathione, superoxide dismutase (SOD), and glutathione peroxidase (Ito et al. 1992; Choi et al. 2013) (AlZahrani et al. 2014). Moreover, carnosine reduces NO production in murine macrophages. Increased NO production by cells of the immunological system is associated with cell damage due to oxidative or nitrosative stress (Caruso et al., 2017). Increased production of NO by the immunological system has also been suggested to lead to compromised neuronal and vascular functions due to a decrease in the bioavailability of arginine, the precursor of NO (Ali-Sisto et al., 2018).

MDD has been repeatedly associated with a mild chronic inflammatory state (Milenkovic et al., 2019). Carnosine has anti-inflammatory effects. It reduces the levels of TNF-α and the transcription of other pro-inflammatory cytokines, chemokines and cellular adhesion molecules (Banerjee et al., 2018; Los et al., 1995) (Caruso et al., 2017; Houjeghani et al., 2018). Carnosine has also been suggested to modulate the HPA axis (Tsoi et al., 2011), which has altered ways to react to stress during MDD (Zorn et al., 2017).

Carnosine permeates the blood-brain barrier, thus allowing it to modify processes in the CNS (Yamashita et al., 2018). In the CNS, decreased levels of both brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) have been associated with MDD (Chen et al., 2015; Polyakova et al., 2015). Carnosine has been shown to activate glial cells to secrete these two growth factors (Yamashita et al., 2018). Moreover, decreased CNS levels of GABA have also been associated with MDD (Schür et al., 2016). Animal models and *in vitro* studies suggest that carnosine could ameliorate the decreased levels of GABA in the CNS, possibly due to upregulation of glutamate transporter 1 (GLT-1) (Chen et al., 2014; Ouyang et al., 2016; Schür et al., 2016;

Smart and Stephenson, 2019; Zhao et al., 2012), which has also been shown to have decreased expression in animal models (Chen et al., 2014).

Recent studies also suggest that carnosine can increase the release of transforming growth factor beta (TGF-β1) from microglial cells. Carnosine appears to protect microglial cells from oxidative damage and alleviate neuroinflammation through several mechanisms. A recent in vitro study demonstrated the ability of carnosine to increase the expression of TGF-β1, as well as to suppress the secretion of pro-inflammatory IL-1β and decrease NO and superoxide ion (O□-•) intracellular levels in BV-2 microglial cells (Caruso et al., 2019). This is potentially relevant for understanding the somatic comorbidity linked to MDD, as deficiency of TGF-β1 signaling appears to contribute to neuroinflammation (Caraci et al., 2018) and may partially mediate the higher cardiovascular risk associated with MDD (Caruso et al., 2020), and neuroinflammation (Caraci et al., 2018)

The strengths of the present study include a relatively large sample size and a possibility to investigate the roles of several factors that might potentially bias our findings, such as medications. An even larger sample would have allowed a more detailed examination of the associations between carnosine, histidine, and different types of psychiatric medications. Nevertheless, we were able to investigate some of the factors explaining the complex associations between MDD and carnosine, such as the serum levels of HbA1c. Moreover, our patient sample represented tertiary care patients, the majority of whom had a relatively long disease history, and these findings cannot, therefore, be directly generalized to primary care patients with depression.

Some reports (e.g., Więdłocha et al., 2018) indicate that different SSRI preparations may have different, even opposite effects on inflammatory markers. However, as our longitudinal patient sample was not very large, the numbers of patients using specific SSRI preparations was very

modest. Consequently, it was not statistically feasible to run individual analyses for each SSRI, even though this would have been the most meaningful and interesting approach. Therefore, we grouped the different SSRI preparations together to obtain a general estimate of their net impact.

