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Pilot study indicates that a gluten-free diet lowers oxidative stress for gluten-sensitive persons with schizophrenia

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ABSTRACT

One-third of people with schizophrenia have elevated levels of anti-gliadin antibodies (AGA IgG). A 5-week randomized double-blind pilot study was performed in 2014–2017 in an inpatient setting to test the effect of a gluten-free diet (GFD) on participants with schizophrenia or schizoaffective disorder who also had elevated AGA IgG (≥ 20 U) but were negative for celiac disease. This earlier pilot study reported that the GFD-group showed improved gastrointestinal and psychiatric symptoms, and also improvements in TNF-α and the inflammatory cytokine IL-23. Here, we performed measurements of these banked plasma samples to detect levels of oxidative stress (OxSt) using a recently developed iridium (Ir)-reducing capacity assay. Triplicate measurements of these samples showed an Intraclass Correlation Coefficient of 0.84 which indicates good reproducibility. Further, a comparison of the OxSt measurements at the baseline and 5-week end-point for this small sample size shows that the GFD-group ($N = 7$) had lowered OxSt levels compared to the gluten-containing diet group (GCD; $N = 9$; $p = 0.05$). Finally, we showed that improvements in OxSt over these 5 weeks were correlated to improvements in gastrointestinal ($r = +0.64$, $p = 0.0073$) and psychiatric ($r = +0.52$, $p = 0.039$) symptoms. Also, we showed a possible association between the decrease in OxSt and the lowered levels of IL-23 ($r = +0.44$, $p = 0.087$), although without statistical significance. Thus, the Ir-reducing capacity assay provides a simple, objective measure of OxSt with the results providing further evidence that inflammation, redox dysregulation and OxSt may mediate interactions between the gut and brain.

1. Introduction

There is increasing evidence for linkages between diet and gut dysbiosis and mental health (Mitrea et al., 2022; Nemani et al., 2015; Shi et al., 2023; Vafadari, 2021). Epidemiological evidence indicated that consumption of gluten-containing foods (e.g., wheat) was linked to hospital admissions for schizophrenia (SCZ) (Dohan, 1966a, 1966b; Dohan et al., 1984), and several studies indicated that adherence to a gluten free diet (GFD) was associated with improvements in psychotic symptoms (Levinta et al., 2018). In addition, approximately 30 % of persons diagnosed SCZ are sensitive to gluten and have antibodies against the gluten protein gliadin (anti-gliadin antibody; AGA IgG) (Cascella et al., 2009; Čiháková et al., 2018; Dickerson et al., 2010; Jin et al., 2012; Okusaga et al., 2013). Animal models of gluten sensitivity show that AGA IgG are associated with proinflammatory signaling (Vijaykrishnaraj et al., 2017) and the elevated AGA IgG in patients with SCZ are correlated with the peripheral and central makers of inflammation (Kelly et al., 2018; Rowland et al., 2017). We previously

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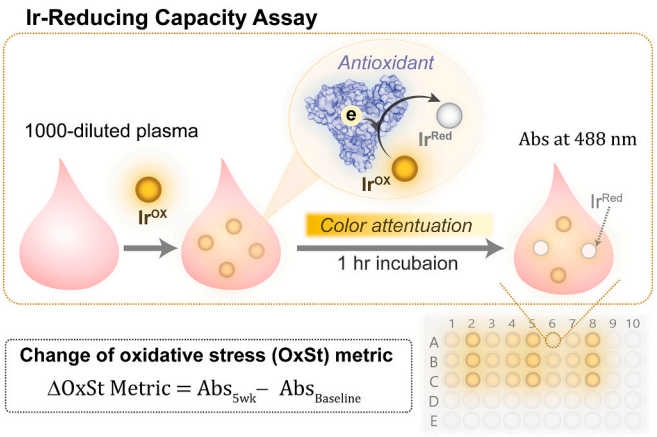
performed a 5-week randomized double-blind pilot study in an inpatient setting to test the effect of a GFD on participants with SCZ or schizoaffective disorder who also had elevated AGA IgG (≥ 20 U) but were negative for celiac disease (Kelly et al., 2019b). After 5-weeks, GFD group showed moderate improvements in psychiatric symptoms, and specifically negative symptoms (Cohen's d = −0.53), and also showed decreases in inflammatory cytokines (Friendshuh et al., 2020; Kelly et al., 2019b).

