Measurement of Brain Regional \(\alpha-[^{11}\text{C}]\text{Methyl-L-Tryptophan} \) Trapping as a Measure of Serotonin Synthesis in Medication-Free Patients With Major Depression

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**Context:** The serotonin hypothesis of depression invokes a relative or absolute deficit of serotonin neurotransmission. Reduced synthesis of serotonin in the brain pathways mediating the expression of mood (ie, the limbic cortex) is a plausible candidate mechanism.

**Objectives:** To measure and compare, using the \(\alpha-[^{11}\text{C}]\text{methyl-L-tryptophan} \) positron emission tomography method, the brain trapping constant of \(\alpha-[^{11}\text{C}]\text{methyl-L-tryptophan} \) (K*, milliliters per gram per minute), an index of serotonin synthesis, in brain areas involved in the regulation of mood in patients with major depression (MD) and age- and sex-matched controls.

**Design:** Between-group comparison.

**Setting:** Department of Psychiatry and Montreal Neurological Institute, McGill University.

**Participants:** Seventeen medication-free outpatients with a current episode of MD (9 women: mean±SD age, 41±11 years; 8 men: mean±SD age, 41±11 years) and 17 controls (9 women: mean±SD age, 37±15 years; 8 men: mean±SD age, 32.5±9.9 years).

**Main Outcome Measure:** Normalized K*, normalized to the global mean, was measured in the dorsolateral prefrontal, anterior cingulate, and mesial temporal cortices; the thalamus; and the caudate nucleus.

**Results:** Compared with age- and sex-matched controls, normalized K* was significantly decreased bilaterally in female patients with MD in the anterior cingulate cortex, in the left anterior cingulate cortex in male patients with MD, and in the left mesial temporal cortex in male and female patients with MD \((P<.001 \text{ for all})\). Exploratory analyses identified additional patient-control differences for normalized K* (eg, inferior frontal gyrus and superior parietal lobule), most of which, once corrected for 38 multiple comparisons, lost their significance. Morphometric measurements of the cingulate cortex divisions confirmed that the reduction of normalized K* in depressed patients was not attributable to a reduction in gray matter volume. Normalized K* in the anterior cingulate cortex did not correlate with ratings of depression severity collected at the time of scan.

**Conclusions:** Reduction of normalized K*, an index of serotonin synthesis, in parts of the limbic and paralimbic cortices may contribute to the biochemical alterations associated with MD.

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The serotonin hypothesis of major depression (MD) postulates that an absolute or relative deficit in serotonergic neurotransmission in the brain pathways regulating mood may serve as a vulnerability diathesis for MD.\(^1\) With the development of selective radioactive probes for various components of the serotonin system, functional neuroimaging studies of specific neurochemical processes across disease stages are now deemed possible.

Reduced serotonin neurotransmission could result from any of the following mechanisms, alone or in combination: reduced availability of the precursor L-tryptophan (Trp) for serotonin synthesis; reduced metabolism, release, or both; altered neuronal reuptake; impaired receptor(s) function(s); and transduction(s). Evidence supporting a reduction of serotonin and serotonin\(_{1A}\) receptor binding potential\(^9,10\) and serotonin transporter\(^10\) availability in the brain of medication-free patients with MD has been recently reported; whether such alterations are primary or secondary, compensating for dysfunctions in other neural systems, is unknown.

The synthesis of serotonin from the essential amino acid Trp is a 2-step enzymatic process catalyzed by tryptophan hydroxylase,\(^11\) which is considered to be the
rate-limiting enzyme for serotonin synthesis; in the brain, it is found exclusively in serotonin neurons. The product of the Trp hydroxylation reaction, 5-hydroxy-L-Trp, undergoes decarboxylation catalyzed by aromatic amino acid decarboxylase. Serotonin is metabolized into 5-hydroxyindolacetic acid, which is removed from the brain into the cerebrospinal fluid and eventually into the blood by a probenecid-sensitive system.