The findings of the present study raise a few questions for future studies to address. If quetiapine and antipsychotics in general decrease levels of carnosine and this finding is reproducible, it could imply that care should be taken when using antipsychotics in patients with diabetes, as carnosine has properties protecting from glycation. Furthermore, the mechanisms by which serum levels of carnosine may rise during longer depressive episodes are unclear. Better understanding of these mechanisms could facilitate general understanding of the etiopathogenesis of MDD. From a clinical perspective, it would be crucial to investigate whether supplementation with carnosine or its precursors could either ameliorate symptoms of depression or provide protection against somatic comorbidities associated with depressive states.

CONCLUSIONS

To the best of our knowledge, no human studies on carnosine levels during MDD have previously been conducted. Antipsychotic medications, in particular quetiapine, were associated with lowered serum levels of carnosine among patients with MDD. Furthermore, when MDD patients using antipsychotics were excluded, our findings suggested that depression may be accompanied by increased levels of carnosine, particularly after a long depressive episode; we observed the increase in carnosine only after a depressive episode had lasted 40 months. Thus, the serum levels of

carnosine may represent a dynamic marker having a temporal association with the illness phase of the disease rather than a marker of the depressive state itself.

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Table 1. Characteristics of the study groups at baseline. Values are medians (interquartile ranges) unless otherwise stated.

	MDD n = 99	Remitted in the MDD group follow-up n = 33	Non-remitted in the MDD group follow-up n = 45	Non-depressed controls n = 230	Test statistics test value (p-value) MDD vs controls	Test statistics test value (p-value) Remitted vs non-remitted
Age, mean (SD)	39.41 (11.94)	37.76 (12.82)	43.42 (10.13)	55.28 (10.08)	t = 12.60 (<0.001)[a]	t = 2.17 (0.080)[a]
Female, n (%)	56 (56.6)	17 (51.5)	28 (62.2)	129 (51.0)	χ^2 = 0.89 (0.346)[b]	χ^2 = 0.89 (0.344)[b]
Married or living with a partner, n (%)	84 (84.4)	18 (54.5)	20 (44.4)	213 (84.2)	χ^2 = 0.02 (0.878)[b]	χ^2 = 0.78 (0.378)[b]
University, polytechnic or college education, n (%)	36 (36.4)	6 (18.8)	16 (35.6)	88 (34.8)	χ^2 = 0.08 (0.780)[b]	χ^2 = 2.59 (0.108)[b]
Regular smoking, n (%)	29 (29.3)	14 (42.4)	12 (26.7)	26 (10.3)	χ^2 = 19.52 (< 0.001)[b]	χ^2 = 2.13 (0.145)[b]
Significant alcohol usage, n (%)	22 (22.2)	12 (36.4)	17 (37.8)	36 (14.2)	χ^2 = 3.10 (0.069)[b]	χ^2 = 0.016 (0.898)[b]
Regular exercise, n (%)	43 (42.4)	28 (84.8)	21 (46.7)	243 (96.0)	χ^2 = 132.77 (< 0.001)[b]	χ^2 = 11.88 (0.001)[b]
HbA1C (%)	4.9 (4.3-5.5)	--	--	5.6 (5.4-5.9)	Z = -8.02 (< 0.001)[c]	--
HDL-C (mmol/l)	1.0 (0.7-1.5)	1.56 (1.19-1.99)	1.39 (1.15-1.83)	1.4 (1.1-1.7)	Z = -5.20 (< 0.001)[c]	Z = -1.03 (0.302)[c]
Diabetes, n (%)	5 (5.1)	1 (3.0)	3 (6.7)	21 (8.2)	χ^2 = 1.10 (0.295)[b]	χ^2 = 0.52 (0.472)[b]
Arterial hypertension, n (%)	32 (32.3)	6 (18.2)	19 (42.2)	78 (30.8)	χ^2 = 0.07 (0.786)[b]	χ^2 = 5.05 (0.025)[b]
Coronary artery disease, n (%)	3 (3.0)	1 (3.0)	2 (4.4)	7 (2.8)	χ^2 = 0.18 (0.894)[b]	χ^2 = 0.10 (0.748)[b]
Asthma, n (%)	12 (12.1)	2 (6.1)	7 (15.6)	22 (8.6)	χ^2 = 0.95 (0.328)[b]	χ^2 = 1.68 (0.195)[b]
BDI scores	29 (19.0-36.0)	16 (7-23)	32 (20-39)	2.0 (0.0-4.0)	Z = -14.12 (< 0.001)[c]	Z = -5.05 (< 0.001)[c]

