HHS Public Access

Author manuscript

Psychoneuroendocrinology. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Psychoneuroendocrinology. 2018 April ; 90: 157–164. doi:10.1016/j.psyneuen.2018.02.015.

Prospective Association between Major Depressive Disorder and Leukocyte Telomere Length over Two Years

Mary C. Vance, MD[1,2], **Eric Bui, MD, PhD**[3,4], **Susanne S. Hoeppner, PhD**[3,4], **Benjamin Kovachy, BA**[4], **Jennifer Prescott, PhD**[4], **David Mischoulon, MD**[3,4], **Zandra E. Walton**[6,8], **Melissa Dong**[3], **Mireya F. Nadal, MD, PhD**[3,4], **John J. Worthington, MD**[3,4], **Elizabeth A. Hoge, MD**[5], **Paolo Cassano, MD, PhD**[3,4], **Esther H. Orr, BS**[9], **Maurizio Fava, MD**[3,4], **Immaculata de Vivo, PhD**[4], **Kwok-Kin Wong, MD, PhD**[4,6,7], and **Naomi M. Simon, MD, MSc**[3,4,7]

[1]University of Michigan

[2]VA Ann Arbor Healthcare System

[3]Massachusetts General Hospital

[4]Harvard Medical School

[5]Georgetown University Medical Center

[6]Dana Farber Cancer Institute

[7]New York University Medical School

[8]University of Pennsylvania

[9]Brigham and Women's Hospital

Abstract

Background—Reduced leukocyte telomere length (LTL) has been found to be associated with multiple common age-related diseases, including heart disease, diabetes, and cancer. A link has also been suggested between shortened LTL and major depressive disorder (MDD), suggesting that MDD may be a disease of accelerated aging. This prospective, longitudinal study examined the association between depression diagnosis at baseline and change in LTL over two years in a well-characterized sample of *N*=117 adults with or without MDD at baseline, using rigorous entry criteria.

[*]**Corresponding Author:** Mary C. Vance, MD, University of Michigan Institute for Healthcare Policy and Innovation, National Clinician Scholars Program, 2800 Plymouth Road, Building 14, Suite G100, Ann Arbor, MI 48109-2800, marycv@med.umich.edu, Phone: (585) 766-2498.

Conflicts of interest: On behalf of all authors, the corresponding author wishes to declare the following potential conflicts of interest:
Dr. Maurizio Fava - For a list of lifetime disclosures, please see http://mghcme.org/faculty/faculty-detail/maurizio_fava.
Dr. Paolo Cassano – His past 12-month disclosures include the following: Litecure Inc – unrestricted grant (ELATED-3); Niraxx Light Therapeutics Inc – co-founder & member of the board of directors & shares-holder; Janssen – consultation fee, plus reimbursement for travel; Litecure Inc – unrestricted grant (NIR-flow); Janssen – grant; filing of patent related to the use of near-infrared light in psychiatry.

Methods—Participants aged 18 to 70 were assessed with validated instruments by trained, doctoral-level clinician raters at baseline and at two-year follow-up, and blood samples were obtained at both visits. LTL was assayed under identical methods using quantitative polymerase chain reaction (qPCR). The effect of an MDD diagnosis at baseline on change in LTL over two years was examined via hierarchical mixed models, which included potential confounders.

Results—Individuals with MDD at baseline had greater LTL shortening over two years than individuals without MDD ($p = 0.03$), even after controlling for differences in age, sex, and body mass index (BMI). In the sub-sample of individuals with MDD diagnoses at baseline, no significant associations between LTL change and symptom severity or duration were found.

Conclusion—A baseline diagnosis of MDD prospectively predicted LTL shortening over two years. Our results provide further support for MDD as a disease associated with accelerated aging in a well-characterized sample using validated, clinician-rated measures.

Keywords

telomeres; depression; aging; prospective; stress; anxiety

1. INTRODUCTION

Major depressive disorder (MDD) is associated with elevated morbidity and mortality from multiple age-related medical illnesses, including cardiovascular disease and cancer (Carney and Freedland, 2017; Cosci et al., 2015). This risk remains after controlling for potential health behavior confounders, such as smoking and exercise (Penninx et al., 1998). Investigations into biological mechanisms suggest that dysregulated stress and immune responses are present in individuals with depression (Chrousos, 1998; Pariante and Miller, 2001; Raison and Miller, 2003; Simon et al., 2006), that they may accelerate aging by causing chronic “wear and tear” in cells and tissues, and that they result in increased vulnerability to age-related medical disorders (Kop et al., 2010; Maes et al., 2011). Genetic predispositions to accelerated biological aging may also confer increased risk for depression (Michalek et al., 2017).

Emerging evidence supports telomere length as a marker of cellular aging. Telomeres are nucleoprotein complexes comprised of long, double-stranded TTAGGG repeats that cap the ends of chromosomal DNA. By preventing end-to-end recombination at chromosomal termini during cell division, telomeres protect DNA and thereby maintain chromosomal integrity (Blackburn, 2010). Telomeres shorten with repeated cell divisions, due to incomplete replication of the telomere ends and stress-related oxidative damage (Wolkowitz et al., 2011). Therefore, telomere shortening could reflect a stress response.

Leukocyte telomere length (LTL) has typically been measured for clinical studies, because peripheral blood is readily accessible (Price et al., 2013). LTL is an ideal marker of stress-related cellular aging, due to the sensitivity of telomeres to stress-related oxidative damage (Hovatta, 2015; Zhang et al., 2014). Multiple studies have assessed the relationship between MDD and LTL, but results from individual studies so far have been mixed (e.g., see Lindqvist et al., 2015; Schutte and Malouff, 2015 for reviews).

Four recent meta-analyses found significant associations between depression and shorter LTL. A meta-analysis by Schutte and Malouff (2015), pooling 25 cross-sectional and longitudinal studies (N= 21,040), found depression to be significantly associated with shorter telomere length (r = −0.12, 95% CI = −0.17 to −0.07, Cohen's d not reported). Lin and colleagues' meta-analysis (2016) of 16 case-control studies pooled N=7207 participants and cross-sectionally compared telomere length between depressed individuals and healthy controls, revealing significantly shorter telomere length in the depressed group (Hedges' g = −0.42 corresponding to r = −0.21, 95% CI = −0.60 to −0.25). Ridout et al. (2016) pooled 38 cross-sectional, case-control, and cohort studies involving N=34,347 subjects and found a significant association between depression and telomere length (Cohen's d = −0.205, 95% CI = −0.288 to −0.122). Finally, Darrow et al.'s meta-analysis (2016), which examined 27 studies utilizing various designs and pooled N=14,827 participants, found that psychiatric disorders overall (including depressive disorders, PTSD, anxiety disorder, psychotic disorder, and bipolar disorders) were associated with significantly shorter LTL (Hedges' g = −0.50, 95% CI = −0.70 to −0.30). Furthermore, the subgroup effect size of depressive disorders was moderate (Hedges' g = −0.55, 95% CI = −0.92 to 0.18), although this result did not reach statistical significance.

Cross-sectional studies are limited by substantial variability in telomere length across individuals regardless of the presence of disease (Aviv et al., 2006; Takubo et al., 2002). Inter-individual variability reduces the power and reliability of cross-sectional studies, and it has been estimated that five times fewer subjects would be required to detect an association between depression and telomere length in a longitudinal study (Aviv et al., 2006).

