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Lipid peroxidation biomarkers in adolescents with or at high-risk for bipolar disorder

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Abstract

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Conflict of interest

Authors declare no conflicts of interest.

Contributions

All authors contributed to the conception and design of the study. Drs. McNamara and DelBello managed the clinical characterization of participants and collection of samples. Drs. Scola and Andreazza performed laboratory analytics. Ms. Geske and Dr. Biernacka performed statistical analyses. Drs. Scola, McNamara, Croarkin, Leffer, Cullen, Geske, Biernacka, Frye, DelBello, and Andreazza contributed to writing the manuscript and gave final approval of the manuscript.

Disclosures

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Background—Prior work suggests that adult bipolar disorder (BD) is associated with increased oxidative stress and inflammation. This exploratory study examined markers of lipid and protein oxidation and inflammation in adolescents with and at varying risk for BD type I (BD-I).

Methods—Blood was obtained from four groups of adolescents (9–20 years of age): (1) healthy comparison subjects with no personal or family history of psychiatric disorders ($n = 13$), (2) subjects with no psychiatric diagnosis and at least one parent with BD-I ('high-risk', $n = 15$), (3) subjects with at least one parent with BD-I and a diagnosis of depressive disorder not-otherwise-specified ('ultra-high-risk', $n = 20$), and (4) first-episode patients exhibiting mixed or manic symptoms that received a diagnosis of BD-I ($n = 16$). Plasma levels of lipid peroxidation (LPH, 4-HNE, 8-ISO), protein carbonyl, and inflammation (IL-1 α - β , IL-6, IL-10, IFN γ TNF α) were assessed using analysis of variance and covariance models.

Results—LPH was lower in adolescents with fully syndromal BD than controls, while LPH levels in the at-risk groups were between healthy controls and fully syndromal BD. Post-hoc analysis showed a non-significant increase in the (4-HNE+8-ISO)/LPH ratio suggesting a potential conversion of LPH into late-stage markers of lipid peroxidation. There were no significant differences among protein carbonyl content and inflammatory markers.

Conclusions—In adolescents, fully syndromal BD is associated with significant reductions in LPH levels, and LPH levels decrease along the spectrum of risk for BD-I. Quantifying lipid peroxidation in longitudinal studies may help clarify the role of LPH in BD risk progression.

Keywords

Adolescent bipolar disorder; At-risk adolescents; Lipid peroxidation; Oxidative stress markers

1. Introduction

The initial onset of mania, and by definition bipolar I disorder (BD), typically occurs during adolescence and is associated with significant psychosocial morbidity and increased risk for suicide (Perlis et al., 2004, 2009). Misdiagnosis, heterogeneity of clinical symptoms, profound functional impairment, and identification of effective pharmacologic treatments are common challenges for youth and clinicians in treatment planning. The pathophysiology of BD is multifaceted and incompletely understood. Heritability estimates for BD range from 60% to 87% (Smoller and Finn, 2003), and family studies suggest that having a first-degree relative with BD substantially increases risk for developing bipolar disorder (DelBello and Geller, 2001; Mortensen et al., 2003). Retrospective and prospective longitudinal studies additionally suggest that mood symptoms, including episodic subsyndromal depressive symptoms and major depressive disorder (MDD), frequently precede the initial onset of mania by several years (Correll et al., 2014; Egeland et al., 2000; Howes et al., 2011; Skjelstad et al., 2010), and increase risk for developing mania in adolescent bipolar offspring (Axelson et al., 2015). While emerging retrospective and prospective evidence suggests that adolescent age, positive family history, and prodromal mood symptoms can serve as criteria to identify individuals at increased risk for developing bipolar disorder (Bechdolf et al., 2010, 2014), the addition of prodromal biomarkers may augment prognostic accuracy as

well as inform early intervention strategies (Conus et al., 2008; McGorry et al., 2014; McNamara et al., 2010).

