Alterations in circadian rhythms are associated with increased lipid peroxidation in females with bipolar disorder

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Abstract

Disturbances in both circadian rhythms and oxidative stress systems have been implicated in the pathophysiology of bipolar disorder (BD), yet no studies have investigated the relationship between these systems in BD. We studied the impact of circadian rhythm disruption on lipid damage in 52 depressed or euthymic BD females, while controlling for age, severity of depressive symptoms and number of psychotropic medications, compared to 30 healthy controls. Circadian rhythm disruption was determined by a self-report measure (Biological Rhythm Interview of Assessment in Neuropsychiatry; BRIAN), which measures behaviours such as sleep, eating patterns, social rhythms and general activity. Malondialdehyde (MDA) levels were measured as a proxy of lipid peroxidation. We also measured the activity of total and extracellular superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST). Multiple linear regressions showed that circadian rhythm disturbance was independently associated with increased lipid peroxidation in females with BD (p<0.05). We found decreased extracellular SOD (p<0.05), but no differences in total SOD, CAT or GST activity between bipolar females and controls. Circadian rhythms were not associated with lipid peroxidation in healthy controls, where aging was the only significant predictor. These results suggest an interaction between the circadian system and redox metabolism, in that greater disruption in daily rhythms was associated with increased lipid peroxidation in BD only. Antioxidant enzymes have been shown to follow a circadian pattern of expression, and it is possible that disturbance of sleep and daily rhythms experienced in BD may result in decreased antioxidant defence and therefore increased lipid peroxidation. This study provides a basis for further investigation of the links between oxidative stress and circadian rhythms in the neurobiology of BD.

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Key words: Bipolar disorder, circadian rhythms, oxidative stress.

Introduction

Bipolar Disorder (BD) is a chronic illness consisting of episodes of mania and depression, affecting nearly four per cent of the population (Merikangas et al., 2007). Abnormalities in circadian rhythms, such as sleep, daily activity, social rhythms and eating behaviour are commonly observed not only during mood episodes but also during periods of euthymia (Harvey et al., 2005). There is a growing body of evidence suggesting complex associations between BD and circadian activity (McClung, 2013). Disruptions in the sleep/wake cycle are well known triggers of affective episodes in patients with BD (Proudfoot et al., 2011), and indeed one of the most effective psychotherapy interventions in BD targets the maintenance of stable biological rhythms as one of its core goals (Frank et al., 2007). In fact, virtually all treatments for mood disorders have an influence on circadian rhythms, in particular chronotherapeutics such as light therapy, sleep deprivation and sleep phase advancement (Wehr et al., 1979; Benedetti, 2012). From a molecular perspective, there are several lines of evidence supporting that circadian rhythms are sensitive to disruption in BD patients, such as abnormalities in circadian genes and circadian endocrine markers (Milhiet et al., 2011; McClung, 2013).

Circadian rhythm disruption has been shown to have negative consequences in numerous biological systems
in healthy subjects, in particular; immune, inflammatory and oxidative stress systems (Faraut et al., 2012). Multiple mechanisms have been proposed to explain the correlation between sleep and oxidative stress, including sleep-related changes in transcriptional responses of genes involved in oxidative stress in peripheral tissues (Anafi et al., 2013). The endogenous timekeeper in mammals is the suprachiasmatic nucleus (SCN) in the hypothalamus, which regulates biological rhythms of endocrine secretion, body temperature, sleep/wake cycles and other behaviours including cognition (Kyriacou and Hastings, 2010) in a period close to twenty-four hours (Reppert and Weaver, 2002). Almost all peripheral tissues exhibit circadian oscillations that are synchronized by the SCN (Cermakian and Boivin, 2009). The molecular mechanisms of circadian rhythm are tightly linked with transcription – translation feedback loops of circadian genes such as CLOCK and BMAL1 in the SCN, which activate transcription of other regulatory circadian genes (Reppert and Weaver, 2002). Notably, many of these genes (e.g. PER, CRY, REV-ERBα and GSK3β) have been considered among the top candidate genes for BD and some have been implicated in treatment response (Etain et al., 2011). Animal models of mania also implicate circadian rhythms and sleep disruptions in BD, in that CLOCK mutations and sleep-deprived mice demonstrate a manic-like behavioural profile (McClung, 2007).