To our knowledge, only six prospective longitudinal studies have assessed the relationship between MDD and LTL (Hoen et al., 2011; Hoen et al., 2013; Rius-Ottenheim et al., 2012; Shalev et al., 2014; Verhoeven et al., 2016; Verhoeven et al., 2017), yielding mixed results. These measured LTL using quantitative polymerase chain reaction (qPCR) and controlled for a range of potential confounds, including age, sex, antidepressant use, smoking, activity level, physical health, body mass index (BMI), adverse life events, and educational level. However, they were all secondary analyses of banked blood collected for other purposes, and as such they may not have treated samples under ideal clinical collection conditions. Additionally, some did not use doctoral-level trained interviewers to assess psychiatric diagnoses. For example, three studies (Rius-Ottenheim et al., 2012; Hoen et al., 2013; Verhoeven et al., 2017) used self-rated scales, and one study (Hoen et al., 2011) used a clinician-rated scale administered by research assistants.

Hoen et al. (2011) used data from the Heart and Soul study, assessing N=952 participants at baseline and n=608 at follow-up; they found MDD was associated with shorter LTL at baseline but not at 5-year follow-up. Hoen et al. (2013) used data from the Prevention of Renal and Vascular End-stage study and found that anxiety, but not depressive, disorders were associated with shorter LTL at follow-up in N=974 participants. Shalev et al. (2014) found that persistence of "internalizing disorders" (MDD, generalized anxiety disorder, posttraumatic stress disorder) from ages 11 to 38 predicted shorter LTL at follow-up in a dose-dependent manner only among men. Rius-Ottenheim et al. (2012) found no association between LTL shortening over a 7-year period and depression symptom severity in elderly

men from the Netherlands (n=203) and Greece (n=123). Verhoeven et al. (2016) found that, compared to controls, those with a current or remitted depressive or anxiety disorder had shorter LTL at both baseline and 6-year follow-up; however, neither baseline diagnosis nor symptom change were associated with the LTL shortening rate. Verhoeven et al. (2017) found that higher average scores of depression across a 10-year period were associated with shorter LTL, but within-subject increases in self-reported depression were not associated with LTL change. In summary, existing prospective studies did not consistently find an association between psychiatric disorders, including MDD or depressive symptoms, and telomere shortening, and those found varied in their persistence over time and population impacted. These mixed data indicate a need for additional longitudinal research.

Consideration of potential confounders such as age and sex (Aubert and Lansdorp, 2008; Gardner et al., 2014) as well as BMI (Müezzinler et al., 2014) is also important in studies of MDD and LTL. Exposure to traumatic events and early-life stress should also be assessed, as they are associated with both psychiatric disorders (Heim and Nemeroff, 2001) and telomere shortening (Price et al., 2013; Shalev et al., 2013) Of note, one study found that individuals with PTSD had shorter telomeres than controls, and childhood trauma exposure largely accounted for this finding (O'Donovan et al., 2011).

We used a prospective, longitudinal design to examine the association between a current MDD diagnosis at baseline in participants whose depression had an onset at least five years prior and relative LTL change over two years. We extensively characterized our sample at baseline and follow-up (see Simon et al., 2015 for baseline data), including for age; sex; race; ethnicity; educational level; living situation; BMI; smoking pack-years; exercise level; antidepressant use; trauma exposure during childhood and adulthood; depression symptom severity; general anxiety symptom severity; and perceived stress. Individuals with characteristics previously linked to shorter telomere length were excluded, such as severe obesity (Muezzinler et al., 2014) and severe ongoing medical illnesses (Kong et al., 2013). Psychiatric diagnosis was assessed by doctoral-level interviewers using the *Structured Clinical Interview for DSM-IV* (SCID; First et al., 2002). We hypothesized that MDD diagnosis at baseline would be independently predictive of greater LTL shortening over two years, even after taking into account a range of potential confounders.

2. MATERIALS AND METHODS

2.1 Participants

Adults aged 18 to 70 years with a primary diagnosis of MDD and healthy controls were recruited to the Center for Anxiety and Traumatic Stress Disorders at the Massachusetts General Hospital (MGH) through referral and advertisement. After initial screening by telephone, potentially eligible individuals were assessed in person by trained, experienced doctoral-level (MD or PhD) clinicians for psychiatric disorders using the SCID for *DSM-IV* (First et al., 2002). Participants meeting entry criteria completed interviewer-rated and self-report questionnaires, and also underwent phlebotomy, at both baseline and two-year follow-up. Participants provided written informed consent and were compensated $75 at baseline and $75 at follow-up for study participation. All study procedures were approved by the Institutional Review Board at MGH. The participants in this study were a longitudinal

follow-up subsample of our previous cross-sectional study assessing the association between MDD and LTL in n=50 out of 166 adults with MDD who had signed consent and agreed to two-year follow-up and n=67 out of 166 age- and sex-matched controls (see Simon et al., 2015). In this follow-up cohort, cases and controls were not one-to-one age- and sex-matched due to loss to follow-up.

Entry criteria for the study included current chronic or recurrent MDD, with the first lifetime episode of depression diagnosed at least five years prior to study enrollment and meeting full criteria for a current major depressive episode at the time of study entry. Participants may have had a previous remission, but must have been in a current major depressive episode during screen and phlebotomy. Lifetime psychosis, bipolar disorder, mental retardation, and organic medical disorders were exclusionary, as were current eating disorders and current substance use disorders (including alcohol use disorder). Control participants did not meet diagnostic criteria for any lifetime *DSM-IV* Axis I psychiatric disorder. In both groups, a history of lifetime substance use disorders was allowable if criteria had not been met for at least the past 12 months. In order to prevent selective survival bias (i.e., the most severely depressed participants with the shortest telomeres may have a greater risk for earlier death and thus be missing from comparisons between older age groups, biasing the MDD sample towards those less impacted), the maximum age of participants at baseline was limited to 70. Current cancer, chronic inflammatory disorders, diabetes, unstable cardiovascular illness, epilepsy, surgery within the past four weeks, severe obesity (BMI > 35), and pregnancy were exclusionary to reduce potential confounders. Use of any psychiatric medication in the two weeks (four weeks for fluoxetine) prior to screen, or of anti-inflammatory medications in the three days prior to phlebotomy, was exclusionary. Lifetime psychiatric medication use was not exclusionary. If participants were using antibiotics or undergoing an acute infectious process such as an upper respiratory infection, they returned for phlebotomy one week after symptom resolution or completion of antibiotics.

2.2 Measures

Demographic and clinical information, including BMI, exercise frequency, smoking history, medical history, and a battery of self-rated and clinician-rated questionnaires were administered at baseline and follow-up. Trained study psychiatrists and psychologists used the *Structured Clinical Interview for DSM-IV* (SCID; First et al., 2002) to assess current and lifetime psychiatric disorders. The 10-item *Montgomery-Asberg Depression Rating Scale* (MADRS; Montgomery and Asberg, 1979), a validated, clinician-administered measure of depression with total scores ranging from 0 to 60, was used to assess depressive symptom severity. The 14-item *Hamilton Anxiety Rating Scale* (HAM-A; Hamilton, 1959) was used to measure the severity of general anxiety symptoms. Lifetime exposure to antidepressant medication was formally assessed and categorized as less than 6 months or greater than 6 months of cumulative exposure. The 10-item, self-report *Perceived Stress Scale* (PSS; Cohen et al., 1983) was used to measure subjective stress during the last month.

