Association between the indole pathway of tryptophan metabolism and subclinical depressive symptoms in obesity: a preliminary study

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Abstract

Converging data support the role of chronic low-grade inflammation in depressive symptomatology in obesity. One mechanism likely to be involved relies on the effects of inflammation on tryptophan (TRP) metabolism. While recent data document alterations in the indole pathway of TRP metabolism in obesity, the relevance of this mechanism to obesity-related depressive symptoms has not been investigated. The aim of this preliminary study was to assess the association between plasma levels of TRP and indole metabolites and depressive symptoms in forty-four subjects with severe or morbid obesity, free of clinically relevant neuropsychiatric disorders. The interaction effect of inflammation, reflected in serum high-sensitive C-reactive protein (hsCRP) levels, and indoles on depressive symptoms was also determined.

Higher serum levels of hsCRP and lower concentrations of tryptophan (TRP) and indoles, particularly indole-3-carboxaldehyde (IAld), correlated with more severe depressive symptoms. Interestingly, the effect of high hsCRP levels in predicting greater depressive symptoms was potentiated by low IAld levels.

These results comfort the link between inflammation, the indole pathway of TRP metabolism, and obesity-related depressive symptoms.

Introduction

Depressive symptoms are frequently reported in subjects with obesity (1). Converging data supports the role of chronic low-grade inflammation, a key component of obesity, in this effect (1,2), consistent with the well documented association between chronically activated inflammatory processes and the development of depressive symptoms in clinical populations (3,4). One mechanism likely to be involved relies on the effect of inflammatory processes on the metabolism of tryptophan (TRP), an essential amino acid precursor of serotonin that is highly involved in the regulation of mood (5,6). Whereas a large amount of data highlights the involvement of the enzyme indoleamine 2,3-dioxygenase in inflammation-driven TRP catabolism and its impact on depressive morbidity (6,7), recent data obtained in our group indicate significant associations between systemic inflammation and the indole pathway of TRP metabolism in subjects with obesity (8). Nevertheless, the relevance of this pathway to obesity-related depressive symptoms remains to be determined.

TRP is partly catabolized by specific gut bacteria into indole and its derivatives, including indole-3-acetic acid (IAA), indole-3-carboxaldehyde (IAld), indole-3-lactic acid (ILA), and indole-3-propionic acid (IPA) (9). Overall, indole metabolites are considered beneficial since they prevent a leaky intestinal epithelial barrier, contrast neuronal damage, and exert anti-inflammatory effects (9–11). Consistent with this notion, the indole metabolic pathway is altered in individuals with obesity, who exhibit lower plasma levels of TRP and indoles than non-obese controls (8). Recent metabolomic data showed that reductions in indoles were associated with obesity-related memory alterations (12), a result in line with preclinical findings indicating an attenuation of cognitive deficits after IPA administration in a genetic mouse model of

diabetes (13). Albeit restrained to cognitive function, these results suggest that alterations in the indole pathway of TRP metabolism may more largely contribute to neuropsychiatric symptomatology in obesity.

The primary aim of this preliminary study was to investigate the association of plasma levels of TRP and indole metabolites with subclinical depressive symptoms in subjects with severe or morbid obesity. The secondary aim was to determine the interaction effect between inflammation and indoles on these symptoms.

Methods

Participants

Forty-four adult subjects with severe or morbid obesity (body mass index, BMI $\geq$ 35 kg/m^2 or $\geq$ 40 kg/m^2), awaiting gastric surgery and free of clinically relevant neuropsychiatric disorders, were recruited from two private clinics in the Bordeaux region. Exclusion criteria were: age > 65 years old; infections within the last two months preceding study entry; acute or chronic inflammatory conditions (except obesity or obesity-related comorbidities); current diagnosis of severe or uncontrolled medical illness or psychiatric disease; current treatment with anti-inflammatory, antidepressants, or any other psychotropic drugs. In addition, subjects with a diagnosis of current psychiatric disorder, including major depression (MDD), were excluded to avoid a potential effect of antidepressant treatment on the variables of interest. Similarly, subjects with a personal history of MDD were not included to primarily target obesity-related incident depressive symptoms and eliminate potential recurrent or chronic depressive symptoms. Diagnosis of current and past major depressive disorder was determined using the MINI-International Neuropsychiatric Interview, according to

Diagnostic and Statistical Manual of Mental Disorders criteria (14). The study was approved by the Institutional Committee of Protection of Persons of Bordeaux, France.

