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Elevated Plasma Oxidative Stress Markers in Individuals With Intermittent Explosive Disorder and Correlation With Aggression in Humans

Emil F. Coccaro, Royce Lee, and David Gozal

ABSTRACT

BACKGROUND: Animal and clinical studies suggest a link between inflammation and oxidative stress. Because oxidative stress is an inherent part of inflammation, and inflammation is associated with behavioral aggression in lower mammals and humans, we hypothesized that markers of oxidative stress would be related to aggression in human subjects. In this case-control study, markers of oxidative stress and aggression were assessed in human subjects with histories of recurrent, problematic, impulsive aggressive behavior and in nonaggressive comparator subjects.

METHODS: Plasma levels of 8-hydroxy-2′-deoxyguanosine and 8-isoprostane were examined in the context of measures of aggression and impulsivity in physically healthy subjects with intermittent explosive disorder ($n = 69$), nonaggressive subjects with Axis I or II disorders ($n = 61$), and nonaggressive subjects with no history of Axis I or II disorders ($n = 67$).

RESULTS: Levels of plasma 8-hydroxy-2′-deoxyguanosine and 8-isoprostane were significantly higher in subjects with intermittent explosive disorder compared with psychiatric or normal control subjects. In addition, both oxidative stress markers correlated with a composite measure of aggression; more specifically, 8-hydroxy-2′-deoxyguanosine correlated with measures reflecting a history of actual aggressive behavior in all subjects.

CONCLUSIONS: These data suggest a positive relationship between plasma markers of oxidative stress and aggression in human subjects. This finding adds to the complex picture of the central neuromodulatory role of aggression in human subjects.

Keywords: Aggression, 8-ISO, 8-OH-DG, Impulsivity, Inflammation, Oxidative stress

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Animal (1–4) and human (5–14) studies suggest that behavioral traits related to hostility, anger, aggressive tendencies, and aggressive behavior are associated with elevations in inflammatory markers. In addition to the proaggressive effects that inflammatory proteins may have on brain circuits underlying aggressive behavior (15,16), inflammation can be associated with a state of heightened oxidative stress (17). In the context of this process, activated phagocytes are significant sources of reactive oxidative species that function as a cytotoxic response to the source of the inflammatory process (17). Animal models of inflammation suggest that systematic oxidative processes involving critical molecules (e.g., nucleic acids, lipids) are part of the host response to inflammation (18).

Oxidative stress is important in clinical neuroscience because the brain is particularly vulnerable to oxidative stress owing to its high consumption of oxygen, modest antioxidant defenses, and lipid-rich makeup (19,20). Oxidative stress can lead to damage in neuronal membranes that are dense in lipids leading to a reduction in membrane fluidity and deactivation of receptors, enzymes, and ion channels, all of which can alter neurotransmission and neuronal function (21–24). Over the last decade, evidence of increased oxidative stress has been reported in various psychiatric disorders, such as schizophrenia (25), depression (26–30), and anxiety disorder (31,32). Evidence of increased oxidative stress in the context of psychological stress has also been reported in studies of animals and humans (33). Finally, some behaviors, such as smoking and alcohol consumption, particularly prevalent in psychiatric conditions, are also associated with oxidative stress (34–36).

Although inflammatory processes have been studied previously in relation to hostility, anger, and aggression, no study of oxidative stress markers has been conducted so far in subjects with psychiatric disorders and prominent histories of recurrent, problematic, impulsive aggressive behavior. In this study, we hypothesized that plasma levels of markers of oxidative stress, similar to plasma inflammatory markers (14), would be elevated in individuals with a current diagnosis of intermittent explosive disorder (IED), a disorder of recurrent, problematic, impulsive aggressive behavior (37) and would correlate directly with measures of aggression (or impulsivity or both) in healthy subjects and subjects with psychiatric disorders. In this first study of oxidative stress in aggression, we chose to measure reliable and ubiquitous reporters of

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oxidative stress—8-hydroxy-2′-deoxyguanosine (8-OH-DG) as a measure of nucleic acid oxidation (38) and 8-isoprostane (8-ISO) as a measure of lipid oxidation (39).

METHODS AND MATERIALS

Subjects

Study participants included 197 physically healthy subjects. Subjects were recruited from clinical settings and through newspaper advertisements, seeking out individuals who reported psychosocial difficulty related to one or more Axis I and Axis II conditions or who had little evidence of psychopathology. All subjects gave signed informed consent as approved by our institutional review board. Subjects with a history of bipolar disorder, schizophrenia, or mental retardation were excluded. Medical health of subjects was documented by a comprehensive medical history and physical examination, which included a screen for drugs of abuse (subjects testing positive were excluded).

Diagnostic Assessment

Axis I and Axis II diagnoses were made based on DSM-IV (40); diagnoses of IED were made by research criteria (37). Diagnoses were made using information from the following: 1) Structured Clinical Interview for DSM (41) and Structured Interview for the Diagnosis of DSM Personality Disorder (42), 2) clinical interview by a research psychiatrist, and 3) review of all available clinical data. Research assessments were conducted by individuals with a master's or doctoral degree in clinical psychology. Raters underwent a rigorous training program until they were deemed reliable by the trainer; this resulted in good to excellent interrater reliabilities (mean κ, .84 $\pm$ .05; range, .79–.93) across mood, anxiety, substance use, impulse control, and personality disorders. Final diagnoses were assigned by team best-estimate consensus procedures as previously described (43).

Criteria for current IED were met by 69 subjects, criteria for current or lifetime Axis I or Axis II disorder were met by 61 subjects (psychiatric control [PC] subjects), and 67 subjects had no evidence of any DSM-IV psychopathology (healthy control [HC] subjects). Among IED and PC subjects, 112 had a current history of an Axis I disorder, 125 had a lifetime history of an Axis I disorder, and 105 had an Axis II personality disorder. In addition, 54% of PC and IED subjects had a history of formal psychiatric treatment or history of behavioral disturbance for which they (or others) thought they should seek mental health services. Specific diagnoses for IED and PC subjects are listed in Supplement 1.