Two inventories were used to assess the effect of traumatic life experiences. The *Early Trauma Inventory Self-Report* short form (ETISR-SF; Bremner et al., 2007) assessed trauma exposure before age 18, including four categories: general trauma (11 items), physical abuse

(5 items), emotional abuse (5 items), and sexual abuse (6 items). The *Traumatic Events Questionnaire* (TEQ; Vrana and Lauterbach, 1994) assessed cumulative exposure to 11 categories of childhood and adult traumatic life events in a self-rated format.

2.3 Collection of blood samples and DNA extraction

Participants underwent phlebotomy at rest between 9 AM and 7 PM during their baseline and follow-up visits. Total DNA for telomere assays was isolated from anticoagulated whole blood (stored at −20 degrees Celsius) using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, cat no. 69506).

2.4 Assessment of telomere length

Relative LTL was assessed using quantitative polymerase chain reaction (qPCR), a well-validated method that has been shown to correlate well with the gold standard of Southern blot and that is now commonly used (e.g., Cawthon, 2009). The ratio of telomere repeat copy number to a single gene copy number (T/S) was determined using a modified, high-throughput version of the qPCR telomere assay (Cawthon, 2002) that has been previously described (Wang et al., 2008), run on the Applied Biosystems 7900HT Sequence Detection System (Foster City, CA, USA). Triplicate reactions of each assay were performed for each sample. Relative LTL was reported as relative T/S (T/S of the sample divided by the T/S of a within-plate reference sample) exponentiated ratios (Cawthorn, 2009). The telomere and single-gene assay coefficients of variation (CVs) for triplicates were <2.5% for baseline and <2% for follow-up. CVs for the exponentiated T/S ratio at baseline and follow-up were 13.4% and 11.7%, respectively.

Although procedures were identical, baseline and follow-up samples were not run within the same qPCR batches and thus used different within-plate reference samples. To minimize the impact of a batch effect, we calculated the natural logarithm of relative LTL to improve normality, checked for outliers using the generalized extreme studentized deviate many-outlier procedure (Rosner, 1983; no outliers were detected at $\alpha = 0.05$), and computed z-scores of the log-transformed relative LTLs among samples taken at each time point.

2.5 Statistical analysis

To assess for baseline demographic and clinical differences between individuals with MDD and healthy controls, we used t-tests for continuous variables and Fisher's Exact Tests for categorical variables. We used hierarchical mixed models with relative LTL as the outcome variable, baseline MDD diagnosis, time, and the interaction of MDD diagnosis with time as predictors, and modeling repeated measures over time per person with an unstructured covariance matrix. The main outcome of interest was relative LTL change from baseline to follow-up (i.e. interactions with time). We followed up this basic model with an extended model that accounted for previously reported effects of age, sex, and BMI by adding age, sex, and baseline BMI as additional covariates and slope moderators to the mixed model. We then screened all the remaining demographic and clinical variables listed in Table 1 (with the exception of MADRS scores, anti-depressant use, and months of MDD, because these were MDD-specific or highly correlated) in models analogous to the base model, but replacing MDD and the MDD by time interactions with each predictor and predictor by time

interaction in turn. Any potential predictors that had significant moderating effects on time at $p < 0.1$ were also entered into the extended model. Lastly, to explore whether trauma exposure might explain any of the variation in relative LTL, we also ran models with childhood trauma exposure (ETISR-SF total scores at baseline), and trauma exposure at baseline (TEQ total scores at baseline) or in the intervening years (TEQ total scores at follow-up) as additional baseline covariates and moderators, both individually and in combination. We calculated the effect size (*ES*) of the group difference in relative LTL change scores using the standard deviation of the change scores in each group to reflect within-group changes over time without removing the effect of pretest-posttest correlations (Feingold, 2009): $ES = (\text{M change-MDD} / SD_{\text{change-MDD}}) - (M_{\text{change-HC}} / SD_{\text{change-HC}})$, where $M_{\text{change-MDD}}$ and $M_{\text{change-HC}}$ are the mean change scores for the group with MDD at baseline and healthy controls, respectively, while $SD_{\text{change-MDD}}$ and $SD_{\text{change-HC}}$ are the standard deviations of their respective change scores. As an additional follow-up analysis, we then screened all variables that assessed symptom severity at baseline, across the intervening years, or at follow-up in the sub-sample of people with MDD at baseline (i.e., MADRS, HAM-A, and PSS scores at baseline and follow-up, anti-depressant use for more than 6 months at baseline or during the follow-up period, months of MDD at baseline or during the follow-up period) using the basic mixed model described above to assess if symptom severity had any association with LTL change. The level of statistical significance was set to $\alpha = 0.05$ (two-tailed) for all analyses, unless otherwise specified. All analyses were performed using SAS (Version 9.4 of the SAS System for Windows).

3. RESULTS

3.1 Demographic and clinical characteristics

Table 1 shows the baseline, follow-up, and change in demographic and clinical characteristics of the N=117 individuals who completed study follow-up, according to their MDD diagnosis at baseline (n=67 adults without MDD and n=50 with MDD). Table 2 presents the simple correlations for potential predictors and LTL at baseline and at year 2.

3.1.1 Baseline characteristics—There were no significant differences in age, sex, race, ethnicity, or highest educational level between the baseline depressed and non-depressed groups. Marital status did differ significantly between the two groups: non-depressed individuals were more likely to be married or living with a partner at baseline compared to individuals with MDD. In the MDD group, the mean ± *SD* duration of MDD at baseline was 128.9±122.5 months. Participants with baseline MDD also had significantly higher MADRS total scores, HAM-A total scores, and PSS total scores at baseline than study participants without MDD, and were also more likely to report early traumatic events at baseline on the ETISR-SF and traumatic events at baseline on the TEQ (Table 1). With respect to health behaviors, participants with baseline depression had greater BMIs on average than healthy controls (though truncated by exclusion criteria requiring BMI below 35) and were less likely to report exercising more than once per week during the previous year. Depressed participants were not more likely to report lifetime alcohol or substance use diagnoses and did not differ in smoking pack-years from the non-depressed participants.

3.1.2 Change in characteristics between baseline and follow-up—At the follow-up visit, one individual in the healthy control group at baseline received an MDD diagnosis, and 33 individuals with baseline MDD no longer had a current MDD diagnosis, resulting in only 30% with a current full diagnosis of a major depressive episode (Table 1). Of note, 9 participants had incomplete or missing SCID diagnostic data (3 MDD and 6 controls). Despite this reduction in follow-up MDD diagnoses, participants with baseline MDD still had higher MADRS, HAM-A, and PSS scores at follow-up compared to the non-depressed control group. Participants in the baseline MDD group were, furthermore, more likely to report a higher number of traumatic events they had experienced in the intervening two years, were more likely to be diagnosed with a new alcohol or substance use disorder, and had smoked more during the intervening two years than participants without MDD at baseline (Table 1). The groups no longer differed in the proportion of people who exercised more than once a week, but the differences in BMI observed at baseline persisted.

