



Acetyl-L-carnitine deficiency in patients with major depressive disorder

Carla Nasca^{a,1}, Benedetta Bigio^{a,b}, Francis S. Lee^{c,d}, Sarah P. Young^{e,f}, Marin M. Kautz^g, Ashly Albright^d, James Beasley^f, David S. Millington^{e,f}, Aleksander A. Mathé^h, James H. Kocsis^d, James W. Murrough^g, Bruce S. McEwen^{a,1}, and Natalie Rasgon^{a,i}

^aHarold and Margaret Milliken Hatch Laboratory of Neuroendocrinology, The Rockefeller University, New York, NY 10065; ^bBiostatistics and Experimental Research Design, Center for Clinical and Translational Science, The Rockefeller University, New York, NY 10065; ^cSackler Institute for Developmental Psychobiology, Weill Cornell Medical College, New York, NY 10065; ^dDepartment of Psychiatry, Weill Cornell Medical College, New York, NY 10065; ^eDivision of Medical Genetics, Department of Pediatrics, Duke University School of Medicine, Durham, NC 27710; ^fBiochemical Genetics Laboratory, Duke University Health System, Durham, NC 27710; ^gMood and Anxiety Disorders Program, Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY 10029; ^hDepartment of Clinical Neuroscience, Karolinska Institutet, SE-171 77 Stockholm, Sweden; and ⁱCenter for Neuroscience in Women's Health, Stanford University, Palo Alto, CA 94305

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The lack of biomarkers to identify target populations greatly limits the promise of precision medicine for major depressive disorder (MDD), a primary cause of ill health and disability. The endogenously produced molecule acetyl-L-carnitine (LAC) is critical for hippocampal function and several behavioral domains. In rodents with depressive-like traits, LAC levels are markedly decreased and signal abnormal hippocampal glutamatergic function and dendritic plasticity. LAC supplementation induces rapid and lasting antidepressant-like effects via epigenetic mechanisms of histone acetylation. This mechanistic model led us to evaluate LAC levels in humans. We found that LAC levels, and not those of free carnitine, were decreased in patients with MDD compared with age- and sex-matched healthy controls in two independent study centers. Secondary exploratory analyses showed that the degree of LAC deficiency reflected both the severity and age of onset of MDD. Moreover, these analyses showed that the decrease in LAC was larger in patients with a history of treatment-resistant depression (TRD), among whom childhood trauma and, specifically, a history of emotional neglect and being female, predicted the decreased LAC. These findings suggest that LAC may serve as a candidate biomarker to help diagnose a clinical endophenotype of MDD characterized by decreased LAC, greater severity, and earlier onset as well as a history of childhood trauma in patients with TRD. Together with studies in rodents, these translational findings support further exploration of LAC as a therapeutic target that may help to define individualized treatments in biologically based depression subtype consistent with the spirit of precision medicine.

epigenetic | glutamate | treatment-resistant depression | childhood trauma | mGlu2

Major depressive disorder (MDD) is among the leading causes of illness and disability worldwide (1, 2). MDD is a severe and life-threatening disease, which is also associated with other major illnesses, such as diabetes, cardiovascular disorders, and Alzheimer's disease (3, 4). A known risk factor for MDD is childhood trauma, which occurs at alarmingly high rates and has been associated with poorer responses to available antidepressant medications as well as with treatment-resistant depression (TRD) (5). The pathophysiology of MDD remains poorly understood, with a consequent lack of biological targets that can guide the development of diagnostics and improved therapeutics (6, 7).

In rodent models, epigenetic agents such as histone deacetylase inhibitors and the acetylating molecule acetyl-L-carnitine (LAC, Fig. 1A) have been shown to promote rapid antidepressant responses (8–15). Converging evidence from our group and others has shown that supplementation of LAC exerts rapid antidepressant actions, at least in part, by acetylating histones to regulate the expression of key genes important for synaptic plasticity, including the proneurogenic molecule brain-derived

neurotrophic factor (BDNF) and a critical regulator of synaptic glutamate release, the metabotropic glutamate receptor of class-2, mGlu2 (10, 16–18). In several animal models, LAC supplementation has been shown to ameliorate glutamatergic dysfunction and associated neuronal atrophy in brain regions such as the hippocampus and medial amygdala (13, 16, 19–21). LAC is an endogenous short-chain acetyl ester of free carnitine that crosses the blood–brain barrier (17, 22, 23). Notably, we found that these animals that rapidly responded to LAC supplementation (10–12, 19) show an endogenous decrease in LAC in plasma and in mood regulatory brain regions (i.e., the hippocampus and prefrontal cortex) (13, 17). Furthermore, the deficiency in LAC was associated with insulin resistance (IR), which was ameliorated by supplementation of LAC (19).