[a] Student's t-test; [b] Chi–squared test; [c] Mann–Whitney U-test

Abbreviations: MDD, major depressive disorder; SD, standard deviation; HbAC1, glycated hemoglobin; HDL-C, serum high-density lipoprotein cholesterol; BDI, beck depression inventory

Table 2. Characteristics of the study groups. Values are medians (interquartile ranges).

		MDD n = 99	Remitted in the MDD group follow-up n = 33	Non-remitted in the MDD group follow-up n = 45	Non-depressed controls n = 253	Test statistics[a] test value (p-value) MDD vs non-depressed	Remitted vs non-remitted
Carnosine	Baseline	0.10 (0.10–0.17)	0.13 (0.09–0.16)	0.14 (0.10–1.17)	0.13 (0.09–0.18)	Z = -0.27 (0.790)[c]	Z = -1.28 (0.201)
	Follow-up	--	0.135 (0.083–0.162)	0.118 (0.096–0.164)	--	--	Z = -0.14 (0.891)
	Change	--	0.0095 (0.396–0.0340)	-0.0136 (-0.073–0.0356)	--	--	Z = -0.86 (0.393)
Histidine	Baseline	77.33 (63.69–87.53)	77.33 (66.76–87.89)	79.07 (62.77–88.64)	80.87 (66.14–92.66)	Z = -1.84 (0.065)[c]	Z = -0.329 (0.742)
	Follow-up	--	84.214 (66.647–94.073)	80.932 (61.394–91.569)	--	--	Z = -1.39 (0.164)
	Change	--	5.144 (-9.6887–21.1635)	-5.8309 (-19.[illegible]997–16.94[illegible])	--	--	Z = -1.44 (0.150)

[a] Mann–Whitney U-test
Abbreviations: MDD, major depressive disorder

Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for the likelihood of belonging to the major depressive disorder group vs. the non-depressed group for each one-unit increase in serum levels of each metabolite.

		OR	95% CI	p value	Goodness of fit[a]
Carnosine	Model 1	0.88	0.85–1.51	0.382	0.292
	Model 2	1.45	1.02–2.07	0.038	0.452
	Model 3	1.45	1.02–2.06	0.040	0.452
	Model 4	1.40	0.95–2.06	0.091	0.491
	Model 5	1.44	1.01–2.05	0.046	0.453
Histidine	Model 1	0.75	0.56–1.01	0.057	0.298
	Model 2	0.83	0.58–1.18	0.297	0.447
	Model 3	0.83	0.57–1.19	0.297	0.447
	Model 4	0,88	0.59–1,31	0.522	0.487
	Model 5	0.82	0.57–1.18	0.279	0.448

Model 1: Adjusted for age and sex
Model 2: Model 1 further adjusted for regular smoking, significant alcohol use, and regular exercise
Model 3: Model 2 further adjusted for education and cohabitation
Model 4: Model 3 further adjusted for HbA1c
Model 5: Model 3 further adjusted for HDL
[a] Cox and Snell R-square
Abbreviations: OR, odds ratio; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high density lipoprotein

Table 4. Odds ratios (OR) and 95% confidence intervals (CI) for the likelihood of belonging to remitted group vs. the non-remitted group for each one-unit increase in serum levels of each metabolite.