Melatonin is the primary circadian signalling molecule, which has increased secretion in the dark and is inhibited in the light (Nölte et al., 2009). Several studies suggest BD patients have irregular melatonin secretion. For example, the inhibition of melatonin synthesis in light has been shown to be impaired in BD patients compared to controls (Nathan et al., 1999). In addition, lower nocturnal melatonin levels have been observed during depression and euthymia (Kennedy et al., 1996). A recent study showed that patients with depression have an increased number of melatonin receptors in the SCN (Wu et al., 2013). An increase in melatonin receptors may be a compensatory mechanism for the reduced melatonin levels in patients with mood disorders, and these receptors have been recently suggested as targets of novel antidepressant agents (Fornaro et al., 2013).

Besides its role in circadian rhythm control, melatonin has antioxidant properties, acting as an electron donor in scavenging free radicals to protect against oxidative damage to lipids, proteins and DNA (Reiter et al., 1995). Oxidative damage occurs when there is a disturbance in the oxidant–antioxidant balance, as a result of an overproduction of reactive oxygen species (ROS) and/or insufficient antioxidant defence (Halilwali, 2012). Melatonin is more effective at neutralising ROS than other intracellular antioxidants such as glutathione (GSH) and also stimulates antioxidant enzyme activity (Reiter et al., 1995; Wang et al., 2013). Evidence has shown that oxidative stress may be important in the pathophysiology of BD, particularly with respect to lipid peroxidation. A meta-analysis of studies on peripheral markers of oxidative stress showed that lipid peroxidation was significantly increased in BD (Andreazza et al., 2008), as indicated by increased thiobarbituric acid reactive substances (TBARS) (Draper and Hadley, 1990). Lipid peroxidation was found to be increased across all mood states, and has been considered a trait marker of BD (Andreazza et al., 2008). Several recent studies also show an increase in end products of lipid peroxidation in BD peripheral blood (Versace et al., 2013) and in post-mortem brain tissue in BD (Andreazza et al., 2013). Together, these results suggest an imbalance toward a pro-oxidant state in BD, however, the factors leading to an altered redox metabolism remain unknown.

The above-mentioned studies indicate there are disturbances in both circadian rhythms and oxidative stress systems in BD, yet it remains unclear whether there is any relationship between these systems within the disorder. Thus, the aim of the present study is to determine whether disruptions in circadian rhythms have an impact on levels of lipid peroxidation or in antioxidant enzymes in depressed and euthymic subjects with BD, as compared to matched controls. Sex differences have been reported in a number of circadian rhythm measures (Mong et al., 2011). For instance, women display different timings of sleep from childhood until menopause (Roenneberg et al., 2007), phase-advanced endogenous temperature and melatonin rhythms (Cain et al., 2010) and shorter circadian period as compared to men (Duffy et al., 2011). Animal models also suggest sex differences in the circadian rhythms of activity, neuronal physiology, and gene expression (Kuljis et al., 2013). Therefore, we have restricted this initial study to the female population. Other variables that may impact oxidative stress levels such as age, severity of depression and psychotropic medications in BD were also investigated. We hypothesized that circadian rhythm disturbances would negatively affect lipid peroxidation levels in individuals with BD.

Method

Participants and study design

Fifty-two females with BD (37 BD Type I and 15 BD Type II) and 30 age-matched healthy controls were recruited from the Mood Disorders Program and the Women’s Health Concerns Clinic, St. Joseph’s Healthcare Hamilton, Ontario. All subjects gave written informed consent to take part in the study, as approved by the ethics committees of St. Joseph’s Healthcare Hamilton and Hamilton Health Sciences. The diagnosis of BD was confirmed with the Structured Clinical Interview for the DSM-IV (SCID-I). Patients with BD were included in the study if they either met criteria for a current major depressive episode (n=44) or if they did not meet criteria for any current mood episode (n=32).
according to the SCID-I. Participants were excluded if they met criteria for a hypomanic, manic or mixed episode. Control participants were excluded if they met criteria for current or lifetime history of any psychiatric illness according to the SCID-I.

Severity of depressive symptoms was measured with the Montgomery-Åsberg Depression Rating Scale (MADRS). Circadian rhythms were measured with the Biological Rhythm Interview of Assessment in Neuropsychiatry (BRIAN), a self-report questionnaire composed of 18-items measuring sleep, general activities, social rhythm and eating behaviour scored from 1 (no difficulties) to 4 (serious difficulties), with greater scores indicating greater circadian rhythm disruption. This scale has been validated in BD subjects in its ability to discriminate euthymic BD and controls (Giglio et al., 2009). Psychiatric medications were recorded for each participant and are listed in Table 1. Psychotropic medications such as mood stabilizers, antidepressants, antipsychotics and anxiolytic medications were included in the total number of medications participants were taking.