Therefore, the animal models provide a conceptual platform that is consistent with a known role of glutamatergic dysfunction, altered trophic environment, and proinflammatory states in humans with depression (3, 21, 24–31). Here, using such a biological target- and mechanistically driven approach, we evaluated the role of LAC in MDD in humans.

Significance

Identifying biological targets in major depressive disorder (MDD) is a critical step for development of effective mechanism-based medications. The epigenetic agent acetyl-L-carnitine (LAC) has rapid and enduring antidepressant-like effects in LAC-deficient rodents. Here, we found that LAC levels were decreased in patients with MDD versus age- and sex-matched healthy controls in two independent study centers. In subsequent exploratory analyses, the degree of LAC deficiency reflected both the severity and age of onset of MDD. Furthermore, the lowest LAC levels were found in patients with treatment-resistant depression, whereby history of emotional neglect and being female predicted decreased LAC levels. These translational findings suggest that LAC may serve as a candidate biomarker to help the diagnosis of a clinical endophenotype of MDD.

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¹To whom correspondence may be addressed. Email: cnasca@rockefeller.edu or mcewen@rockefeller.edu.

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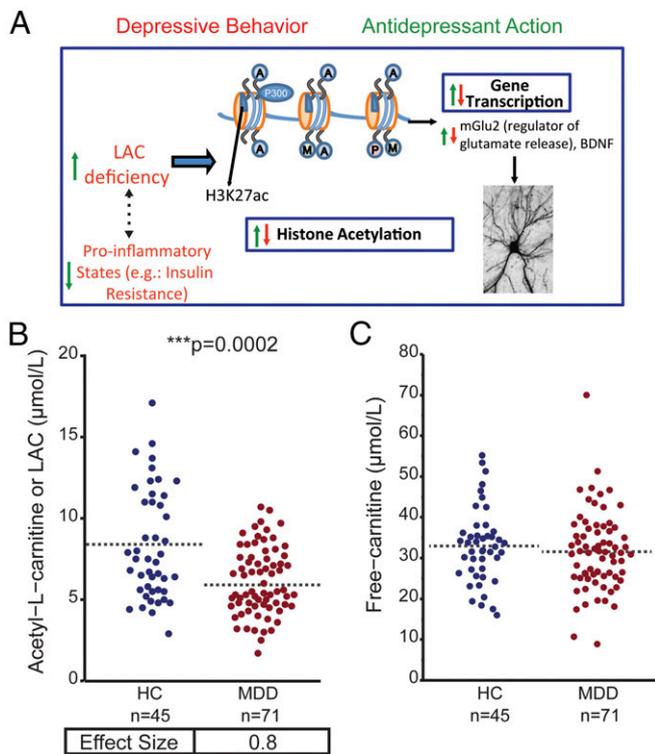


Fig. 1. Decreased Acetyl-L-carnitine (LAC) Levels in patients with MDD compared with HC. (A) Schematic model featuring our innovative framework: The endogenously produced molecule acetyl-L-carnitine (LAC) is critical for hippocampal function and several behavioral domains. In rodents with depressive-like traits, LAC levels are markedly decreased and accompanied by abnormal hippocampal glutamatergic function, decreased expression of the neurotrophic factor BDNF, and dendritic plasticity as well as by systemic inflammation, including insulin resistance. LAC supplementation rescues those deficits and induces rapid and lasting epigenetic antidepressant-like effects via acetylation of histones. (B and C) Plasma LAC (B) and free-carnitine (C) concentrations in HC and in patients with MDD in acute depressive episodes during study participation as assessed by ultraperformance liquid chromatography–electrospray–tandem mass spectrometry (UPLC-MS/MS). See also *SI Appendix, Figs. S1 and S3*. *Significant comparisons with HC. *** $P < 0.001$ in Student's two-tailed t tests ($\alpha = 0.05$). Dashed bars indicate group mean.