		OR	95% CI	p value	Goodness of fit[a]
Carnosine	Model 1	0.89	0.55–1.43	0.627	0.068
	Model 2	0.88	0.54–1.42	0.592	0.094
	Model 3	0.82	0.50–1.36	0.448	0.134
	Model 4	0.85	0.52–1.39	0.518	0.089
	Model 5	0.89	0.55–1.44	0.639	0.072
Histidine	Model 1	0.69	0.42–1.15	0.156	0.090
	Model 2	0.71	0.43–1.19	0.193	0.111
	Model 3	0.67	0.40–1.13	0.136	0.153
	Model 4	0.70	0.42–1.16	0.166	0.107
	Model 5	0.66	0.39–1.11	0.118	0.099

Model 1: Adjusted for age and sex
Model 2: Model 1 further adjusted for regular smoking
Model 3: Model 1 further adjusted for regular exercise
Model 4: Model 1 further adjusted for HbA1C
Model 5: Model 1 further adjusted for HDL
[a] Cox and Snell R-square
Abbreviations: OR, odds ratio; CI, confidence interval; HbA1c, glycated hemoglobin; HDL, high density lipoprotein

Table 5. Longitudinal changes in metabolite levels from baseline to follow-up within the remitted and non-remitted MDD groups.

	MDD group (n = 78), Z (p-value)*	Non-remitted (n = 45), Z (p-value)*	Remitted (n = 33), Z (p-value)*
Carnosine	-1.52 (p = 0.129)	-1.45 (p = 0.147)	-0.42 (p = 0.675)
Histidine	-0.44 (p = 0.664)	-0.39 (p = 0.697)	-1.49 (p = 0.136)

* Wilcoxon signed-rank test
Abbreviations: MDD, major depressive disorder

Figure 1. Serum carnosine in MDD group (n = 99) by duration of depressive episode (months). Patients with of any antipsychotic medication (n = 36) excluded. Error bars: 95% CI

Author Statement

Contributors

TAS participated in the design of the study, performed the statistical analyses, interpreted the data, and wrote the manuscript. TT and SML designed the study and interpreted the data. SLK participated in performing the statistical analyses and writing the manuscript. PM, MVK, HKH, KH, AR, and SML participated in collecting the study sample. VV and performed the metabolomics analyses and participated in writing the manuscript. All the authors critically revised the manuscript for important intellectual content and have approved the final version to be published.

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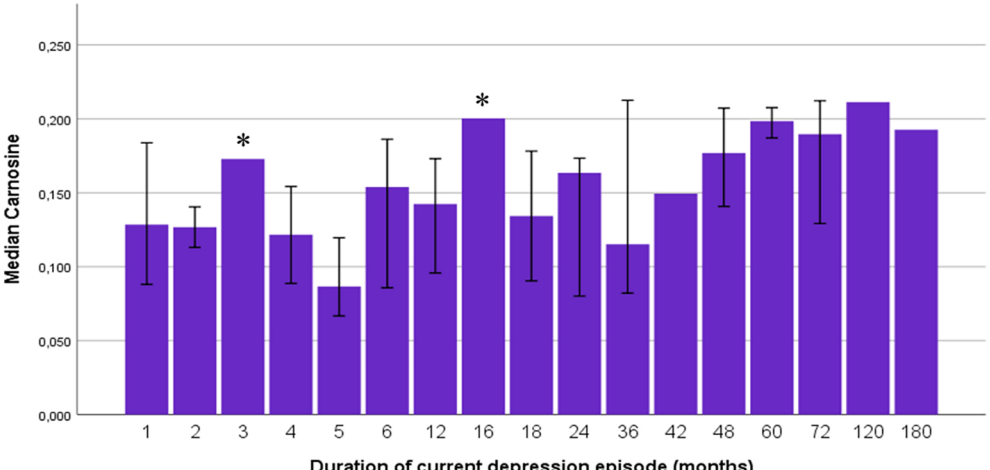
Conflicts of interest

None declared

Highlights

- An increased serum level of carnosine was associated with a longer duration of depression.
- No significant differences were observed between remitted vs. non-remitted groups.
- The use of quetiapine was associated with a decrease in the serum levels of carnosine.



* n = 1, no error bar

Figure 1