Psychometric Measures of Relevant Aggression, Impulsivity, and Related Behavioral Dimensions

Aggression was assessed by the Aggression Scale from Life History of Aggression (LHA) (44) and the Buss-Perry Aggression Questionnaire (BPAQ) (45). Impulsivity was assessed by the Life History of Impulsive Behavior (LHIB) (46) and Barratt Impulsivity Scale (BIS-11) (47). Self-directed aggression was assessed by history of suicidal behavior and self-injurious behavior as assessed during the Structured Clinical Interview for DSM interviews. Other assessments included Beck Depression Inventory (BDI-II) (48) for state depression, Life Experiences Survey (LES) (49) for stressful life events over the past 6 months, and Eysenck Personality Questionnaire (Neuroticism, Extraversion, Psychoticism) (50) as control dimensions of general personality. Global Assessment of Function (40) scale served as the variable for psychosocial functioning.

Assessment of Plasma Markers of Oxidative Stress

All subjects were medication free at time of recruitment and were $\geq$4 weeks medication free at the time of the study. After resting for at least 30 minutes, whole blood, anticoagulated with ethylenediamine tetraacetate, was obtained between 9:00 AM and 11:00 AM through venipuncture of a forearm vein. Plasma processed after centrifugation was stored immediately at -80°C and kept frozen until assay. Levels of 8-OH-DG were measured by a commercially available immunoassay (Cayman Chemical, Ann Arbor, Michigan) of all three oxidized guanine species (i.e., 8-hydroxy-2′-deoxyguanosine from DNA, 8-hydroxyguanosine from RNA, and 8-hydroxyguanine from either DNA or RNA). The limit of detection of this assay was 30 pg/mL; intra-assay and interassay coefficients of variation were $<$6.7% and $<$9.2%, respectively. Levels of 8-ISO were also measured by a commercially available immunoassay (Cayman Chemical). The limit of detection of this assay was 2.7 pg/mL; intra-assay and interassay coefficients of variation were $<$4.7% and $<$5.6%, respectively. Levels of the oxidative stress markers represent the mean of the two assay determinations per subject.

Statistical Analysis and Data Reduction

Comparisons between groups were performed by *t* test, multivariate analysis of variance (MANOVA) and covariance, and χ^2 tests. Correlational analyses included Pearson's correlation, partial correlation, and multiple regression. α values $\leq$ .05 denoted statistical significance. Plasma 8-OH-DG levels were normally distributed, but 8-ISO levels were not, and logarithm transformation was applied to 8-ISO levels for analysis (Log 8-ISO). Data were first analyzed without any potential covariates and then with relevant demographic (age, gender, race, socioeconomic status), psychometric (state depression score, recent psychosocial stress score), and lifestyle variables (body mass index, current alcohol and cigarette consumption) as covariates. Composite variables for "aggression" and "impulsivity" were created in a data-reduction step by taking the average of each subject's *z* scores for the primary measure (i.e., LHA and BPAQ; LHIB and BIS-11) as in our previous studies (51).

RESULTS

Demographic, Lifestyle, and Psychometric Characteristics of Subjects

Subjects did not differ by gender or race but did differ in age and Hollingshead socioeconomic status (Table 1). The HC subjects were modestly younger than the PC and IED subjects, and the HC and PC subjects had a greater proportion in the higher socioeconomic status categories

Table 1. Demographic, Behavioral, and Lifestyle Variables Among the Groups

	Healthy Control Subjects (n = 67)	Psychiatric Control Subjects (n = 61)	Intermittent Explosive Disorder Subjects (n = 69)	Group Differences
Demographic Variables				
Age (years), ± SD	32.0 ± 9.1	35.8 ± 7.4	35.1 ± 7.9	HC < PC = IED[a]
Gender (M/F)	32/35	28/36	37/32	HC = PC = IED[b]
Race (white/nonwhite)	43/24	49/13	43/26	HC = PC = IED[b]
SES category (I/II/III/IV/V)	9/41/6/4/7	18/29/9/2/3	7/28/19/12/3	HC = PC[c] HC ≠ IED[b]
BMI, ± SD	27.2 ± 3.9	26.2 ± 5.6	27.8 ± 5.2	HC = PC = IED[a]
Behavioral Variables				
Psychosocial function (GAF), ± SD	83.6 ± 4.5	65.7 ± 10.2	53.7 ± 8.0	HC > PC > IED[a]
LHA aggression, ± SD	4.4 ± 3.0	8.9 ± 5.6	20.1 ± 3.2	HC < PC < IED[a]
BPAQ aggression, ± SD	41.5 ± 14.9	47.7 ± 14.9	74.3 ± 17.9	HC = PC < IED[a]
LHIB impulsivity, ± SD	21.9 ± 15.7	45.5 ± 16.0	56.0 ± 17.7	HC < PC < IED[a]
BIS-11 impulsivity, ± SD	54.0 ± 8.5	63.2 ± 10.0	69.4 ± 11.6	HC < PC < IED[a]
BDI-II state depression, ± SD	2.2 ± 8.5	7.1 ± 7.9	16.2 ± 11.8	HC < PC < IED[a]
Stressful life experiences (LES: past 6 months), ± SD	5.3 ± 5.1	6.5 ± 5.8	13.7 ± 10.6	HC = PC < IED[d]
Lifestyle Variables				
Obesity (BMI >30.0) (yes/no)	15/52	12/49	14/55	HC = PC = IED[c]
Current alcohol consumption (yes/no)	42/24	41/21	42/27	HC = PC = IED[c]
Subjects with >2 alcoholic drinks/day (yes/no)	2/65	1/60	10/59	HC = PC < IED[b]
Mean alcoholic drinks/day (among those drinking currently), ± SD	.8 ± .6	.5 ± .6	1.3 ± 3.1	HC = PC < IED[a]
Current smoking (yes/no)	9/57	15/47	19/50	HC = PC = IED[c]
Mean packs/day (among those smoking currently), ± SD	.2 ± .2	.5 ± .4	.4 ± .3	HC = PC = IED[e]

ANOVA, analysis of variance; BDI-II, Beck Depression Inventory; BIS-11, Barratt Impulsivity Scale; BMI, body mass index; BPAQ, Buss-Perry Aggression Questionnaire; F, female; GAF, Global Assessment of Function; HC, healthy control; IED, intermittent explosive disorder; LES, Life Experiences Survey; LHA, Life History of Aggression; LHIB, Life History of Impulsive Behavior; M, male; PC, psychiatric control; SES, socioeconomic status.