Results

LAC Levels Differ Between Healthy Controls and Patients with MDD.

In a sample of 116 participants, no difference was observed between healthy controls (HC) ($n = 45$) and patients with MDD ($n = 71$) with respect to demographic characteristics, including age and sex (*SI Appendix, Tables S1 and S2*). All patients were in an acute depressive episode during study participation.

LAC levels (Fig. 1A) in plasma, as the most accessible biospecimen in a clinical setting, were measured by UPLC-MS/MS (ultraperformance liquid chromatography–electrospray–tandem mass spectrometry) and ESI-MS/MS (stable isotope dilution electrospray–tandem mass spectrometry) as previously described (10, 32). LAC differed significantly between HC and patients with MDD with mean concentrations lower in the MDD group (Fig. 1B, $P < 0.0001$, effect size = 0.8; HC: $8.3 \mu\text{mol/L} \pm 0.4$, MDD: $6.1 \mu\text{mol/L} \pm 0.3$; *SI Appendix, Fig. S1*). No significant difference was observed in free-carnitine concentrations between HC and patients with MDD (Fig. 1C). The association between MDD and LAC held when stratifying by sex (*SI Appendix, Fig. S2*). Furthermore, LAC levels were similarly decreased in patients with MDD compared with age- and sex-matched HC in both independent study centers [Weill Cornell (C) and Mount Sinai School of Medicine (S)] (Fig. 2).

LAC Levels Within the MDD Group. Within the group of patients with MDD, no difference was observed in LAC concentrations in relation to use of psychotropic medications (*SI Appendix, Fig. S3*). Given the primary findings that showed a decrease in LAC levels in patients with MDD compared with age- and sex-matched HC in two independent study centers, we evaluated the contribution of clinical characteristics of MDD, such as depression severity and age of onset, on LAC levels by performing exploratory analyses. In subjects with mild MDD no correlation was observed between LAC levels and the severity of the disease using Hamilton Depression-Rating Scale (HDRS-17) (33). Among subjects with moderate to severe MDD, we observed a significant negative correlation between LAC concentrations and severity scores at HDRS-17 (33), whereby the higher the severity the lower the concentrations of LAC ($P = 0.04$, $r = 0.35$) (*SI Appendix, Fig. S4*). This relationship remained significant upon multiple regression analysis controlling for number of past episodes ($t = -2.13$, $P = 0.04$) and length of current episode ($t = -2.57$, $P = 0.017$) as well as controlling for sex ($P < 0.0001$) and age ($P = 0.0001$). Furthermore, prediction model analysis showed that LAC predicted HDRS-17 severity scores among subjects with moderate to severe MDD ($P = 0.04$, $r = 0.35$, *SI Appendix, Fig. S5*). Indeed, the HDRS-17 severity scores statistically inferred from LAC measures were consistent with the rater-administered HDRS-17 severity scores (*SI Appendix, Fig. S5*). Pearson correlation analysis also showed a positive correlation between LAC concentrations and age of onset of depression ($P = 0.04$, $r = 0.32$); that is, earlier age of onset correlated with lower concentrations of LAC (*SI Appendix, Fig. S6*).

LAC Levels and Treatment-Resistant Depression: Role of Childhood Trauma.

Driven by the findings above showing an LAC deficiency in patients with MDD in two study centers, we evaluated LAC levels across HC subjects and patients with MDD with or without history of TRD. Consistent with lower LAC in patients with MDD that were also characterized by greater severity and earlier onset of the illness, we found that the decrease in LAC