There are at least 3 lines of evidence suggesting that impairment of serotonin neurotransmission in patients with MD could result from altered Trp uptake or metabolism, leading to reduced conversion of plasma Trp into brain serotonin: (1) depressive symptoms can be induced in vulnerable individuals by a procedure known to acutely reduce serotonin content in the brain, that is, Trp depletion; (2) inhibition of tryptophan hydroxylase by p-chlorophenylalanine, in patients’ remission of depressive symptoms, can trigger a rapid relapse of the depressive state; and (3) likewise, vulnerable patients remitted from an episode of MD were reported to experience a relapse of depression after acute Trp depletion.

A more recent method for estimating brain regional serotonin synthesis relies on the use of $\alpha-[11C]methyl-L$-tryptophan as a tracer. However, besides its rapid conversion into 5-hydroxy-$\alpha-[11C]$tryptamine, this tracer has the main disadvantage of bypassing the rate-limiting enzyme tryptophan hydroxylase for serotonin synthesis, hence being a substrate for all brain monoamine aromatic amino acid decarboxylase.

In the present study, measurements of brain regional serotonin synthesis were obtained and compared in medication-free patients with current MD (n = 17) and controls (n = 17) matched for age and sex. Brain regional K* was measured bilaterally in 5 areas (10 volumes of interest [VOIs]) selected on the basis of the current literature on the functional brain correlates of mood, cognitive, and autonomic dysregulations that characterize MD: the dorsolateral prefrontal cortex (DLPFC), cingulate gyrus, mesial temporal lobe, caudate nucleus, and thalamus.

The PET studies of cerebral blood flow or glucose metabolism in patients with primary depression identified a pattern of corticalcortic regional dysfunctions believed to underlie the pathogenesis or expression of MD: reduced frontal lobe function (dorsolateral and ventrolateral PFC) is perhaps the most consistently reported abnormality, together with changes in cingulate cortex (CC), limbic and paralimbic areas (anterior temporal, amygdala, and insula), and subcortical nuclei (basal ganglia and thalamus). Particularly relevant to the a priori selection of the brain areas to be examined prospectively in this study is the recent observation that serotonin,α and serotonin, in vivo binding potentials and the metabolic response to serotonin agonists are reportedly reduced in patients with MD in some of the same brain regions (DLPFC, medial prefrontal cortex, and anterior CC [ACC]).

**METHODS**

**PARTICIPANTS**

Primary inclusion criteria were as follows: (1) current MD, as per the Structured Clinical Interview for the DSM-IV Axis I Disorders; (2) Hamilton Depression Rating Scale score of 18 or higher; (3) medication free for 2 weeks or for more than 5 elimination half-lives of the drug, whichever was more; (4) no current substance abuse; and (5) never having used the putative serotonin neurotoxins 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxymethamphetamine. Twenty-nine consecutive patients with a provisional diagnosis of MD referred by Montreal-area psychiatrists and general practitioners were assessed for study participation; 17 met the entry criteria. Reasons for exclusion included hypothyroidism (n = 2), hypertrophydism (n = 1), a history of severe brain trauma (n = 1), severe diabetes mellitus (n = 1), current substance abuse (n = 1), and (5) for the diagnostic criteria for current MD (n = 2), and technical breakdown (n = 3).

All controls, recruited via newspaper advertisements, were interviewed using the Structured Clinical Interview for the DSM-IV Axis I Disorders. All subjects were physically healthy, as determined by a physical examination and standard laboratory tests. Exclusion criteria included a personal history of past or current DSM-IV Axis I psychiatric disorder, a DSM-IV Axis I psychiatric disorder in a first-degree relative, a Beck Depression Inventory score greater than 10, and past use of 3,4-methylenedioxymethamphetamine or 3,4-methylenedioxymethamphetamine. Seventeen controls were selected and matched to the patient group for age and sex.

On the day of the PET study, all participants had negative findings on a urine drug screen sensitive to cocaine, opiates, phencyclidine, piperidine, tetrahydrocannabinol, barbiturates, benzodiazepines, and amphetamines (Triage Panel for Drugs of Abuse, Biosite Diagnostics Inc, San Diego, Calif). All women of fertile age were scanned during their follicular phase. Written informed consent was obtained from all participants. The study was carried out in accordance with the Declaration of Helsinki, and it was approved by the research ethics committee of the Montreal Neurological Institute and by the institutional review board of McGill University.