Growing evidence links inflammation, redox dysregulation and oxidative stress (OxSt) to SCZ pathology (Bitanihirwe and Woo, 2011; Dwir et al., 2020; Flatow et al., 2013; Giangreco et al., 2023; Koga et al., 2016; Perkins et al., 2020), with OxSt being suggested as a "hub of convergence between genetic and environmental risk factors" for SCZ (Cuenod et al., 2022; Dwir et al., 2023). OxSt is generally believed to reflect an imbalance between pro- and anti-oxidant activities (Go and Jones, 2014; Sies, 2018, 2019). Typically, OxSt is believed to be initiated by the generation of reactive oxygen/nitrogen species (ROS or RNS) through mitochondrial (Stein et al., 2023) or immune cell activities (Halliwell, 2023). Under homeostatic conditions, the generation of these reactive pro-oxidants can be balanced by antioxidant defenses (e.g., ROS-degrading enzymes or antioxidant scavenging molecules such as glutathione (GSH)), and these reactive species can serve as signaling molecules that can control gene expression through redox-responsive transcription factors (e.g., the master regulator, NF-kB) (Jennings et al., 2019; Schiavone and Trabace, 2017; Sies et al., 2022; Sies and Jones, 2020). When the generation of these reactive oxidants exceeds the antioxidant defense capabilities, then sensitive molecular (e.g., proteins) and cellular structures (e.g., membranes) can become oxidatively damaged (Colombo et al., 2012; Marrocco et al., 2017; Paramasivan et al., 2020).

A variety of biomarkers have been considered for detecting and characterizing OxSt (Dalle-Donne et al., 2005; Frijhoff et al., 2015; Klein et al., 2002; Lowe, 2014; Marrocco et al., 2017; Więdłocha et al., 2023). Molecular biomarkers include the reactive oxidants, immune signaling molecules (e.g., pro/anti-inflammatory cytokines) (Dunleavy et al., 2022; Rudkowski et al., 2023), antioxidant enzymes (e.g., superoxide dismutase), small molecule antioxidants (e.g., GSH) (Coughlin et al., 2021; Frustaci et al., 2012; Jones et al., 2000; Rossi et al., 2006) or the oxidation of susceptible amino acid residues (e.g., of serum albumin) or nucleic acids residues (Colombo et al., 2012; Niki, 2018). In addition to measuring individual molecules or residues, some methods aim to provide a systems-level measure of OxSt (e.g., Total Antioxidant Capacity) (Apak et al., 2016; Laher, 2014; Lowe, 2014). While many of these biomarkers have shown some success in correlating to clinical assessments of disease, there have been many contradictory findings (Frijhoff et al., 2015; Więdłocha et al., 2023) and there does not yet appear to be a single, accepted measurement of OxSt.

Previously, we reported an Ir-reducing-capacity assay (Ir-RCA) as a global, system-level measurement of OxSt (Kim et al., 2019; Kim et al., 2017). In this assay, the oxidized Ir mediator (Ir^{Ox}) is used to probe a sample for molecular species (e.g., antioxidants) that can donate electrons to reduce Ir^{Ox} and attenuate its yellow color as illustrated in Scheme 1. In essence, the Ir mediator is surveying the sample for its historical record of oxidation (e.g., oxidation of amino acid residues of the protein pool). There are several important features of the Ir-RCA. First, while the Ir-RCA is similar to other antioxidant capacity assays (e.g., Total Antioxidant Capacity (Apak et al., 2016; Gupta et al., 2021; Silvestrini et al., 2023)), the Ir mediator has been shown to be especially sensitive to sulfur-containing compounds (e.g. GSH) (Bhattarai and Stanbury, 2012; Kim et al., 2017) which are physiologically-relevant antioxidants (Kim et al., 2017). Second, the Ir-RCA is detecting stable features in serum samples as triplicate measurements of OxSt performed at different times (spaced months apart) yielded comparable results (N = 118; intraclass correlation coefficient of 0.687)(Kim et al., 2019). This indicates both that the measurement is not detecting transient and reactive species, and that precautions are not needed to exclude oxygen during sampling, storage and measurement. Third, the single endpoint measurement detected greater levels of OxSt for a SCZ group vs healthy controls (N = 118; AUC = 0.89; $p < 0.0001$) (Kim et al., 2019) which supports the suspected link between OxSt and SCZ. Finally, an expanded Ir-RCA that coupled optical and electrochemical methods demonstrated higher levels of OxSt 90 min after volunteers were exposed to psychosocial stress(Kim et al., 2021). Thus, the Ir-RCA of OxSt appears to provide a reliable and movable biomarker.



Scheme 1. Iridium reducing capacity assay (Ir-RCA). A redox-mediator (K_2IrCl_6, Ir^{OX}) is used to probe for reducing capacities of biochemical components in plasma through the attenuation of optical signal after 1 h incubation in plasma. A change of OxSt metric (ΔOxSt Metric) is the difference of absorbance at 488 nm (Abs) between baseline and 5 weeks.