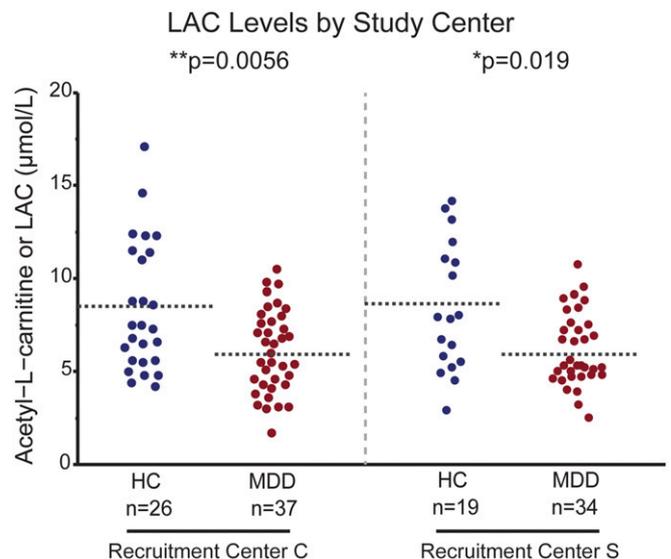


Fig. 2. LAC levels differ between HC and MDD groups in two independent study centers. Plasma LAC levels were similarly decreased in patients with MDD compared with age- and sex-matched HC in both study centers (C, Cornell; S, Sinai) as assessed by UPLC-MS/MS. *Significant comparisons with HC. ** $P < 0.01$ in Student's two-tailed t tests ($\alpha = 0.05$). Dashed bars indicate group mean.

was greater in subjects with MDD and a history of TRD, $F(2, 53) = 4.3$, $P = 0.01$ (Fig. 3 and *SI Appendix*, Fig. S7).

As childhood trauma has been associated with depression severity and treatment resistance, we conducted exploratory analyses to evaluate the contribution of childhood trauma on LAC levels. First, we found that the reported rates of childhood trauma assessed by the Childhood Trauma Questionnaire (CTQ) (34) differed from HC and patients with MDD (Fig. 4). Within the group of patients with MDD, we found that rates of emotional abuse, physical neglect, and emotional neglect were significantly higher either in patients with TRD or in patients with MDD non-TRD compared with HC, while physical and sexual abuse were significantly higher only in patients with TRD versus HC (Fig. 4 A–E): emotional abuse, $F(2, 52) = 17.08$, $P < 0.0001$; physical abuse, $F(2, 52) = 6.88$, $P = 0.0023$; sexual abuse, $F(2, 52) = 2.35$, $P = 0.1$; emotional neglect, $F(2, 52) = 13.75$, $P < 0.0001$; physical neglect, $F(2, 52) = 6.26$, $P = 0.0038$.

Furthermore, multiple regression analyses showed that LAC levels were predicted by a history of childhood trauma in patients with TRD; that is, emotional neglect by sex interaction predicted LAC levels ($P = 0.04$, $r = 0.66$, Fig. 4F). Specifically, models stratified by sex showed emotional neglect as a predictor of LAC concentrations only in women with TRD ($P = 0.02$, $r = 0.89$, Fig. 4G), but not in men ($P = 0.59$, $r = 0.40$, Fig. 4H). No interaction of LAC with emotional neglect was observed in women HC ($P = 0.77$) or in women with MDD non-TRD ($P = 0.84$).

Discussion

We report that a deficiency of the epigenetic agent acetyl-L-carnitine (LAC) occurs in humans who have major depressive disorder. These translational findings are an outgrowth of a mechanistic model in rodents with depressive-like traits, wherein