Assessments

Clinical and biological assessments occurred at the clinic during a medical visit with surgeons to discuss the possibility and modalities of future bariatric surgery. Socio-demographic, clinical characteristics and blood samples were collected during this visit. BMI was calculated as self-reported weight (kg)/height $(m)^2$.

Depressive symptoms were assessed using the Montgomery-Asberg Depression Rating Scale (MADRS), a 10-item clinician-administered scale assessing depressive symptoms, including sadness, inner tension, concentration difficulties, lassitude, inability to feel, pessimistic thoughts, suicidal thoughts, reduced sleep and appetite (15).

Fasting blood samples were collected in plain or EDTA-containing tubes for serum and plasma, respectively. After 30–45min at room temperature, samples were centrifuged (4.000 rpm, 20min at 4◦C for plasma and 3.200 rpm, 10min at 4◦C for serum) and stored immediately at -80◦C until further analysis. Plasma concentrations of TRP, IAA, IAld, ILA, IPA, were determined by Ultra High Performance Liquid Chromatography-ElectroSpay-Ionization-Tandem Mass Spectrometry, as described elsewhere (16).

Serum concentrations of high-sensitive C-reactive protein (hsCRP) were determined by enzyme-linked immunosorbent assay (CYT298, manufacture: Millipore, Billerica, Massachusetts, USA). Assay sensitivity and intra-/inter-assay variability were, respectively, 0.20 ng/mL, ± 4.6%, and ± 6.0%.

Data analysis

Values of TRP, indole metabolites and hsCRP were log-transformed due to non-normality, as assessed by the Shapiro-Wilk test. One participant was excluded from analyses on log-transformed IAA because of extreme value (> 3SD above the mean). Multiple regression analyses controlling for comorbidities (hypertension, type-2 diabetes, obstructive sleep apnea, or dysthyroidism) were performed to assess the relationship between biological markers and depressive symptoms. The significant associations were reported before and after Bonferroni correction for multiple testing. The interaction effect of inflammation (hsCRP levels) and markers of the indole pathway of TRP metabolism on MADRS total scores was assessed using multiple regression analysis controlling for comorbidities and entering the interaction term in the model. Statistical analyses were performed with SPSS statistics, version 25. All probabilities were two-sided, with the degree of significance set at $p<0.05$.

Results

Characteristics of study participants are shown in **Supplementary table 1**.

Biological markers were highly inter-related. In particular, hsCRP levels were significantly associated with TRP levels ($\beta= -0.312$, $p= 0.04$). No significant association was found between hsCRP and indole levels. Regarding the indole pathway of TRP metabolism, ILA levels were significantly associated with TRP ($\beta= 0.712$, $p<0.001$) and IAld concentrations ($\beta= 0.673$, $p<0.001$). Plasma levels of IPA and IAA were not significantly associated with TRP or indole metabolites.

As expected, higher levels of hsCRP correlated with more severe depressive symptoms, as reflected in MADRS total scores (**Table 1**). Higher MADRS total scores were also associated with lower plasma concentrations of TRP, IAld, and ILA.

Correlations with individual depressive symptoms are presented in **Table 1**. Levels of hsCRP levels were positively associated with scores of *inner tension* whereas lower TRP levels were associated with higher *suicidal thoughts.* Levels of indole metabolites correlated with several depressive symptoms, including *concentration difficulties* that were associated with IAld, IPA and ILA levels, and *lassitude* and *inability to feel* that correlated with IAld and ILA. Interestingly, lower IAld levels were also associated with greater symptoms of *reported sadness*, *pessimistic thoughts* and *suicidal thoughts.* Nevertheless, when the correlations were corrected for multiple testing (Bonferroni correction), only the association between IAld and MADRS total scores remained significant.

