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A five-year follow-up study of antioxidants, oxidative stress and polyunsaturated fatty acids in schizophrenia

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Original Article

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Abstract

Objective: Oxidative stress and dysregulated antioxidant defence may be involved in the pathophysiology of schizophrenia. In the present study, we investigated changes in antioxidants and oxidative stress from an acute to a later stable phase. We hypothesised that the levels of oxidative markers are increased in schizophrenia compared with healthy controls; change from the acute to the stable phase; and are associated with the levels of membrane polyunsaturated fatty acids (PUFAs) and symptom severity. *Methods:* Fifty-five patients with schizophrenia spectrum disorders, assessed during an acute phase and 5 years later during a stable phase, and 51 healthy controls were included. We measured antioxidants (α-tocopherol, uric acid, albumin and bilirubin), markers of oxidative stress (F2-isoprostane and reactive oxygen metabolites) and membrane fatty acids. Antioxidants and oxidative stress markers were compared in schizophrenia versus healthy controls, adjusting for differences in sex, age and smoking, and changes over time. Associations between symptoms and PUFA were also investigated. *Results:* In the acute phase, α-tocopherol was significantly higher ($p < 0.001$), while albumin was lower ($p < 0.001$) compared with the stable phase. Changes in α-tocopherol were associated with PUFA levels in the acute phase. In the stable phase, schizophrenia patients had higher uric acid ($p = 0.009$) and lower bilirubin ($p = 0.046$) than healthy controls. CRP was higher in patients in the stable phase ($p < 0.001$), and there was no significant change from the acute phase. *Conclusion:* The present findings of change in antioxidant levels in the acute versus stable phase of schizophrenia the present findings suggest that redox regulation is dynamic and changes during different phases of the disorder.

Significant outcomes

- The levels of α-tocopherol were significantly higher and the levels of albumin significantly lower in the acute phase compared with the stable phase of schizophrenia.
- The change in α-tocopherol was associated with membrane fatty acid (PUFA) levels in the acute phase.
- In the stable phase of schizophrenia the levels of bilirubin were lower and uric acid higher compared with healthy controls after adjusting for sex, age and smoking.

Limitations

- The relatively small sample size reduces statistical power and increases the risk of type II errors.
- Body weight of the participants, diet and dietary supplements that may influence the levels of membrane fatty acids and antioxidants were not controlled for.
- The group of patients not using antipsychotic medication was small, and information of adherence was not obtained, making it difficult to assess the effect of medication.

Introduction

Schizophrenia is regarded as a complex syndrome of developmental defects caused by genetic and environmental factors (van Os *et al.*, 2008; Howes & Murray, 2014), but the underlying pathological mechanisms are still mainly unknown. Antioxidant defence systems may be disturbed in schizophrenia (Bitanihirwe & Woo, 2011; Emiliani *et al.*, 2014), and oxidative stress may play a role in the underlying disease mechanisms (Zhang & Yao, 2013).

Under normal physiological conditions free-radical damage is controlled by the antioxidant defence systems, comprising a series of enzymatic components, including glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT), and non-enzymatic components, including glutathione (GSH), α-tocopherol (vitamin E) and ascorbic acid (vitamin C) (Yao & Keshavan, 2011; Pisoschi & Pop, 2015). Oxidative stress is the imbalance of free radicals generated from both normal metabolism, including neurotransmitters associated with schizophrenia, such as dopamine and glutamate, and from various environmental exposures. Several lines of evidence suggest increased oxidative stress in schizophrenia (Boskovic *et al.*, 2011; Lai *et al.*, 2016). In addition, stress-induced signalling cascades, including those involving inflammatory processes and oxidative stress, will modulate the development and maintenance of synaptic connectivity, and further contribute to the abnormalities seen in schizophrenia (Boulanger, 2009; Do *et al.*, 2009). However, the findings are not consistent and the manner by which free radicals influence schizophrenia pathophysiology is still unclear (Herken *et al.*, 2001; Pandya *et al.*, 2013; Bulbul *et al.*, 2014; Koga *et al.*, 2016).

Impaired levels of non-enzymatic antioxidants, including glutathione, bilirubin, uric acid, albumin, ascorbic acid and α-tocopherol, have been suggested as a pathophysiological mechanism in schizophrenia (Suboticanec *et al.*, 1990; McCreadie *et al.*, 1995; Pae *et al.*, 2004; Dadheech et al., 2006; Ben *et al.*, 2008; Labad *et al.*, 2015; Steullet *et al.*, 2016), and antioxidant levels have been associated with clinical severity in schizophrenia (Li *et al.*, 2011; Bentsen *et al.*, 2012; Widschwendter *et al.*, 2016). Further, several studies implicate oxidative stress to be related to symptom severity in schizophrenia (Lai *et al.*, 2016). Oxidative stress may change during the course of schizophrenia, and can be related to symptom severity and medication (Katsuta *et al.*, 2014). Oxidative DNA damage was found to be higher in patients with non-remission schizophrenia (Sertan *et al.*, 2015). The levels of protein oxidation were shown to be higher in patients with acute psychosis than in remission and healthy controls (Tuncel *et al.*, 2015).

Membrane polyunsaturated fatty acids (PUFAs) are among the cellular components highly susceptible to oxidative damage, resulting in impaired membrane structure, fluidity and signal transduction (Reddy & Yao, 1996). Lipid peroxidation may be associated with different phases of schizophrenia (Mico *et al.*, 2011; Tuncel *et al.*, 2015), and heterogeneity of lipid levels may reflect the phase of disease, in addition to other modifiers (Medema *et al.*, 2015). Dysregulation of antioxidant defence is a possible modifier that can explain variation in lipid levels in different phases of the disease.

Aims of the study

The first aim was to identify changes in antioxidants and markers of oxidative stress in schizophrenia across a 5-year period, from the acute phase to a later stable phase. The second aim was to explore differences between patients and healthy controls in the stable phase. We hypothesised that the levels of oxidative markers increase in schizophrenia compared with healthy controls; change from the acute to the stable phase; and are associated with the levels of membrane PUFAs and symptom severity.

Material and methods

Study design and patient population

The current observational study includes longitudinal data on antioxidants and markers of oxidative stress from patients with schizophrenia spectrum disorders and from a group of healthy controls. The current study is part of a larger longitudinal study of schizophrenia patients. They were first investigated in an acute phase of the disease and followed up on average 5 years later in a stable phase, and compared with a healthy control group. The patients were examined in the acute phase from 2001 to 2004, and in the stable phase from 2006 to 2010.

Patients with schizophrenia spectrum disorders were recruited to a randomised controlled trial of an omega-3 fatty acid and antioxidants (Bentsen *et al.*, 2013). At inclusion, the patients were admitted to emergency psychiatric wards in southern Norway, considered to be in an acute phase. The patients were re-assessed 5 years later, and at this follow-up, the patients received treatment at outpatient clinics or at psychiatric long-term care facilities, considered to be in a stable phase of the disease (Solberg *et al.*, 2016). The patients were screened for somatic illness both at inclusion and at follow-up.

In addition to longitudinal monitoring and comparison within the patient group, healthy controls were included in the stable phase to provide normal biochemical values, including the levels of antioxidants and oxidative stress markers to compare with the schizophrenia group.

The study was approved by the Regional Committee for Medical Research Ethics. All participants gave written informed consent.

We have previously reported lipid and antioxidant results from the acute phase of the study (Bentsen *et al.*, 2011, 2013). Further, we have published associations between lipid profiles and clinical characteristics in the stable phase (Solberg *et al.*, 2015), and shown that PUFA levels changed from the acute to the stable phase (Solberg *et al.*, 2016). In the present report, we investigated the markers of oxidative stress in the stable phase, also including novel laboratory measures not reported earlier.

Clinical assessment

All patients were diagnosed with the Structural Clinical Interview for DSM-IV (SCID) at inclusion (acute phase), and the diagnosis was verified during follow-up assessment (stable phase) (Solberg *et al.*, 2016). Those with an initial diagnosis of schizophrenia spectrum disorders were re-diagnosed at follow-up according to ICD-10, similar to DSM-IV. In the following, we use 'schizophrenia' to describe schizophrenia, schizoaffective and schizophreniform disorders. The Positive and Negative Syndrome Scale (PANSS), Structured Interview Version, and the Global Assessment of Functioning (GAF), Split version, including Symptom (GAF-S) and Functioning (GAF-F) scales, were used for clinical assessments.