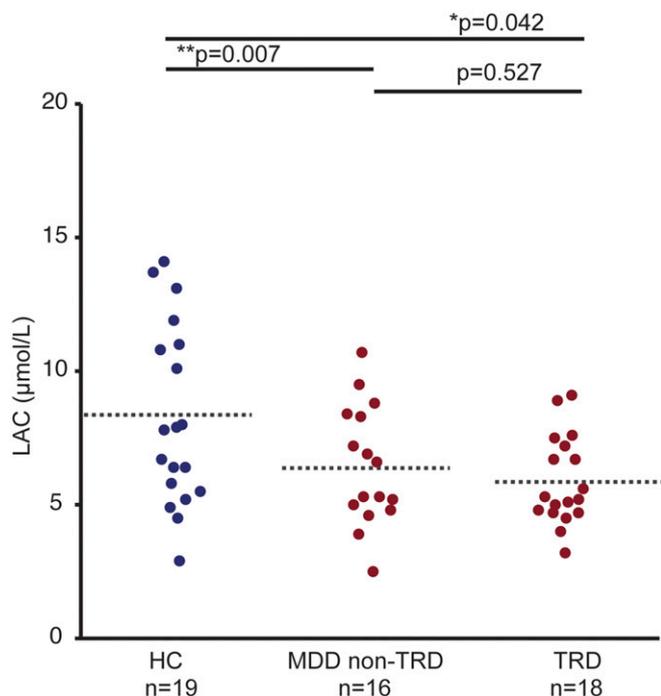


Fig. 3. The LAC deficiency is greater in patients with MDD and history of treatment-resistant depression (TRD). Plasma LAC concentrations across HC, patients with MDD without history of TRD (MDD non-TRD), and patients with MDD and with history of TRD for study center S. $F(2, 53) = 4.3$, $P = 0.01$. *Significant comparisons with HC. $*P < 0.05$, $**P < 0.01$ in Student's two-tailed t tests ($\alpha = 0.05$). Dashed bars indicate group mean. *SI Appendix*, Fig. S7 also shows a consistency of this stepwise in study center C.

LAC levels are markedly decreased and signal abnormal brain functions as well as metabolic dysregulation (*SI Appendix*, Fig. S8). Furthermore, hypotheses-generating exploratory analyses reported herein reveal that (i) the degree of LAC deficiency reflected both the severity and age of onset of MDD, and (ii) the LAC deficiency was associated with a history of childhood trauma in patients with treatment-resistant depression (*SI Appendix*, Fig. S8). These findings compel further research on the potential role of LAC as a candidate biomarker that together with clinical characteristics can aid the diagnosis and identification of a clinical endophenotype of MDD. Furthermore, LAC deficiency may represent an innovative biological therapeutic target in treatment of depression.

The decreased levels of LAC in patients with MDD is particularly important because LAC is an essential molecule for systemic and neural functions (13, 17, 22). LAC plays a central role in the transport of fatty acids into the mitochondria for beta oxidation to sustain energy metabolism in the brain and the rest of the body. LAC also facilitates elimination of oxidative products, provides acetyl groups to regulate expression of neurotrophins and glutamate genes that contain spontaneous glutamate release, and protects from excitotoxicity, therefore interacting with mechanisms that contribute to the pathophysiology of MDD (10, 13, 17, 21). In rodent models with depressive-like traits, LAC levels are markedly decreased and accompanied by hippocampal glutamatergic dysfunction as well as abnormal dendritic plasticity in the hippocampus, among other brain regions (13, 16, 20). LAC supplementation ameliorates these deficits. The specificity of changes in LAC and lack of changes in free carnitine suggest that the relationship between LAC and MDD is independent of potential dietary changes (17, 35). Of interest is also the previously reported positive correlation between peripheral and CNS LAC concentrations (17). Of note, LAC levels were similarly decreased in patients with MDD compared with age- and sex-matched HC in both study sites, providing an initial replication of our results. Furthermore, the use of two different methods to assess LAC in the same 116 samples supports the reliability of our results. Future studies will also be needed to assess whether decreased LAC levels in patients with MDD are sensitive to unhealthy lifestyle choices, such as physical inactivity, poor dietary habits, and lack of adequate sleep.

Furthermore, LAC appears to act as a state-dependent marker, given that all patients were in an acute depressive episode at the time of study participation and that the presence of medications did not influence LAC levels. However, the cross-sectional design of our study does not allow establishing whether the decrease in LAC levels in patients with MDD may also be a trait biomarker. Further studies may help in elucidating trait-dependent LAC levels. In addition, given the high comorbidity of MDD with other psychiatric disorders, future studies with larger cohorts will be needed to investigate whether decreased LAC levels may represent a specific signature for MDD or is a general marker of affective disorders. Moreover, the finding that the decrease in LAC levels in patients with MDD is independent of psychotropic drug treatment raises the possibility that increasing LAC levels may be needed to induce antidepressant effects.

LAC levels were significantly correlated with depression severity and with age of onset of MDD. Although these were exploratory analyses, the strength of such correlations remained after controlling for sex, number of depressive episodes, and duration of the current episode and was independent of use of psychotropic medications. Consistent with these results is the observation of decreased LAC in patients with history of treatment-resistant depression (TRD). The greater decrease in LAC in more severe forms of MDD and in patients with TRD is akin to a “kindling-like” progression of MDD in that earlier age at onset and/or the presence of early life adversity, such as childhood trauma, conveys liability to more severe and treatment-resistant

also be important to investigate the association of LAC deficiency with insulin resistance.