[a]Significant ($p < .05$) after ANOVA (post hoc $p < .05$).

[b]Significant ($p < .05$) after χ^2 test (post hoc by serial χ^2).

[c]Not significant after χ^2 test.

[d]Significant ($p < .05$) after ANOVA of log-transformed values (post hoc $p < .05$).

[e]Not significant after ANOVA.

(e.g., I and II) compared with the IED subjects (76% vs. 77% vs. 51%). Neither mean body mass index nor proportion of obese subjects (i.e., body mass index ≥30) differed across groups. In addition, the groups did not differ in the rates, or the degree, of current cigarette smoking. Although the groups did not differ in the proportion currently consuming alcohol, the IED subjects reported a higher mean number of drinks per day. As expected, subject groups differed in LHA and BPAQ Aggression, LHIB and BIS-11 Impulsivity, BDI-II and Inventory to Diagnose Depression, and LES scores (IED > PC > HC) and differed in Global Assessment of Function scores (IED < PC < HC).

Oxidative Stress Markers in IED and Control Subjects

A significant difference was revealed by MANOVA among the HC, PC, and IED groups with both markers of oxidative stress (Wilks $\lambda = .79$, $F_{4,386} = 11.99$, $p < .001$; 8-OH-DG, $F_{2,194} = 10.92$, $p < .001$; Log 8-ISO, $F_{2,194} = 17.63$, $p < .001$). The IED subjects displayed higher levels of 8-OH-DG (Figure 1A, left) and Log 8-ISO (Figure 1B, left) levels than either HC or PC subjects. Adding relevant demographic, psychometric, and lifestyle variables to the MANOVA model did not change these results (Wilks $\lambda = .85$, $F_{4,352} = 7.41$, $p < .001$; 8-OH-DG, $F_{2,176} = 5.91$, $p = .003$; Log 8-ISO, $F_{2,176} = 10.46$, $p < .001$).

Oxidative Stress Markers as a Function of Axis I and Axis II Disorders and as a Function of Prior Psychiatric Evaluation and Treatment

Among PC and IED subjects, MANOVA analysis confirmed higher oxidative stress markers in the IED subjects compared with the PC subjects when current Axis I (Wilks $\lambda = .71$, $F_{2,124} = 26.01$, $p < .001$), lifetime Axis I (Wilks $\lambda = .73$, $F_{2,123} = 22.86$, $p < .001$), or Axis II Cluster (Wilks $\lambda = .81$, $F_{2,123} = 14.06$, $p < .001$) disorders were included in the model. Adding the relevant demographic, psychometric, and lifestyle variables did not alter this finding (Table S1 in Supplement 1). A separate MANOVA revealed elevated oxidative stress markers, as a function of IED, even when prior history of evaluation and treatment for a behavioral condition was included in the model (Wilks $\lambda = .76$, $F_{2,122} = 19.21$, $p < .001$); the latter variable had no effect on the oxidative stress marker levels (Wilks $\lambda = .99$, $F_{2,122} = .82$, $p = .444$).

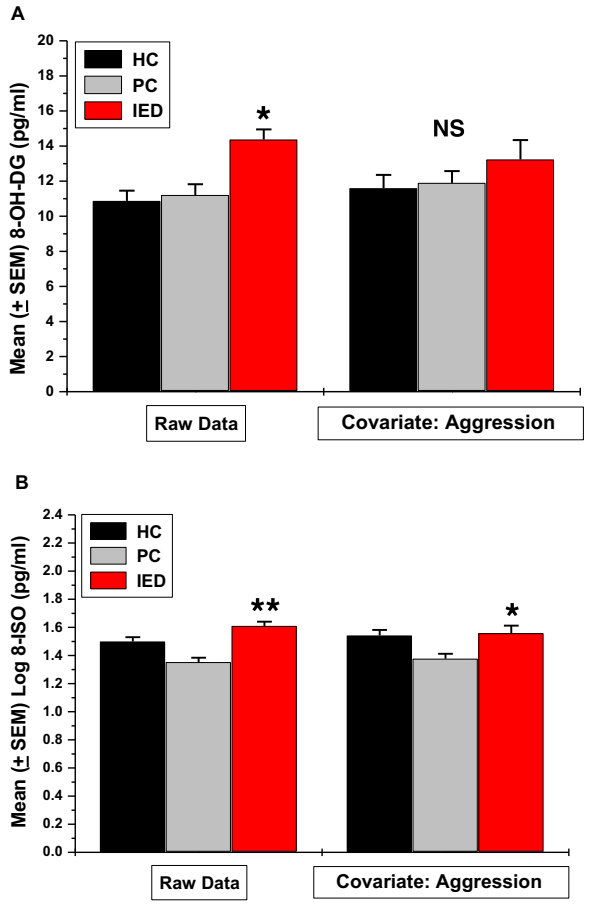

Figure 1. **(A)** Plasma 8-hydroxy-2′-deoxyguanosine (8-OH-DG) as a function of subject status. *$p < .05$ different from healthy control (HC) and psychiatric control (PC) subjects. **(B)** Plasma logarithm (Log 10) 8-isoprostane (8-ISO) as a function of subject status. *$p < .05$ different from PC subjects; p = not significant from HC subjects. IED, intermittent explosive disorder.

Oxidative Stress and Plasma Inflammatory Markers

In addition to the two oxidative stress makers, data for two plasma inflammatory markers were available for the study subjects (14). The 8-OH-DG levels correlated significantly, but weakly, with both C-reactive protein ($r = .17$, $n = 197$, $p = .015$) and interleukin-6 ($r = .15$, $n = 195$, $p = .039$). The Log 8-ISO levels correlated weakly with C-reactive protein ($r = .11$, $n = 197$, $p = .111$) but more strongly with interleukin-6 ($r = .31$, $n = 195$, $p < .001$). However, adding these inflammatory markers to the statistical models did not change the above-reported results for IED.