Several candidate biomarkers are emerging and hold the promise of elucidating relevant biologic underpinnings of BD, identifying subsets, facilitating early identification, characterizing staging approaches, personalizing treatment, and improving outcomes. One compelling approach focuses on peripheral measures of oxidative stress and inflammation. Prior work suggests that mitochondrial dysfunction and oxidative stress play a central role in the etiopathology of BD (Scola et al., 2013; Versace et al., 2014). Recent meta-analytic studies and systematic reviews indicate that lipid peroxidation is increased in adult BD (Brown et al., 2014). Lipid peroxidation is initiated by reactive oxygen species, thereby forming lipid hydroperoxides (LPH), 4-hydroxy-2-nonenal (4-HNE), and 8-isoprostane (8-ISO) (Halliwell, 2007). Independent studies have consistently demonstrated elevations in these indices of oxidative stress in adult BD (Andreazza et al., 2013; Versace et al., 2014; Wang et al., 2009). Inflammation also orchestrates important molecular changes that may be involved in the pathophysiology of BD (Scola and Andreazza, 2014). For example, proinflammatory cytokines, such as IL-1 α - β , IL-6 and TNF α , are increased in adult BD (aged 35 and older) with BD (Goldstein et al., 2009; Hope et al., 2011; Rao et al., 2010; Scola and Andreazza, 2014) as well as children and adolescents with BD (Mitchell and Goldstein, 2014).

There is currently a dearth of research focused on measures of oxidative stress in adolescents and at-risk populations. Magalhães and colleagues reported that young adults (aged 18–24) with BD had higher protein carbonyl content compared to healthy controls (Magalhães et al., 2012). Decreased glutathione levels (a marker of oxidative stress) were also observed in the anterior cingulate cortex of patients with evolving BD by proton magnetic resonance spectroscopy (Chitty et al., 2015). More recently, Hatch and colleagues found that adolescents with BD had lower LPH as compared to prior samples of adult patients with BD (Hatch et al., 2015). While prior research suggests that lipid peroxidation and inflammation are increased in adult patients with BD, it is not known whether these abnormalities exist during acute illness or if it represents a risk biomarker for BD. In the present, exploratory, cross-sectional study, we determined levels of lipid peroxidation and inflammatory biomarkers in adolescents with and at varying risk for BD. Based on extant evidence, our primary hypothesis was that lipid peroxidation and inflammatory biomarkers would be elevated in first-episode BD patients compared with healthy adolescents, and these increases would be graded in asymptomatic and symptomatic high risk adolescents.

2. Methods

2.1. Study participants

Study participants were recruited from inpatient units and outpatient clinics at Cincinnati Children's Hospital Medical Center and University of Cincinnati Medical Center. Demographically matched healthy comparison participants were recruited from the communities in which the other participants resided. The cohort consisted of 4 groups of adolescents (9–20 years of age): (1) healthy comparison (HC) participants with no personal or family history of psychiatric disorders, (2) participants with no *DSM-IV-TR* Axis I

