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Patients with Gaucher disease display systemic oxidative stress dependent on therapy status

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ABSTRACT

Gaucher disease is an autosomal recessive metabolic disorder caused by mutations in *GBA1*, which encodes for the lysosomal hydrolase enzyme, β-glucocerebrosidase. The resulting misfolded protein can trigger endoplasmic reticulum stress and an unfolded protein response within the affected cells. The enzyme deficiency leads to accumulation of its substrates, glucosylceramide and glucosylsphingosine, within macrophage lysosomes and with prominent disease manifestations in macrophage rich tissues. Resultant lysosomal pathology and impaired autophagy leads to redox imbalance, mitochondrial dysfunction and intracellular oxidative stress. Here we have systematically examined a role for oxidative stress in individuals affected by Gaucher disease. We compared multiple oxidative stress biomarkers in plasma and red blood cell samples from patients who are currently untreated, with those who are stable on standard-of-care therapy, and with healthy controls. We found significant differences in key oxidative stress biomarkers in untreated patients compared to healthy control. In treated patients, results generally fell between the controls and the untreated patients. Interestingly, even asymptomatic and minimally symptomatic untreated patients had evidence of significant systemic oxidative stress. We conclude that underlying oxidative stress may contribute to Gaucher disease pathophysiology including long-term adverse outcomes such as Parkinsonism and malignancies. Therapies targeting oxidative stress may prove useful as adjuvant treatments for Gaucher disease and other lysosomal storage disorders.

1. Introduction

Gaucher disease (GD), one of most common lysosomal storage disorders, is caused by a deficiency in lysosomal glucocerebrosidase (GCase) activity resulting from homozygous or biallelic mutations in the *GBA1* gene. This leads to the accumulation of glycosphingolipids in multiple organs including spleen, liver and bone marrow, which in turn, significantly affects the quality of life [13]. Patients with GD can display a spectrum of phenotypes, and are broadly classified into 3 subtypes, depending on the degree of neurological involvement [16]. The most common subtype, type 1 GD (GD1) is found in children and adults who have signs and symptoms that generally do not involve the central nervous system (CNS), but can produce chronic and progressive disease manifestations. The less common neuronopathic subtypes, types 2 and 3 (GD2 and GD3) primarily affect newborns and infants (GD2) or children and adolescents (GD3) and are associated with a more severe

Abbreviations: ACE, angiotensin converting enzyme; CHITO, chitotriosidase; CNS, central nervous system; ERT, enzyme replacement therapy; GCase, glucocerebrosidase; GD, Gaucher disease; GD1, Type 1 Gaucher disease; GD2, Type 2 Gaucher disease; GD3, Type 3 Gaucher disease; GPG, Glycine-Proline-Glutamate; GPx, glutathione peroxidase; GSH, glutathione; GSSG, inactive, oxidized form of glutathione; HPLC, high performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; Lyso-GL1, glucosylsphingosine; MDA, malondialdehyde; NYU, New York University; RBC, red blood cell; ROS, reactive oxygen species; SOD, superoxide dismutase; SRT, substrate reduction therapy; TAC, total antioxidant capacity; TBARS, thiobarbituric acid reactive substances; TRAP, tartrate resistant acid phosphatase; UMN, University of Minnesota

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phenotype that include prominent involvement of the CNS. Systemic treatment enables some GD3 patients to function well in society and survive to at least middle age [31]. In the United States, five treatments are FDA-approved for the non-neuronopathic manifestations of GD. These include imiglucerase, velaglucerase alfa, taliglucerase alfa (intravenous enzyme replacement therapy), miglustat and eliglustat (oral inhibitors of glucosylceramide synthase). None of these addresses the neurological manifestations of GD2 and GD3.

Current therapies alleviate many of the symptoms associated with GD1 but many patients continue to report unresolved pain and fatigue. It is generally believed that these chronic complaints may reflect unabated inflammation associated with release of pro-inflammatory cytokines and other mediators either directly from Gaucher macrophages or indirectly by crosstalk between Gaucher cells and immunomodulatory lymphocytes [12,24,25]. However, it has also been hypothesized that accumulation of toxic glycosphingolipids within cells leads to the production of reactive oxygen species (ROS) and an imbalance between the pro-oxidants and the antioxidant reserve, resulting in oxidative stress and inflammation [19].