Laboratory assays
Participants provided blood samples collected by venipuncture. We obtained serum by centrifugation at 3000 g for 15 min and kept samples frozen at −80 °C until biochemical assays were performed. Malondialdehyde (MDA) levels were obtained as a measure of lipid peroxidation, with higher MDA levels representing greater lipid oxidative damage. Specifically, lipid peroxidation was measured via colorimetric detection of the malondialdehyde-thiobarbituric (MDA-TBA) adduct with TBARS assay kit (Cayman Chem, USA). Plates were read at the kit specified wavelength of 535 nm using an automated reader (Spectra Max Plus 384, Molecular Devices, Plate Reader).

The activity of two key antioxidant enzymes was analysed in whole blood: catalase (CAT) and total superoxide dismutase (SOD). Catalase (EC 1.11.1.6; CAT) activity was assayed by measuring the rate of decrease in hydrogen peroxide (H2O2) absorbance in a spectrophotometer at 240 nm (Aebi, 1984) and superoxide dismutase (EC 1.15.1.1, SOD) activity was assessed by quantifying the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer at 480 nm (Misra and Fridovich, 1972). We also analysed extracellular SOD (EC-SOD) in serum samples by quantifying the inhibition of superoxide-dependent adrenaline auto-oxidation in a spectrophotometer at 480 nm (Misra and Fridovich, 1972).

Glutathione S-transferase (GST, E.C. 2.5.1.18) activity was determined spectrophotometrically at 340 nm by measuring the formation of the conjugate of GSH (glutathione) and CDNB (chloro-dinitro benzene) as previously described (Habig and Jakoby, 1981). Enzyme activity was determined by adding GSH 20 mM to a buffer and the sample. The reaction started by the addition of CDNB 20 mM was carried out at 30 °C, and monitored spectrophotometrically for 3 min. Corrections of the spontaneous reaction were made by measuring and subtracting the rate in the absence of enzyme.

Statistical analyses
All analyses were performed with R (Version 2.14.2, R Development Core Team, 2012). In the BD sample,
multiple linear regression analysis was performed using BRIAN, MADRS, age and number of psychiatric medications as predictors, and MDA as the dependent variable. A multiple linear regression was also performed in the healthy control sample with only age and BRIAN as predictors and MDA as the dependent variable, since depression severity and psychiatric medications were not relevant to this population. Differences in MDA, total SOD, EC-SOD, CAT and GST levels between bipolar subjects and healthy controls were tested with independent t-tests. Because we found lower levels of EC-SOD in bipolar subjects as compared to healthy controls, we have added EC-SOD as a predictor in the multiple linear regression models.

Assumptions of linear regression were tested with the Shapiro-Wilk test (normality), a partial residuals plot (linearity), Durbin-Watson test (independence of errors), non-constant error variance (heteroscedasticity) and variance inflation factor test (multicollinearity). MDA and GST levels were square root transformed to lead to a normal distribution. All regression models met all of the regression assumptions. A p value of <0.05 was used to indicate statistical significance.

**Results**

Demographic and clinical data for BD and control subjects are displayed in Table 1. As expected, MADRS and BRIAN scores were significantly higher in BD patients than controls. We did not find differences in MDA, total SOD, CAT or GST levels between bipolar subjects and controls (all \( p \geq 0.05 \)). Bipolar subjects had lower EC-SOD levels than controls (\( p < 0.05 \)). In the BD group, higher levels of lipid oxidative damage were correlated with increased circadian rhythm disruption (BRIAN, \( r_p = 0.33, \text{ CI} [0.06, 0.56], p < 0.05 \)) and greater number of psychiatric medications (\( r_p = 0.29, \text{ CI} [0.02, 0.52], p < 0.05 \)), but were not related to depression severity or age.