**PET AND MAGNETIC RESONANCE IMAGING**

The $\alpha-[11C]MTrp$ was prepared as described in a previous article. All patients observed an overnight fast (water allowed ad libitum), preceded by a low-protein diet, to reduce interindividual variability in plasma amino acid concentrations. All PET studies were performed between 11 AM and 2 PM using a whole-body scanner (ECAT HR+; CTI Molecular Imaging, Inc, Siemens, Knoxville, Tenn). All images were collected and reconstructed using a 3-dimensional mode with an intrinsic resolution of $5 \times 5 \times 5$ mm full width at half maximum. Transmission scans for attenuation correction were performed using a $^{68}$Ge/Ga source. After the intravenous injection of 10 to 20 mCi of $\alpha-[11C]MTrp$, dose not scaled to body weight, administered as a 2-minute slow infusion, 60-minute dynamic PET data were acquired. During each PET scan, 13 blood samples were drawn from the antecubital vein to compute the $\alpha-[11C]MTrp$ half-life.
input function. The input function was derived from sinus radioactivity (0-20 minutes) and venous plasma (20-60 minutes) as described in previous publications. Three 2-mL venous blood samples were collected from each participant during the PET study. All samples were centrifuged, and ultrafiltrates were stored at −80°C for measurement of free plasma Trp concentrations using high-performance liquid chromatography. Two additional plasma samples were treated with trichloroacetic acid (2:1) for determination by high-performance liquid chromatography of total plasma Trp concentrations.

All participants underwent high-resolution magnetic resonance imaging using a 1.5-T superconducting magnet system (Philips Gyroscan; Philips Medical Systems, Eindhoven, the Netherlands). Images were collected using 3-dimensional volume acquisition, T1 weighted (3-dimensional fast-field echo scan: repetition time, 18 milliseconds; echo time, 10 milliseconds; and flip angle, 30°), over the whole brain. Magnetic resonance imaging data were corrected for field inhomogeneities and normalized by the mean global K* to 100. Volumetric measurements were performed in brain volumes in Talairach space using the software DISPLAY, which allows for simultaneous labeling of a target structure in multiple planes.

### STATISTICAL ANALYSIS

All statistical procedures were performed using STATISTICA (version 4.1; Statsoft Inc, Tulsa, Okla), with the main comparisons based on the use of repeated-measures analyses of variance, with hemisphere (left vs right) as a within-subject factor (repeated-measure factor) and sex and group (controls vs patients with MD) as between-subject factors. When the group factor was significant (P<.05), post hoc analysis using the Student-Newman-Keuls test was performed. All comparisons were carried out only between patients and controls of the same sex. Significance was assessed at P<.05. Correlations between regional normalized K* values in VOIs and demographic and clinical variables, including age, duration of illness, number of relatives with affective illness, free plasma Trp concentration, and Hamilton Depression Rating Scale and Beck Depression Inventory scores, were examined using the Pearson product moment correlation. In the exploratory analysis, all probabilities were corrected for multiple comparisons (n=38), a procedure that, in this instance, might be deemed conservative given the high degree of regional correlation among variables.

### RESULTS

### DEMOGRAPHICS

A total of 17 patients (9 women: mean±SD age, 41±11 years; and 8 men: mean±SD age, 41±11 years) and 17 controls (9 women: mean±SD age, 37±15 years; and 8 men: mean±SD age, 32.5±9.9 years) participated in the study (Table 1). Three patients were diagnosed as having single-episode MD, 12 (5 women and 7 men) had major depressive disorder, and 2 met the criteria for bipolar disorder type II. Mean±SD Hamilton Depression Rating Scale scores were 27.3±6.6 for women with MD and
There were no group differences between unmedicated patients and controls in the left mesial temporal cortex (F1,30 = 4.75; P = .04) and sex (F1,30 = 7.73; P < .01) and a group × sex × hemisphere interaction (F30 = 7.52; P < .01). The post hoc Student-Newman-Keuls analysis confirmed that the K* was reduced bilaterally in the CC in women with MD and on the left side in men with MD (P < .001 for both) (Table 2). As illustrated by a group × hemisphere interaction (F1,30 = 7.50; P < .02), normalized K* was also significantly reduced in the left mesial temporal cortex in patients with MD relative to controls (P < .001). The clinical severity of depression did not correlate with normalized K* values in any of the a priori–defined VOIs.