Since IAld showed the strongest associations with depressive symptoms, the interaction effect of this marker with hsCRP levels on MADRS total scores was tested using multiple regression analyses. As shown in **Table 2**, the individual effect of hsCRP on MADRS total scores was significantly increased by IAld plasma levels. More precisely, while higher hsCRP levels predicted greater MADRS scores in the study population, this effect was potentiated by low levels of IAld.

Discussion

This preliminary study shows for the first time an association between markers of the indole pathway of TRP metabolism, particularly IAld, and depressive symptoms in obesity. These results also reveal an interaction effect between high hsCRP and low IAld concentrations on the severity of this symptomatology.

Consistent with previous data obtained in a clinical model of inflammation-driven depression (e.g., interferon-alpha therapy) (17), low plasma concentrations of TRP were

associated with more severe depressive symptoms in the present study. Interestingly, hsCRP levels were significantly and negatively associated with TRP levels, supporting the close link between inflammation-related reductions in TRP and depressive symptoms. In contrast, no significant associations were found between indole metabolites and hsCRP. This result may be due to a lack of power to detect significant correlations. Importantly, indole metabolites, except IAA, were related to the intensity of depressive symptoms, with lower levels of indoles correlating with greater symptom severity. Nevertheless, when the analyses were corrected for multiple testing, only the association of IAld with MADRS total scores remained significant.

Research on the behavioral effects of indoles is still scarce, especially in obesity. Noteworthy, the limited body of data available on this topic has uniformly highlighted a behavioral role for IPA, notably with respect to cognitive function (12,13,18). Consistent with these data, we found a significant negative correlation between plasma levels of IPA and the MADRS item assessing *concentration difficulties*. However, this association did not survive to Bonferroni corrections for multiple testing. Interestingly, our results point to IAld, as a crucial marker in obesity-related depressive symptoms. Indeed, this marker correlated significantly with MADRS scores, even after Bonferroni corrections.

Interestingly, an interaction effect between high hsCRP and low IAld levels was found with respect to the intensity of depressive symptoms. Although the mechanisms underlying this interaction cannot be disentangled at this stage, different scenario can be considered. IAld is a bioactive metabolite proposed to have a signaling role in the gut-brain axis. It can regulate host immunity via activation of the aryl hydrocarbon receptor, at both peripheral and central levels, respectively by strengthening the epithelial barrier of the intestinal wall and by modulating astrocytes activity within the central nervous

system (10,11,19,20). Low IAld levels may thus reflect alterations in the microbiota-gut-brain axis, promoting likely endotoxemia and astrocyte activation, which may participate in the effect of obesity-related inflammation on mood.

The present preliminary study bears some limitations. First, the small sample size and the exclusion of patients clinical MDD may have limited statistical power. Second, the design and preliminary nature of the study do not allow to establish causal associations nor to provide a precise characterization of the clinical depressive phenotype of subjects with obesity. Complementary investigations are needed to address these issues. Third, the assessments performed in the present study did not include the measurement of fecal levels of indoles nor a description of the microbiota, limiting thus inferences on the potential clinical impact of low IAld plasmatic concentrations. Finally, in the absence of nutritional information, TRP intake could not be determined.

In conclusion, this study provides insights on the relationship between inflammation, alterations in the indole pathway of TRP metabolism, and depressive symptoms in severe or morbid obesity. Preclinical and large longitudinal cohort studies with comprehensive measures of the indole pathway, inflammation, and behavior will help to expand these results and to start identifying the underlying mechanisms.

Author contributions

ID and LC conceived and designed the work that led to the submission and were involved in writing the manuscript. ID, SC, BA, AAn, FM and LC critically contributed to the interpretation of the results. CB, DF, PL, and EM enrolled obese participants in the study and performed the medical examinations. SD performed study inclusions and

was involved in patients' follow up. ID and AAu conducted the biological experiments. ID and SC performed data analysis. AAn and FM assisted with the target metabolomic. All authors critically revised the manuscript, agreed on all aspects of the work, and approved the final version.