diagnosis and at least one parent with bipolar I disorder (high-risk; HR), (3) adolescents with at least one parent with bipolar I disorder and a *DSM-IV-TR* Axis I diagnosis of MDD or depressive disorder not-otherwise-specified ('ultra-high-risk' UHR), and (4) first-episode patients exhibiting mixed or manic symptoms that received a *DSM-IV-TR* Axis I of bipolar I disorder. *DSM-IV-TR* diagnoses were determined using the Washington University in St. Louis Kiddie-Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (Geller et al., 2001). Parental diagnoses were determined using the Structured Clinical Interview for DSM-IV-TR (SCID) (First et al., 1995) and confirmed using the Family Interview for Genetic Studies (FIGS) (Maxwell, 1999). All participants were assessed by board-certified psychiatrists with established inter-rater reliabilities ($\kappa > 0.9$). IQ was estimated using the Wechsler Abbreviated Scale of Intelligence (WASI) (Wechsler, 1997). Symptom severity ratings were obtained with the Young Mania Rating Scale (YMRS) (Young et al., 1978) for mania and the 28-item Hamilton Depression Rating Scale (HDRS/HAM-D) (Hamilton, 1960) for depression. Pubertal development was measured using a self-rated Tanner Scale. Exclusion criteria included a diagnosis of substance dependence within the previous 3 months (McLellan et al., 1992), any major medical or neurological illnesses (e.g., head trauma resulting in loss of consciousness for more than 5 min), an IQ < 70, and a positive urine pregnancy test in females. Cigarette use in the past 30 days was documented but not exclusionary. All patients were free of psychotropic medications or medications with central nervous system effects, at the time of the blood draw, with the exception of psychostimulants in participants with ADHD. Ultra-high risk patients were required to be anti-depressant-free for at least 5 days prior to the blood draw (or at least one month for fluoxetine because of its long half-life). In first-episode patients, the blood draw occurred prior to initial treatment with antipsychotics or mood stabilizer medication. Written informed consent and assent were provided by a legal guardian and the patient, respectively. This study was approved by the Institutional Review Boards of University of Cincinnati College of Medicine.

2.2. Lipid peroxidation assessments

Serum levels of lipid peroxidation (lipid hydroperoxides, LPH; 8-isoprostane, 8-Iso; 4-hydroxy-2-nonenal, 4-HNE) were measured. LPH was assessed using the colorimetric LPH assay kit (Cayman Chemical item number 705002) according to manufacturer's instructions. The sensitivity of this kit is in between 0.25 and 5 nmol hydroperoxides. The intra-assay presented a coefficient of variation (CV) in between 2% and 15% and the inter-assay presented a CV of 9%. Data are presented as the amount of LPH (μM). Levels of 4-HNE proteins via Michael addition to lysine (Lys), histidine (His) or cysteine (Cys) were measured using ELISA as previously described with slight modifications. For each sample, 30 $\mu\text{g/mL}$ of total proteins were used. The sensitivity of this assay is in between 0.5 and 10 $\mu\text{g/mL}$ of 4-HNE. The intra-assay presented a coefficient of variation in between 1% and 16% and the inter-assay presented a CV of 7.5%. Results were expressed as fmol/ μg of protein. 8-ISO levels were quantified using a standard competitive sandwich ELISA (Cayman Chemical item number 516351) according to manufacturer's instructions. The sensitivity of this assay is in between 0.8 and 500 pg/mL of 8-ISO. The intra-assay presented a coefficient of variation in between 1% and 19% and the inter-assay presented a CV of 9.1%. Results were expressed as fmol/ μg of protein or pg/mL.

2.3. Protein Carbonyl groups

Protein Carbonyl Content Assay Kit (Abcam item number ab126287) was used to assess the protein oxidation amount according to manufacturer's instructions. The lowest detection limit of this kit is 0.015 nmol of carbonyl groups. The intra-assay presented a coefficient of variation in between 4% and 19% and the inter-assay presented a CV of 15%. Data are presented as the amount of protein carbonyl groups (nmol/mg).

2.4. Inflammatory assessments

Multiplexed microsphere suspension ELISA platform (Luminex, US) was used to assess the levels of expression of inflammatory interleukins (IL-1 α - β , IL-6, IL-10, IFN γ and TNF α) and trophic factor (VEGF) according to manufacturer's instructions (EMD Millipore item number HCYTOMAG-60K). The sensitivity of this kit is in between 3.2 and 2000 pg/mL for each distinct molecule. The average between all markers in the intra-assay analysis presented a coefficient of variation in between 0.6% and 20% and their inter-assay presented an average of 9% CV. The investigators were blind to group identity, diagnosis and demographic variables of participants during all experiments and measurements. Samples were coded numerically in a random manner and the code lifted only during data analyses after all experiments were completed.