The major antioxidant enzymes responsible for ROS-scavenging pathways are catalase, superoxide dismutase (SOD) and glutathione peroxidase (GPx) [15,33]. In the non-enzymatic antioxidant defense system, glutathione (GSH) is an efficient, endogenous, low molecular weight thiol antioxidant that, when not bound to proteins, can react directly with ROS. It exists as GSH (an active, reduced form) and GSSG (the inactive, oxidized form). The ratio of GSH:GSSG is an established indicator of intracellular redox environment, with a low ratio of GSH:GSSG being indicative of oxidative stress [28,30]. GSH has several important functions within biological systems including its role in the rearrangement of protein disulfide bonds and control of intracellular thiol redox state [4]. In addition, ROS are highly reactive and the resultant modifications of lipids by peroxidation and proteins via oxidation have been linked with the pathogenesis of several diseases in humans [11,14].

Although oxidative stress is implicated in GD1 pathophysiology, the extent of oxidative damage and changes in enzymatic and non-enzymatic antioxidant defenses in patients undergoing therapy is not well understood. The occurrence of endoplasmic reticulum stress associated with the unfolded protein response, triggered by misfolding of mutated GCase proteins, was demonstrated in GD patient-derived fibroblasts [18,34]. Inhibition of GCase activity also led to mitochondrial dysfunction and free radical damage in a human dopaminergic cell line [6]. There is also emerging evidence of systemic oxidative stress in patients with GD1 [19,29]. We designed a study to systematically evaluate oxidative stress biomarkers in participants with GD1 at three independent time points. We included participants with GD1 who are currently stable on therapy, participants who are untreated for GD, and healthy controls. By analyzing the biomarker levels between these three groups, we can gain further insights into the role of oxidative stress in GD1 pathophysiology and the potential benefits of using antioxidants as adjunctive therapies.

2. Methods

The study protocols were approved by the Human Research Protection Programs at the University of Minnesota (UMN) and New York University (NYU) and was listed on ClinicalTrials.gov: NCT02437396 and NCT02583672. All participants gave written informed consent before enrollment.

2.1. Study participants

Eighteen participants with genetically and/or metabolically confirmed GD1 were recruited from the UMN, NYU and nationally with the assistance from National Gaucher Foundation and study investigators. Age- and gender-matched healthy controls were recruited using the UMN Study Finder website (https://studyfinder.umn.edu/). All participants were 18 years of age or older, living in the United States, and were enrolled between 2015 and 2018. Age, ethnicity, sex, and routinely used medications were recorded for all participants on enrollment. When available for individuals with GD1, information related to *GBA1* mutation and GD diagnostic biomarkers - chitotriosidase (CHITO), angiotensin converting enzyme (ACE) or tartrate resistant acid phosphatase (TRAP) were recorded from past medical records. Participants with GD1 who are treated were required to be on a specific enzyme replacement therapy (ERT) or substrate reduction therapy (SRT) regimen for at least 2 years prior to enrollment to ensure disease stability at enrollment. If, during that 2-year period, they had a change in therapy, they were enrolled only after at least 6 months elapsed since the change. All participants were current non-smokers without any concurrent medical conditions that might interfere with study completion or integrity of the data collected.

2.2. Blood sample collection and processing

Peripheral venous blood samples were collected from all participants at 3 different time points over a three-month period and processed to separate plasma and red blood cells (RBC). These were aliquoted, snap frozen and stored in a −80 °C freezer until analysis. For antioxidant enzyme analysis, the RBC were hemolyzed in four times its volume of ice-cold HPLC-grade water and centrifuged at 10,000 g for 15 min at 4 °C, and the supernatant of the lysates were collected and stored frozen.

2.3. Assessing antioxidant activity and modifications in lipids and proteins

The blood samples were tested for the following biomarkers: 1) intracellular glutathione (GSH) status measured as total GSH and redox ratio of reduced/oxidized glutathione (GSH/GSSG) in RBC; 2) activity of intracellular antioxidant enzymes-catalase, SOD and GPx in RBC; 3) plasma lipid peroxidation profile as determined by malondialdehyde (MDA) levels; 4) oxidative modification of proteins determined as protein carbonylation levels in plasma.

Catalase, SOD, GPx, MDA (measured as Thiobarbituric Acid Reactive Substances, TBARS) and protein carbonylation assays were measured using commercially available kits (Cayman Chemical, Ann Arbor, MI, USA) following manufacturer's instructions with minor modifications (e.g., sample dilution for SOD was 1:5000). All biomarkers except GSH status were analyzed colorimetrically using Synergy™ 2 multi-mode microplate reader and Gen5 microplate data collection and analysis software (BioTek Instruments Inc., Winooski, VT, USA). These values were normalized to protein concentrations determined by Bradford method. Total GSH and GSH redox status were measured using liquid chromatography–tandem mass spectrometry (LC-MS/MS) methods.