Oxidative Stress Markers, Aggression, and Impulsivity

Across all subjects, the core feature of IED, aggression, displayed a positive relationship with both 8-OH-DG and Log 8-ISO levels (Figure 2A,B). The 8-OH-DG and Log 8-ISO levels were also significantly correlated ($r = .31$, $n = 197$, $p < .001$). Subsequent hierarchical multiple regression analysis with each oxidative stress marker as a dependent variable and demographic variables as independent variables at step 1, followed by psychometric and lifestyle variables as independent variables at step 2, and composite aggression as an independent variable at step 3 revealed a significant contribution for composite aggression for both 8-OH-DG and Log 8-ISO values above and beyond that for all variables included at steps 1 and 2 (Table 2).

Composite impulsivity scores also displayed a positive relationship with both 8-OH-DG and Log 8-ISO levels (8-OH-DG, $r = .27$, $n = 176$, $p < .001$; Log 8-ISO, $r = .15$, $n = 176$, $p = .041$). However, both composite aggression and composite impulsivity scores were highly correlated ($r = .67$, $n = 165$, $p < .001$), and multiple regression analysis ($F_{2,162} = 11.53$, $p < .001$) revealed a unique contribution for composite aggression ($B = 1.74 \pm .55$, $\beta = .31$, $p = .002$) but not for

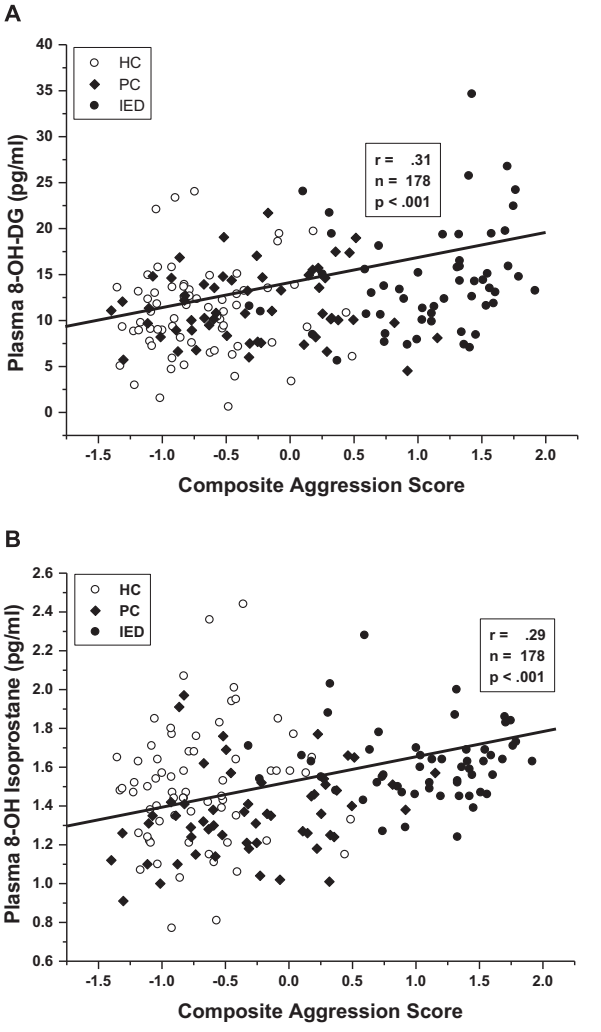

Figure 2. **(A)** Life History of Aggression (LHA) aggression score with plasma 8-hydroxy-2′-deoxyguanosine (8-OH-DG) in all subjects. **(B)** LHA aggression score with plasma logarithm (Log) 8-isoprostane (8-ISO) in all subjects. HC, healthy control; IED, intermittent explosive disorder; PC, psychiatric control.

Table 2. Hierarchical Linear Regression Analysis for Plasma 8-OH-DG and Log 8-ISO

	R	R^2	R^2 Change	*F* Change	*df1/df2*	Significance of *F* Change
8-OH-DG						
Model 1[a]	.251	.063	.063	2.315	5/172	.046
Model 2[b]	.351	.123	.060	11.741	1/171	.001
Log 8-ISO						
Model 1[c]	.219	.020	.048	1.732	5/172	.130
Model 2[d]	.310	.096	.064	9.105	1/171	.003
			B ± SE	β	Partial *r*	*p*
Model 1 for 8-OH-DG[a]						
BMI			−.005 ± .076	−.005	−.005	.949
Composite state depression			.866 ± .378	.175	.172	.023
Psychosocial stress			−.799 ± .760	−.080	−.080	.294
Current drinking			1.184 ± .774	.118	.116	.128
Current smoking			.456 ± .520	.067	.067	.650
Model 2 for 8-OH-DG[b]						
BMI			−.028 + .074	−.028	−.029	.702
Composite state depression			.157 ± .421	.032	.027	.710
Psychosocial stress			−1.077 ± .742	−.108	−.110	.148
Current drinking			.943 ± .754	.094	.095	.213
Current smoking			.231 ± .509	.034	.033	.650
Composite aggression			1.609 ± .470	.295	.253	.001
Model 1 for Log 8-ISO[c]						
BMI			.000 ± .004	.001	.001	.993
Composite state depression			.037 ± .021	.137	.135	.077
Psychosocial stress			.029 ± .042	.052	.052	.057
Current drinking			.073 ± .043	.133	.130	.088
Current smoking			.014 ± .029	.038	.038	.074
Model 2 for Log 8-ISO[d]						
BMI			−.001 ± .004	−.020	−.021	.787
Composite state depression			.002 ± .023	.009	.008	.918
Psychosocial stress			.015 ± .041	.027	.028	.148
Current drinking			.061 ± .042	.112	.111	.145
Current smoking			.003 ± .028	.009	.009	.909
Composite aggression			.079 ± .470	.264	.225	.003

BDI-II, Beck Depression Inventory; BMI, body mass index; BPAQ, Buss-Perry Aggression Questionnaire; 8-ISO, 8-isoprostane; 8-OH-DG, 8-hydroxy-2′-deoxyguanosine; IDD, Inventory to Diagnose Depression; LES, Life Experiences Survey; LHA, Life History of Aggression; Log, logarithm.