2.5. Statistical analyses

The analyses for this exploratory study were performed as follows. Sample means and standard deviations for continuous demographic and baseline clinical characteristics were calculated. Categorical variables were described with frequencies and percentages. Demographic characteristics across the groups were examined with analysis of variance. Analysis of variance and covariance models adjusting for age and gender were used to test for overall difference in the biomarker levels across the 4 risk level groups. An additional analysis was conducted with the 2 symptomatic groups (ultra-high risk, BD-I) and healthy subjects (3 groups analysis). These analyses were also run in the sample including only patients of Caucasian ancestry for 4 group ($n = 46$) and 3 group ($n = 32$) comparisons. Finally, we examined Spearman's correlations of LPH and symptom severity (HAMD and YMRS), age, and body mass index. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Inc., Cary, NC). The level of significance was set at $\alpha = 0.05$ (two-tailed) and *post hoc* pairwise p -values adjusted using Bonferroni's method.

3. Results

Participant demographics are presented in Table 1. Four group unadjusted ($p = 0.009$, Table 2) and age/gender adjusted ($p = 0.016$) group comparisons of lipid peroxidation (LPH) demonstrated significant differences (see Table 2 and Fig. 1). There were no significant differences among 4-HNE, 8-ISO, and Carbonyl group. However, a non-significant increase in the levels of 4-HNE and 8-ISO were found. These findings were further analyzed through the ratios between the early marker (LPH) and late markers (4-HNE and 8-ISO) of lipid peroxidation. There were no significant differences among 4-HNE/LPH and 8-ISO/LPH ratios. On the other hand, a non-significant increase was found for the (4-HNE + 8-ISO)/LPH ratio for unadjusted ($p = 0.055$, Table 1) and age/gender adjusted ($p = 0.053$) 4

group comparisons (Table 2). Analysis using the 2 symptomatic groups (ultra-high risk and BD-I) and healthy subjects (3 groups analysis) demonstrated significant differences for LPH levels (adjusted $p = 0.0132$, Table 2) and the (4-HNE + 8-ISO)/LPH ratio (adjusted $p = 0.0363$). Four group ($n = 46$) and three group ($n = 32$) analyses were repeated excluding patients who were not of Caucasian ancestry. Overall results did not change with this approach; however, the significant difference was lost on (4-HNE + 8-ISO)/LPH ratio (Table 2). Post-hoc pairwise comparisons showed that healthy controls and first-episode BD were significantly different after Bonferroni adjustment for multiple comparisons with LPH mean difference (95% CI): 12.6 (2.7, 22.5) for all participants and 13.1 (1.1, 25.1) for Caucasians only (Table 3). The (4-HNE + 8-ISO)/LPH ratio also demonstrated significant differences between healthy controls and first-episode for BD group in all subjects with mean difference (95% CI): 0.40 (0.004, 0.797) (Table 3).

Among all subjects, depression severity (HAMD score) was correlated with LPH ($r = -0.457$, $p = 0.002$), ISO-8/LPH ($r = 0.299$, $p = 0.046$), 4-HNE/LPH ($r = 0.343$, $p = 0.021$), and (4-HNE+ISO-8)/LPH ($r = 0.316$, $p = 0.034$) (Fig. 2). Manic symptom severity (YMRS) was correlated with ISO-8/LPH ($r = 0.346$, $p = 0.006$) and with (4-HNE+ISO-8)/LPH ($r = 0.330$, $p = 0.008$). Within the first-episode BD group, manic symptom severity was positively correlated with LPH ($r = 0.77$, $p = 0.0005$), and negatively correlated with the 8-ISO/LPH ($r = -0.76$, $p = 0.0007$) and 4-HNE+8-ISO/LPH ($r = -0.76$, $p = 0.0007$) ratios (Supplemental Table 1). Age and BMI did not have a statistically significant correlation with markers of oxidative stress.

Protein carbonyl content did not show significant differences between the groups (Table 2). Multiplex analysis for inflammatory markers showed no significant differences among the different groups (Table 2).