Information about the current and previous use of antipsychotics was obtained via interviews and access to medical records.

Neither the healthy subjects nor their first-degree relatives had any ongoing or past severe psychiatric disorder. This was determined by an interview assessing mental illness, and screening for ongoing and past psychiatric disorder using the Mini International Neuropsychiatric Interview (MINI). In addition, their physical health was assessed with self-report and a short screening interview addressing current and previous somatic illnesses (Solberg *et al.*, 2016).

Biochemical analyses

Biochemical parameters were compared between inclusion (acute phase) and the follow-up visit (stable phase). α-Tocopherol,

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albumin, uric acid and PUFA were analysed in both phases, while the other outcome variables – bilirubin, F2-isoprostane and reactive oxygen metabolites (ROMs) – were only analysed at follow-up (stable phase).

All blood samples for biochemical analyses were drawn after overnight fasting, and samples for analyses of antioxidants, oxidative stress and PUFAs were stored at –80°C and transported frozen (shipped with dry ice) to the analysing laboratory.

Serum α-tocopherol in the acute phase was analysed at the Nutrition Laboratory, Oslo University Hospital, Aker, Oslo, Norway, with kits from Bio-Rad Lab, GmBH (Munich, Germany), by high-performance liquid chromatography (HPLC) and ultraviolet light detection. In the stable phase, serum α-tocopherol was analysed by Vitas AS, Oslo, Norway. Serum was diluted with 2-propanol containing the internal standard tocol and butylhydroxytoluene as an antioxidant. The same HPLC methods were used both in the acute and stable phases and can be considered equivalent. The lipid-adjusted term [α-tocopherol/(triglycerides + cholesterol)] was used in statistical analyses to better define the vitamin E status in groups with elevated serum lipid concentrations (Ford *et al.*, 2006).

Uric acid, albumin, total bilirubin and CRP were analysed in serum using standard methods. In the acute phase, analyses were carried out at the hospital to which the patient was submitted. In the stable phase, the analyses were carried out at Diakonhjemmet Hospital, Oslo, Norway.

The test for Diacron reactive oxygen metabolites (D-ROMs) was performed according to the information provided by the manufacturer (Diacron International, Grosetto, Italy). In brief, heparin plasma was diluted in an acidic buffer solution (pH 4.8). Iron present in the sample catalysed the breakdown of plasma hydroperoxides to alkoxyl and peroxyl radicals, which generate a coloured complex, and was quantified with a photometer. The results are denominated by arbitrary units, Carratelli Units (CARR U), and 1 CARR U corresponds to 0.08 mg H_2O_2/100 ml. The concentration of D-ROMs was used as an index for the production of reactive oxygen species (ROS), with higher values indicating higher oxidative stress (Cesarone *et al.*, 1999).

The quantification of 8-iso-prostaglandin F2α (8-IsoPGF2α) was done by triple-stage liquid chromatography tandem mass spectrometry (LC/MS/MS). An Applied Biosystems 4000 Q TRAP LC/MS/MS system with ESI was operated in multiple reaction monitoring (MRM) mode. The mass limit of detection (mLOD) was 1 pg of analyte eluting from the column (Bastani *et al.*, 2009). 8-IsoPGF2α was analysed during the acute phase but with another method (enzyme-linked immunoassay). This yields results that cannot be directly related to those obtained by the method used for the stable phase. Therefore, this variable was not included in longitudinal analyses.

For the analyses of membrane PUFAs, washed red blood cells (RBCs) were stored at −80°C and sent within 3 months in dry ice to Mylnefield Research Services Ltd (Dundee, United Kingdom). For the analyses of membrane PUFAs, lipids were extracted, converted into fatty acid methyl esters, and analysed by gas chromatography, yielding fatty acid profiles (Bentsen *et al.*, 2011). Fatty acids were reported as micrograms per gram of RBCs. The sum of PUFAs with 20 or 22 carbon atoms is classified as long-chain PUFAs (LCPUFAs). We have previously reported that membrane PUFA levels were bimodally distributed among patients in the acute phase (Bentsen *et al.*, 2011). In the present study, we defined low and high PUFA groups by PUFA levels in the acute phase. We have previously reported (Bentsen *et al.*, 2011) that saturated and monounsaturated fatty acids were normally distributed, while fatty acids with at least three double bonds were bimodally distributed in the acute phase. Thus, the data represented a mixture of two distributions assumed to be normal (Bentsen *et al.*, 2011). For PUFAs in the current sample, we found that the point with the lowest density of observations was 183 μg/g RBC, which was used to split the sample into two groups. Twenty-nine per cent of the patients had a value at or below this cut-off score (low PUFA group); 71% had a value above the threshold (high PUFA group).

Table 1. Characteristics of schizophrenia patients and healthy controls

	Patients*	Healthy controls
Age, years†	31.3 (5.7)	33 (5.7)
Sex, male‡	38 (69.1)	28 (54.9)
Smokers‡	29 (52.7)	9 (17.6)
Education‡		
Primary school	9 (17)	1 (2)
High school	30 (46.6)	8 (16)
University/college	14 (26.4)	41 (82)
PANSS†	81.4 (24.1)	–
GAF-S†	47.7 (12.5)	–
GAF-F†	49.2 (12.7)	–

$n = 55$ schizophrenia patients and $n = 51$ healthy controls (except for education: $n = 53$ and 50, respectively).
SD, standard deviation; PANSS, Positive and Negative Syndrome Scale (total score); GAF, Global Assessment of Functioning (S = Symptoms, F = Functioning).
*Data from stable phase, †mean (SD), ‡n (%).

Statistics

The Wilcoxon signed-rank test was used to evaluate possible changes in the levels of antioxidants from the acute to the stable phase of schizophrenia. The Mann–Whitney test was used to compare the levels of antioxidants and markers of oxidative stress in stabilised patients with those of healthy controls. Subsequently, multiple linear regression analyses were performed for each of the antioxidants and markers of oxidative stress with patient status ('healthy = 0', 'patient = 1') tested as explanatory variable, including sex, age and smoking habit as potential covariates. The selection of covariates in the final multivariate models was based on sequential backward elimination by excluding covariates with $p > 0.1$. Multiple regression analyses were also used to explore the influences of phase (acute, stable) and duration of follow-up on changes in α-tocopherol. Finally, Spearman correlation tests were used to evaluate the relationship between levels of antioxidants, markers of oxidative stress and membrane PUFAs.

All statistical analyses were performed using SPSS version 23. The significance level was set to $p < 0.05$ (two-sided).

Results

Patient characteristics

Fifty-five schizophrenia patients and 51 healthy controls were included in the study. The patient group had a significantly higher number of current smokers, low education levels and a higher number of males compared with the control group. Demographics of the included schizophrenia patients and healthy controls are shown in Table 1.

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Table 2. Univariate analysis of antioxidants and markers of oxidative stress

Measure	Healthy controls	Acute phase	Stable phase
α-tocopherol	4.8 (4.3, 5.4)	5.2 (3.9, 7.5)	4.5 (4.0, 4.9)#,***
albumin	47.0 (44.5, 48.5)	42.0 (39.0, 45.3)###	47.0 (45.0, 49.0)***
uric acid	295.0 (240.8, 359.0)	327.0 (238.5, 381.5)	342.0 (263.5, 412.5)#
bilirubin	10.0 (7.75, 13.0)	–	7.0 (5.0, 9.0)###
8-IsoPGF2α	39 (28.5, 56)	–	46 (32, 58)
ROMs	341.0 (295.9, 395.3)	–	371.8 (329.2, 403.5)

α-tocopherol (adjusted): α-tocopherol / (triglycerides + cholesterol).
P-value: *#0.01 < $P \leq 0.05$; **##0.001 < $P \leq 0.01$; ***###<0.001 (median, 25, 75 quartiles).
Schizophrenia $n = 50$–55; healthy controls $n = 46$–51. Units: α-tocopherol: µmol/mmol; albumin: g/L; bilirubin: µmol/l; uric acid: µmol/l; 8-IsoPGF2α: pg/mL; ROMs: Carratelli Units.
8-IsoPGF2α: 8-iso-prostaglandin F2α; ROMs: derivatives of reactive oxygen metabolites.
#*P*-value vs. healthy controls, Mann–Whitney *U* test.
**P*-value vs. acute phase, related samples Wilcoxon signed-rank test.