3.2 Telomere length changes by MDD diagnosis

In the basic hierarchical mixed model that used only MDD diagnosis at baseline as a covariate and slope moderator, MDD was associated with greater relative LTL shortening over two years ($b = -0.55 \pm 0.24$, $F_{(1, 115)} = 5.14$, $p = 0.03$). The mean relative telomere length change (M ±*SD*) was 0.233±1.323 among individuals without MDD diagnosis at baseline and −0.313±1.240 among individuals with MDD at baseline (Table 1). The overall correlation between relative LTL at baseline and follow-up was low ($r = 0.14$), and the effect of a baseline MDD diagnosis on relative LTL change was small to medium ($ES = 0.43$).

3.3 Assessment of potential confounders

Baseline depression was a significant predictor of LTL change even after accounting for potential confounders. Of the potential covariates considered, only age, anxiety severity (HAM-A), and a lifetime diagnosis of alcohol or substance use disorders at baseline moderated the simple effect of time on relative LTL at a significance level of $p < 0.1$. Of these, HAM-A could not be entered into the exploratory mixed models due to its high correlation with MDD at baseline ($r = 0.90$). The addition of age, sex, and baseline BMI as covariates and moderators of the time effect (Table 3, Model 2) had little impact on the moderating effect of baseline MDD diagnoses on relative LTL change over time ($b = -0.54 \pm 0.25$, $F_{(1, 112)} = 4.90$, $p = 0.03$). In this extended model, age emerged as a baseline covariate of relative LTL ($b = -0.02 \pm 0.01$, $F_{(1, 112)} = 5.17$, $p = 0.02$), but did not moderate relative LTL change over time ($F_{(1, 112)} = 3.14$, $p = 0.08$), and neither sex nor BMI were significantly associated with relative LTL or change in relative LTL (Table 3, Model 2). In our exploratory models, none of the additional predictors added (i.e., lifetime diagnosis of alcohol or substance use disorders at baseline, baseline ETISR-SF total scores, TEQ total scores at baseline, and TEQ total scores at follow-up) emerged as significant covariates of relative LTL or moderators of change over time in relative LTL (Table 3, Models 3–7), and MDD diagnosis at baseline ranged from being barely non-significant ($b = -0.57 \pm 0.30$, $F_{(1, 106)} = 3.79$, $p = 0.05$; Table 3, Model 4) to being a significant moderator ($b = -0.73 \pm 0.27$, $F_{(1, 98)} = 7.20$, $p < 0.01$; Table 3, Model 5) with a fairly stable negative effect on relative LTL over time. Finally, additional follow-up analyses supported that neither adjustment for the moderating effect of new onset of alcohol and substance use disorders,

nor for the moderating effects of current smoking (past 2-year pack-years) altered the significance of the MDD-LTL change relationship, and neither of these moderators was a significant moderator of LTL change (data not shown).

3.4 Telomere length changes and symptom severity among individuals with MDD

Among only the study participants with MDD at baseline (n=50), none of the measures of symptom severity examined showed any significant association with LTL change scores (all $p > 0.3$). These included depression severity (MADRS), anxiety severity (HAM-A), perceived stress severity (PSS), antidepressant use measures, and depression duration measures.

4. DISCUSSION

Using z-scores to standardize LTL across the baseline and follow-up assays, we found significantly greater relative LTL shortening over two years in participants with an MDD diagnosis at baseline compared to baseline healthy controls, indicating that LTL decreased more in the MDD group than in the healthy controls. Similar to our baseline paper (Simon et al., 2015), our prospective study of an extensively characterized sample of adults with and without MDD found no significant differences in LTL as measured by qPCR between groups at baseline. However, at two-year follow up, the MDD group had significantly shorter LTL. A range of potential correlates of LTL change were examined in the whole sample, and only age, lifetime alcohol or substance use disorder at baseline, and symptom severity moderated the simple effect of time on relative LTL. The addition of age, sex, and BMI into the model as covariates and moderators of the time effect did not have a significant impact on the effect of baseline MDD diagnoses on relative LTL change over time, and neither did exploratory confounders such as lifetime diagnosis of alcohol or substance use disorders at baseline, baseline ETISR-SF total scores, TEQ total scores at baseline, or TEQ total scores at follow-up. In the MDD group, rates of current depression were relatively low at year 2, with many patients who remitted over the 2 years. Indices of depression severity or duration did not, however, show any significant associations with LTL change within the MDD group. Our data, therefore, support the hypothesis that an association exists between MDD and telomere shortening, and that this relationship is more likely to be a threshold than a linear relationship. That MDD independently predicts telomere erosion, even after careful entry criteria excluding severe obesity (BMI > 35) and other potential confounds as well as adjustment for other potential confounds, suggest the potential for a mechanism that is not accounted for by the potential confounds assessed in our well-characterized sample.

This study builds upon the existing literature on the association between depression and telomere shortening in several ways. Its prospective design controls for the intra-individual variability in telomere length that could not be adequately assessed cross-sectionally. The use of the well-validated, doctorate-level-clinician-administered SCID to diagnose depression supports the clinical reliability of the MDD diagnosis. Further, we used rigorous enrollment criteria, requiring at least five years since the onset of the first major depressive episode as well as a current depressive episode, in order to ensure that MDD was a primary diagnosis of sufficient severity and length to potentially impact telomere length. We also

performed extensive formal characterization of lifetime comorbidities, environmental stressors, and medical illnesses, and recruited a sample with careful selection criteria, limiting potential confounding by factors, such as severe obesity, that may not be sufficiently adjusted for by statistical approaches alone. Our study cannot fully rule out, however, whether the MDD-LTL association might be explained by residual unmeasured confounding across a range of other factors associated with depression that may together have cumulative effects contributing to telomere shortening.

It is unclear why differences in LTL length between the MDD and control groups would not have been present at baseline, but developed over a two-year period. This finding stands in contrast to Verhoeven et al. (2016), who found that differences were present at baseline between those with depressive or anxiety disorders and healthy controls, but that the rate of telomere erosion did not change over a 6-year follow-up. One consideration is that our rigorous study entry criteria developed to limit confounding, including the exclusion of some medical conditions, may have selected an unrepresentative cohort of patients with MDD. For example, enrolled patients were required to be free of antidepressants at study entry in order not to interfere with experimental assays, such as telomerase examined in the baseline sample (Simon et al., 2015), and this may have limited the chronicity and severity of the sample. In line with this explanation is the finding from a prior report on the same cohort showing no difference in inflammatory markers at baseline (Cassano et al., 2017). A hypothesis to explain the lack of a significant finding in our baseline results is that the load of additional depression chronicity during the study period accelerated telomere erosion. However, neither lifetime duration of depression nor duration of depressive symptoms from baseline to follow-up predicted LTL change in the MDD group, suggesting that the chronicity of depression is not linearly associated with LTL erosion. Additionally, it could be hypothesized that the severity of the psychopathology increased from baseline to follow-up in our sample, leading to accelerated cellular aging and LTL shortening. However, a large proportion of the people with MDD at baseline no longer had a current MDD diagnosis at follow-up, average depression and anxiety symptom severity at follow-up was lower than or equal to symptom severity at baseline (Table 1), and neither depression nor anxiety severity predicted LTL change in the MDD group.