In Exploratory VOIs

Exploratory analyses were performed in 38 additional VOIs. The comparisons between patients with MD and controls suggested significantly decreased normalized K* values in some brain structures, although most of those group differences (except in the superior parietal lobule bilaterally in women [uncorrected P < .001]) disappeared after correction for multiple comparisons (critical value of P = .001).

VOLUMETRIC ANALYSIS OF THE CINGULATE GYRUS

Volumetric analysis of the CC was performed to determine whether the reduction in normalized K* was secondary to volume reduction in the CC in patients with MD, as suggested by Drevets et al.46 Because automatic segmentation methods only provide total volume of the cingulate formation, further segmentation was performed, distinguishing between posterior, anterodorsal, subgenual, and subcallosal cingulate gyri. Gray and white matter boundaries were determined using an automatic tissue classification procedure.48 All measurements were performed by the same rater (N.G.) masked to age, sex, and diagnosis. Repeated-measures analysis of variance was performed to examine group differences, taking into account sex and hemisphere. Patients with MD did not differ statistically from controls on morphometric measurements of the segmented cingulate formation.

A refined VOI analysis for normalized K* measurements performed for each individual subdivision of the CC indicated that patients with MD, relative to controls, exhibited statistically significantly lower normalized K* values in the anterodorsal cingulate, bilaterally.

Table 2. Brain Trapping Constant of α-[11C]MTrp (K*) for Volumes of Interest (VOIs) Normalized by the Global Mean (Absolute Values of the Global Mean Are Given) in 10 A Priori–Selected VOIs

<table>
<thead>
<tr>
<th>VOI and Hemisphere</th>
<th>Healthy Women (n = 9)</th>
<th>Women With MDE (n = 9)</th>
<th>Healthy Men (n = 8)</th>
<th>Men With MDE (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Global (range), µL/g/min</strong></td>
<td>4.79 ± 2.07 (2.4-5.7)</td>
<td>4.69 ± 1.29 (2.4-5.7)</td>
<td>3.84 ± 1.82 (2.0-6.7)</td>
<td>3.99 ± 1.21 (2.1-5.7)</td>
</tr>
<tr>
<td><strong>DLPFC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>121 ± 25</td>
<td>116 ± 36</td>
<td>132 ± 29</td>
<td>120 ± 28</td>
</tr>
<tr>
<td>Left</td>
<td>118 ± 22</td>
<td>103 ± 37</td>
<td>132 ± 27</td>
<td>121 ± 22</td>
</tr>
<tr>
<td><strong>Cingulate cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>132 ± 21</td>
<td>108 ± 23*</td>
<td>140 ± 21</td>
<td>136 ± 17</td>
</tr>
<tr>
<td>Left</td>
<td>129 ± 12</td>
<td>114 ± 20*</td>
<td>147 ± 24</td>
<td>134 ± 21*</td>
</tr>
<tr>
<td><strong>Mesial temporal lobe</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>171 ± 12</td>
<td>156 ± 31</td>
<td>188 ± 37</td>
<td>197 ± 39</td>
</tr>
<tr>
<td>Left†</td>
<td>176 ± 12</td>
<td>156 ± 34*</td>
<td>207 ± 43</td>
<td>189 ± 27</td>
</tr>
<tr>
<td><strong>Thalamus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>128 ± 16</td>
<td>109 ± 23</td>
<td>136 ± 36</td>
<td>133 ± 20</td>
</tr>
<tr>
<td>Left</td>
<td>128 ± 16</td>
<td>109 ± 21</td>
<td>136 ± 36</td>
<td>133 ± 20</td>
</tr>
<tr>
<td><strong>Caudate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td>138 ± 15</td>
<td>121 ± 23</td>
<td>148 ± 39</td>
<td>143 ± 30</td>
</tr>
<tr>
<td>Left</td>
<td>120 ± 15</td>
<td>121 ± 25</td>
<td>136 ± 29</td>
<td>133 ± 24</td>
</tr>
</tbody>
</table>