Table 3. α-tocopherol in schizophrenia during acute and stable phases stratified by PUFA levels in the acute phase

Acute phase group	α-tocopherol, acute	α-tocopherol, stable	*P*-value*
Low PUFA	4.21 (3.59, 4.84)	4.27 (3.87, 4.67)	0.28
High PUFA	6.42 (5.38, 7.46)	4.55 (4.29, 4.82)	<0.001
P-value#	0.0004	0.27	

α-tocopherol, adjusted = α-tocopherol / (triglycerides + cholesterol) (µmol/l)/(mmol/l); mean, 95% confidence interval.
Low PUFA $n = 13$, high PUFA $n = 37$. Low and high PUFA groups: acute-phase PUFA <183 and ≥183 µg/g RBC, respectively.
PUFA, omega-3 + omega-6 polyunsaturated fatty acids in red blood cells.
#Student's *t*-test.
*Related samples Wilcoxon signed-rank test.

The mean follow-up time for the 55 patients from the acute phase was 61 months. We intended to follow up everyone in the acute phase sample ($n = 99$), but 21.2% did not wish to participate, 12.1% could not be located, 9.1% were dead, and 2.0% had moved to other regions of Norway (Solberg *et al.*, 2016). The follow-up sample did not differ significantly from the remaining patients in the original acute phase sample with respect to relevant characteristics.

Eleven patients did not use any antipsychotic medication at follow-up. Those who received antipsychotics were compliant with the treatment, as defined by measures of blood levels within the normal concentration range. The results of PUFA and antioxidant investigations from the acute phase have been published earlier (Bentsen *et al.*, 2012, 2013).

Antioxidants

The levels of α-tocopherol in the schizophrenia group decreased significantly from the acute to the stable phase (−1.3 µmol/mmol, 95% CI −2.1 to −0.6 µmol/mmol, $p < 0.001$) (Tables 2 and 3). α-Tocopherol remained stable in the low-PUFA acute phase group. In contrast, in the high-PUFA acute phase group, α-tocopherol levels decreased significantly ($p < 0.001$) towards the stable phase, while PUFA levels remained the same (Table 3, Fig. 1). The PUFA group (high, low) had a modifying effect on changes in α-tocopherol (linear mixed model interaction term PUFA × α-tocopherol × time, $p = 0.02$). In the acute phase, the difference in α-tocopherol in the high and low PUFA groups remained significant after adjusting for sex, age and smoking habits. In the stable phase, the two acute-phase PUFA groups had similar α-tocopherol and PUFA levels (Table 3, Fig. 1, Suppl. Fig. 1A–B). The duration of illness did not predict α-tocopherol levels in the acute or stable phases, and the duration of follow-up did not predict changes in α-tocopherol.

The levels of albumin increased significantly from the acute to the stable phase ($p < 0.001$) (Table 2). Changes in albumin were not significantly associated with the PUFA acute phase group. There was no significant difference between the levels of uric acid in the acute and stable phases of schizophrenia. Bilirubin was only measured in the stable phase.

When patients in the stable phase were compared with healthy controls in a multivariate model, adjusting for sex, age and smoking, bilirubin was significantly lower ($p = 0.046$), uric acid was significantly higher ($p = 0.009$), while α-tocopherol was non-significantly lower ($p = 0.06$) in the patients compared with healthy controls (Table 4).

Albumin level was significantly associated with total PANSS score ($r = 0.28$, $p = 0.04$) in the stable phase. There was no other significant associations between the levels of antioxidants and symptoms or functioning. Albumin was significantly associated with LCPUFA in healthy controls only ($p = 0.004$). There was no other significant associations between the levels of antioxidants and PUFAs in patients or in healthy controls (Table 5).

Markers of oxidative stress

No significant differences were found in 8-IsoPGF2α and ROMs between patients in the stable phase and healthy controls (Table 2). In the multivariate model, adjusting for sex, age and smoking, the levels of ROMs were non-significantly ($p = 0.09$) associated with schizophrenia (Table 4).

No significant associations between markers of oxidative stress and measures of symptom severity were found. The associations between the levels of ROMs and PUFA ($r = 0.39$, $p = 0.007$) and

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Table 4. Estimated effects of patient status, sex, age and smoking habits on antioxidants and markers of oxidative stress in the stable phase of schizophrenia vs. healthy controls in a multiple linear regression analysis

Variable	α-tocopherol	*P*-value	Bilirubin	*P*-value	Albumin	*P*-value	Uric acid	*P*-value	ROMs	*P*-value
Constant	*3.74*		*12.4*		*47.9*		*285.9*		*325.0*	
Patients vs. healthy	−0.30	0.06	−2.5	0.046*	−0.3	0.66	36.9	0.009**	31.2	0.09
Females vs. males	0.19	0.30	−3.3	0.01*	−3.1	<0.001***	109.6	<0.001***	75.4	<0.001***
Age, per year[a]	0.03	0.02*	0.1	0.30	−0.1	0.27	2.0	0.06	0.4	0.78
Smoking vs. non-smoking	0.19	0.31	−1.8	0.19	−0.1	0.85	−25.0	0.08	−32.9	0.12

P-values: *0.01 < *P* ≤ 0.05; **0.001 < *P* ≤ 0.01; ***<0.001.
Numbers are absolute effect of the respective variables on the concentrations of α-tocopherol, bilirubin, albumin, uric acid and ROMs. The selection of covariates in the final models was based on a sequential backward elimination by excluding covariates with $p > 0.10$. All constants were significantly different from zero ($p < 0.001$).
Units: 8-IsoPGF2α: pg/mL (no significant effects in the model); α-tocopherol: μmol/mmol; bilirubin: μmol/l; albumin: g/l; uric acid: μmol/l; ROMs: Carratelli Units.
ROMs, reactive oxygen metabolites
[a]Years above 21.

Table 5. Associations between antioxidants, markers of oxidative stress and membrane PUFAs, stable phase

	Schizophrenia[a]		Healthy controls	
	PUFA	LCPUFA	PUFA	LCPUFA
α-tocopherol	−0.01	−0.04	−0.06	−0.05
bilirubin	0.01	−0.03	−0.11	−0.12
uric acid	0.25	0.27	−0.01	−0.14
albumin	0.06	0.12	−0.27	−0.41**
8-IsoPGF2α	0.01	−0.17	−0.16	−0.13
ROMs	0.13	0.10	0.39 **	0.42 **

α-tocopherol: α-tocopherol / (triglycerides + cholesterol).
Schizophrenia $n = 50–55$; healthy controls $n = 46–51$. Spearman's correlation coefficients are reported. PUFA and LCPUFA are non-normally distributed.
PUFA, omega-3 + omega-6 polyunsaturated fatty acids in red blood cells; LCPUFA, long-chain PUFA with 20–24 carbon atoms in red blood cells; PGF2α, isoprostane-8-epi prostaglandin F2 α; ROMs, reactive oxygen metabolites.
[a]Data from the stable phase.
*0.01 < *P* ≤ 0.05; **0.001 < *P* ≤ 0.01.