Some explanations for our finding of no significant interactions between most of the potential confounders we measured in the groups with and without baseline depression and change over time in LTL can also be posited. First, two years of follow-up may not be sufficient time to allow for the adequate assessment of some of the factors that impact change in LTL. Our finding of a lack of association when shorter prospective time frames are used is in line with what currently exists in the literature. In their two-year study, Hoen et al. (2013) found that anxiety disorders predicted shortened LTL, but that the association was not explained by adverse life events, lifestyle factors, educational level, or antidepressant use. On the other hand, in their twelve-year study, Shalev et al. (2014) found that men with internalizing disorders showed accelerated LTL erosion and that a number of alternative explanatory variables (childhood maltreatment, cigarette smoking, substance use disorders, psychiatric medication use, physical health problems, and socioeconomic status) were all significantly correlated with LTL shortening. Therefore, the effect of these confounds on LTL erosion may be small and require a larger sample size or more than two years to

become statistically detectable using current methods. Our main finding was fairly robust to the addition of a range of potential confounders to our model (Table 3).

The present investigation had several limitations. Although we extensively characterized our sample, it is impossible to be fully comprehensive. The assessment of other potential confounds that could explain the association between MDD and telomere shortening, such as parental age and genetic contributors, may have been missed. In addition, we had a relatively small sample size compared to some other investigations of LTL over time in depression (Hoen et al., 2011; Hoen et al., 2013; Shalev et al., 2014; Verhoeven et al., 2016; Verhoeven et al., 2017). Further, although the baseline and follow-up telomere length qPCR assays were performed in the same laboratory, and all samples were processed in the same batch at each time point, some degree of "batch drift" between the two time points could have occurred and contributed to differences in the measured LTL. Thus, while the same conditions were present for the MDD and control samples each time, relative telomere length at baseline and year 2 could not be compared side-by-side due to the difference in composition between reference samples in the baseline and follow-up assays. This necessitated the transformation of the LTL T/S ratio using z-scores, which has been previously established as a methodology in the literature (Du et al., 2012), but might obscure significant differences across the longitudinal assays. Additionally, the use of qPCR (as opposed to the Southern blot technique) is associated with larger measurement error, which is exacerbated by relatively shorter follow-up periods (Steenstrup et al., 2013). These phenomena, including a relatively low simple correlation of baseline and year 2 LTL (r = 0.14), may explain why LTL in healthy controls appeared to have increased slightly (though significantly) over the two-year study period, as well as some of the differences in associations of predictors with baseline and 2-year LTL (Table 2).

In summary, despite some limitations, our prospective, two-year study of a well-characterized sample with chronic MDD and healthy controls adds to previous literature suggesting that major depression at baseline independently predicts LTL shortening prospectively over time. This finding lends further support to the hypothesis that MDD is a disease of accelerated aging. It is of note, however, that we were unable to include anxiety or perceived stress into our model due to their very high correlation with depression. Depression may be a marker for higher levels of anxiety, stress, and a range of potentially overlapping psychological states (and potentially health-related behaviors and physiological responses) that may together contribute to cumulative effects on telomere erosion. Future research might examine cross-diagnostic mechanisms by which mood, anxiety, and stress together may accelerate telomere shortening, as well as potential immune and neurobiological mediators and their change with biological and psychological depression treatments.

Acknowledgments

This study was funded by grant from the National Institute of Mental Health (R01MH077700) to NMS at Massachusetts General Hospital, with additional support from the Highland Street Foundation. The funding sponsor was not involved in study design; in the collection, analysis and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

References

Aubert G, Lansdorp PM. Telomeres and aging. Physiol. Rev. 2008; 88:557–579. https://doi.org/10.1152/physrev.00026.2007. [PubMed: 18391173]

Aviv A, Valdes AM, Spector TD. Human telomere biology: pitfalls of moving from the laboratory to epidemiology. International journal of epidemiology. 2006; 35:1424–1429. [PubMed: 16997848]

Blackburn EH. Telomeres and telomerase: the means to the end (Nobel lecture). Angewandte Chemie. 2010; 49:7405–7421. [PubMed: 20821774]

Bremner JD, Bolus R, Mayer EA. Psychometric properties of the Early Trauma Inventory-Self Report. J Nerv Ment Dis. 2007; 195:211–218. [PubMed: 17468680]

Cassano P, Bui E, Rogers AH, Walton ZE, Ross R, Zeng M, Nadal-Vicens M, Mischoulon D, Baker AW, Keshaviah A, Worthington J, Hoge EA, Alpert J, Fava M, Wong KK, Simon NM. Inflammatory cytokines in major depressive disorder: A case-control study. Aust N Z J Psychiatry. 2017; 51:23–31. [PubMed: 27313138]

Carney RM, Freedland KE. Depression and coronary heart disease. Nat Rev Cardiol. 2017; 14:145–155. [PubMed: 27853162]

Cawthon RM. Telomere measurement by quantitative PCR. Nucleic acids research. 2002; 30:e47. [PubMed: 12000852]

Cawthon RM. Telomere length measurement by a novel monochrome multiplex quantitative PCR method. Nucleic acids research. 2009; 37:e21. [PubMed: 19129229]

Chrousos GP. Stressors, stress, and neuroendocrine integration of the adaptive response. The 1997 Hans Selye Memorial Lecture. Ann N Y Acad Sci. 1998; 851:311–335. [PubMed: 9668623]

Cohen, JE. Statistical Power Analysis for the Behavioral Sciences. Hillsdale, New Jersey: 1988.

Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. J Health Soc Behav. 1983; 24:385–396. [PubMed: 6668417]

Cosci F, Fava GA, Sonino N. Mood and Anxiety Disorders as Early Manifestations of Medical Illness: A Systematic Review. Psychotherapy and Psychosomatics. 2015; 84:22–29. [PubMed: 25547421]

Darrow SM, Verhoeven JE, Révész D, Lindqvist D, Penninx BWJH, Delucchi KL, Wolkowitz OM, Mathews CA. The association between psychiatric disorders and telomere length: a meta-analysis involving 14,827 persons. Psychosom. Med. 2016; 78:776–787. https://doi.org/10.1097/PSY.0000000000000356. [PubMed: 27359174]

Du M, Prescott J, Kraft P, Han J, Giovannucci E, Hankinson SE, De Vivo I. Physical Activity, Sedentary Behavior, and Leukocyte Telomere Length in Women. American Journal of Epidemiology. 2012; 175:414–422. [PubMed: 22302075]

Feingold A. Effect sizes for growth-modeling analysis for controlled clinical trials in the same metric as for classical analysis. Psychol. Methods. 2009; 14:43–53. https://doi.org/10.1037/a0014699. [PubMed: 19271847]

First, MB., Spitzer, RL., Gibbon, M., Williams, JBW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition. (SCID-I/P). New York State Psychiatric Institute Biometrics Research Department; New York: 2002.