Here, we used the Ir-RCA to measure OxSt levels in the banked plasma samples from the previously completed pilot feasibility study and compared changes in the OxSt levels for the GFD relative to GCD groups and also how the changes in OxSt levels were related to changes in gastrointestinal, psychiatric symptoms and inflammation biomarker levels.

2. Methods

2.1. Study procedures

In the parent trial conducted from 2014 to 2017 (Kelly et al., 2019b), participants were recruited to have a DSM-IV diagnosis of schizophrenia or schizoaffective disorder, not currently on a gluten-free diet and were between the ages of 18 and 64 years. They were screened by laboratory tests including AGA lgG, AGA lgA, and tTG. However, they were excluded if participants tested positive for tTG due to the possibility of celiac disease. Sixteen participants who tested positive for AGA IgG (> 20 U) were eligible for study enrollment. More details of screening process were previously described (Kelly et al., 2019b). The study involved 5 weeks of randomized, double-blind treatment with a GFD (N = 7) or GCD (N = 9) in an inpatient setting with strict dietary control. Briefly, all participants received a GFD with 3 meals and 2 snacks daily. All participants were randomized to 10 g of rice flour (GFD) or gluten flour (GCD) to a protein shake taken once daily and all were maintained in the blind. We maintained a strict regimen and oversight for maintaining a gluten-free diet. All staff in the inpatient setting were required to ensure that all participants remained gluten-free. All medications remained stable throughout the course of the 5-week study.

2.2. Clinical study assessments

In our previous study (Kelly et al., 2019b), psychiatric symptoms were weekly measured over the 5-week study using the Sale for the Assessment of Negative Symptoms (SANS) (Andreasen, 1982), the Brief

Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962) and MATRICS Consensus Cognitive Battery (MCCB) (August et al., 2012). We assessed negative symptoms using the total score of the SANS. All raters were trained and reliable, with an intraclass correlation coefficient of >0.7. Also, gastrointestinal effects were measured using the Gastrointestinal Symptom Rating Scale (GSRS) at the baseline and endpoint (5 week).

Previously, for the laboratory assessments, blood was drawn at baseline and endpoint (5 weeks) and plasma samples were prepared for assaying the peripheral cytokines (Friendshuh et al., 2020; Kelly et al., 2019b). Peripheral cytokines were assessed using ELISA cytokine kits from R&D Systems. All assays were conducted at the John Hopkins Čiháková's Laboratory. Cytokines measured included IL-1β (cat # DLB50), IL-8 (cat# D8000C), IL-4 (cat# D4050), TNF-α (cat# DTA00C), and highly sensitive C reactive protein (cat# DCRP00). IL-17 (cat# D1700) and IL-23 (cat# D2300B) which were selected due to their linkage to gut inflammation (Friendshuh et al., 2020; Kelly et al., 2019b).

2.3. Chemicals

The following were purchased from Sigma-Aldrich: K_2IrCl_6 (IV), and phosphate buffered saline (PBS, pH 7.4). The water (>18 MΩ) used in this study was obtained from a Super Q water system (Millipore). A stock solution of 10 mM K_2IrCl_6 (Ir^{OX}) was prepared in phosphate buffer saline (PBS; pH 7.4) and its aliquot was stored in −80 °C freezer.

2.4. Ir-reducing capacity assay

Before the measurement, we thawed each aliquot of plasma samples and a 10 mM Ir^{OX} stock solution that had all been frozen at −80 °C. Sample solution was prepared by diluting plasma (100-fold) with 0.1 M PBS. In each well of a 96 well microplate, 20 μL of 100-fold diluted plasma sample was added into 170 μL PBS and then 10 μL of Ir^{OX} (10 mM) was added (final plasma dilution:1000; and final Ir level: 0.5 mM). Scheme 1 illustrates that after 1-hour incubation at room temperature, the attenuated optical absorbance (Abs) was measured at 488 nm using a standard absorbance microplate reader (Spark, Tecan). The change of oxidative stress metric (Δ OxSt Metric) was calculated by subtracting the absorbance (Abs_{5wk}) of the end point (5-week) sample from the absorbance of the baseline sample ($Abs_{Baseline}$) for each sample. Three independent analyses of 32 plasma samples were performed over a 60-day period. At each analysis, each sample was measured in triplicate and the triplicate measurements were averaged for further data analysis. The person performing the Ir-RCA was blinded to all clinical information until all assays were completed.