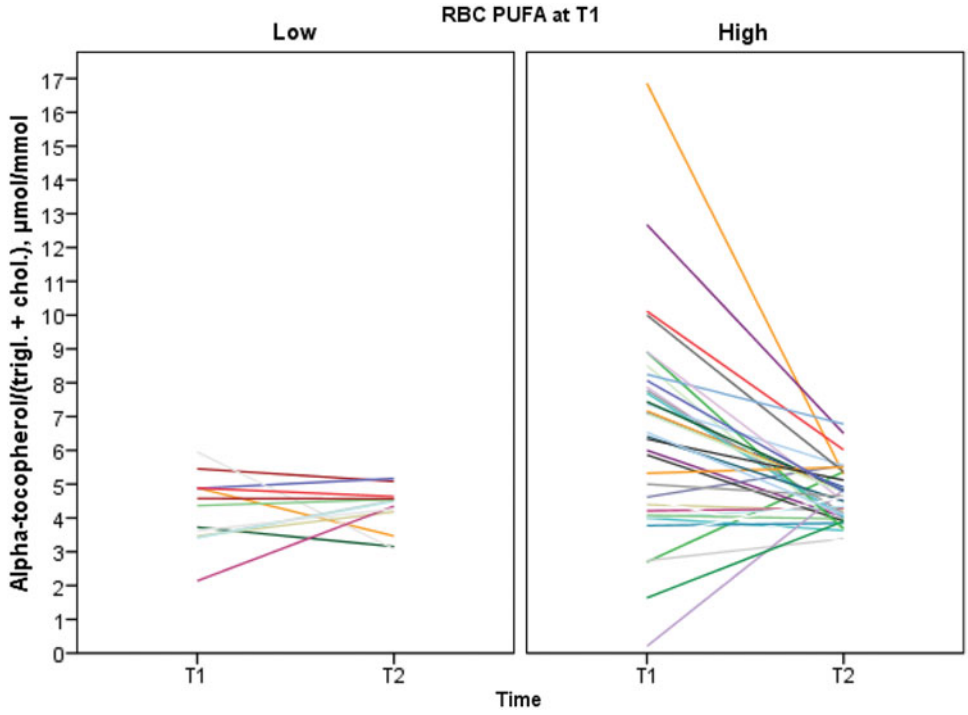

Fig. 1. Changes of lipid-adjusted serum α-tocopherol from T1 (baseline, acute phase) to T2 (5 years follow-up, stable phase) in 50 schizophrenia patients. Five subjects had missing baseline α-tocopherol and were excluded. Red blood cells Polyunsaturated fatty acids at T1, red blood cell polyunsaturated fatty acids at baseline, acute phase. Low: patients with ≤183 μg PUFA/g RBC at T1; high: patients with >183 μg PUFA/g RBC at T1.

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LCPUFA ($r = 0.42$, $p = 0.003$) were significantly positive in healthy controls, but not significant in schizophrenia patients (Table 5). There were no significant associations between 8-IsoPGF2α and PUFAs (Table 5).

C-reactive protein

CRP was significantly higher in patients than in healthy controls in the stable phase ($p < 0.001$). In the multivariate model, the difference between patients and healthy controls remained significant after adjusting for sex, age, smoking and use of antipsychotic medication. There was no significant change in patients from the acute to the stable phase ($p = 0.81$).

Discussion

We found significantly higher levels of α-tocopherol and lower levels of albumin in the acute phase compared with the stable phase of schizophrenia. The change in α-tocopherol was associated with membrane PUFA levels in the acute phase. Further, we found lower levels of bilirubin and higher levels of uric acid in the stable phase of schizophrenia compared with healthy controls when adjusting for covariates. These findings suggest that dysregulation of antioxidants and oxidative stress may be dynamic and related to different phases of the illness.

Antioxidants

The course of α-tocopherol from the acute to the stable phase was heterogeneous, but overall the level was falling and values converging, as shown in Fig. 1. This may indicate dysregulation of α-tocopherol during the acute phase.

In the acute phase, α-tocopherol was positively associated with the levels of membrane PUFAs (Bentsen *et al*., 2011). We have previously reported that PUFA levels were bimodally distributed in the acute phase of schizophrenia in the original larger sample (Bentsen *et al*., 2011; Solberg *et al*., 2016). Membrane PUFAs in the low PUFA group increased from the acute to the stable phase in schizophrenia, whereas levels in the high PUFA group remained unchanged (Solberg *et al*., 2015). Here we showed that α-tocopherol levels in the high PUFA group of the acute phase decreased, while in the low PUFA group, levels of α-tocopherol did not change (Fig. 1). Acute psychosis has been associated with inflammation (Bergink *et al*., 2014), and increased serum levels of α-tocopherol may result from catabolism by CYP3A4 being inhibited by inflammatory cytokines (Traber, 2007; Christensen & Hermann, 2012; Wollmann *et al*., 2017). The reduction in α-tocopherol levels in the patient group with high PUFA in the acute phase is consistent with lower inflammation activity in the stable phase. In contrast, a lower level of α-tocopherol in the low PUFA group during the acute phase may result from oxidative stress (Raederstorff *et al*., 2015; Koga *et al*., 2016). The stability of α-tocopherol among patients with low PUFAs in the acute phase (Fig. 1, Suppl. Fig. 1A–B) could indicate that this group had an enduring deficit in redox regulation, as well as a lack of inflammation indicated by the adverse effect of eicosapentaenoic acid (EPA), 2 g/d, on this group of patients, presumably a pro-oxidant effect (Bentsen *et al*., 2013).

The assessment of vitamin E status in critically ill patients with systemic inflammation has been discussed by Vasilaki *et al*. (2009). They found that adjustment for cholesterol alone yielded higher levels of α-tocopherol than in healthy controls, whereas adjustment for triglycerides yielded lower levels. Patients' cholesterol levels were lower, while triglyceride levels were similar to those of healthy controls. In our study, we adjusted for both cholesterol and triglycerides.

While the uric acid level did not significantly change between the acute and stable phases of schizophrenia in the present study, we found significantly higher levels of uric acid in the stable phase of schizophrenia than in healthy controls after adjusting for age, sex and smoking habits. Earlier studies have somewhat conflicting findings regarding the potential change in uric acid level in schizophrenia (Yao *et al*., 1998; Dadheech *et al*., 2006). The uric acid's antioxidative properties would be assumed to deplete during the acute phase of schizophrenia, in conditions of oxidative stress. Still, as with bilirubin, the exact relationship between schizophrenia, oxidative stress and the levels of endogenous antioxidants remains unclear and warrants further study.

The present study showed lower albumin levels in patients in the acute phase compared with the stable phase. In the latter, there was no difference between schizophrenia patients and healthy controls. Earlier studies have shown similar findings, linking low levels of albumin to the acute phase of schizophrenia (Reddy *et al*., 2003). Other studies have shown no difference in albumin levels between first episode and chronic schizophrenia (Pae *et al*., 2004). Low albumin may be an effect of malnutrition and inflammation (Don & Kaysen, 2004); both conditions may be present in the acute phase of schizophrenia. In our study, patients in the acute phase were recently admitted to a psychiatric hospital and were at risk of somatic and social stress. Low albumin may also be an effect of oxidative stress and related to the underlying pathogenesis of psychosis (Labad *et al*., 2015).

We found that bilirubin was lower in the schizophrenia group compared with healthy controls in the stable phase, independent of smoking status. Low bilirubin concentration confers a potent antioxidant redox amplification cycle and antioxidant protection via recycling of bilirubin to biliverdin (Jansen & Daiber, 2012). Altered levels of bilirubin have been shown in schizophrenia. One study has found increased levels in first-episode schizophrenia (Reddy *et al*., 2003), while other studies on patients in the stable phase have conflicting findings (Yao *et al*., 2000; Semnani *et al*., 2010). Differences in bilirubin levels between patients and healthy controls have been found independent of smoking status and antipsychotic medication (Yao *et al*., 2000; Reddy *et al*., 2003). Bilirubin is metabolised by UGT1A1, in which genetic variation might lead to idiopatic unconjugated hyperbilirubinemia (Gilbert's syndrome). This syndrome is more prevalent among schizophrenia patients than in the general population (Yao *et al*., 2000). Altered bilirubin levels during the course of schizophrenia may indicate that oxidative stress is related to symptom severity and need to be further examined in longitudinal studies.

Measures of antioxidants and oxidant markers only partially correlate, regarding change over time as well as differences between healthy controls and patients in the stable phase. This is in line with earlier reports of inconsistent findings of oxidative stress markers and antioxidants in schizophrenia (Ciobica *et al*., 2011). Both antioxidants and markers of oxidative stress are indirect measurements of free radical production, as *in vivo* measurement of free radical concentrations is impractical because their reactive nature results in short half-lives and low levels. It is possible that oxidative stress and alterations in antioxidant enzyme activities may be involved in the pathophysiology of specific subtypes of schizophrenia. The difference in the direction of changes may be attributed to clinical symptoms, age, BMI and use of antipsychotic medication, but also to the dynamic status of antioxidant enzymes, which have an

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intricate balance with other biological pathways and systems (Zhang *et al.*, 2006; Wu *et al.*, 2013).