In the multiple linear regression model, circadian rhythms disruption (\( \beta = 0.46, t = 2.56, p < 0.05 \)) and number of psychiatric medications (\( \beta = 0.28, t = 2.10, p < 0.05 \)) were independent predictors of lipid damage in the BD sample (\( F_{4,47} = 3.54; p < 0.05, \text{ Table 2} \)). This relationship between MDA levels and circadian rhythms disruption and number of psychiatric medications was independent of EC-SOD (\( F_{5,46} = 2.95; p < 0.05, \text{ Table 3} \)). In order to investigate whether these results were due to circadian fluctuations of MDA levels, we correlated MDA with time of blood draw in the BD sample and found no significant relationship (\( r_p = -0.03, \text{ CI} [-0.26, 0.29], p = 0.92 \)). None of the antioxidant enzymes measured in this study were correlated with time of blood collection (all \( p > 0.05 \)). In addition, there was no difference in the timing of blood draws between BD and control subjects (\( p > 0.05; \text{ Table 1} \)). In healthy controls, higher MDA levels were correlated with age only (\( r_p = 0.40, \text{ CI} [0.05, 0.67], p < 0.05 \)), but not with BRIAN. In the multiple linear

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**Table 2. Predictors of lipid oxidative damage in BD subjects**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Standardized coefficients (( \beta ))</th>
<th>Unstandardized coefficients (( B ))</th>
<th>S.E.</th>
<th>( t )-value</th>
<th>Pearson’s ( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.1029</td>
<td>0.0011</td>
<td>0.0015</td>
<td>0.494</td>
<td>0.15</td>
</tr>
<tr>
<td>MADRS</td>
<td>−0.2347</td>
<td>−0.0028</td>
<td>0.0019</td>
<td>−1.288</td>
<td>0.08</td>
</tr>
<tr>
<td>BRIAN</td>
<td>0.4568*</td>
<td>0.0064*</td>
<td>0.0022</td>
<td>2.563</td>
<td>0.33*</td>
</tr>
<tr>
<td># of psychotropic medications</td>
<td>0.2799*</td>
<td>0.0249*</td>
<td>0.0121</td>
<td>2.099</td>
<td>0.29*</td>
</tr>
</tbody>
</table>

Adj. \( R^2 = 0.165; F = 3.54; df = 4.47; p < 0.05 \).
* \( p < 0.05 \).

**Table 3. Predictors of lipid oxidative damage in BD subjects**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Standardized coefficients (( \beta ))</th>
<th>Unstandardized coefficients (( B ))</th>
<th>S.E.</th>
<th>( t )-value</th>
<th>Pearson’s ( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular SOD</td>
<td>−0.2160</td>
<td>−0.0132</td>
<td>0.0081</td>
<td>−1.633</td>
<td>−0.13</td>
</tr>
<tr>
<td>Age</td>
<td>0.0840</td>
<td>0.0009</td>
<td>0.0015</td>
<td>−0.610</td>
<td>0.15</td>
</tr>
<tr>
<td>MADRS</td>
<td>−0.2594</td>
<td>−0.0031</td>
<td>0.0019</td>
<td>−1.604</td>
<td>0.08</td>
</tr>
<tr>
<td>BRIAN</td>
<td>0.4640*</td>
<td>0.0064*</td>
<td>0.0022</td>
<td>2.908</td>
<td>0.33*</td>
</tr>
<tr>
<td># of psychotropic medications</td>
<td>0.2922*</td>
<td>0.0260*</td>
<td>0.0081</td>
<td>2.134</td>
<td>0.29*</td>
</tr>
</tbody>
</table>

Adj. \( R^2 = 0.161; F = 2.95; df = 5, 46; p < 0.05 \).
* \( p < 0.05 \).
regression model, age ($\beta=0.004$, $t=2.50$, $p<0.05$) was the only significant predictor of lipid peroxidation ($F_{2,27}=3.92$, $p<0.05$) in healthy controls (Tables 4 and 5).

### Discussion

**Lipid peroxidation levels are influenced by circadian rhythms in BD**

The main finding of the present study is that severity of circadian rhythm disruption in BD is associated with increased lipid oxidative damage independent of age, severity of depressive symptoms and use of psychotropic medications. Formation of lipid peroxidation (i.e. MDA, TBARS) indicates an imbalance favouring the formation of ROS and leading to lipid damage. Cellular defence against ROS depends on non-enzymatic antioxidants (i.e. GSH and vitamins) and protective enzymes, such as SOD and CAT (Halliwell, 2012). Notably, there is evidence that many of these antioxidant defence mechanisms follow circadian rhythms in various organisms and tissues (Kondratova and Kondratov, 2012). For instance, circadian fluctuations of SOD have been observed in animal tissues and human blood plasma (Hardeland et al., 2003). It has been suggested that circadian timing of these protective enzymes is to compensate for times of increased ROS formation (Hardeland et al., 2003). Therefore, it is conceivable that a disruption in the circadian expression of antioxidant enzymes may result in a redox imbalance leading to increased formation of oxidative molecules (i.e. MDA, TBARS) in BD patients with greater rhythm disruptions. Future studies measuring a wider range of markers of oxidative stress at various time points during the twenty-four hours are needed to investigate this hypothesis.