2.5. Statistical analysis

Statistical analyses were performed using the software package R. Group differences in demographic, clinical information and in the change of OxSt metric were assessed using Kruskal-Wallis test. The intraclass correlation coefficient (ICC) was calculated using the 'irr' package based on a consistency and two-ways random effect analysis of variance (ANOVA) model (Bartko et al., 1966; Hallgren, 2012; Koo and Li, 2016). Cohen's d was calculated using the change in OxSt metric from baseline to endpoint with a GFD relative to a GCD group (Kelly et al., 2019b). Spearman's correlation coefficients were calculated to examine the correlation of the change of OxSt metric with the change of gastrointestinal, inflammation markers and psychiatric symptom scales.

3. Results

Table 1 lists the demographic and clinical characteristics of the GCD ($N = 9$) and GFD ($N = 7$) groups at baseline. Table 1 shows that there is no significant difference between GCD and GFD groups in both demographic and clinical characteristics ($p > 0.05$). Also, all participants maintained their doses of anti-psychotics and other treatments during the 5-week study.

Table 1
Demographic and clinical information.

Characteristic	Gluten-containing diet (GCD, $N = 9$)	Gluten-free diet (GFD, $N = 7$)	Test[a] statistics
Mean age, yr	42.0 ± 14.6	32.5 ± 9.7	p = 0.22
Sex (M/F, no. (%))	5/4 (56 / 44)	4/3 (57 / 43)	p = 0.22
Race (White/AA[b], no. (%))	2/7 (22 / 78)	2/5 (29 / 71)	p = 0.95
Body mass index (kg/m^2)	28.5 ± 4.7	31.4 ± 8.9	p = 0.71
Smoker Y/N, no. (%)	5/4 (56/44)	6/1 (86/14)	p = 0.21
Level of education, yr	11.8 ± 1.3	12.4 ± 2.1	p = 0.29
Medications no. (%)			
Antipsychotic			p = 0.78
FGA[c]	2 (22.2)	2 (28.6)	
SGA[d]	2 (22.2)	2 (28.6)	
FGA + SGA	3 (33.4)	2 (28.6)	
Clozapine	2 (22.2)	1 (14.2)	
Anti-depressant	6 (66.7)	3 (42.9)	p = 0.36
Anti-cholinergic	7 (77.8)	6 (85.7)	p = 0.70
Anti-inflammatory	2 (22.2)	2 (28.6)	p = 0.78
Diabetes[e] no. (%)	1 (11.1)	0 (0)	p = 0.19
Baseline AGA- lgG, U	55.8 ± 28.6	43.8 ± 12.2	p = 0.56
GSRS total score	26.9 ± 8.1	31.0 ± 16.5	p = 1
SANS total score	25.2 ± 5.0	32.7 ± 14.2	p = 0.24

[a] The group difference was assessed using Kruskal-Wallis test.
[b] AA: African American.
[c] FGA: first-generation antipsychotic.
[d] SGA: second-generation antipsychotic.
[e] Diabetes is for Type II.

To check the reliability of Ir-reducing capacity assay (Ir-RCA), we calculated the intraclass correlation coefficient (ICC) for three replicated absorbance (Abs) measurements of the same sample (Bartko et al., 1966; Koo and Li, 2016; Shrout and Fleiss, 1979) (Note: 3-dimensional plot of Fig. S1 shows the three replicated Abs measurements for the 16 participants at baseline and 5 weeks ($N = 32$ samples)). The calculated ICC was +0.84 which indicates a good agreement for the three replicate measurements. Importantly, the agreement in these replicates indicates that the Ir-RCA is measuring stable features in the plasma (i.e., it is not measuring transiently-appearing or unstable molecular species), and the method is not sensitive to the presence/absence of air as no precautions were made to exclude oxygen when drawing the blood, or when storing (i.e., freezing), processing (i.e., thawing) or assaying the plasma (Kim et al., 2019; Kim et al., 2021).

To assess the effect of GFD on the OxSt in plasma, we examined the changes in the OxSt metric after 5 weeks (Abs_{5wk}) compared with the OxSt metric Abs at baseline ($Abs_{Baseline}$) for each participant as described in Scheme 1. Fig. 1 compares the change of OxSt metric (Δ OxSt Metric) for the participants from GFD (N = 7) and GCD (N = 9) groups. This plot shows that the OxSt levels for the participants from the GFD group were lowered over the course of this 5-week study, compared to increases in the OxSt levels observed for the GCD group (Cohen's d = −0.71).