References

(1) Capuron L, Lasselin J, Castanon N. Role of Adiposity-Driven Inflammation in Depressive Morbidity. Neuropsychopharmacology 2017;42:1–14. https://doi.org/10.1038/npp.2016.123.

(2) Delgado I, Huet L, Dexpert S, Beau C, Forestier D, Ledaguenel P, et al. Depressive symptoms in obesity: Relative contribution of low-grade inflammation and metabolic health. Psychoneuroendocrinology 2018;91:55–61. https://doi.org/10.1016/j.psyneuen.2018.02.030.

(3) Capuron L, Miller AH. Immune System to Brain Signaling: Neuropsychopharmacological Implications. Pharmacol Ther 2011;130:226–38. https://doi.org/10.1016/j.pharmthera.2011.01.014.Immune.

(4) Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol 2006;27:24–31. https://doi.org/10.1016/j.it.2005.11.006.

(5) Wang Q, Liu D, Song P, Zou MH. Tryptophan-kynurenine pathway is dysregulated in inflammation, and immune activation. Front Biosci - Landmark 2015;20:1116–43. https://doi.org/10.2741/4363.

(6) Hunt C, Macedo e Cordeiro T, Suchting R, de Dios C, Cuellar Leal VA, Soares JC, et al. Effect of immune activation on the kynurenine pathway and depression symptoms – A systematic review and meta-analysis. Neurosci Biobehav Rev 2020;118:514–23. https://doi.org/10.1016/j.neubiorev.2020.08.010.

(7) O'Connor JC, Lawson MA, André C, Moreau M, Lestage J, Castanon N, et al. Lipopolysaccharide-induced depressive-like behavior is mediated by indoleamine

2,3-dioxygenase activation in mice. Mol Psychiatry 2009;14:511–22. https://doi.org/10.1038/sj.mp.4002148.

(8) Cussotto S, Delgado I, Anesi A, Dexpert S, Aubert A, Beau C, et al. Tryptophan Metabolic Pathways Are Altered in Obesity and Are Associated With Systemic Inflammation. Front Immunol 2020;11:1–7. https://doi.org/10.3389/fimmu.2020.00557.

(9) Roager HM, Licht TR. Microbial tryptophan catabolites in health and disease. Nat Commun 2018;9:1–10. https://doi.org/10.1038/s41467-018-05470-4.

(10) Zelante T, Puccetti M, Giovagnoli S, Romani L. Regulation of host physiology and immunity by microbial indole-3-aldehyde. Curr Opin Immunol 2021;70:27–32. https://doi.org/10.1016/j.coi.2020.12.004.

(11) Zhang LS, Davies SS. Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. Genome Med 2016:1–18. https://doi.org/10.1186/s13073-016-0296-x.

(12) Arnoriaga-Rodríguez M, Mayneris-Perxachs J, Burokas A, Contreras-Rodríguez O, Blasco G, Coll C, et al. Obesity Impairs Short-Term and Working Memory through Gut Microbial Metabolism of Aromatic Amino Acids. Cell Metab 2020;32:548-560.e7. https://doi.org/10.1016/j.cmet.2020.09.002.

(13) Liu Z, Dai X, Zhang H, Shi R, Hui Y, Jin X, et al. Gut microbiota mediates intermittent-fasting alleviation of diabetes-induced cognitive impairment. Nat Commun 2020;11:1–14. https://doi.org/10.1038/s41467-020-14676-4.

(14) Sheehan D V., Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): The development

and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J Clin Psychiatry 1998;59:22–33. https://doi.org/10.1016/S0924-9338(99)80239-9.

(15) Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry 1979;134:382–9. https://doi.org/10.1192/bjp.134.4.382.