4. Discussion

Previous studies have suggested that lipid alterations play an important role in the pathophysiology of BD (Brown et al., 2014), though it is not known whether these alterations also occur early in the course of illness progression. Therefore, this preliminary cross-sectional study explored biomarkers of lipid peroxidation, protein carbonyl profile, and inflammation in adolescents with and at varying risk for BD. In contrast to our hypothesis and prior findings from adult BD studies finding elevated LPH levels (early-stage lipid peroxidation marker), we found that LPH was significantly lower in adolescents with first-episode BD compared to controls, while the at-risk groups' levels were not significantly different from healthy controls. Additionally, no significant alterations on protein carbonylation or inflammatory markers were found.

Although our findings did not support our hypothesis, lipid peroxidation is a complex process with structural alterations and degradation of lipids (Halliwell, 2007; Halliwell and Gutteridge, 2007). Lipid peroxides and hydroperoxides (early-stage marker) are formed through reactions with lipid radicals and phospholipids when homeostasis is dysregulated. Notably, the levels of LPH were reduced in symptomatic adolescents with BD compared to healthy controls; however, a non-significant increase of late stage markers is evident. When

coupled with genetic and environmental factors, this initial imbalance may promote further oxidative stress and mood dysregulation (Berk et al., 2011). This is supported by the observation that late-stage markers of lipid oxidation were similarly elevated, albeit non-significantly, in the high risk and ultra-high risk groups. In order to investigate whether early-stage markers of lipid peroxidation are being converted into late-stage markers, we also determined ratios between late-stage markers 4-HNE and 8-ISO and the early-stage marker LPH (i.e. (4HNE+8ISO)/LPH). Our results demonstrated a significant difference between healthy controls and first-episode BD subjects which is consistent with elevated LPH conversion, though these exploratory findings will need to be confirmed in a larger cohort.

The lipid subproduct 4-HNE is highly reactive and has great affinity to create Michael adducts to Cys, His and Lys residues, causing important alterations to cellular dynamics which can lead to disruption of Ca^{2+} homeostasis, ATPase activity (mitochondrial dysfunction) and activation of caspase pathways (Esterbauer et al., 1991; Pugazhenth et al., 2006). On the other hand, 8-ISO is a secondary molecule formed from lipid peroxidation and is released in free form by phospholipase action that provides indication for progressive oxidative stress (Montuschi et al., 2004). Moreover, higher levels of this compound are associated with cardiovascular diseases (Montuschi et al., 2004) and, more importantly, to depression (Black et al., 2015).

While exploratory, an eventual progress of lipid peroxidation results in specific late-stage lipid byproducts that can exert signaling effects. For example, 4-HNE is able to regulate a substantial amount of transcription factors related to lipid metabolism, mitochondria and antioxidant effects. Such factors include Nrf2, AP-1, PPAR and NF- κ B, which have a great impact on gene transcription (Forman et al., 2003; Kim and Yang, 2013; Lim et al., 2006; Miller et al., 2013). Moreover, this molecule could have an important role in the pathophysiology of BD since it could also stimulate protein modification and activation of Erk1/2, JNK and p38 MAP kinases, as well as phosphoinositide-3-kinase (PI3)/Akt (Lin et al., 2014; Zheng et al., 2014). All of these alterations have been observed in adults with BD (Andreazza et al., 2013; Kerner et al., 2013). Additionally, the LPH ratios between the late-stage markers (ISO-8/LPH, HNE-4/LPH and HNE4+ISO-8/LPH) and symptom severity (HAMD and YMRS) conferred a stage-related indication in this youth cohort (Table 1). While preliminary and exploratory, these data contributed with further evidence for the role of lipid oxidation in BD.