Next, we examined whether the changes in the OxSt levels (as measured by the Δ OxSt Metric) were correlated to previously observed changes in the severity of gastrointestinal symptoms as measured by the Gastrointestinal Symptom Rating Scale (GSRS) during the 5-week trial. In the previous study (Kelly et al., 2019b), the GFD group showed a robust improvement in total gastrointestinal symptom, as measured by total GSRS score (Cohen's d = −0.81). Fig. 2 shows that over this 5-week period, there was a positive correlation (r = +0.64) between the change in the levels of OxSt (Δ OxSt Metric) and the change in GSRS (Δ GSRS) for both GFD and GCD groups. This suggests that the lowered OxSt levels observed for the GFD group may be associated with the improvements in gastrointestinal symptoms.

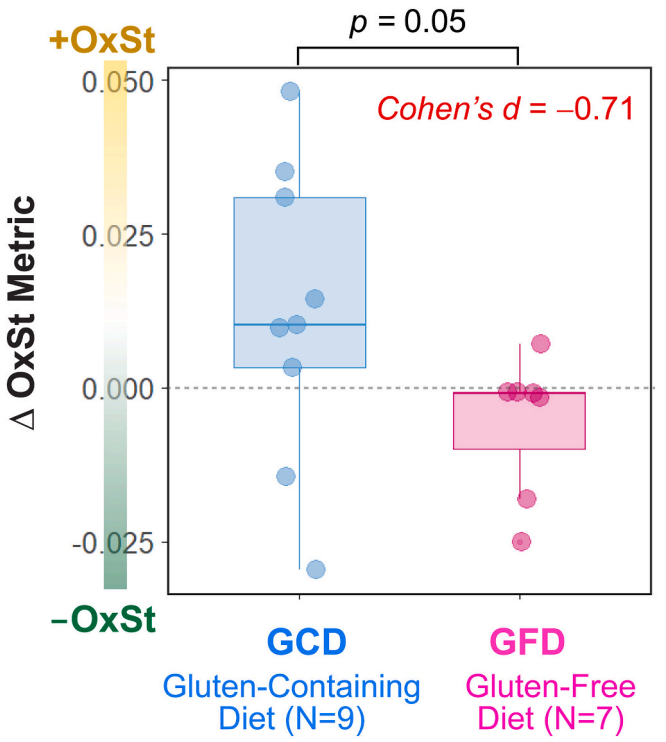


Fig. 1. Oxidative stress (OxSt) is lowered in gluten free diet group. The GFD group shows lowered levels of OxSt compared to the GCD group.

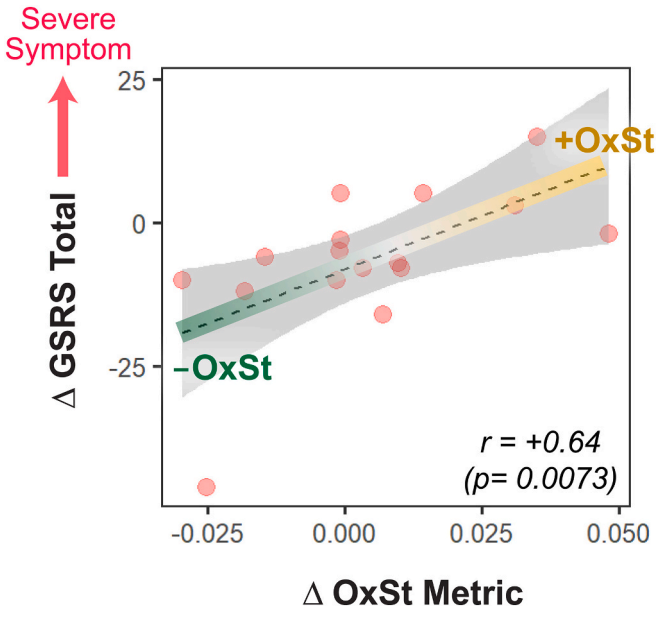


Fig. 2. Oxidative stress and gastrointestinal symptoms. Changes in the severity of gastrointestinal symptoms correlates to changes in levels of OxSt.

We then examined whether the changes in the OxSt levels correlated to previously-observed changes in the levels of inflammatory cytokines (Friendshuh et al., 2020). In the previous study(Friendshuh et al., 2020), the GFD group showed decreases (i.e., improvements) in the levels of TNF-α (Cohen's d = −0.93) and IL-23 (Cohen's d = −1.65) relative to the GCD group. We found no significant correlation between the change in the OxSt metric and the change in inflammatory cytokines (Note: Fig. S2 in Supplementary Material provides the correlation heat map for these correlations). Fig. 3 shows a weak positive correlation (r = +0.44)