2.4. Total GSH and GSH/GSSG redox assays

The redox ratio in RBC was determined by simultaneous detection and quantitation of GSH and GSSG concentrations using LC-MS/MS and tripeptide Glycine-Proline-Glutamate (GPG) as the internal standard. GSH (concentrations ranging from 50 μg/mL to 800 μg/mL) and GSSG (6.25 μg/mL to 100 μg/mL) calibration standards were prepared at the time of the analysis in ammonium formate buffer (pH 3.5) Both calibration standards were linear with an r^2 of 0.99. Samples were processed with addition of 0.025 mL RBC lysis buffer and 3 mL of 10 mM ammonium formate buffer (pH 3.5) to 0.025 mL RBC samples. RBC samples were vortexed after addition of each buffer solution and finally filtered using 0.2 μm acrodisc filters into HPLC vial for analysis. Total GSH was determined by adding 0.1 mL of 50 mM TCEP to 0.025 mL of RBC sample after addition of GPG internal standard and 0.025 mL RBC lysis buffer. Finally, the blood sample was diluted with 3 mL of formate buffer, vortexed, and filtered into HPLC vials for LC-MS/MS analysis. The total GSH was quantitated using GPG as the internal standard and GSH calibration standards (concentrations ranging from 62.5 μg/mL to 1000 μg/mL) prepared in buffer. GSH calibration standard was linear with r^2 of 0.99.

RBC samples were analyzed using TSQ Quantum MAX (Thermo Scientific, MA), a triple quadrupole mass spectrometer with electrospray

ionization. The LC system consisted of Dionex Ultimate 3000 and the thiol compounds were separated using a Zorbax Eclipse (Agilent Technologies, Santa Clara, CA, USA) XDB C18 column (150 × 3.0 mm, 3.5 μm particle size) with a mobile phase consisting of 10 mM ammonium formate buffer (pH 3.5) and acetonitrile (Sigma; 98:2 v/v). The mass spectrometer is operated at a capillary voltage of 3200 V; ion transfer tube temperature of 350 °C and vaporizing temperature of 250 °C. The analytes were detected in positive mode with quantitation ions at *m/z* 309 for GSH and m/z 613 for GSSG. The flow rate was 0.2 mL/min or lower to achieve separation of GSH and GSSG, 10 μL blood injections were made into the system and the run time was 10 min.

2.5. Statistical analysis

Descriptive statistics were tabulated overall and by group, including the mean and standard deviation for continuous variables and frequency with percentage for categorical variables. Mean outcomes within each group and differences in mean outcomes between groups were estimated using generalized estimating equations with exchangeable working correlation to account for dependence between observations from the same patient. Robust variance estimation was used to calculate *p*-values and confidence intervals. Analyses were performed using R version 3.6.1 [27].

3. Results

3.1. Participant characteristics

Thirty-four participants completed the study:16 healthy volunteers and 18 participants with GD1. Of these 18 participants, 5 were treatment naïve and 13 were on stable therapy. Demographics for all participants and the clinical profile for participants with GD1 are summarized in Table 1. Mutation information was known for 16 participants, of whom nine were homozygous N370S (p.N409S), while the rest were compound heterozygotes. L444P (p.L483P) was the most common second allele. The average age of healthy volunteers was 38.8 (range 21–70) years, while that of participants with GD1 was 50.6 (range 26–75) years. Although we were able to age-match treated GD1 participants with healthy controls, the untreated participants were slightly older with a mean age of 60.0 (range 51–75) years. Among treated participants, six were on intravenous ERT and seven were on oral eliglustat (an SRT). On average, patients had been on therapy for 16 years (range 3–25 years). Most patients currently receiving eliglustat had been switched from prior ERT. Details on complete blood counts and GD1 diagnostic biomarkers (plasma CHITO, serum ACE and/or serum TRAP) were recorded when available. As expected, CHITO activity was markedly increased in untreated patients relative to treated patients who nonetheless often had residual increased activity compared to the normal range.

3.2. Individuals diagnosed with Gaucher disease, whether treated or not, exhibit significant systemic oxidative stress

As shown in Table 2, oxidative stress measures in blood revealed significant and consistent differences between individuals with GD1 and healthy volunteers. Among the intracellular antioxidants, participants with GD1 showed altered GSH status and lower catalase enzyme activity, indicating redox imbalance. MDA, a reliable marker of lipid peroxidation was elevated in participants with GD1. These observations are consistent with the occurrence of increased oxidative stress in patients with GD1.