[a]Model 1 for 8-OH-DG ($F_{5,172}$ = 2.32, p = .046): 8-OH-DG as dependent variable with BMI, composite state depression score (BDI-II + IDD), recent psychosocial stress score (LES score), current alcohol consumption (drinks per day), and current cigarette consumption (packs per day).

[b]Model 2 for 8-OH-DG ($F_{6,171}$ = 4.01, p = .001): Adds composite aggression (LHA + BPAQ) score to model.

[c]Model 1 for Log 8-ISO ($F_{5,172}$ = 1.73, p = .130): Log 8-ISO as dependent variable with BMI, composite state depression score (BDI-II + IDD), recent psychosocial stress score (LES score), current alcohol consumption (drinks per day), and current cigarette consumption (packs per day).

[d]Model 2 for Log 8-ISO ($F_{6,171}$ = 3.03, p = .003): Adds composite aggression (LHA + BPAQ) score to model.

composite impulsivity (B = .33 ± .56, β = .06, p = .560) for 8-OH-DG levels. The same was true for Log 8-ISO levels ($F_{2,162}$ = 7.74, p = .001; composite aggression, B = .10 ± .03, β = .34, p = .001; composite impulsivity, B = −.02 ± .03, β = −.07, p = .480). Adding composite aggression scores to the MANCOVA model for the two oxidative stress markers, as a function of subject status, eliminated any significant difference among HC, PC, and IED subjects for 8-OH-DG (Figure 1A, right) and eliminated the significant difference among HC and IED subjects for Log 8-ISO (Figure 1B, right).

History of Actual Aggressive Behavior Versus Aggression as a Personality Trait

Within the composite aggression variable, multiple regression analysis ($F_{2,175}$ = 9.50, p < .001) revealed a unique contribution of LHA aggression score (B = .140 ± .064, β = .22, p = .03) but not of BPAQ aggression score (B = .029 ± .023, β = .12, p = .209) for 8-OH-DG levels. However, similar analysis with Log 8-ISO levels did not demonstrate a unique effect for either LHA aggression score or BPAQ aggression score.

Aggression and Other Nonaggressive Personality Variables

Hierarchical multiple regression analysis, with each oxidative stress marker as a dependent variable and the three Eysenck Personality Questionnaire personality scale scores as independent variables at step 1, followed by composite aggression at step 2, revealed a significant relationship for composite aggression for both 8-OH-DG and Log 8-ISO levels above and beyond that for the Eysenck Personality Questionnaire variables included at step 1 (Table 3). Adding the relevant demographic, psychometric, or lifestyle variables to the model did not change this result.

Self-Directed Aggression Variables

Among PC and IED subjects, MANOVA revealed no effect of history of suicide attempt (Wilks $\lambda = .99$, $F_{2,125} = .13$, $p = .880$), or of self-injurious behavior (Wilks $\lambda = .97$, $F_{2,125} = 1.96$, $p = .145$).

DISCUSSION

This is the first study to examine systemic oxidative stress markers as a function of aggression or impulsivity in human subjects. In addition, this is the first study to examine evidence of oxidative stress in well-characterized subjects with current history of recurrent, problematic, impulsive aggression, diagnosed as IED, and to examine psychometric, non–personality-based measures of aggression and impulsivity. In this sample, plasma measures of oxidative stress were elevated in IED subjects and displayed a positive relationship with dimensional measures of aggression above and beyond relationships with other variables examined. Adding aggression to the statistical models eliminated the IED and control subject differences in both oxidative stress markers.

Although elevation of these oxidative markers may be associated with the presence of an inflammatory process in these subjects (14), correlations between the markers were weak in all cases except for Log 8-ISO where the correlation with interleukin-6 was of modest size ($r = .31$). However, until a more complete panel of inflammatory markers is studied in conjunction with oxidative stress markers, it is premature to conclude that this finding is mediated by the upstream presence of inflammation in these subjects. The possibility that oxidative stress may operate as the driver of downstream activation of inflammatory pathways cannot be ignored at this time.

Table 3. Hierarchical Linear Regression Analysis for Plasma 8-OH-DG and Log 8-ISO With Regard to Aggression and Dimensions of General Personality

	R	R^2	R^2 Change	*F* Change	*df1/df2*	Significance of *F* Change
8-OH-DG						
Model 1[a]	.318	.101	.101	6.23	3/166	<.001
Model 2[b]	.379	.143	.042	8.13	1/165	.005
8-ISO						
Model 1[c]	.245	.060	.043	3.53	3/166	.016
Model 2[d]	.349	.122	.062	11.68	1/165	.001
			B ± SE	*B*	Partial *r*	*P*
Model 1 for 8-OH-DG[a]						
EPQ Scale: Neuroticism			.493 ± .142	.269	.261	.001
EPQ Scale: Extraversion			.176 ± .171	.080	.080	.304
EPQ Scale: Psychoticism			−.423 ± .189	−.168	−.172	.026
Model 2 for 8-OH-DG[b]						
EPQ Scale: Neuroticism			.253 ± .162	.138	.121	.120
EPQ Scale: Extraversion			.100 ± .169	.045	.046	.556
EPQ Scale: Psychoticism			−.404 ± .185	−.161	−.168	.030
Composite Aggression			1.364 ± .489	.250	.217	.005
Model 1 for 8-ISO[c]						
EPQ Scale: Neuroticism			.011 ± .008	.108	.106	.172
EPQ Scale: Extraversion			.022 ± .010	.181	.175	.023
EPQ Scale: Psychoticism			−.017 ± .011	−.122	−.122	.114
Model 2 for 8-ISO[d]						
EPQ Scale: Neuroticism			−.005 ± .009	−.050	−.044	.574
EPQ Scale: Extraversion			.017 ± .009	.139	.139	.074
EPQ Scale: Psychoticism			−.015 ± .010	−.113	−.117	.132
Composite Aggression			.092 ± .027	.304	.257	.001

8-ISO, 8-isoprostane; 8-OH-DG, 8-hydroxy-2′-deoxyguanosine; EPQ, Eysenck Personality Questionnaire.