Antioxidants, markers of oxidative stress and association to symptom severity

The current analyses revealed no significant association between symptom severity and levels of antioxidants or markers of oxidative stress in the stable phase. This is in line with earlier studies of chronic schizophrenia (Lee *et al.*, 2016). We have earlier demonstrated that F2-isoprostane levels were associated with negative symptoms in the same patient group in the acute phase (Bentsen *et al.*, 2012). Persistent low levels of antioxidants may be toxic and not compatible with sustainable life, and any associations with symptoms will more likely be present intermittently during the course of the disease.

Antioxidants and markers of oxidative stress, and association with membrane PUFA

The levels of ROMs and albumin were associated with those of PUFAs in healthy controls, but not in patients in the stable phase. Dysregulation of antioxidant defence through a reduction in expression and activity has been implicated in disease pathophysiology (Miller *et al.*, 2008). PUFAs are especially prone to lipid peroxidation, and one would expect a stable relationship between PUFAs, antioxidants and markers of oxidative stress. During episodes of higher symptom intensity, the levels of membrane lipids may be influenced by neuroinflammation, oxidative stress and lipid peroxidation (Bitanihirwe & Woo, 2011; Muller *et al.*, 2015). Taken together, discrepancies in oxidant markers may indicate a dysregulation of antioxidant defences and suggest that oxidative stress and excessive free radical production may be involved in the pathophysiology of schizophrenia.

The levels of α-tocopherol were associated with those of PUFAs in patients and were lower in the low PUFA group in the acute phase (Bentsen *et al.*, 2011). We showed that changes in α-tocopherol from the acute to stable phase were different between the two groups. Add-on treatment with EPA had different effects between the PUFA groups in the acute phase, leading to increased psychotic symptoms in the low PUFA group (Bentsen *et al.*, 2013). The fact that the bimodality was no longer present in the stable phase (Solberg *et al.*, 2016) suggests a possible stage-specific biological effect. Together, this may indicate a dysregulation of redox regulation in schizophrenia affecting PUFA levels, with different patterns in the acute and stable phases.

Oxidative stress and lifestyle factors

Antioxidant status and oxidative stress are related to lifestyle factors and diet, which have been found to be poorer in schizophrenia patients than in the general population (Dipasquale *et al.*, 2013). Nutrients, including PUFAs and vitamins, have anti-inflammatory and antioxidant mechanisms of action, and an unhealthy diet may contribute to the observed differences between patients and healthy controls (Mitra *et al.*, 2017). Though studies have shown diet inadequacy among schizophrenia patients, this may not alone explain the differences in antioxidants and polyunsaturated acids observed between patients and healthy controls (Strassnig *et al.*, 2005; Ballesteros *et al.*, 2013).

The changes in antioxidant levels in our study were independent of smoking status. Tobacco smoking is known to increase oxidative stress and lipid peroxidation (Kharb & Singh, 2000; Van't Erve *et al.*, 2016). Among schizophrenia patients, higher levels of oxidative stress have been shown among first-episode patients that smoked (Jordan *et al.*, 2018), but lipid peroxidation has been lower among patients who smoked in a stable phase (Zhang *et al.*, 2006). The levels of antioxidants were lower in first-episode schizophrenia independent of smoking status (Yao *et al.*, 2000; Reddy *et al.*, 2003). Smoking was associated with higher glutathione levels in controls, while smoking in patients was not associated with this effect (Ballesteros *et al.*, 2013). Oxidative stress from smoking may stimulate the antioxidant defence, and increased antioxidant activity with greater cigarette consumption is consistent with tobacco smoke, leading to oxidative stress and the stimulation of protective actions of enzymes such as superoxide dismutase and catalase to reverse this stress (Zhang *et al.*, 2007). Some components of tobacco smoke also appear to inhibit monoamine oxidase, and this might decrease free radical formation (Mazzio *et al.*, 2005). Taken together, this indicates that though smoking may lead to increased oxidative stress, the changes in antioxidant levels observed in schizophrenia patients may be independent of smoking status and related to the pathophysiology of schizophrenia.

Oxidative stress and inflammation

Several lines of evidence suggest that the pathophysiology of schizophrenia involves immune and inflammatory pathways that may have important aetiological and therapeutic implications (Anderson & Maes, 2013; Muller *et al.*, 2015; Muller, 2018). A possible association between schizophrenia and the immune system is supported by epidemiological studies suggesting links with infection and systemic inflammation (Khandaker *et al.*, 2015). The use of antipsychotic medication may also affect the inflammatory state, and antipsychotics can impact the expression of genes that code for inflammatory cytokines and immune cells (Chen *et al.*, 2013; Debnath, 2015). However, in clinical trials, results are mixed and may reflect different properties of each medication (Roge *et al.*, 2012; de Witte *et al.*, 2014). Aberrant cytokine levels have been observed in schizophrenia independent of antipsychotic medication and associated both to state and trait (Miller *et al.*, 2011; Hope *et al.*, 2013; Morch *et al.*, 2017). However, several genome-wide association studies have implicated immune genes among the strongest genetic risk factors (Shi *et al.*, 2009; Sekar *et al.*, 2016; Miller & Goldsmith, 2017).

We found higher levels of CRP in patients in the stable phase, similar to previous findings in the acute phase (Bentsen *et al.*, 2012). This is in line with earlier studies (Fernandes *et al.*, 2016; Johnsen *et al.*, 2016) and may indicate a low-grade inflammation among patients, which could play a role in disease progression, either by itself or as a modulator of oxidative stress. Oxidative stress and inflammation are intricately linked (Barron *et al.*, 2017) and have been suggested to reflect developmental redox dysregulation (Do *et al.*, 2009). Oxidative stress induces inflammation via the activation of rapid-acting transcriptional activators of inflammatory response that can also lead to the production of more free radicals (Liu *et al.*, 2008; Bitanihirwe & Woo, 2011). In the brain, the activation of microglia to destroy pathogens involves the generation of reactive superoxides, which can also damage neurons if not balanced with antioxidants (Block *et al.*, 2007). Dysregulation of neuro-immune and redox systems due to genetic and early-life environmental risk factors may contribute to CNS anomalies observed in schizophrenia, and dysregulation of more than one of these systems may be particularly deleterious

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(Steullet *et al.*, 2016). In general, studies of oxidative stress and inflammation in schizophrenia have provided conflicting results, but some studies have suggested poorer outcome among patients with higher oxidative stress and greater inflammation (Fraguas *et al.*, 2017). These processes may be dynamic and related to different phases of schizophrenia, and apply to subgroups of patients and need to be studied further.

Oxidative stress related to different phases of schizophrenia

The current findings are in line with the notion that different pathological processes may be involved in different phases of schizophrenia. Several lines of evidence from basic neuroscience support this concept. Krystal *et al.* describe an imbalance in cortical excitation and inhibition that vary throughout the development and progression of schizophrenia across different phases (prodrome, predrome, syndrome and chronic illness) (Krystal & Anticevic, 2015; Krystal *et al.*, 2017). This may lead to successive allostatic neuroadaptions that ultimately affect network integrity and function (Krystal & Anticevic, 2015).