Abbreviations: α-[11C]MTrp, α-[11C]methyl-L-tryptophan; DLPFC, dorsolateral prefrontal cortex; MDE, major depression.

*Three-way repeated-measures analysis of variance with diagnosis, sex, and hemisphere as grouping factors. Post hoc Student-Newman-Keuls test (P < .001).
†There was a significant difference for diagnoses × hemisphere interaction. The post hoc Student-Newman-Keuls test indicates a significant difference between patients and controls in the left mesial temporal cortex (P < .001).

In A Priori–Selected VOIs

There were no group differences between unmedicated patients with MD and age- and sex-matched controls in plasma concentrations of total or free Trp or in global K* values. The repeated-measures analyses of variance for the regional normalized K* values, focusing on a priori–defined VOIs, found for the CC main effects of group (F1,30 = 4.75; P = .04) and sex (F1,30 = 7.73; P < .01) and a group × sex × hemisphere interaction (F30 = 7.52; P < .01). The post hoc Student-Newman-Keuls analysis confirmed that the K* was reduced bilaterally in the CC in women with MD and on the left side in men with MD (P < .001 for both) (Table 2). As illustrated by a group × hemisphere interaction (F1,30 = 7.50; P < .02), normalized K* was also significantly reduced in the left mesial temporal cortex in patients with MD relative to controls (P < .001). The clinical severity of depression did not correlate with normalized K* values in any of the a priori–defined VOIs.

25.8 ± 5.2 for men with MD. There was no statistically significant change in the normalized global K* with age.

All but 4 patients (2 men and 2 women) had a documented positive family history of mood disorder in first-degree relatives, and 2 patients were adopted. Only 1 patient reported a history of suicidal attempt. All patients with MD, except 1, and all controls were right handed. There were no correlations between the severity of the index episode and duration of the medication-free period, number of previous episodes, or number of affected relatives.

COMPARISON OF NORMALIZED REGIONAL K* VALUES IN PATIENTS WITH MD VS CONTROLS

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A refined VOI analysis for normalized K* measurements performed for each individual subdivision of the CC indicated that patients with MD, relative to controls, exhibited statistically significantly lower normalized K* values in the anterodorsal cingulate, bilaterally.
in women and on the left in men. Normalized $K^*$ values in the ACC did not correlate with Beck Depression Inventory or Hamilton Depression Rating Scale scores.

**COMMENT**

In this study, medication-free depressed patients exhibited a statistically significant reduction of the regional normalized trapping constant ($K^*$) of $\alpha$-[11C]MTrp, a proxy for serotonin synthesis, in the CC. The difference in the CC was statistically significantly more robust in women, and, after segmentation, the differences between patients with MD and controls in normalized $K^*$ values are primarily in the ACC (Figure 1).

The serotonin hypothesis of depression predicts that a reduced availability of serotonin in certain brain systems involved in the regulation of mood mediates aspects of vulnerability to mood disorders. The present finding of reduced normalized $K^*$ values in parts of the paralimbic cortex but not in the DLPFC, orbital frontal cortex (OFC), or caudate provides some preliminary indication about the potential serotonin subsystems that may be relevant in the modulation of various facets of the depression phenotype. Germene to the finding of reduced normalized $K^*$ values in the ACC is the relatively consistent observation that this paralimbic structure is activated during induced negative mood states in controls and deactivated in patients with MD or alexithymia. Whereas other interconnected limbic structures, such as the amygdala and the hippocampus, are known to be involved in emotional responses to simple perceptual stimuli, the ACC is thought to play a larger role in the cognitive evaluation of more complex affectively relevant events and mood regulation. It receives abundant ascending serotonin neuron projections from the median and dorsal raphe and projects to the OFC and limbic caudate, forming a circuit hypothesized to be functionally deficient in patients with MD. Measurements of cerebral blood flow during acute Trp depletion in patients recovering from an episode of MD show that the severity of depressive symptoms, elicited by the reduced serotonin neurotransmission, correlates with the inhibition of neuronal activity in several cortical, subcortical, and limbic brain regions, including the subgenual ACC. The possibility that a reduction in serotonin synthesis in serotonergic fibers innervating the ACC accounts, in part, for the reduced positive emotional salience of external and internal stimuli (anhedonia) often experienced by patients with MD, although speculative, is tantalizing.