In conclusion, the current findings of LAC deficiency in MDD suggest a possible endophenotype of depression, characterized by history of childhood trauma, greater depression severity, and earlier age at onset. Future prospective, placebo-controlled studies will be needed to address some of the limitations inherent in our cross-sectional cohorts. Further study with larger cohorts is also needed to better understand the role of LAC in clinically distinct populations of patients with depressive disorders.

Methods

The Rockefeller University Institutional Review Board and the respective Institutional Review Boards of the collaborating Institutions (Weill Cornell Medicine, Icahn School of Medicine at Mount Sinai, and Duke University) approved the current study in its entirety.

Participants. Following an initial phone screen, potential participants were evaluated in person to determine study eligibility. All participants determined to be eligible to join the study provided written informed consent before study enrollment. Study participants, ranging between 20 and 70 y old, were recruited at two independent sites, the Affective Disorders Research Program at Weill Cornell Medicine and the Mood and Anxiety Disorders Program at the Icahn School of Medicine at Mount Sinai. At both study sites, study clinicians or trained coordinators conducted the Structured Clinical Interview for DSM-IV (SCID) or Mini International Neuropsychiatric Interview (MINI) to confirm MDD diagnosis and rule out exclusionary comorbid conditions.

Inclusion and exclusion criteria were similar at both recruitment sites. Inclusion criteria included a primary diagnosis of MDD in a current major depressive episode. Inclusion criteria in the TRD group also included having at least moderate severity and a history of nonresponse to at least two therapeutic trials of an antidepressant according to the Antidepressant Treatment History Form (ATHF) or Antidepressant Treatment Record (ATR) during their lifetime. Exclusion criteria included a presence of neurologic or other physical illness, such as diabetes, alcohol or substance abuse in the last 6 mo, or an unstable medical illness. Current medication use was assessed at screening for all study participants. Participants were free of current substances of abuse as determined by a urine toxicology test at the time of screening. HC were free of lifetime psychiatric illness and significant medical conditions. Participants were free of active infections and systemic illness as confirmed by medical history at the time of study evaluation. Blood samples were obtained via antecubital venous collection using standard techniques and were drawn after a period of fasting (>6 h). Participants were asked not to exercise for >6 h before blood draw.

Clinical and Psychiatric Assessment. Clinical assessment consisted of a physical examination, including measures of height, weight, and body mass index (BMI). Other data collected included current medication use and history of failed antidepressant trials. Demographic information, including sex, was also recorded from the participants (S1 Appendix, Tables S1 and S2). The psychiatric examination at screening included SCID or MINI to confirm MDD diagnosis, and trained raters administered the structured depression-rating scales: 17-item Hamilton Depression-Rating Scale (HDRS-17) and the Montgomery-Asberg Depression Rating Scale (MADRS). Among participants, two were identified as having eating disorders and one as having Crohn's disease and, therefore, were excluded from the analyses. Cutoff scores of 19 and 34 were used to stratify for depression severity at the HDRS-17 and MADRS, respectively (33). As a result, among the 71 patients with MDD, 28 patients had moderate depression (HDRS-17 < 19, MADRS < 34) and 43 patients had severe depression at the time of study evaluation (HDRS-17 ≥ 19, MADRS ≥ 34). With regard to medication use, 53 patients were free of antidepressant medications at the time of study participation and 18 patients were on psychotropic medications. A subgroup of 18 patients with MDD recruited at Icahn School of Medicine at Mount Sinai reported history of TRD defined by at least two failed antidepressant trials (41, 42). All participants (i.e., HC, MDD non-TRD, and TRD) at Icahn School of Medicine at Mount Sinai completed the Childhood Trauma Questionnaire (CTQ) (43) to assess for childhood traumatic experiences in five specific areas: physical, sexual, and emotional abuse and physical and emotional neglect. Some information about subjects from the Icahn School of Medicine at Mount Sinai included in the current study was previously reported (44).