A similar scenario may explain why not all markers of oxidative stress increase constantly in schizophrenia, as suggested by the current findings. Oxidative stress may also be a dynamic process, related to different phases of schizophrenia. Oxidative stress abnormalities in first-episode psychosis suggest an effect independent of antipsychotic medications (Zhang *et al.*, 2009; Jordan *et al.*, 2018). It is not clear if later variations are results of disease progression or disease phase. α-Tocopherol has been suggested as a biomarker in schizophrenia (Liu *et al.*, 2014). Our results suggest that changes in α-tocopherol and albumin were associated with phase rather than duration of illness, supporting the role of some biomarkers being state markers for acute exacerbations of psychosis, while others might be trait markers (Flatow *et al.*, 2013). Taken together, the possibility of alternating pathobiological processes involving oxidative stress may explain the conflicting findings regarding the levels of antioxidants and antioxidant therapy in schizophrenia, and warrants further longitudinal studies. Disease phase should be taken into account when studying redox regulators in schizophrenia

Strength and limitations

To the best of our knowledge, this is the first longitudinal study of antioxidants in schizophrenia that includes an acute and a stabilised phase. The main strength of the study is the comparison of biomarkers (bilirubin, albumin, uric acid) over time, and the relationship between antioxidants and markers of oxidative stress based on PUFA levels. Changes in the levels of antioxidants from the acute to the stable phase of schizophrenia suggest dysregulation of antioxidant defences. Further, changes in the levels of biomarkers related to disease phase and related to other biomarkers – in our study, PUFA levels in the acute phase – may give insights into conflicting findings of biomarkers in schizophrenia, and indicate dynamic alterations of redox regulation during different phases of schizophrenia.

The study has some limitations. The relatively small sample size reduces statistical power, which makes the results from subgroups harder to interpret and reduces the opportunities for analysing confounders and effect modifiers. Small sample sizes also increase the risk of bias, making the sample size less representative of the population from which it has been drawn. The weight of participants was not obtained, and thus obesity as a factor in relation to lipid and antioxidant levels cannot be adjusted for. Diet, dietary supplements and other lifestyle factors that may influence the levels of membrane fatty acids and antioxidants were not controlled for. Bilirubin and D-ROMs were only analysed at follow-up (stable phase), and the method used to analyse F2-isoprostane in the stable phase differed compared with the acute phase, reducing the possibility of discovering longitudinal differences.

The control group was not fully matched for smoking habits and other lifestyle factors, nor for education level – parameters that may influence membrane fatty acids and antioxidant levels. In a naturalistic study, the long period of time (5 years) between the acute and the stable phase increases the possibility of other factors not adjusted for affecting the lipid and antioxidant levels, including age, BMI and treatment with antipsychotic medication, and the lack of clinical information of the sample over the follow-up period makes it hard to exclude association with other long-term outcomes. Positive and negative symptoms of schizophrenia fluctuate over time, which makes it difficult to identify associations. This will most probably induce noise in the analysis, while systematic bias is less probable, making type II errors more likely than type I errors. Further, the group of patients not using antipsychotic medication in the stable phase was small, and information on adherence to medication prior to evaluation in the acute phase and during follow-up was not obtained, making it difficult to assess the effect of medication on antioxidants and oxidative stress. Thus, the current findings should be confirmed in independent samples.

Concluding remark

The present findings of changing α-tocopherol levels in the acute versus stable phase of schizophrenia suggests that redox regulation is dynamic and changes during different phases of the disorder. Abnormal levels of antioxidants in the stable phase indicate persisting redox dysregulation. The acute versus stable phase should be taken into account when studying redox regulators in schizophrenia.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/neu.2019.14.

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References

Anderson G and Maes M (2013) Schizophrenia: linking prenatal infection to cytokines, the tryptophan catabolite (TRYCAT) pathway, NMDA receptor hypofunction, neurodevelopment and neuroprogression. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **42**, 5–19.

Ballesteros A, Jiang P, Summerfelt A, Du X, Chiappelli J, O'Donnell P, Kochunov P and Hong LE. (2013) No evidence of exogenous origin for the abnormal glutathione redox state in schizophrenia. *Schizophrenia Research* **146**, 184–189.

Barron H, Hafizi S, Andreazza AC and Mizrahi R (2017) Neuroinflammation and oxidative stress in psychosis and psychosis risk. *International Journal of Molecular Sciences* **18**, pii: E651.

Bastani NE, Gundersen TE and Blomhoff R (2009) Determination of 8-epi PGF(2alpha) concentrations as a biomarker of oxidative stress using triple-stage liquid chromatography/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry* **23**, 2885–2890.

Ben OL, Mechri A, Fendri C, Bost M, Chazot G, Gaha L and Kerkeni A. (2008) Altered antioxidant defense system in clinically stable patients with schizophrenia and their unaffected siblings. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **32**, 155–159.

Bentsen H, Osnes K, Refsum H, Solberg DK and Bohmer T (2013) A randomized placebo-controlled trial of an omega-3 fatty acid and vitamins E+C in schizophrenia. *Translational Psychiatry* **3**, e335.

Bentsen H, Solberg DK, Refsum H and Bohmer T (2012) Clinical and biochemical validation of two endophenotypes of schizophrenia defined by levels of polyunsaturated fatty acids in red blood cells. *Prostaglandins, Leukotrienes & Essential Fatty Acids* **87**, 35–41.

Bentsen H, Solberg DK, Refsum H, Gran JM, Bohmer T, Torjesen PA, Halvorsen O and Lingjaerde O. (2011) Bimodal distribution of polyunsaturated fatty acids in schizophrenia suggests two endophenotypes of the disorder. *Biological Psychiatry* **70**, 97–105.

Bergink V, Gibney SM and Drexhage HA (2014) Autoimmunity, inflammation, and psychosis: a search for peripheral markers. *Biological Psychiatry* **75**, 324–321.

Bitanihirwe BK and Woo TU (2011) Oxidative stress in schizophrenia: an integrated approach. *Neuroscience & Biobehavioral Reviews* **35**, 878–893.

Block ML, Zecca L and Hong JS (2007) Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nature Reviews Neuroscience* **8**, 57–69.

Boskovic M, Vovk T, Kores PB and Grabnar I (2011) Oxidative stress in schizophrenia. *Current Neuropharmacology* **9**, 301–312.

Boulanger LM (2009) Immune proteins in brain development and synaptic plasticity. *Neuron* **64**, 93–109.

Bulbul F, Virit O, Alpak G, Unal A, Bulut M, Kaya MC, Altindag A, Celik H and Savas HA. (2014) Are oxidative stress markers useful to distinguish schizoaffective disorder from schizophrenia and bipolar disorder? *Acta Neuropsychiatrica* **26**, 120–124.

Cesarone MR, Belcaro G, Carratelli M, Cornelli U, De Sanctis MT, Incandela L, Barsotti A, Terranova R and Nicolaides A. (1999) A simple test to monitor oxidative stress. *International Angiology* **18**, 127–130.

Chen ML, Wu S, Tsai TC, Wang LK and Tsai FM (2013) Regulation of macrophage immune responses by antipsychotic drugs. *Immunopharmacol Immunotoxicol* **35**, 573–580.

Christensen H and Hermann M (2012) Immunological response as a source to variability in drug metabolism and transport. *Frontiers in Pharmacology* **3**, 8.

Ciobica A, Padurariu M, Dobrin I, Stefanescu C and Dobrin R (2011) Oxidative stress in schizophrenia - focusing on the main markers. *Psychiatria Danubina* **23**, 237–245.

Dadheech G, Mishra S, Gautam S and Sharma P (2006) Oxidative stress, alpha-tocopherol, ascorbic acid and reduced glutathione status in schizophrenics. *Indian Journal of Clinical Biochemistry* **21**, 34–38.

de Witte L, Tomasik J, Schwarz E, Guest PC, Rahmoune H, Kahn RS and Bahn S. (2014) Cytokine alterations in first-episode schizophrenia patients before and after antipsychotic treatment. *Schizophrenia Research* **154**, 23–29.

Debnath M (2015) Adaptive immunity in schizophrenia: functional implications of T cells in the etiology, course and treatment. *Journal of Neuroimmune Pharmacology* **10**, 610–619.

Dipasquale S, Pariante CM, Dazzan P, Aguglia E, McGuire P and Mondelli V (2013) The dietary pattern of patients with schizophrenia: a systematic review. *Journal of Psychiatric Research* **47**, 197–207.

Do KQ, Cabungcal JH, Frank A, Steullet P and Cuenod M (2009) Redox dysregulation, neurodevelopment, and schizophrenia. *Current Opinion in Neurobiology* **19**, 220–230.

Don BR and Kaysen G (2004) Serum albumin: relationship to inflammation and nutrition. *Seminars in Dialysis* **17**, 432–437.