Gardner M, Bann D, Wiley L, Cooper R, Hardy R, Nitsch D, Martin-Ruiz C, Shiels P, Sayer AA, Barbieri M, Bekaert S, Bischoff C, Brooks-Wilson A, Chen W, Cooper C, Christensen K, De Meyer T, Deary I, Der G, Diez Roux A, Fitzpatrick A, Hajat A, Halaschek-Wiener J, Harris S, Hunt SC, Jagger C, Jeon H-S, Kaplan R, Kimura M, Lansdorp P, Li C, Maeda T, Mangino M, Nawrot TS, Nilsson P, Nordfjall K, Paolisso G, Ren F, Riabowol K, Robertson T, Roos G, Staessen JA, Spector T, Tang N, Unryn B, van der Harst P, Woo J, Xing C, Yadegarfar ME, Park JY, Young N, Kuh D, von Zglinicki T, Ben-Shlomo Y. Halcyon study team. Gender and telomere length: systematic review and meta-analysis. Exp. Gerontol. 2014; 51:15–27. https://doi.org/10.1016/j.exger.2013.12.004. [PubMed: 24365661]

Hamilton M. The assessment of anxiety states by rating. The British journal of medical psychology. 1959; 32:50–55. [PubMed: 13638508]

Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. Biol. Psychiatry. 2001; 49:1023–1039. [PubMed: 11430844]

Hoen PW, de Jonge P, Na BY, Farzaneh-Far R, Epel E, Lin J, Blackburn E, Whooley MA. Depression and leukocyte telomere length in patients with coronary heart disease: data from the Heart and Soul Study. Psychosomatic medicine. 2011; 73:541–547. [PubMed: 21597035]

Hoen PW, Rosmalen JG, Schoevers RA, Huzen J, van der Harst P, de Jonge P. Association between anxiety but not depressive disorders and leukocyte telomere length after 2 years of follow-up in a population-based sample. Psychological medicine. 2013; 43:689–697. [PubMed: 22877856]

Hovatta I. Genetics: Dynamic Cellular Aging Markers Associated with Major Depression. Current Biology. 2015; 25:R409–R411. [PubMed: 25989078]

Kong CM, Lee XW, Wang X. Telomere shortening in human diseases. The FEBS journal. 2013; 280:3180–3193. [PubMed: 23647631]

Kop WJ, Stein PK, Tracy RP, Barzilay JI, Schulz R, Gottdiener JS. Autonomic nervous system dysfunction and inflammation contribute to the increased cardiovascular mortality risk associated with depression. Psychosomatic medicine. 2010; 72:626–635. [PubMed: 20639389]

Lin P-Y, Huang Y-C, Hung C-F. Shortened telomere length in patients with depression: A meta-analytic study. J. Psychiatr. Res. 2016; 76:84–93. https://doi.org/10.1016/j.jpsychires.2016.01.015. [PubMed: 26919486]

Lindqvist D, Epel ES, Mellon SH, Penninx BW, Revesz D, Verhoeven JE, Reus VI, Lin J, Mahan L, Hough CM, Rosser R, Bersani FS, Blackburn EH, Wolkowitz OM. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neuroscience and biobehavioral reviews. 2015; 55:333–364. [PubMed: 25999120]

Maes M, Mihaylova I, Kubera M, Leunis JC, Geffard M. IgM-mediated autoimmune responses directed against multiple neoepitopes in depression: new pathways that underpin the inflammatory and neuroprogressive pathophysiology. Journal of affective disorders. 2011; 135:414–418. [PubMed: 21930301]

Michalek JE, Kepa A, Vincent J, Frissa S, Goodwin L, Hotopf M, Hatch SL, Breen G, Powell TR. Genetic predisposition to advanced biological ageing increases risk for childhood-onset recurrent major depressive disorder in a large UK sample. Journal of Affective Disorders. 2017; 213:207–213. [PubMed: 28233563]

Montgomery SA, Asberg M. A new depression scale designed to be sensitive to change. Br J Psychiatry. 1979; 134:382–389. [PubMed: 444788]

Müezzinler A, Zaineddin AK, Brenner H. Body mass index and leukocyte telomere length in adults: a systematic review and meta-analysis. Obes. Rev. Off. J. Int. Assoc. Study Obes. 2014; 15:192–201. https://doi.org/10.1111/obr.12126.

O'Donovan A, Epel E, Lin J, Wolkowitz O, Cohen B, Maguen S, Metzler T, Lenoci M, Blackburn E, Neylan TC. Childhood trauma associated with short leukocyte telomere length in post-traumatic stress disorder. Biol. Psychiatry. 2011; 70:465–471. https://doi.org/10.1016/j.biopsych.2011.01.035. [PubMed: 21489410]

Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatry. 2001; 49:391–404. [PubMed: 11274650]

Penninx BW, Guralnik JM, Pahor M, Ferrucci L, Cerhan JR, Wallace RB, Havlik RJ. Chronically depressed mood and cancer risk in older persons. J Natl Cancer Inst. 1998; 90:1888–1893. [PubMed: 9862626]

Price LH, Kao HT, Burgers DE, Carpenter LL, Tyrka AR. Telomeres and early-life stress: an overview. Biol Psychiatry. 2013; 73:15–23. [PubMed: 22831981]

Raison CL, Miller AH. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am J Psychiatry. 2003; 160:1554–1565. [PubMed: 12944327]

Rosner B. Percentage points for a generalized ESD many-outlier procedure. Technometrics. 1983; 25:165–172.

Ridout KK, Ridout SJ, Price LH, Sen S, Tyrka AR. Depression and telomere length: A meta-analysis. Journal of affective disorders. 2016; 191:237–247. [PubMed: 26688493]

Rius-Ottenheim N, Houben JM, Kromhout D, Kafatos A, van der Mast RC, Zitman FG, Geleijnse JM, Hageman GJ, Giltay EJ. Telomere length and mental well-being in elderly men from the Netherlands and Greece. Behavior genetics. 2012; 42:278–286. [PubMed: 21870178]

Schutte NS, Malouff JM. The Association Between Depression And Leukocyte Telomere Length: A Meta-analysis. Depress Anxiety. 2015

Selya AS, Rose JS, Dierker LC, Hedeker D, Mermelstein RJ. A Practical Guide to Calculating Cohen's f2, a Measure of Local Effect Size, from PROC MIXED. Frontiers in Psycholology. 2012; 3:111.

Shalev I, Moffitt TE, Braithwaite AW, Danese A, Fleming NI, Goldman-Mellor S, Harrington HL, Houts RM, Israel S, Poulton R, Robertson SP, Sugden K, Williams B, Caspi A. Internalizing disorders and leukocyte telomere erosion: a prospective study of depression, generalized anxiety disorder and post-traumatic stress disorder. Mol Psychiatry. 2014; 19:1163–1170. [PubMed: 24419039]

Simon NM, Smoller JW, McNamara KL, Maser RS, Zalta AK, Pollack MH, Nierenberg AA, Fava M, Wong KK. Telomere shortening and mood disorders: preliminary support for a chronic stress model of accelerated aging. Biol Psychiatry. 2006; 60:432–435. [PubMed: 16581033]

Simon NM, Walton ZE, Bui E, Prescott J, Hoge E, Keshaviah A, Schwarz N, Dryman T, Ojserkis RA, Kovachy B, Mischoulon D, Worthington J, DeVivo I, Fava M, Wong KK. Telomere length and telomerase in a well-characterized sample of individuals with major depressive disorder compared to controls. Psychoneuroendocrinology. 2015; 58:9–22. [PubMed: 25932992]