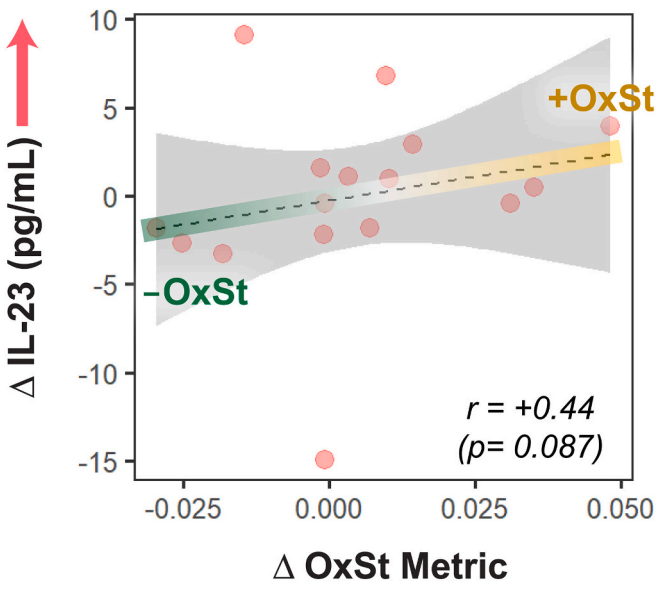


Fig. 3. Oxidative stress and inflammatory cytokines. Changes in plasma levels of the inflammatory cytokine (IL-23) correlates to changes in levels of OxSt (note: the *p* value indicates this correlation is not statistically significant).

between the change in the OxSt metric (Δ OxSt Metric) and the change in the IL-23 levels (Δ IL-23), although this correlation is not statistically significant ($p = 0.087$). Although this result is not statistically significant, the trend is consistent with the expectation that increasing levels of OxSt could be associated with increasing levels of inflammation (Czarny et al., 2018; Yu et al., 2022).

Finally, we examined whether the changes in the OxSt levels were correlated to previously-observed changes in various psychiatric symptoms (Note: Fig. S3 in Supplementary Material provides the correlation heat map between the changes in OxSt and changes in various psychiatric symptoms). We found that the changes in OxSt are significantly correlated only with the changes in negative psychiatric symptoms, measured by the Sale for the Assessment of Negative Symptoms (SANS) method. In the previous study (Kelly et al., 2019b), the GFD group showed a decrease of total SANS scores (Cohen's d = −0.53), indicating improvements in negative symptoms, compared to the GCD group. Fig. 4 shows the positive correlation (r = +0.52) between the change in the oxidative stress metric (Δ OxSt Metric) and the change in total SANS (Δ SANS total) for both GFD and GCD groups. This correlation suggests that higher levels of OxSt are associated with more severe negative symptoms.

Overall, these results complement previous measurements and indicate that for persons with SCZ and who are also gluten intolerant, a GFD can lower levels of OxSt, and this appears to be associated with improvements in both gastrointestinal and psychiatric symptoms.

4. Discussion

Here, we observed that persons with SCZ who are also sensitive to gluten (i.e., elevated levels of anti-gliadin antibodies; AGA IgG) had lowered levels of oxidative stress (OxSt) after 5 weeks on a gluten free diet (GFD). This lowering of OxSt for the GFD group was correlated to previously-observed (Friendshuh et al., 2020; Kelly et al., 2019b): (i) improvements in gastrointestinal symptoms (as measured by GSRS); (ii) improvements in negative psychiatric symptoms (as measured by SANS); and (iii) decreases in the levels of the inflammatory cytokine IL-23 (note: the correlation between OxSt and IL-23 was not statistically significant). These results are consistent with recent hypotheses that redox dysregulation and oxidative stress (OxSt) are involved in interactions between the immune, digestive and nervous systems

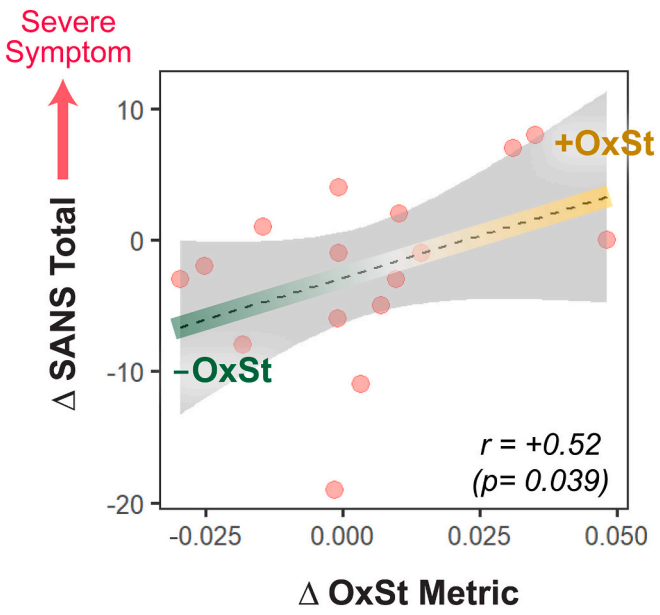


Fig. 4. Oxidative stress and negative psychiatric symptoms. Changes in the severity of negative psychiatric symptoms correlates to changes in levels of OxSt.