Emiliani FE, Sedlak TW and Sawa A (2014) Oxidative stress and schizophrenia: recent breakthroughs from an old story. *Current Opinion in Psychiatry* **27**, 185–190.

Fernandes BS, Steiner J, Bernstein HG, Dodd S, Pasco JA, Dean OM, Nardin P, Goncalves CA and Berk M. (2016) C-reactive protein is increased in schizophrenia but is not altered by antipsychotics: meta-analysis and implications. *Molecular Psychiatry* **21**, 554–564.

Flatow J, Buckley P and Miller BJ (2013) Meta-analysis of oxidative stress in schizophrenia. *Biological Psychiatry* **74**, 400–409.

Ford L, Farr J, Morris P and Berg J (2006) The value of measuring serum cholesterol-adjusted vitamin E in routine practice. *Annals of Clinical Biochemistry* **43**, 130–134.

Fraguas D, Diaz-Caneja CM, Rodriguez-Quiroga A and Arango C (2017) Oxidative stress and inflammation in early onset first episode psychosis: a systematic review and meta-analysis. *The International Journal of Neuropsychopharmacology* **20**, 435–444.

Herken H, Uz E, Ozyurt H, Sogut S, Virit O and Akyol O (2001) Evidence that the activities of erythrocyte free radical scavenging enzymes and the products of lipid peroxidation are increased in different forms of schizophrenia. *Molecular Psychiatry* **6**, 66–73.

Hope S, Ueland T, Steen NE, Dieset I, Lorentzen S, Berg AO, Agartz I, Aukrust P and Andreassen OA. (2013) Interleukin 1 receptor antagonist and soluble tumor necrosis factor receptor 1 are associated with general severity and psychotic symptoms in schizophrenia and bipolar disorder. *Schizophrenia Research* **145**, 36–42.

Howes OD and Murray RM (2014) Schizophrenia: an integrated sociodevelopmental-cognitive model. *Lancet* **383**, 1677–1687.

Jansen T and Daiber A (2012) Direct antioxidant properties of bilirubin and biliverdin. Is there a role for Biliverdin Reductase? *Frontiers in Pharmacology* **3**, 30.

Johnsen E, Fathian F, Kroken RA, Steen VM, Jorgensen HA, Gjestad R and Loberg EM. (2016) The serum level of C-reactive protein (CRP) is associated with cognitive performance in acute phase psychosis. *BMC Psychiatry* **16**, 60.

Jordan W, Dobrowolny H, Bahn S, Bernstein HG, Brigadski T, Frodl T, Isermann B, Lessmann V, Pilz J, Rodenbeck A, Schiltz K, Schwedhelm E, Tumani H, Wiltfang J, Guest PC and Steiner J. (2018) Oxidative stress in drug-naive first episode patients with schizophrenia and major depression: effects of disease acuity and potential confounders. *European Archives of Psychiatry and Clinical Neuroscience* **268**, 129–143.

Katsuta N, Ohnuma T, Maeshima H, Takebayashi Y, Higa M, Takeda M, Nakamura T, Nishimon S, Sannohe T, Hotta Y, Hanzawa R, Higashiyama R, Shibata N and Arai H. (2014) Significance of measurements of peripheral carbonyl stress markers in a cross-sectional and longitudinal study in patients with acute-stage schizophrenia. *Schizophrenia Bulletin* **40**, 1366–1373.

Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R and Jones PB (2015) Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet Psychiatry* **2**, 258–270.

Kharb S and Singh GP (2000) Effect of smoking on lipid profile, lipid peroxidation and antioxidant status in normal subjects and in patients during and after acute myocardial infarction. *Clinica Chimica Acta* **302**, 213–219.

Koga M, Serritella AV, Sawa A and Sedlak TW (2016) Implications for reactive oxygen species in schizophrenia pathogenesis. *Schizophrenia Research* **176**, 52–71.

Krystal JH and Anticevic A (2015) Toward illness phase-specific pharmacotherapy for schizophrenia. *Biological Psychiatry* **78**, 738–740.

https://doi.org/10.1017/neu.2019.14 Published online by Cambridge University Press

Krystal JH, Anticevic A, Yang GJ, Dragoi G, Driesen NR, Wang XJ and Murray JD. (2017) Impaired tuning of neural ensembles and the pathophysiology of schizophrenia: a translational and computational neuroscience perspective. *Biological Psychiatry* **81**, 874–885.

Labad J, Stojanovic-Perez A, Montalvo I, Sole M, Cabezas A, Ortega L, Moreno I, Vilella E, Martorell L, Reynolds RM and Gutierrez-Zotes A. (2015) Stress biomarkers as predictors of transition to psychosis in at-risk mental states: roles for cortisol, prolactin and albumin. *Journal of Psychiatric Research* **60**, 163–169.

Lai CY, Scarr E, Udawela M, Everall I, Chen WJ and Dean B (2016) Biomarkers in schizophrenia: a focus on blood based diagnostics and theranostics. *World Journal of Psychiatry* **6**, 102–117.

Lee EE, Eyler LT, Wolkowitz OM, Martin AS, Reuter C, Kraemer H, *et al*. (2016) Elevated plasma F2-isoprostane levels in schizophrenia. *Schizophrenia Research* **176**, 320–326.

Li XF, Zheng YL, Xiu MH, Chen DC, Kosten TR and Zhang XY (2011) Reduced plasma total antioxidant status in first-episode drug-naive patients with schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **35**, 1064–1067.

Liu GH, Qu J and Shen X (2008) NF-kappaB/p65 antagonizes Nrf2-ARE pathway by depriving CBP from Nrf2 and facilitating recruitment of HDAC3 to MafK. *Biochimica et Biophysica Acta* **1783**, 713–727.

Liu ML, Zheng P, Liu Z, Xu Y, Mu J, Guo J, *et al*. (2014) GC-MS based metabolomics identification of possible novel biomarkers for schizophrenia in peripheral blood mononuclear cells. *Molecular BioSystems* **10**, 2398–2406.

Mazzio EA, Kolta MG, Reams RR and Soliman KF (2005) Inhibitory effects of cigarette smoke on glial inducible nitric oxide synthase and lack of protective properties against oxidative neurotoxins in vitro. *Neurotoxicology* **26**, 49–62.

McCreadie RG, Macdonald E, Wiles D, Campbell G and Paterson JR (1995) The Nithsdale Schizophrenia Surveys. XIV: Plasma lipid peroxide and serum vitamin E levels in patients with and without tardive dyskinesia, and in normal subjects. *British Journal of Psychiatry* **167**, 610–617.

Medema S, Mocking RJ, Koeter MW, Vaz FM, Meijer C, de HL, *et al*. (2015) Levels of red blood cell fatty acids in patients with psychosis, their unaffected siblings, and healthy controls. *Schizophrenia Bulletin* **42**, 358–368.

Mico JA, Rojas-Corrales MO, Gibert-Rahola J, Parellada M, Moreno D, Fraguas D, *et al*. (2011) Reduced antioxidant defense in early onset first-episode psychosis: a case-control study. *BMC Psychiatry* **11**, 26.

Miller BJ, Buckley P, Seabolt W, Mellor A and Kirkpatrick B (2011) Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. *Biological Psychiatry* **70**, 663–671.

Miller BJ and Goldsmith DR (2017) Towards an immunophenotype of schizophrenia: progress, potential mechanisms, and future directions. *Neuropsychopharmacology* **42**, 299–317.

Miller JD, Chu Y, Brooks RM, Richenbacher WE, Pena-Silva R and Heistad DD (2008) Dysregulation of antioxidant mechanisms contributes to increased oxidative stress in calcific aortic valvular stenosis in humans. *Journal of the American College of Cardiology* **52**, 843–850.

Mitra S, Natarajan R, Ziedonis D and Fan X (2017) Antioxidant and anti-inflammatory nutrient status, supplementation, and mechanisms in patients with schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **78**, 1–11.

Miyaoka T, Seno H, Itoga M, Iijima M, Inagaki T and Horiguchi J (2000) Schizophrenia-associated idiopathic unconjugated hyperbilirubinemia (Gilbert's syndrome). *The Journal of Clinical Psychiatry* **61**, 868–871.