[a]Model 1 for 8-OH-DG ($F_{3,166} = 6.23$, $p < .001$): 8-OH-DG as dependent variable with EPQ scales as independent variables.

[b]Model 2 for 8-OH-DG ($F_{4,165} = 6.91$, $p < .001$): Adds composite aggression.

[c]Model 1 for 8-ISO ($F_{3,166} = 3.53$, $p = .016$): 8-ISO as dependent variable with EPQ scales as independent variables.

[d]Model 2 for 8-ISO ($F_{4,165} = 5.74$, $p < .001$): Adds composite aggression.

These results likely are not due to common confounding factors. Subjects were physically healthy, free of systemic illness or physical injury, free of any medication for >4 weeks, and not using illicit drugs of any kind. These relationships were not changed when adjusted for demographic, psychometric, or lifestyle variables that could confound the results. These results also were not due to the presence of other Axis I or Axis II conditions other than IED, which is the categorical expression of the construct of impulsive aggression.

Elevation of oxidative stress markers in IED subjects may be related to recent physiologic or psychological stress (33). However, subjects were physically healthy and uninjured, subjects were studied at rest, and no subject reported any meaningful stressor on the day of the sample collection. Finally, although our measure of stressful life events may not have captured potential stresses close in time to collection of the blood sample, this measure did show clear elevation of stressful life events in the IED subjects versus PC or HC subjects, and controlling for this variable did not change the statistical significance of the findings.

The oxidative stress markers in this study were associated with aggressive behavior (LHA) in the case of 8-OH-DG and aggressive behavior and aggression as a personality trait (BPAQ) in the case of Log 8-ISO levels, rather than with impulsivity as a behavior (LHIB) or as a personality trait (BIS-11). Despite the univariate correlation between LHA and BPAQ measures and LHIB and BIS measures and oxidative stress markers, the observed relationship with both is through the shared variance with aggression. In addition, the oxidative stress markers were associated with aggression above and beyond what might be associated with general personality traits, such as neuroticism, extraversion, and psychoticism.

These data are consistent with previous human studies that report an increase in oxidative stress markers in schizophrenia (25), depression (26–30), obsessive-compulsive disorder (31), and panic disorder (32). In addition, a postmortem study of the prefrontal cortex of schizophrenic subjects reported that alterations of transcript, protein, and metabolic levels were associated with energy metabolism and oxidative stress response (52). Animal models of mania also demonstrate increased levels of protein oxidation and lipid peroxidation markers in the brain (53). Finally, animal models of stress-induced depression demonstrate an increase in lipid peroxidation markers in the brain (54). Oxidative stress may play a role in the pathophysiology of several neuropsychiatric disorders despite differences in phenotype (19).

The mechanism by which oxidative stress is associated with aggression may be mediated by damage to nucleic acids or lipids, which could degrade basic cellular functions in the brain, such as membrane fluidity, receptors and second messengers signaling, and enzyme function (20). There are many neuronal based mechanisms associated with aggression, including serotonin (55), dopamine (55), glutamate (56), and numerous peptides (57–60), any or all of which may be affected by oxidative stress.

The strengths of this study include a well-characterized sample, multiple validated measures of aggression and impulsivity, and a standardized approach to drug-free status and subject activity to minimize the effect of extraneous factors on oxidative stress marker levels. There are some limitations. First, this is the first study of its kind, and replication is required. This was cross-sectional study, and no causal conclusions can be made from these data. Further experimental work would be necessary to establish causal relationships. Second, because these subjects volunteered for research studies, rather than for clinical treatment, these results may not be generalizable to the clinic. However, analyses revealed that history of behavioral conditions played no significant role in these findings. Additionally, subjects were recruited based on the presence of self-identified problematic symptoms. Third, this study focused on selective markers of oxidative stress and did not assess the status of antioxidative enzymes or other substances that can counter oxidative stress. It is unknown if the balance of the two processes is tilted one way or the other. Future studies should assess markers involving both oxidative and antioxidative processes.

In conclusion, we report a direct relationship between two plasma oxidative stress markers and aggression in human subjects, particularly in individuals with IED. This relationship was not accounted for by any factors studied, such as body mass index, state depression, stressful life events, or other possible confounders. In addition, these findings were not accounted for by the presence of other psychiatric disorders or general personality factors other than aggression. Given that IED, a disorder of impulsive aggression, displays a 2%–3% 1-year prevalence rate in the United States (61) and that currently available psychotropic treatments lead to remission in <50% of individuals treated (62), additional strategies for the examination and intervention of impulsive aggression in human subjects is needed. If this finding is replicated, such strategies could include testing the efficacy of antioxidant agents on aggression and testing the efficacy of anti-inflammatory agents that would reduce inflammation and, as a consequence, oxidative stress.

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ARTICLE INFORMATION

From the Clinical Neuroscience Research Unit (EFC, RL), Department of Psychiatry and Behavioral Neuroscience, Pritzker School of Medicine, The University of Chicago, Chicago, Illinois; and Department of Pediatrics (DG), Pritzker School of Medicine, The University of Chicago, Chicago, Illinois.

Address correspondence to Emil F. Coccaro, M.D., Clinical Neuroscience Research Unit, Department of Psychiatry and Behavioral Neuroscience, The University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637; E-mail: ecoccaro@bsd.uchicago.edu.