(Dzikowski et al., 2020), and are also linked to inflammation (Dudzińska et al., 2022; Murray et al., 2021) and risk factors for SCZ (Cuenod et al., 2022; Dwir et al., 2023).

It is not surprising that the original study observed that persons with elevated AGA IgG benefited from a GFD. Previously, we found that after 5 weeks, the AGA-IgG levels decreased more for the GFD group (34 % decrease) than the GCD group (16 % decrease) (Cohen's d = −0.34) (Kelly et al., 2019a). Also, it is also not surprising that these improved gastrointestinal symptoms are associated with lowered levels of OxSt. Several studies report that, for sensitive individuals, gluten-ingestion can increase inflammation and OxSt (Dzikowski et al., 2020; Kaplan et al., 2017; Lerner et al., 2017; Levescot et al., 2022; Moretti et al., 2018; Stamnaes et al., 2021).

Plasma samples from the original pilot study (Friendshuh et al., 2020) were previously analyzed for changes in inflammation cytokines. These previous analyses showed no robust changes for many of the putative inflammatory cytokines, although there were large decreases in TNF-α (Cohen's d = −0.93) and IL-23 (Cohen's d = −1.65), and moderate decreases in IL-4 (Cohen's d = −0.73) and IL-17 (Cohen's d = −0.44). Here, we found that only one of the inflammatory cytokines, IL-23, showed changes that had a weak, but not significant, correlation to changes of OxSt levels (r = +0.44, $p = 0.087$). IL-23, a member of IL-12 family, is a pro-inflammatory cytokine which has been shown to be associated with inflammatory diseases, such as inflammatory bowel disease, psoriasis, and coronary heart disease (Chan et al., 2006; Zhang et al., 2014b), and is associated with gluten-ingestion for gluten sensitive people (Harris et al., 2008). Newer data suggest this interleukin and its pathway play a critical role in ROS production (Chen et al., 2020; Itoh et al., 2011; Müller et al., 2020; Zhang et al., 2014a; Zhu et al., 2013) and higher IL-23 levels have been reported in schizophrenia patients with enduring negative symptoms (Al-Hakeim et al., 2022). Interestingly, a recent Mediterranean diet study found that changes in oxidative stress were associated with changes in IL-23 (Tüccar and Akbulut, 2023). Thus, the trend between IL-23 and OxSt observed in Fig. 3, although without statistical significance, is consistent with expectations that inflammation is positively correlated with OxSt (Fan et al., 2007; Miller et al., 2023; Pedraz-Petrozzi et al., 2020; Savitz and Savitz, 2019).

In the original 5-week GFD pilot study (Kelly et al., 2019b), we measured several psychiatric symptoms. For the GFD group relative to gluten-containing diet (GCD) group, we observed improvements in the negative symptoms (SANS; Cohen d = −0.53), but no notable improvement in the other psychiatric scales such as Brief Psychiatric Rating Scale (BPRS) and MATRICS Consensus Cognitive Battery (MCCB). Here, we observed significant correlations between the 5-week changes in SANS scale for negative symptoms and changes in OxSt levels (r = +0.52, $p = 0.039$), but we did not find significant correlations for changes in BPRS and MCCB.

Our results are consistent with previous studies that suggest a relationship between negative symptoms and OxSt (Gunes et al., 2017; Matsuzawa et al., 2008; Murray et al., 2021). Importantly, even though negative symptoms are associated with the greatest functional disability in SCZ, there are no current treatments for negative symptoms (Chang et al., 2016; Tamminga et al., 1998). The observation that a GFD improved both OxSt and negative symptoms for this gluten-sensitive subgroup is consistent with other studies investigating the use of antioxidants. For instance, the antioxidant, *N*-acetylcysteine, has been shown to reverse oxidative stress (Otte et al., 2011), and diminish depressive symptoms (Berk et al., 2008; Farokhnia et al., 2013; Zheng et al., 2018), and, more recently, to improve negative symptoms and working memory in people with SCZ (Yolland et al., 2020). The focus in our study of people with SCZ and high AGA IgG levels represents an important subgroup that has been shown to have high elevated peripheral cytokines (Kelly et al., 2018; Rowland et al., 2017) and potential brain inflammation (Rowland et al., 2017). This subgroup is known to have lower positive symptoms and thus, this subgroup may offer a unique opportunity to better-understand the relationship among inflammation, OxSt and negative symptoms.