Morch RH, Dieset I, Faerden A, Hope S, Aas M, Nerhus M, *et al*. (2017) Persistent increase in TNF and IL-1 markers in severe mental disorders suggests trait-related inflammation: a one year follow-up study. *Acta Psychiatrica Scandinavica* **136**, 400–408.

Muller N (2018) Inflammation in Schizophrenia: pathogenetic Aspects and Therapeutic Considerations. *Schizophrenia Bulletin* **44**, 973–982.

Muller N, Weidinger E, Leitner B and Schwarz MJ (2015) The role of inflammation in schizophrenia. *Frontiers in Neuroscience* **9**, 372.

Pae CU, Paik IH, Lee C, Lee SJ, Kim JJ and Lee CU (2004) Decreased plasma antioxidants in schizophrenia. *Neuropsychobiology* **50**, 54–56.

Pandya CD, Howell KR and Pillai A (2013) Antioxidants as potential therapeutics for neuropsychiatric disorders. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **46**, 214–223.

Pisoschi AM and Pop A (2015) The role of antioxidants in the chemistry of oxidative stress: a review. *European Journal of Medicinal Chemistry* **97**, 55–74.

Raederstorff D, Wyss A, Calder PC, Weber P and Eggersdorfer M (2015) Vitamin E function and requirements in relation to PUFA. *British Journal of Nutrition* **114**, 1113–1122.

Reddy R, Keshavan M and Yao JK (2003) Reduced plasma antioxidants in first-episode patients with schizophrenia. *Schizophrenia Research* **62**, 205–212.

Reddy RD and Yao JK (1996) Free radical pathology in schizophrenia: a review. *Prostaglandins Leukotrienes & Essential Fatty Acids* **55**, 33–43.

Roge R, Moller BK, Andersen CR, Correll CU and Nielsen J (2012) Immunomodulatory effects of clozapine and their clinical implications: what have we learned so far? *Schizophrenia Research* **140**, 204–213.

Sekar A, Bialas AR, de Rivera H, Davis A, Hammond TR, Kamitaki N, Tooley K, Presumey J, Baum M, Van Doren V, Genovese G, Rose SA, Handsaker RE, Schizophrenia Working Group of the Psychiatric Genomics C, Daly MJ, Carroll MC, Stevens B and McCarroll SA. (2016) Schizophrenia risk from complex variation of complement component 4. *Nature* **530**, 177–183.

Semnani Y, Nazemi F, Azariyam A and Ardakani MJ (2010) Alteration of serum bilirubin level in schizophrenia. *International Journal of Psychiatry in Clinical Practice* **14**, 262–267.

Sertan CU, Virit O, Hanifi KM, Orkmez M, Bulbul F, Binnur EA, Semiz M, Alpak G, Unal A, Ari M and Savas HA. (2015) Increased oxidative stress and oxidative DNA damage in non-remission schizophrenia patients. *Psychiatry Research* **229**, 200–205.

Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, Dudbridge F, Holmans PA, Whittemore AS, Mowry BJ, Olincy A, Amin F, Cloninger CR, Silverman JM, Buccola NG, Byerley WF, Black DW, Crowe RR, Oksenberg JR, Mirel DB Kendler KS Freedman R and Gejman PV. (2009) Common variants on chromosome 6p22.1 are associated with schizophrenia. *Nature* **460**, 753–757.

Solberg DK, Bentsen H, Refsum H and Andreassen OA (2015) Association between serum lipids and membrane fatty acids and clinical characteristics in patients with schizophrenia. *Acta Psychiatrica Scandinavica* **132**, 293–300.

Solberg DK, Bentsen H, Refsum H and Andreassen OA (2016) Lipid profiles in schizophrenia associated with clinical traits: a five year follow-up study. *BMC Psychiatry* **16**, 299.

Steullet P, Cabungcal JH, Monin A, Dwir D, O'Donnell P, Cuenod M and Do KQ. (2016) Redox dysregulation, neuroinflammation, and NMDA receptor hypofunction: a "central hub" in schizophrenia pathophysiology? *Schizophrenia Research* **176**, 41–51.

Strassnig M, Singh Brar J and Ganguli R (2005) Dietary fatty acid and antioxidant intake in community-dwelling patients suffering from schizophrenia. *Schizophrenia Research* **76**, 343–351.

Suboticanec K, Folnegovic-Smalc V, Korbar M, Mestrovic B and Buzina R (1990) Vitamin C status in chronic schizophrenia. *Biological Psychiatry* **28**, 959–966.

Traber MG (2007) Vitamin E regulatory mechanisms. *Annual Review of Nutrition* **27**, 347–362.

Tuncel OK, Sarisoy G, Bilgici B, Pazvantoglu O, Cetin E, Unverdi E, Avci B and Boke O. (2015) Oxidative stress in bipolar and schizophrenia patients. *Psychiatry Research* **228**, 688–694.

van Os J, Rutten BP and Poulton R (2008) Gene-environment interactions in schizophrenia: review of epidemiological findings and future directions. *Schizophr Bulletin* **34**, 1066–1082.

Van't Erve TJ, Lih FB, Jelsema C, Deterding LJ, Eling TE, Mason RP and Kadiiska MB. (2016) Reinterpreting the best biomarker of oxidative stress: the 8-iso-prostaglandin F2alpha/prostaglandin F2alpha ratio shows complex origins of lipid peroxidation biomarkers in animal models. *Free Radical Biology and Medicine* **95**, 65–73.

Vasilaki AT, Leivaditi D, Talwar D, Kinsella J, Duncan A, O'Reilly DS and McMillan DC. (2009) Assessment of vitamin E status in patients with

https://doi.org/10.1017/neu.2019.14 Published online by Cambridge University Press

systemic inflammatory response syndrome: plasma, plasma corrected for lipids or red blood cell measurements? *Clinica Chimica Acta* **409**, 41–45.

Widschwendter CG, Rettenbacher MA, Kemmler G, Edlinger M, Baumgartner S, Fleischhacker WW and Hofer A. (2016) Bilirubin concentration correlates with positive symptoms in patients with schizophrenia. *The Journal of Clinical Psychiatry* **77**, 512–516.

Wollmann BM, Syversen SW, Lie E, Gjestad C, Mehus LL, Olsen IC and Molden E. (2017) 4beta-Hydroxycholesterol Level in Patients With Rheumatoid Arthritis Before vs. After Initiation of bDMARDs and Correlation With Inflammatory State. *Clinical and Translational Science* **10**, 42–49.

Wu JQ, Kosten TR and Zhang XY (2013) Free radicals, antioxidant defense systems, and schizophrenia. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **46**, 200–206.

Yao JK and Keshavan MS (2011) Antioxidants, redox signaling, and pathophysiology in schizophrenia: an integrative view. *Antioxidants & Redox Signaling* **15**, 2011–2035.

Yao JK, Reddy R, McElhinny LG and van Kammen DP (1998) Reduced status of plasma total antioxidant capacity in schizophrenia. *Schizophrenia Research* **32**, 1–8.

Yao JK, Reddy R and van Kammen DP (2000) Abnormal age-related changes of plasma antioxidant proteins in schizophrenia. *Psychiatry Research* **97**, 137–151.

Zhang XY and Yao JK (2013) Oxidative stress and therapeutic implications in psychiatric disorders. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **46**, 197–199.

Zhang XY, Chen DC, Xiu MH, Wang F, Qi LY, Sun HQ, Chen S, He SC, Wu GY, Haile CN, Kosten TA, Lu L and Kosten TR. (2009) The novel oxidative stress marker thioredoxin is increased in first-episode schizophrenic patients. *Schizophrenia Research* **113**, 151–157.

Zhang XY, Tan YL, Cao LY, Wu GY, Xu Q, Shen Y and Zhou DF. (2006) Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. *Schizophrenia Research* **81**, 291–300.

Zhang XY, Tan YL, Zhou DF, Haile CN, Wu GY, Cao LY, Kosten TA and Kosten TR. (2007) Nicotine dependence, symptoms and oxidative stress in male patients with schizophrenia. *Neuropsychopharmacology* **32**, 2020–2024.

https://doi.org/10.1017/neu.2019.14 Published online by Cambridge University Press