LAC Measures by Ultrapformance Liquid Chromatography–Electrospray–Tandem Mass Spectrometry. LAC and free carnitine in plasma were analyzed using ultrapformance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) with electrospray ionization in positive ion mode on an Xevo-TQD or a TQD tandem mass spectrometer equipped with Acquity UPLC system (Waters Corp.). For the determination of free L-carnitine and LAC, plasma samples were spiked with [²H₃]-free carnitine and acetyl-²H₃-carnitine internal standards. The total concentration of carnitine (sum of free and acylated carnitine) was determined in a second aliquot of each sample mixed with [²H₃]-free carnitine internal standard. These second aliquots were subjected to base hydrolysis of the acylcarnitine species by incubation with 1 mol/L KOH at 65 °C for 15 min, followed by neutralization with 1 mol/L HCl. Protein was precipitated in all aliquots using 0.1% formic acid in acetonitrile and removed by centrifugation. The sample extracts were dried and reconstituted in 0.1% formic acid and 7.5 mmol/L ammonium formate in 18:82 (vol/vol) acetonitrile:deionized water. L-Carnitine and LAC were separated on an Acquity BEH HILIC, 2.1 mm × 100 mm, 1.7-μm column (Waters Corp.) with gradient elution using 0.1% formic acid and 7.5 mmol/L ammonium formate in acetonitrile:deionized water as the mobile phase and detected using selected reaction monitoring. The ratios of signal intensities for the transitions *m/z* 162 > 103 (free carnitine) and 165 > 103 ([²H₃]-carnitine) and *m/z* 204 > 85 (acetyl-carnitine) and 207 > 85 (acetyl-²H₃-carnitine) were converted to a concentration by means of a calibration curve. Materials: L-carnitine.HCl and LAC hydrochloride (Sigma Chemical Co.); ²H₃-L-carnitine.HCl and acetyl-²H₃-L-carnitine.HCl (Cambridge Isotope Laboratories, Inc.); all other reagents, solvents, and solvent additives were purchased from Sigma Chemical Co. or VWR. All groups were evenly divided between the experimental plates to account for any interplate variability.

LAC Measures by Stable Isotope Dilution Electrospray–Tandem Mass Spectrometry. Deuterated internal standards were obtained from Cambridge Isotope Laboratories, Inc. and from Sigma Chemical Co. Three Molar methanolic HCl was obtained from Sigma Chemical Co. General reagents and solvents were obtained from VWR. Plasma acetylcarnitine was analyzed as methyl ester by a semiquantitative method using stable isotope dilution electrospray–tandem mass spectrometry (ESI-MS/MS). Plasma was mixed in an internal standard mixture containing d₃-acetylcarnitine, d₃-propionylcarnitine, d₃-butylcarnitine, d₃-octanoylcarnitine, and d₃-palmitoylcarnitine. Protein was precipitated by the addition of methanol and removed by centrifugation. An aliquot of the supernatant liquid was dried under nitrogen and methylated by incubation with 3 M HCl in methanol at 50 °C for 15 min. The derivatized extract was dried under nitrogen, reconstituted in methanol:dH₂O 85:15 (vol/vol), and analyzed directly by flow injection–MS/MS on a TQD tandem mass spectrometer coupled with an Acquity UPLC system (Waters Corporation). Acetylcarnitine was detected using a precursor ion scan of *m/z* 99, with a scan range of *m/z* 200–500. Concentrations were determined from the ratio of ion intensities of acetylcarnitine species to its specified deuterated internal standard, multiplied by the concentration of the standard. All groups were evenly divided between the experimental plates to account for any interplate variability.

Statistical Analysis. Statistical analyses were conducted using JMP Software from SAS (Statistical Analysis Systems Institute). Two-tailed *t* tests and χ^2 analyses were used to compare, respectively, continuous and categorical demographic and clinical characteristics between HC and MDD subjects. Between- and within-group differences in patient plasma LAC and free-carnitine concentrations were compared using *t* tests. Within-group Pearson correlations were conducted to examine the relationship between LAC concentrations and depression severity or age of onset. Multiple regression analysis was used to control for other clinical characteristics. A one-way ANOVA followed by post hoc Student's *t* tests was used to examine LAC levels upon use of psychotropic medications as well as across HC and subjects with MDD with or without history of TRD. Predictive models were inferred using multiple regression analysis to assess the ability of LAC and CTQ areas to predict the dependent variables, HDRS-17 scores, and LAC concentrations, respectively. Significance was set at 0.05, and data are presented as mean ± SD, unless otherwise specified.

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