Steenstrup T, Hjelmborg JvB, Kark JD, Christensen K, Aviv A. The telomere lengthening conundrum—artifact or biology? Nucleic Acids Research. 2013; 41:e131–e131. [PubMed: 23671336]

Takubo K, Izumiyama-Shimomura N, Honma N, Sawabe M, Arai T, Kato M, Oshimura M, Nakamura K. Telomere lengths are characteristic in each human individual. Experimental gerontology. 2002; 37:523–531. [PubMed: 11830355]

Verhoeven JE, Revesz D, Picard M, Epel EE, Wolkowitz OM, Matthews KA, Penninx BWJH, Puterman E. Depression, telomeres and mitochondrial DNA: between- and within-person associations from a 10-year longitudinal study. Mol Psychiatry. 2017

Verhoeven JE, van Oppen P, Revesz D, Wolkowitz OM, Penninx BW. Depressive and Anxiety Disorders Showing Robust, but Non-Dynamic, 6-Year Longitudinal Association With Short Leukocyte Telomere Length. Am J Psychiatry. 2016 appiajp201515070887.

Vrana S, Lauterbach D. Prevalence of traumatic events and post-traumatic psychological symptoms in a nonclinical sample of college students. J Trauma Stress. 1994; 7:289–302. [PubMed: 8012748]

Wang H, Chen H, Gao X, McGrath M, Deer D, De Vivo I, Schwarzschild MA, Ascherio A. Telomere length and risk of Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society. 2008; 23:302–305. [PubMed: 18044760]

Wolkowitz OM, Reus VI, Mellon SH. Of sound mind and body: depression, disease, and accelerated aging. Dialogues in clinical neuroscience. 2011; 13:25–39. [PubMed: 21485744]

Zhang L, Hu X-Z, Li X, Li H, Smerin S, Russell D, Ursano RJ. Telomere length – A cellular aging marker for depression and Post-traumatic Stress Disorder. Medical Hypotheses. 2014; 83:182–185. [PubMed: 24875221]

Highlights

- A baseline diagnosis of MDD prospectively predicted LTL shortening over two years.
- Hierarchical regression analyses found this MDD-telomere effect versus controls.
- These well-characterized data provide further support for MDD and accelerated aging.

Table 1

Demographic and clinical characteristics of *N*=117 healthy controls and individuals with MDD at baseline.

	Baseline Healthy Controls (n=67†)		**Baseline MDD (n=50†)**		***P*^**
Baseline Demographic Characteristics					
Age in years, mean *(SD)*	44.1	(14.0)	42.7	(13.2)	
Sex, *n* (%) female	39	(58%)	26	(52%)	
Race, *n* (%) white	49	(73%)	41	(82%)	
Ethnicity, *n* (%) Hispanic/Latino	2	(3%)	2	(4%)	
Highest educational level, *n* (%)					
Graduate school	22	(33%)	9	(18%)	
College graduate	28	(42%)	20	(40%)	
Partial college	13	(19%)	14	(28%)	
High school graduate or lower	4	(6%)	7	(14%)	
Marital status, *n* (%) living with partner/married	31	(46%)	9	(18%)	**
Baseline Clinical Characteristics					
MADRS total score, mean *(SD)*	2.4	(2.9)	30.0	(5.6)	***
HAM-A total score, mean *(SD)*	3.2	(2.3)	18.0	(4.7)	***
PSS total score, mean (*SD*) over past month	8.2	(5.9)	25.6	(5.9)	***
ETISR-SF total score, mean (*SD*)	2.7	(2.8)	7.6	(5.3)	***
TEQ total score, mean (*SD*)	1.1	(1.4)	2.3	(2.2)	**
MDD duration total months, mean (*SD*)	n/a		128.9	(122.5)	
Antidepressant use >6 months, *n* (%)	n/a		25	(52%)	
Lifetime alcohol or substance use disorder, *n* (%)	7	(10%)	11	(22%)	
Cigarette smoking pack-years, mean (*SD*)	8.0	(17.3)	6.8	(12.5)	
BMI, mean (*SD*)	24.3	(3.8)	26.0	(4.7)	*
Past year exercise level, *n* (%) more than once a week	61	(91%)	30	(63%)	***
Δ Clinical Characteristics from Baseline to Follow-up					
TEQ (total score, based on past two years), mean (*SD*)	0.2	(0.5)	0.8	(1.2)	**
MDD duration total months (BL to FU), mean (*SD*)	0.4	(2.6)	11.8	(7.6)	***

	Baseline Healthy Controls (n=67†)		Baseline MDD (n=50†)		p^
Antidepressant use >6 months (BL to FU), *n* (%)	0	(0)	15	(37%)	***
Onset of first alcohol or substance use disorder, *n* (%)	0	(0)	7	(15%)	**
Cigarette smoking pack-years (BL to FU), mean (*SD*)	<0.1	(<0.1)	0.2	(0.5)	*
Clinical Characteristics at Follow-up					
Current MDD diagnosis, *n* (%)	1	(2%)	14	(30%)	***
MADRS total score, mean (*SD*)	2.9	(6.1)	16.2	(12.0)	***
HAM-A total score, mean (*SD*)	4.1	(4.9)	15.0	(8.8)	***
PSS total score, mean (*SD*)	9.0	(7.0)	21.3	(6.0)	***
BMI, mean (*SD*)	24.0	(3.8)	26.5	(5.0)	**
Exercised more than once a week, past year, *n* (%)	44	(86%)	34	(77%)	
Biological Outcome					
Baseline zRTL, mean (*SD*)	−0.010	(0.953)	0.013	(1.070)	
Follow-up zRTL, mean (*SD*)	0.223	(0.956)	−0.299	(0.988)	**
Δ zRTL (BL to FU), mean (*SD*)	0.233	(1.323)	−0.313	(1.240)	*

Key: MDD = major depressive disorder, MADRS = Montgomery-Åsberg Depression Rating Scale (range: 0–60, where higher scores indicate greater depression severity), HAM-A = Hamilton Anxiety Rating Scale (range: 0–56, where higher scores indicate greater severity of anxiety), PSS = Perceived Stress Scale (range: 0–40, where higher scores indicate higher perceived stress), ETISR-SF = Early Trauma Inventory Self Report-Short Form, TEQ = Traumatic Events Questionnaire, BMI = body mass index, zRTL = z-score of relative telomere length;

† = across measures, due to small amounts of missing data, sample sizes varied from *n* of 41 to 50 for the baseline MDD sample and from *n* of 51 to 67 for the control sample;

^ = significance of t-test for continuous variables and Fisher's Exact Test for categorical variables,

* where = $p < 0.05$,

** = $p < 0.01$,

*** = $p < 0.001$.

Table 2

Pearson and point-biserial correlation coefficients between variables potentially confounded with depression and mean relative LTL at baseline and follow-up in all study participants (*N* range: 87–116).