The present study also investigated protein oxidation and inflammatory cytokine levels. No alterations on protein carbonylation profile were found. Some studies have shown alteration in the carbonyl content in adults with BD; however, these findings have been inconsistent (Andreazza et al., 2013; Kapczinski et al., 2011). Additionally, there were no significant group differences in plasma levels of IL-1 α - β , IL-6, IL-10, IFN γ or TNF α . Previous studies have identified increased levels of high-sensitivity CRP, but not IL-6 in the serum of adolescents with BD (Goldstein et al., 2011). On the other hand, Padmos et al. (2008) reported that healthy offspring of BD parents exhibited alterations in the expression levels of proinflammatory genes in monocytes, such as IL-6, TNF α , TNFAIP3, PTX3, PTGS2, CCL7, CCL20, and CXCL2 (Padmos et al., 2008). In addition, Mesman et al. (2015)

reported that adolescent bipolar offspring exhibited altered inflammatory gene expression in monocytes independent of psychopathology (Mesman et al., 2015). The present findings would suggest that elevated inflammatory gene expression in monocytes of adolescent bipolar offspring is not associated with elevated cytokine biosynthesis and/or secretion into plasma. Nonetheless, additional studies are needed to assess the dynamics of inflammatory markers in the childhood, adolescence and adulthood to better understand associations with BD progression.

It is also notable that we observed group variability in VEGF in the age/gender adjusted analysis, though this did not reach statistical significance ($p = 0.055$). VEGF is a trophic factor that is produced in different cell types and plays a key role in neuronal dynamics, such as differentiation, protection and plasticity (Scola and Andreazza, 2015). Some studies have shown that lower levels of VEGF are associated with suicide ideation (Isung et al., 2012) and higher levels with depressed mood (Iga et al., 2007; Takebayashi et al., 2010). Prospective longitudinal studies are warranted to determine the role of VEGF in BD progression.

A limitation of this study is the small number of individuals in each group; therefore, the data obtained may not be representative of individuals in each risk category. Moreover, the small sample size limited our ability to adjust for confounding variables beyond age and gender. Second, healthy controls were significantly older than high risk, ultra-high risk, and symptomatic adolescents. While this variable could have some influence in our results since oxidative stress increases with age (Stadtman, 2004), significant group differences persisted after including age/gender as covariates in the analysis. Third, this study investigated only two markers of late-stage lipid peroxidation (4-HNE and 8-ISO), and examining other markers of lipid peroxidation are warranted. Fourth, the cross-sectional design precludes evaluation of causality, and it is not known whether subjects in the at-risk groups will eventually develop mania. While the results of this preliminary cross-sectional study should be interpreted within these limitations, they support a need for future longitudinal studies to clarify the role of lipid peroxidation and inflammation in BD-I risk progression.

In summary, this cross-sectional study suggests that early-stage lipid peroxidation was lower in adolescents with first-episode BD compared with healthy adolescents. Lipid peroxidation in high risk or ultra-high risk samples did not differ from healthy adolescents. It is notable that these findings contrast with previous results reported for adult BD-I patients who were found to exhibit elevated LPH levels, though we did observe non-significant increases in the late stage markers 4-HNE and 8-ISO in high risk, ultra-high risk, and first-episode BD groups. Therefore, lower levels of LPH in adolescents with first-episode BD may suggest that LPH is converted into late stage markers such as 8-ISO to a greater extent in this group. Nevertheless, the present findings add to a growing body of evidence implicating a dysregulation in lipid peroxidation processes in the pathophysiology of BD-I that warrant additional evaluation in future prospective longitudinal studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

4-HNE	4-hydroxy-2-nonenal
8-ISO	8-isoprostane
ADHD	attention deficit hyperactivity disorder
Akt	protein kinase B
AP-1	activator protein 1
BD	bipolar disorder
BMI	body mass index
Cys	cysteine
DD-NOS	depressive disorder not otherwise specified
ELISA	enzyme-linked immunosorbent assay
Erk1/2	extracellular signal-regulated protein kinases 1 and 2
HDRS	Hamilton Depression Rating Scale
His	histidine
IL	interleukins
JNK	c-Jun N-terminal kinases
LPH	lipid hydroperoxides
Lys	lysine
MDD	major depressive disorder
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells

Nrf2	nuclear factor (erythroid-derived 2)-like 2
p38 MAP kinases	p38 mitogen-activated protein kinases
PI3	phosphoinositide-3-kinase
PPAR	peroxisome proliferator-activated receptors
TNFα	tumor necrosis factor α
VEGF	vascular endothelial growth factor
YMRS	Young Mania Rating Scale

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.jad.2015.12.020>.