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REFERENCES

1. Pesce M, Speranza L, Franceschelli S, Ialenti V, Patruno A, Febo MA, *et al*. (2011): Biological role of interleukin-1beta in defensive-aggressive behaviour. J Biol Regul Homeost Agents 25:323–329.
2. Bhatt S, Bhatt R, Zalcman SS, Siegel A (2008): Role of IL-1 beta and 5-HT2 receptors in midbrain periaqueductal gray (PAG) in potentiating defensive rage behavior in cat. Brain Behav Immun 22:224–233.
3. Zalcman SS, Siegel A (2006): The neurobiology of aggression and rage: Role of cytokines. Brain Behav Immun 20:507–514.
4. Patel A, Siegel A, Zalcman SS (2010): Lack of aggression and anxiolytic-like behavior in TNF receptor (TNF-R1 and TNF-R2) deficient mice. Brain Behav Immun 24:1276–1280.
5. Graham JE, Robles TF, Kiecolt-Glaser JK, Malarkey WB, Bissell MG, Glaser R (2006): Hostility and pain are related to inflammation in older adults. Brain Behav Immun 20:389–400.
6. Ranjit N, Diez-Roux AV, Shea S, Cushman M, Seeman T, Jackson SA, *et al*. (2007): Psychosocial factors and inflammation in the multi-ethnic study of atherosclerosis. Arch Intern Med 167:174–181.
7. Suarez EC (2004): C-reactive protein is associated with psychological risk factors of cardiovascular disease in apparently healthy adults. Psychosom Med 66:684–691.
8. Suarez EC (2003): Joint effect of hostility and severity of depressive symptoms on plasma interleukin-6 concentration. Psychosom Med 65:523–527.
9. Coccaro E (2006): Association of C-reactive protein elevation with trait aggression and hostility in personality disordered subjects: A pilot study. J Psychiatr Res 40:460–465.
10. Marsland AL, Prather AA, Petersen KL, Cohen S, Manuck SB (2008): Antagonistic characteristics are positively associated with inflammatory markers independently of trait negative emotionality. Brain Behav Immun 22:753–761.
11. Sjogren E, Leanderson P, Kristenson M, Ernerudh J (2006): Interleukin-6 levels in relation to psychosocial factors: Studies on serum, saliva, and in vitro production by blood mononuclear cells. Brain Behav Immun 20:270–278.
12. Miller GE, Freedland KE, Carney RM, Stetler CA, Banks WA (2003): Cynical hostility, depressive symptoms, and the expression of inflammatory risk markers for coronary heart disease. J Behav Med 26:501–515.
13. Kiecolt-Glaser JK, Loving TJ, Stowell JR, Malarkey WB, Lemeshow S, Dickinson SL, *et al*. (2005): Hostile marital interactions, proinflammatory cytokine production, and wound healing. Arch Gen Psychiatry 62: 1377–1384.
14. Coccaro EF, Lee R, Coussons-Read M (2013): Elevated plasma inflammatory markers in individuals with intermittent explosive disorder and correlation with aggression in humans [published online ahead of print Dec 18]. JAMA Psychiatry.
15. Inagaki TK, Muscatell KA, Irwin MR, Cole SW, Eisenberger NI (2012): Inflammation selectively enhances amygdala activity to socially threatening images. Neuroimage 59:3222–3226.
16. Kullmann JS, Grigoleit JS, Lichte P, Kobbe P, Rosenberger C, Banner C, *et al*. (2013): Neural response to emotional stimuli during experimental human endotoxemia. Hum Brain Mapp 34:2217–2227.
17. Babior BM (2000): The NADPH oxidase of endothelial cells. IUBMB Life 50:267–269.
18. Memon RA, Staprans I, Noor M, Holleran WM, Uchida Y, Moser AH, *et al*. (2000): Infection and inflammation induce LDL oxidation in vivo. Arterioscler Thromb Vasc Biol 20:1536–1542.
19. Ng F, Berk M, Dean O, Bush AI (2008): Oxidative stress in psychiatric disorders: Evidence base and therapeutic implications. Int J Neuropsychopharmacol 11:851–876.
20. Halliwell B (2006): Oxidative stress and neurodegeneration: Where are we now? J Neurochem 97:1634–1658.
21. Chan AS, Ng LW, Poon LS, Chan WW, Wong YH (2007): Dopaminergic and adrenergic toxicities on SK-N-MC human neuroblastoma cells are mediated through G protein signaling and oxidative stress. Apoptosis 12:167–179.
22. Delattre J, Beaudeux JL, Bonnefont-Rousselot D (2005): Radicaux Libres et Stress Oxidant, Aspects Biologiques et Pathologiques. Paris: Editions Medicales internationales.
23. LeBel CP, Bondy SC (1991): Oxygen radicals: Common mediators of neurotoxicity. Neurotoxicol Teratol 13:341–346.
24. Cardozo-Pelaez F, Song S, Parthasarathy A, Hazzi C, Naidu K, Sanchez-Ramos J (1999): Oxidative DNA damage in the aging mouse brain. Mov Disord 14:972–980.
25. Yao JK, Reddy RD, van Kammen DP (2001): Oxidative damage and schizophrenia: An overview of the evidence and its therapeutic implications. CNS Drugs 15:287–310.
26. Bilici M, Efe H, Koroglu MA, Uydu HA, Bekaroglu M, Deger O (2001): Antioxidative enzyme activities and lipid peroxidation in major depression: Alterations by antidepressant treatments. J Affect Disord 64: 43–51.
27. Forlenza MJ, Miller GE (2006): Increased serum levels of 8-hydroxy-2′-deoxyguanosine in clinical depression. Psychosom Med 68:1–7.
28. Irie M, Asami S, Nagata S, Ikeda M, Miyata M, Kasai H (2001): Psychosocial factors as a potential trigger of oxidative DNA damage in human leukocytes. Jpn J Cancer Res 92:367–376.
29. Irie M, Miyata M, Kasai H (2005): Depression and possible cancer risk due to oxidative DNA damage. J Psychiatr Res 39:553–560.
30. Yager S, Forlenza MJ, Miller GE (2010): Depression and oxidative damage to lipids. Psychoneuroendocrinology 35:1356–1362.
31. Kuloglu M, Atmaca M, Tezcan E, Gecici O, Tunckol H, Ustundag B (2002): Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. Neuropsychobiology 46:27–32.
32. Kuloglu M, Atmaca M, Tezcan E, Ustundag B, Bulut S (2002): Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. Neuropsychobiology 46:186–189.
33. Gidron Y, Russ K, Tissarchondou H, Warner J (2006): The relation between psychological factors and DNA-damage: A critical review. Biol Psychol 72:291–304.
34. Mesaros C, Arora JS, Wholer A, Vachani A, Blair IA (2012): 8-Oxo-2'-deoxyguanosine as a biomarker of tobacco-smoking-induced oxidative stress. Free Radic Biol Med 53:610–617.
35. Milne GL, Musiek ES, Morrow JD (2005): F2-isoprostanes as markers of oxidative stress in vivo: An overview. Biomarkers 10(suppl 1): S10–S23.
36. Morrow JD (2005): Quantification of isoprostanes as indices of oxidant stress and the risk of atherosclerosis in humans. Arterioscler Thromb Vasc Biol 25:279–286.
37. Coccaro EF (2012): Intermittent explosive disorder as a disorder of impulsive aggression for DSM-5. Am J Psychiatry 169:577–588.
38. Evans MD, Olinski R, Loft S, Cooke MS (2010): Toward consensus in the analysis of urinary 8-oxo-7,8-dihydro-2'-deoxyguanosine as a noninvasive biomarker of oxidative stress. FASEB J 24: 1249–1260.
39. Halliwell B (2000): Lipid peroxidation, antioxidants and cardiovascular disease: How should we move forward? Cardiovasc Res 47:410–418.
40. American Psychiatric Association (1994): Diagnostic and Statistical Manual of Mental Disorders, 4th ed. Washington, DC: American Psychiatric Press.
41. First MB, Spitzer RL, Gibbon M, Williams JBW (1997): Structured Clinical Interview for DSM-IV Axis I Disorders (SCID). New York: Psychiatric Institute, Biometrics Research.
42. Pfohl B, Blum N, Zimmerman M (1997): Structured Interview for DSM-IV Personality: SIDP-IV. Washington, DC: American Psychiatric Press.
43. Coccaro EF, Nayyer H, McCloskey MS (2012): Personality disorder-not otherwise specified evidence of validity and consideration for DSM-5. Compr Psychiatry 53:907–914.
44. Coccaro EF, Berman ME, Kavoussi RJ (1997): Assessment of life history of aggression: Development and psychometric characteristics. Psychiatry Res 73:147–157.
45. Buss AH, Perry M (1992): The aggression questionnaire. J Pers Soc Psychol 63:452–459.
46. Coccaro EF, Schmidt-Kaplan CA (2012): Life history of impulsive behavior: Development and validation of a new questionnaire. J Psychiatr Res 46:346–352.
47. Patton J, Stanford M, Barratt E (1995): Factor structure of the Barratt impulsiveness scale. J Clin Psychol 51:768–774.