Sleep is a major component of circadian rhythm regulation and has been hypothesized to neutralize ROS produced during the wake cycle (Brown and Naidoo, 2010). Recent animal studies indicate that sleep deprivation results in decreased antioxidant enzymes and increased oxidative stress in certain brain areas such as the hippocampus, thalamus and hypothalamus (Alzoubi et al., 2012). Lungato et al. (2013) showed that CAT was reduced and total SOD activity was increased after sleep deprivation in rats, suggesting that an imbalance of antioxidant enzymes occurs after sleep disturbance. However, the levels of MDA were not associated with sleep deprivation in this latter study (Lungato et al., 2013). A recent study looking at the impact of sleep on whole blood transcriptomes in humans, found that insufficient sleep resulted in a decrease in the expression of a number of circadian rhythm genes (Möller-Levet et al., 2013). Interestingly, this study found that while certain circadian rhythm genes (PER2, PER3 and TIMELESS) were down-regulated after sleep deprivation, some oxidative stress genes (PRDX2 and PRDX5) were up-regulated. Together these studies indicate that sleep loss may lead to dysregulation of the circadian clock and increased oxidative stress (Möller-Levet et al., 2013). Sleep disturbance/insomnia is one of the core symptoms of BD, so it is possible that sleep disturbance may contribute to increased oxidative stress in BD. Future studies should investigate this possibility.

We did not find differences in MDA levels between bipolar females and matched controls. This is in contrast with a number of studies including a meta-analysis

### Table 4. Predictors of lipid oxidative damage in healthy controls

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Standardized coefficients ($\beta$)</th>
<th>Unstandardized coefficients ($b$)</th>
<th>S.E.</th>
<th>$t$-value</th>
<th>Pearson’s $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.4280*</td>
<td>0.0018*</td>
<td>0.0018</td>
<td>2.499</td>
<td>0.40*</td>
</tr>
<tr>
<td>BRIAN</td>
<td>−0.2471</td>
<td>0.0027</td>
<td>0.0027</td>
<td>−1.464</td>
<td>−0.21</td>
</tr>
</tbody>
</table>

Adj. $R^2=0.168$; $F=3.92$; df=2,27; $p<0.05$.

* $p<0.05$.

### Table 5. Predictors of lipid oxidative damage in healthy controls

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Standardized coefficients ($\beta$)</th>
<th>Unstandardized coefficients ($b$)</th>
<th>S.E.</th>
<th>$t$-value</th>
<th>Pearson’s $r$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular SOD</td>
<td>−0.0687</td>
<td>−0.0039</td>
<td>0.0105</td>
<td>−0.372</td>
<td>−0.06</td>
</tr>
<tr>
<td>Age</td>
<td>0.4297*</td>
<td>0.0045*</td>
<td>0.0019</td>
<td>2.423</td>
<td>0.40*</td>
</tr>
<tr>
<td>BRIAN</td>
<td>−0.2664</td>
<td>−0.0038</td>
<td>0.0026</td>
<td>−1.457</td>
<td>−0.21</td>
</tr>
</tbody>
</table>

Adj. $R^2=0.147$; $F=2.61$; df=3,26; $p<0.07$.

* $p<0.05$. 

showing increased lipid peroxidation in BD. However, our study is consistent with two independent studies that also failed to find differences in lipid peroxidation in BD vs. controls (Ranjekar et al., 2003; Gubert et al., 2013). Methodological differences between studies such as sample size, proportion of males/females, chronicity of illness and medications effects (see below) may drive these discrepancies. Consistent with previous studies (Andreazza et al., 2008), we did not find differences in total SOD, CAT or GST activity between bipolar females and controls but we found decreased EC-SOD activity in the bipolar sample. However, EC-SOD activity did not correlate with circadian rhythm disruption and, when added to the linear regression model, it did not affect the association between circadian rhythm disruption and increased lipid peroxidation (our main outcome measure).