Patients with MD did not statistically significantly differ from controls in normalized $K^*$ in the DLPFC and OFC. This is somewhat surprising given the recent study, although not confirmed by others, of a reduced glucose metabolic response to the serotonin-releasing agent fenfluramine hydrochloride in the PFC of medication-free patients with MD. The DLPFC is known to be part of circuit-mediating attentional processes and working memory. Depressed patients with psychomotor retardation and impaired executive function often demonstrate reduced activity in the DLPFC. Because we did not collect measurements of executive function in these patients, we cannot comment on the significance of this apparent negative finding or rule out the possibility of a false-negative finding. This notwithstanding, an association was recently reported in schizophrenic patients between reduced PFC function and enhanced dopaminergic striatal neurotransmission. These findings are consistent with the hypothesis that reduced executive functions and DLPFC activity in patients with MD could, in part, be preferentially mediated, directly or indirectly, by dopamine-related, rather than serotonin, mechanisms.

Reduced metabolic activity and metabolic responsiveness to serotonergic probes in the OFC has recently been associated with impulsive aggression and suicide. The absence of a statistically significant difference in normalized $K^*$ values in the OFC in patients with MD vs controls may, in part, be accounted for by the relatively low incidence of suicidal behavior in this sample of depressed patients (1 of 17 individuals). Consistent with this hypothesis, reduced $\alpha$-[11C]MTrp trapping in the medial OFC was reported recently in patients with cluster B personality disorders endowed with high impulsivity and suicidal morbidity and in patients studied in the immediate aftermath of a severe suicide attempt.

Normalized $K^*$ values were also statistically significantly reduced in the mesial temporal cortex of depressed patients, which included the amygdala and the anterior part of the hippocampus, structures densely innervated by serotonin terminals. The amygdala is a pivotal structure involved in the appraisal of fear stimuli and in the acquisition and expression of anxiety-related responses; the hippocampus, in comparison, plays a role in mediating contextual memory functions. Increased anxiety levels and subjective memory impairments are common expressions of the phenomenology of clinical depression. The amygdala and the hippocampus form important connections with the CC and with the OFC and the DLPFC. Morphometric and metabolic alterations in both areas have been described in patients with MD.

The validity of the finding of reduced normalized $K^*$ values in patients with MD rests on the following methodological considerations: (1) Is the $\alpha$-[11C]MTrp/PET
method a valid technique for estimating serotonin synthesis? (2) Is the VOI approach valid? Why not use statistical parametric mapping? (3) Is the depression sample representative? (4) Could the reduction in normalized K* values reflect a partial volume effect due to anatomic differences between depressed patients and controls? (5) Could the reduction in normalized K* values be an artifact of altered brain hemodynamics, that is, reduced cerebral blood flow, a common biological observation in patients with MD? These considerations are discussed in the following paragraphs.

Some reservations have been expressed about the significance of the α-[11C]MTrp/PET method on the basis of a variety of technical points, including the suggestion that the method might measure blood-brain barrier transport of tryptophan rather than synthesis of serotonin.65 These reservations have been addressed in detail in several related method studies and reviews from our group.65,72 Medication-free patients with MD did not differ statistically significantly from controls in total and free plasma tryptophan concentrations or in free plasma tryptophan fraction, thereby, it is unlikely that reduced normalized K* values reflect group differences in circulating tryptophan concentrations. This is important because changes in plasma tryptophan levels have been related to changes in brain serotonin synthesis.13,16,27,28,38,56 In this study, we elected to report all data as normalized K* values rather than as calculated rates of serotonin synthesis R because the lumped constant required for conversion of K* to R is not known for the human brain.