48. Beck AT, Steer RA, Brown GK (1996): BDI-II, Beck Depression Inventory: Manual, 2nd ed. New York: Harcourt Brace.
49. Sarason I, Johnson J, Siegel J (1978): Assessing the impact of life changes: development of the life experiences survey. J Consult Clin Psychol 46:932–946.
50. Eysenck H Jr, Eysenck SBG (1991): Manual of the Eysenck Personality Scales (EPS Adult). London: Hodder & Stoughton.
51. Coccaro EF, Lee R, Kavoussi RJ (2010): Aggression, suicidality, and intermittent explosive disorder: Serotonergic correlates in personality disorder and healthy control subjects. Neuropsychopharmacology 35: 435–444.
52. Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, *et al*. (2004): Mitochondrial dysfunction in schizophrenia: Evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry 9:684–697.
53. Frey BN, Martins MR, Petronilho FC, Dal-Pizzol F, Quevedo J, Kapczinski F (2006): Increased oxidative stress after repeated amphetamine exposure: Possible relevance as a model of mania. Bipolar Disord 8:275–280.
54. Eren I, Naziroglu M, Demirdas A, Celik O, Uguz AC, Altunbasak A, *et al*. (2007): Venlafaxine modulates depression-induced oxidative stress in brain and medulla of rat. Neurochem Res 32:497–505.
55. Coccaro EF, Lee R (2010): Cerebrospinal fluid 5-hydroxyindolacetic acid and homovanillic acid: Reciprocal relationships with impulsive aggression in human subjects. J Neural Transm 117:241–248.
56. Coccaro EF, Lee R, Vezina P (2013): Cerebrospinal fluid glutamate concentration correlates with impulsive aggression in human subjects. J Psychiatr Res 47:1247–1253.
57. Coccaro EF, Kavoussi RJ, Hauger RL, Cooper TB, Ferris CF (1998): Cerebrospinal fluid vasopressin levels: Correlates with aggression and serotonin function in personality-disordered subjects. Arch Gen Psychiatry 55:708–714.
58. Lee R, Ferris C, Van de Kar LD, Coccaro EF (2009): Cerebrospinal fluid oxytocin, life history of aggression, and personality disorder. Psychoneuroendocrinology 34:1567–1573.
59. Coccaro EF, Lee R, Owens MJ, Kinkead B, Nemeroff CB (2012): Cerebrospinal fluid substance P-like immunoreactivity correlates with aggression in personality disordered subjects. Biol Psychiatry 72: 238–243.
60. Coccaro EF, Lee R, Liu T, Mathé AA (2012): Cerebrospinal fluid neuropeptide Y-like immunoreactivity correlates with impulsive aggression in human subjects. Biol Psychiatry 72: 997–1003.
61. Kessler RC, Coccaro EF, Fava M, Jaeger S, Jin R, Walters E (2006): The prevalence and correlates of DSM-IV intermittent explosive disorder in the National Comorbidity Survey Replication. Arch Gen Psychiatry 63:669–678.
62. Coccaro EF, Lee R, Kavoussi RJ (2009): A double-blind, randomized, placebo-controlled trial of fluoxetine in patients with intermittent explosive disorder. J Clin Psychiatry 70:653–662.