There are strengths and limitations of this study. One strength is that this clinical study was performed in an inpatient setting where food, medications, substance use, exercise and other environmental factors were monitored, controlled and quantified. This represents a unique opportunity to reduce confounds. Also, the subjective ratings were all performed by reliable raters and the study was blinded and randomized to reduce bias. We were able to maintain near 100 % diet adherence to provide the highest level of compliance in this proof-of-principle pilot study. Additionally, as shown in Table 1, the baseline distribution of factors between GCD and GFD groups, possibly impacting oxidative stress, were similar and medications and smoking frequency did not change during the 5 weeks, which might minimize the confounding factors. However, the obvious limitation of this pilot study is the small sample size ($N = 7$ GFD, $N = 9$ GCD) which makes it impossible to develop a definitive understanding of the relationships among diet, inflammation, OxSt and mental health.

We believe the strengths of the Ir-reducing capacity assay (Ir-RCA) are that it is simple, and it measures stable, systems-level molecular features from the sample (i.e., the accumulated historical record of the oxidative modification of the redox interactome). In the validation step of Ir-RCA (Kim et al., 2019), we found that the assay was not affected by long-time storage (~ 2 years) or repeated freezing-thawing steps (Note: Fig. S4 shows that Ir-RCA results are not changed for three repeated freezing-thawing processes (<0.5 %)). We believe the quantitatively most important molecular features being measured by the Ir-RCA are the accumulated oxidative modification of proteins since proteins represent the largest pool of antioxidants in serum and their half-life in circulation is long, measured in days-to-weeks (i.e., 19–25 days for serum albumin) (Colombo et al., 2012). In contrast, levels of metabolic intermediates or signaling molecules (e.g., cytokines) can be dynamically changing with half-lives measured in seconds-to-hours (Fond et al., 2020; Müller, 2018; Sayana et al., 2017). We hypothesize that the measurement of molecular features that are stable (vs transient) and systems-level (vs individual molecules) provides a greater opportunity to detect robust signatures of OxSt. The results from this study support this hypothesis, as statistically-significant correlations were observed between subjective measurements of gastrointestinal or psychiatric symptoms and the objective measurement of OxSt. In comparison to other systems-level

measurements of OxSt (e.g. ferric-reducing ability of plasma and the cupric-reducing antioxidant capacity assays) (Apak et al., 2016; Buico et al., 2009; Gupta et al., 2021; Silvestrini et al., 2023), we believe the Ir-RCA has the advantage that it is particularly sensitive to the sulfur-containing molecules (e.g., GSH, and the cysteine and methionine residues of proteins) which are important targets of oxidative damage (Kim et al., 2017). The obvious limitation of the Ir-RCA is that the individual molecules that contribute to the OxSt measurements are not revealed which makes it more difficult to relate the OxSt measurement to underlying molecular-level pathophysiological mechanisms or to relate serum measurements to brain chemistries believed important to the effects of oxidative stress on mental health.

5. Conclusion

In summary, this study demonstrated that the OxSt levels were lowered for the GFD group compared with the GCD group. This lowering of oxidative stress showed significant correlations to improvements in gastrointestinal (GSRS) and psychiatric (particularly negative) symptoms (SANS), and a weak (non-significant) correlation with the lowering of the levels of the inflammatory cytokine IL-23. These results are of particular interest in the SCZ subgroup with higher AGA IgG levels but also support growing evidence indicating that inflammation, redox dysregulation and OxSt are important mediators of interactions between the gut and brain.

Role of the funding source

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CRediT authorship contribution statement

Eunkyoung Kim: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **Sidney Redwood:** Writing – review & editing, Investigation, Formal analysis. **Fang Liu:** Writing – review & editing, Formal analysis. **Daniel J.O. Roche:** Writing – review & editing, Formal analysis. **Shuo Chen:** Writing – review & editing, Formal analysis. **William W. Eaton:** Writing – review & editing, Investigation, Formal analysis. **Daniela Čiháková:** Writing – review & editing, Investigation, Formal analysis. **Monica V. Talor:** Writing – review & editing, Investigation, Formal analysis. **Deanna L. Kelly:** Writing – review & editing, Writing – original draft, Supervision Conceptualization. **Gregory F. Payne:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization.

Declaration of competing interest

The authors report no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.schres.2024.05.001.

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