Potential predictors	Relative LTL at baseline	Relative LTL at follow-up	
Variables assessed at baseline			
Age, years	−0.07	−0.29	**
Sex, male, y/n	−0.12	−0.11	
Living with partner/married, y/n	−0.01	0.03	
MADRS total score	0.05	−0.21	*
HAM-A total score	0.04	−0.21	*
PSS total score	−0.03	−0.15	
Lifetime MDD duration, months	−0.02	−0.18	
Lifetime antidepressant use, > 6 months, y/n	0.01	−0.18	
ETISR-SF total score	−0.04	−0.09	
TEQ total score	0.00	−0.07	
Lifetime alcohol or substance use disorder, y/n	−0.02	−0.24	*
Lifetime smoking, pack-years	−0.04	−0.16	
BMI	−0.08	−0.25	**
Exercised more than once a week, past year, y/n	−0.02	0.04	
Variables assessed at follow-up			
MADRS total score	n/a	−0.28	**
HAM-A total score	n/a	−0.24	*
PSS total score	n/a	−0.22	*
Lifetime MDD duration, months	n/a	−0.20	*
Lifetime antidepressant use > 6 months, y/n	n/a	−0.23	*
TEQ total score	n/a	−0.03	
Lifetime alcohol or substance use disorder, y/n	n/a	−0.21	*
Lifetime smoking, pack-years	n/a	−0.13	
BMI	n/a	−0.23	*
Exercised more than once a week, past year, y/n	n/a	0.02	

Key:

* $p < 0.05$,

** $p < 0.01$.

MDD = major depressive disorder, MADRS = Montgomery–Åsberg Depression Rating Scale (range: 0–60, where higher scores indicate greater depression severity), HAM-A = Hamilton Anxiety Rating Scale (range: 0–56, where higher scores indicate greater severity of anxiety), PSS = Perceived Stress Scale (range: 0–40, where higher scores indicate higher perceived stress), ETISR-SF = Early Trauma Inventory Self Report-Short Form, TEQ = Traumatic Events Questionnaire, BMI = body mass index, y/n = yes/no.

Table 3

Tests of the effects of potential predictors of relative LTL as baseline covariates or moderators of the effect of time in extended and exploratory mixed models of relative LTL over time, in people with and without MDD diagnoses at baseline.

Model	Effect	Num DF	Den DF	F Value	Pr > F
Model 1: Simple					
	Baseline MDD (MDD_0)	1	115	3.19	0.08
	Time	1	115	0.11	0.74
	Time*MDD_0	**1**	**115**	**5.14**	**0.03**
Model 2: Adjusted by age, gender, and baseline BMI					
	Baseline MDD (MDD_0)	1	112	2.56	0.11
	Time	1	112	2.22	0.14
	Time*MDD_0	**1**	**112**	**4.90**	**0.03**
	Gender	1	112	0.83	0.36
	Time*Gender	1	112	0.34	0.56
	Age	1	112	5.17	0.02
	Time*Age	1	112	3.14	0.08
	Baseline BMI (BMI_0)	1	112	1.54	0.22
	Time*BMI_0	1	112	0.45	0.50
Model 3: Adjusted by age, gender, baseline BMI, and lifetime alcohol/substance use disorder at baseline					
	Baseline MDD (MDD_0)	1	110	2.05	0.15
	Time	1	110	0.94	0.34
	Time*MDD_0	**1**	**110**	**3.82**	**0.05**
	Gender	1	110	0.55	0.46
	Time*Gender	1	110	0.51	0.48
	Age	1	110	4.64	0.03
	Time*Age	1	110	2.58	0.11
	Baseline BMI (BMI_0)	1	110	1.19	0.28
	Time*BMI_0	1	110	0.19	0.66
	Baseline alcohol/substance use disorder, lifetime (alc_drug_0)	1	110	0.81	0.37

Model	Effect	Num DF	Den DF	F Value	Pr > F
	Time*alc_drug_0	1	110	1.56	0.21
Model 4: Adjusted by age, gender, baseline BMI, and baseline ETISR-SF total scores					
	Baseline MDD (MDD_0)	1	106	1.48	0.23
	Time	1	106	1.81	0.18
	Time*MDD_0	**1**	**106**	**3.79**	**0.05**
	Gender	1	106	0.63	0.43
	Time*Gender	1	106	0.52	0.47
	Age	1	106	4.39	0.04
	Time*Age	1	106	2.19	0.14
	Baseline BMI (BMI_0)	1	106	1.94	0.17
	Time*BMI_0	1	106	0.59	0.44
	Baseline ETISR-SF total score (ETISRSF_0)	1	106	0.02	0.90
	Time*ETISRSF_0	1	106	0.32	0.57
Model 5: Adjusted by age, gender, baseline BMI, and TEQ total scores at baseline					
	Baseline MDD (MDD_0)	1	110	2.36	0.13
	Time	1	110	2.66	0.11
	Time*MDD_0	**1**	**110**	**4.12**	**0.04**
	Gender	1	110	0.85	0.36
	Time*Gender	1	110	0.28	0.60
	Age	1	110	5.09	0.03
	Time*Age	1	110	3.02	0.09
	Baseline BMI (BMI_0)	1	110	1.81	0.18
	Time*BMI_0	1	110	0.77	0.38
	Baseline TEQ total score (bl_TEQ)	1	110	0.21	0.65
	Time*bl_TEQ	1	110	0.19	0.67
Model 6: Adjusted by age, gender, baseline BMI, and TEQ total scores at follow-up					
	Baseline MDD (MDD_0)	1	98	2.49	0.12
	Time	1	98	1.83	0.18
	Time*MDD_0	**1**	**98**	**7.20**	**0.01**

Model	Effect	Num DF	Den DF	F Value	Pr > F
	Gender	1	98	0.67	0.41
	Time*Gender	1	98	1.04	0.31
	Age	1	98	5.02	0.03
	Time*Age	1	98	3.74	0.06
	Baseline BMI (BMI_0)	1	98	0.77	0.38
	Time*BMI_0	1	98	0.24	0.63
	Follow-up TEQ total score (fu_TEQ)	1	98	0.04	0.83
	Time*fu_TEQ	1	98	0.45	0.50
Model 7: Adjusted by age, gender, baseline BMI, baseline ETISR-SF total scores, and TEQ total scores at baseline and follow-up					
	Baseline MDD (MDD_0)	1	91	1.63	0.20
	Time	1	91	1.75	0.19
	Time*MDD_0	**1**	**91**	**5.21**	**0.02**
	Gender	1	91	0.54	0.46
	Time*Gender	1	91	0.99	0.32
	Age	1	91	4.16	0.04
	Time*Age	1	91	1.82	0.18
	Baseline BMI (BMI_0)	1	91	1.31	0.25
	Time*BMI_0	1	91	0.62	0.43
	Baseline ETISR-SF total score (ETISRSF_0)	1	91	0.04	0.84
	Time*ETISRSF_0	1	91	0.41	0.52
	Baseline TEQ total score (bl_TEQ)	1	91	0.40	0.53
	Time*bl_TEQ	1	91	0.16	0.69
	Follow-up TEQ total score (fu_TEQ)	1	93	0.01	0.92
	Time*fu_TEQ	1	93	0.71	0.40

Note: The main effect of interest is the time by MDD interaction. Each model assesses whether the inclusion of potential confounders influences this MDD effect on LTL. Abbreviations: ETISR-SF = Early Trauma Inventory Self Report-Short Form, TEQ = Traumatic Events Questionnaire, BMI = body mass index.