Fig. 1.

(a) LPH; (b) 4-HNE/LPH; (c) 8-ISO/LPH; (d) 4-HNE+8-ISO/LPH in healthy controls ($n = 13$), high-risk subjects ($n = 15$), ultra-high risk subjects ($n = 20$), and first-episode BD-I patients ($n = 16$). Note that plasma LPH concentrations were significantly lower in first-episode BD-I subjects compared with healthy subjects ($p < 0.01$). Values are group mean concentration $\pm$ SD.

Fig. 2.

Scatter plots illustrating statistically significant relationships between depressive symptom severity (HAM-D) and lipid peroxidase (LPH) (a), 4-hydroxy-2-nonenal/ lipid peroxidase (4-HNE/LPH) (b), 8-isoprostane/lipid peroxidase, 8-ISO/LPH (c), and 4-hydroxy-2-nonenal +8-isoprostane /lipid peroxidase (4-HNE+8-ISO/LPH) (d). 8-ISO/ LPH and 4-HNE+8-ISO/LPH demonstrated a statistically significant relationship with manic symptom severity (YMRS) (e, f).

Table 1

Comparisons of demographics and clinical characteristics (unadjusted values).

	Controls		High Risk		UH Risk		1st Episode		p-value
	N = 13		N = 15		N = 20		N = 16		
Male	7 (53.9)		8 (53.3)		5 (25.0)		6 (37.5)		0.258
Caucasian	9 (69.2)		14 (93.3)		11 (55.0)		12 (75.0)		0.210
Age	17.8±2.5		12.8±2.7		13.8±2.9		15.5±2.4		<0.0001
BMI	25.5±3.5		20.6±5.0		25.8±8.1		27.3±9.5		0.089
HAMD	1.6±2.4		4.5±2.9		18.8±6.2		12.0±6.6		<0.0001
YMRS	1.4±2.6		5.9±2.2		11.1±5.5		28.8±8.1		<0.0001
LPH (uM)	43.7±12.8		37.2±9.2		36.0±9.5		31.1±7.4		0.0093
4-HNE (fmol/ug)	0.96±0.46		1.15±0.41		1.06±0.44		1.08±0.48		0.7574
4-HNE/LPH	0.023±0.008		0.034±0.017		0.031±0.015		0.036±0.02		0.1241
8-ISO (pg/mL)	29.2±18.7		29.4±10.7		26.6±9.8		30.7±7.6		0.7719
8-ISO/LPH	0.66±0.40		0.82±0.29		0.80±0.43		1.04±0.37		0.0605
(4-HNE+8-ISO)/LPH	0.68±0.40		0.85±0.30		0.83±0.44		1.08±0.39		0.0554
Carbonyl (nmol/mg)	0.20±0.16		0.21±0.11		0.16±0.11		0.19±0.15		0.7971
IFN gamma (pg/mL)	398.6±517.9		505.0±530.9		373.3±363.7		594.0±550.1		0.5409
IL-10 (pg/mL)	7.79±8.08		9.60±12.06		4.36±7.01		16.55±33.31		0.2721
IL-1alpha (pg/mL)	7.45±2.47		11.51±8.43		7.61±2.80		9.71±4.60		0.0928
IL-beta (pg/mL)	327.2±486.6		376.4±511.0		356.1±579.8		472.4±1273.4		0.9605
IL-6 (pg/mL)	7.80±9.12		12.07±13.49		5.72±6.83		11.27±12.63		0.2686
TNF alpha (pg/mL)	16.36±30.66		18.26±19.75		9.14±13.81		103.48±272.03		0.1732
VEGF (pg/mL)	54.87±62.14		159.73±260.88		44.33±72.22		84.62±129.78		0.1388

Table 2

Unadjusted and age/gender adjusted 3-group and 4-group comparisons.