(16) Anesi A, Rubert J, Oluwagbemigun K, Orozco-Ruiz X, Nöthlings U, Breteler MMB, et al. Metabolic profiling of human plasma and urine, targeting tryptophan, tyrosine and branched chain amino acid pathways. Metabolites 2019;9. https://doi.org/10.3390/metabo9110261.

(17) Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M, Dantzer R. Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. Mol Psychiatry 2002;7:468–73. https://doi.org/10.1038/sj.mp.4000995.

(18) Abildgaard A, Elfving B, Hokland M, Wegener G, Lund S. Probiotic treatment reduces depressive-like behaviour in rats independently of diet. Psychoneuroendocrinology 2017;79:40–8. https://doi.org/10.1016/j.psyneuen.2017.02.014.

(19) Ye L, Bae M, Cassilly CD, Jabba S V., Thorpe DW, Martin AM, et al. Enteroendocrine cells sense bacterial tryptophan catabolites to activate enteric and vagal neuronal pathways. Cell Host Microbe 2021. https://doi.org/10.1016/j.chom.2020.11.011.

(20) Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Jessica E, Mayo L, et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and CNS inflammation via the aryl hydrocarbon receptor. Nat Med

2016;22:586–97. https://doi.org/10.1038/nm.4106.Type.

Table legends

Table 1. Association between hsCRP, markers of indole pathway of TRP metabolism and depressive symptoms.

Table 2. Interaction effect between hsCRP and IAld on MADRS total scores.

Table 1. Association between hsCRP, markers of indole pathway of TRP metabolism and depressive symptoms

	hsCRP[a]	**TRP**[a]	**IAld**[a]	**IPA**[a]	**ILA**[a]	**IAA**[a,b]
MADRS Total score	0.307*	-0.367*	-0.527***	-0.180	-0.369*	-0.155
Apparent sadness	0.034	-0.266	-0.160	-0.117	-0.278	0.120
Reported sadness	0.272	-0.219	-0.417*	-0.045	-0.194	-0.214
Inner tension	0.362*	-0.102	-0.153	-0.034	0.024	-0.181
Reduced sleep	0.149	-0.152	-0.183	-0.227	-0.065	0.047
Reduced appetite	0.070	0.069	-0.165	0.004	-0.040	-0.040
Concentration difficulties	0.065	-0.283	-0.385*	-0.445**	-0.354*	-0.293
Lassitude	0.149	-0.198	-0.323*	-0.023	-0.366*	0.084
Inability to feel	0.149	-0.251	-0.337*	0.008	-0.341*	-0.127
Pessimistic thoughts	0.170	-0.282	-0.364*	-0.004	-0.203	-0.197
Suicidal thoughts	0.183	-0.366*	-0.404**	-0.046	-0.270	-0.129

Multiple regression analyses (β coefficients) controlling for comorbidities (hypertension, type-2 diabetes, obstructive sleep apnea, dysthyroidism). The associations reported in the table are uncorrected for multiple testing. N = 44 (except for IAA where n=43).

hsCRP, high-sensitive C-reactive protein; TRP, tryptophan; IAld, indole-3-carboxaldehyde; IPA, indole-3-propionic acid; ILA, indole-3-lactic acid; IAA, indole-3-acetic acid. *p<0.005, **p<0.01, ***p<0.001. [a] Variables were log-transformed for the analyses due to non-normality; [b]one participant was excluded from the analyses due to extreme values (>3SD above the mean).

Table 2. Interaction effect between hsCRP and IAld on MADRS total scores

	Adjusted R^2	β	*t*	*p*
Model				
1. hsCRP	0.081	0.307	2.08	0.04
2. IAld	0.270	-0.527	-4.01	<0.001
3. hsCRP × IAld	0.220	-0.479	-3.52	0.001

Multiple regression analyses (β coefficients) controlling for comorbidities (hypertension, type-2 diabetes, obstructive sleep apnea, dysthyroidism). hsCRP, high-sensitive C-reactive protein; IAld, indole-3-carboxaldehyde. hsCRP and IAld were log-transformed for the analyses due to non-normality. N = 44