In this study, we chose a VOI-based analysis in PET native space for between-group comparisons. This choice was based on the following considerations: (1) the literature on cerebral blood flow and glucose metabolic disturbances in MD permitted the a priori selection of regions of interest, (2) the analysis could follow established neuroanatomy, (3) normalized K* values could be calculated for the purpose of brain-behavior correlations, and (4) the analysis relies on a nonbiased method of brain segmentation and minimizes the potential impact on brain functional measurements of group-related anatomic differences in shape and volume, which are not completely controlled for by the current spatial normalization procedures used in VOI-based analysis.70,71 These techniques are well validated and have been successfully used by other researchers.65,72 Automatic labeling methods, however, have some limitations; in particular, manual corrections may occasionally be necessary, specifically for small VOIs. Statistical parametric maps comparing normalized K* values in depressed patients and controls were also obtained for the purpose of cross validation and confirmed the finding of a reduced normalized K* value in the ACC of patients with MD (t=3.38, z=3.10; P<.001; x=10, y=44, z=24) (Figure 2).

The sample size is modest but well within the range of similar studies in the field. Much attention was focused on preventing contamination of the biological measure of interest by nonspecific factors: although all patients with MD were medication free, 13 of 17 were drug naive at the time of scan, all had negative findings on a comprehensive toxicologic screen the day of scan, and all participants were studied within a 3- to 4-hour period (11 AM to 3 PM); matching for age and sex was rigorous, and women were all tested during their follicular phase. Yet, depression research is often compounded by clinical and biological heterogeneity, and this study is no exception. Until the reported observation is replicated in independent patient samples, caution suggests that the conclusion drawn from this study be applicable only to depressed patients whose clinical characteristics are similar to those of the reported sample: MD, outpatients with moderate to severe symptoms, nonmelancholic type, with no history of previous suicidality, of whom 65% reported a positive family history of mood disorder in 1 or more first-degree relatives.

It has been proposed that reduced tissue volume in the subgenual region of the ACC might confound the results of functional brain imaging studies in patients with MD. The analyses on segmented cingulate did not identify statistically significant differences in tissue volume between patients and matched controls. The absence of statistically significant morphometric differences between patients and controls suggests that the observed difference in the normalized K* values in parts of the ACC is primarily attributable to functional changes, rather than partial volume effects, secondary to anatomic differences between patients and controls.70 We assessed this further by analyzing normalized K* values after corrections for individual differences in tissue volume. After segmentation of the CC, normalized K* values remained lower in the ACC bilaterally in women with MD relative to controls but only on the left in men with MD. Whether this reflects a partial volume effect in men or a true sex-related difference in normalized K* values is unclear.

It is unlikely that the observed group differences in regional K* values are attributable to changes in cerebral blood flow because tracers with a low plasma-brain

![Figure 2](image-url)
rate constant, such as $\alpha$-[11C]MTrp, are insensitive to variations in cerebral blood flow.\textsuperscript{73}

In conclusion, this study reports reduced normalized K\textsubscript{r} values in the ACC and the left mesial temporal cortex in medication-free patients with MD in the absence of gross anatomic defects. Although these findings are highly suggestive, they do not formally prove altered serotonin synthesis or altered serotonergic neurotransmission in MD. More generally, this finding adds to the cumulative evidence supporting serotonergic dysfunction in MD and points to a critical role played by serotonergic innervation to parts of the limbic and paralimbic cortex, such as the ACC, the amygdala, and the hippocampus, in the dysregulation of mood. Finally, these findings raise important questions for future studies: Are the reported alterations of K\textsubscript{r}, an index of serotonin synthesis, trait or state related or genetically or environmentally determined? What, if any, is their developmental time course? Longitudinal measurements in unaffected individuals at risk of major depressive disorder should represent a promising avenue of research in the field.

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