	4 groups		3 groups (no high risk)			4 groups Caucasian (n = 46)			3 group Caucasian (n = 32)		
	unadj*	adj**	unadj	adj	p-value	unadj	adj	p-value	unadj	adj	p-value
LPH	0.0093	0.0164	0.0054	0.0132	0.0378	0.0440	0.0226	0.0426	0.0226	0.0426	0.0426
4-HNE	0.7574	0.2447	0.7815	0.5520	0.4087	0.1484	0.7556	0.6408	0.7556	0.6408	0.6408
8-ISO	0.7719	0.5463	0.6045	0.5406	0.9027	0.5180	0.7784	0.5696	0.7784	0.5696	0.5696
Carbonyl groups	0.7971	0.8028	0.7706	0.8035	0.5376	0.4644	0.4420	0.3101	0.4420	0.3101	0.3101
IFN gamma	0.5409	0.4572	0.3472	0.3184	0.3903	0.1618	0.2532	0.1890	0.2532	0.1890	0.1890
IL-10	0.2721	0.2719	0.1937	0.1934	0.7540	0.7606	0.6692	0.4899	0.6692	0.4899	0.4899
IL-1alpha	0.0928	0.1361	0.1279	0.1077	0.3353	0.5072	0.2396	0.3057	0.2396	0.3057	0.3057
IL-beta	0.9605	0.9466	0.8829	0.8554	0.9914	0.9998	0.9963	0.9833	0.9963	0.9833	0.9833
IL-6	0.2686	0.3151	0.2374	0.2609	0.5850	0.5826	0.3829	0.3628	0.3829	0.3628	0.3628
TNF alpha	0.1732	0.1436	0.1683	0.1336	0.1124	0.0783	0.1595	0.1029	0.1595	0.1029	0.1029
VEGF	0.1388	0.0550	0.4283	0.2850	0.4744	0.2743	0.6563	0.3676	0.6563	0.3676	0.3676
(4-HNE+8-ISO)/LPH	0.0554	0.0535	0.0366	0.0363	0.6276	0.8134	0.4800	0.6417	0.4800	0.6417	0.6417
4-HNE/LPH	0.1241	0.0428	0.0704	0.0631	0.1389	0.0243	0.2072	0.2048	0.2072	0.2048	0.2048
8-ISO/LPH	0.0605	0.0586	0.0404	0.0400	0.6471	0.8327	0.5040	0.6641	0.5040	0.6641	0.6641

* unadjusted

** adjusted

Table 3

Differences in LPH by group.

Group	LPH			4-HNE+8-ISO/LPH					
	All			Caucasian only			All		
	N	Mean (SD)		N	Mean (SD)		N	Mean (SD)	
Healthy controls *	13	43.7(12.8)		9	45.4(11.1)		13	0.68(0.40)	9 0.75(0.41)
High Risk	15	37.2(9.2)		14	36.6(9.3)		15	0.85(0.30)	14 0.87(0.31)
Ultra-high Risk	20	36.0(9.5)		11	37.5(11.1)		20	0.83(0.44)	11 0.89(0.52)
BP 1st episode *	16	31.1(7.4)		12	32.3(8.1)		16	1.08(0.39)	12 0.97(0.29)

* Post hoc pairwise comparisons show healthy controls and BP with 1st episode are significantly different after Bonferroni adjustment for multiple comparisons with LPH mean difference (95% CI): 12.6 (2.7, 22.5) and Caucasians only: 13.1 (1.1, 25.1). Also, 4-HNE+8-ISO/LPH: 0.40 (0.004, 0.797) ratio demonstrates significant differences between healthy controls and first-episode for bipolar disorder.