We also observed that the number of psychotropic medications was positively associated with lipid damage in BD patients. Based on this association it seems that polypharmacy may have some influence on oxidative stress and this finding may be particularly relevant in BD because it is well known that polypharmacy is the rule rather than the exception in BD (Lin et al., 2006; Greil et al., 2012). However, we do not know if this association is a direct effect of psychotropic agents on oxidative stress or a result of greater illness severity or comorbid conditions (Correll et al., 2007). In addition, our study cannot distinguish which medications individually impact lipid damage, since the majority of patients included in the study were on more than one medication. There is evidence suggesting that the mood stabilizers lithium and valproate exert neuroprotective effects against increased oxidative stress in a rodent model in vivo (Frey et al., 2006). Similarly, increased TBARS seen in untreated mania was significantly decreased after treatment with lithium, further corroborating lithium’s potential antioxidant effects (Machado-Vieira et al., 2007). However, a recent study by Toplan et al. (2013) showed that rats treated with lithium had increased levels of both MDA and SOD after thirty days of treatment. The same is true for antidepressant agents where in vitro, animal and human studies show that antidepressants can exert antioxidant or pro-oxidant effects depending on duration of treatment and dosage (Behr et al., 2012). Furthermore, two studies have found that treatment with haloperidol, but not the atypical antipsychotics risperidone, quetiapine, clozapine or olanzapine, increased lipid peroxidation in vitro and in rat brain (Parikh et al., 2003; Dietrich-Muszalska et al., 2011). Unfortunately we are not aware of any study comparing the effects of monotherapy vs. multiple treatments on oxidative stress markers. From a clinical perspective we believe that the positive association between the number of psychotropic medications and lipid peroxidation seen in our study may be due to an additive pro-oxidant effect with the use of multiple medications and/or to the fact that patients requiring a higher number of medications have more severe illness and, as a consequence, may be more susceptible to oxidative stress. Future studies are needed to better discriminate the impact of individual and combination treatments on oxidative stress in humans.

We also found that age was the only significant predictor of lipid damage (MDA levels) in healthy controls, but not in BD subjects. This result is consistent with many previous studies showing that lipid peroxidation increases with healthy aging (Di Massimo et al., 2006; Voss and Siems, 2006). While studies of lipid peroxidation have shown that aging resulted in an increase in TBARS levels in different species, the daily rhythms of TBARS seem to be conserved across different age groups (Manikonda and Jagota, 2012). Together, these studies suggest that, within normal healthy aging, the daily rhythms of redox metabolism remains intact but there is an increase in the amount of lipid peroxidation over time. We believe that we did not observe a correlation between MDA levels and aging in the BD subgroup because the circadian disturbances and medication effects overshadowed the effects of aging on lipid peroxidation.

Limitations and conclusions

To our knowledge, this is the first study to investigate the relationship between circadian rhythm disruption and lipid peroxidation levels in individuals with BD. We found that circadian rhythm disruption as measured by BRIAN has a negative impact on MDA in females with BD. These results suggest an interaction between the circadian system and redox metabolism in BD, in which a measure of daily rhythm disturbances was indicative of increased lipid peroxidation in BD. As suggested during the review of this article, we conducted a post-hoc analysis comparing lipid peroxidation between healthy controls and BD subjects with greater rhythm disruption, as defined as those with BRIAN scores above one standard deviation of the mean (Giglio et al., 2010). In this analysis we found a trend ($t_{111} = -1.88, p=0.09$) towards higher MDA levels in BD subjects with higher BRIAN scores ($N=10$; mean MDA levels=5.53 μM MDA/mg protein) as compared to controls ($N=30$; mean MDA levels=3.53 μM MDA/mg protein). One of the limitations of our study is the lack of objective measures of circadian rhythm disturbances. Future studies should employ the use of actigraphy or dim light melatonin onset to assess the impact of objective measure of circadian rhythm and lipid peroxidation in BD. The finding of an association between the number of psychiatric medications and increased levels of lipid peroxidation in our BD sample deserves further investigation. It is notorious in mood disorder literature that medication effects are often difficult to evaluate/interpret, and are potentially confounded by severity of symptoms, dosage and comorbid conditions (Ranjekar et al., 2003). Future investigation on the impact of individual and combination of medications on
l lipid peroxidation in BD are warranted. Finally, we also found that lipid peroxidation levels seem to be influenced by different variables in BD compared to healthy controls, where only age was a significant predictor. This study provides a basis for further investigation of the links between oxidative stress and circadian rhythms in the pathophysiology of BD.

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Statement of Interest
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References