3.3. Untreated patients with Gaucher disease display elevated oxidative stress

We observed significantly lower total GSH ($p = 0.030$; Fig. 1a), glutathione redox status ($p < 0.001$; Fig. 1b) and catalase activity ($p = 0.003$; Fig. 2a) along with elevated lipid peroxidation levels ($p < 0.001$; Fig. 3a) in this small patient cohort (Table 3). This suggests substantial underlying oxidative stress pathophysiology in these untreated but clinically mild GD1 patients. Although GPx enzyme activity showed a decreasing trend in patients, we did not observe

Table 1
Participant characteristics. Values presented are mean (SD) or N (%) as indicated.

Covariate	Control	Gaucher disease	
	(*N* = 16)	Untreated (*N* = 5)	Treated (*N* = 13)
Age (in years)	38.8 (14.9)	60.0 (9.3)	46.9 (12.0)
Female	9 (50%)	3 (60.0%)	8 (61.5%)
Caucasian	12 (75.0%)	5 (100%)	12 (92.3%)
Mutational status	NA		
N370S/N370S		4 (80%)	5 (38.5%)
N370S/L444P			5 (38.5%)
N370S/unknown			1 (7.7%)
N370S/R463C			1 (7.7%)
Unknown		1 (20%)	1 (7.7%)
Years on therapy	NA	NA	16.1 (8.3)
GD1 therapy			
ERT		NA	6 (46.1%)
SRT		NA	7 (53.8%)
Complete blood count	ND		
Hemoglobin (g/L)		13.6 (1.7)	14.4 (1.7)[2]
Hematocrit (%)		39.4 (5.6)[1]	42.3 (4.9)[2]
Neutrophil (%)		72.5 (0.7)[3]	55.2 (7.4)[6]
Lymphocyte (%)		23.3 (9.2)[2]	34.0 (6.8)[5]
Eosinophils (%)		1.5 (0.7)[3]	2.83 (3.4)[6]
WBC ($\times 10^9$/L)		5.62 (1.0)	6.02 (2.5)[3]
Platelets ($\times 10^9$/L)		122 (28.1)	189 (65.5)[3]
GD biomarkers	NA		
CHITO (nmoles/hr/mL)		3590 (4182.5)[1]	220 (224.2)[7]
ACE (IU/L)		95.6 (86.7)[2]	62.8 (35.2)[5]
TRAP (IU/L)		25.2 (11.7)[3]	7.18 (2.6)[8]

Superscripts indicate number of missing values; ND – Not done; NA – Not applicable.
ERT – enzyme replacement therapy; SRT – substrate reduction therapy; WBC – white blood cells; CHITO – chitotriosidase; ACE – angiotensin converting enzyme; TRAP – Tartrate-resistant acid phosphatase.

Table 2
Oxidative stress measures in participants with Gaucher disease compared to healthy controls. Mean outcome estimates by group with 95% confidence intervals. Estimated using generalized estimating equations with exchangeable working correlation and robust standard errors.

Outcome	Control	Gaucher disease	
		Untreated	Treated
Total GSH (μg/mL)	619.3 (553.1, 685.5)	515.5 (449.2, 581.8)	480.5 (426.1, 534.8)
GSH/GSSG ratio	27.8 (19.8, 35.7)	5.62 (4.8, 6.4)	14.81 (8.6, 21.0)
CAT activity (nmol/min/mg)	575.5 (410.4, 740.6)	321.5 (282.2, 360.8)	245.2 (205.2, 285.3)
SOD activity (U/mg)	6.03 (4.39, 7.68)	4.71 (3.68, 5.73)	6.24 (5.18, 7.29)
GPx activity (nmol/min/mg)	17.8 (15.3, 20.3)	14.1 (10.5, 17.6)	15.3 (13.0, 17.6)
MDA levels (nM/mg)	41.2 (33.8, 48.6)	89.5 (76.7, 102.3)	68.5 (55.1, 81.9)
Protein carbonyl (nmol/mg)	1.53 (0.48, 2.57)	0.43 (0.14, 0.72)	0.54 (0.43, 0.66)

GSH – glutathione; GSH/GSSG – GSH redox ratio; CAT – catalase; SOD – superoxide dismutase; GPx – glutathione peroxidase; MDA – malondialdehyde.

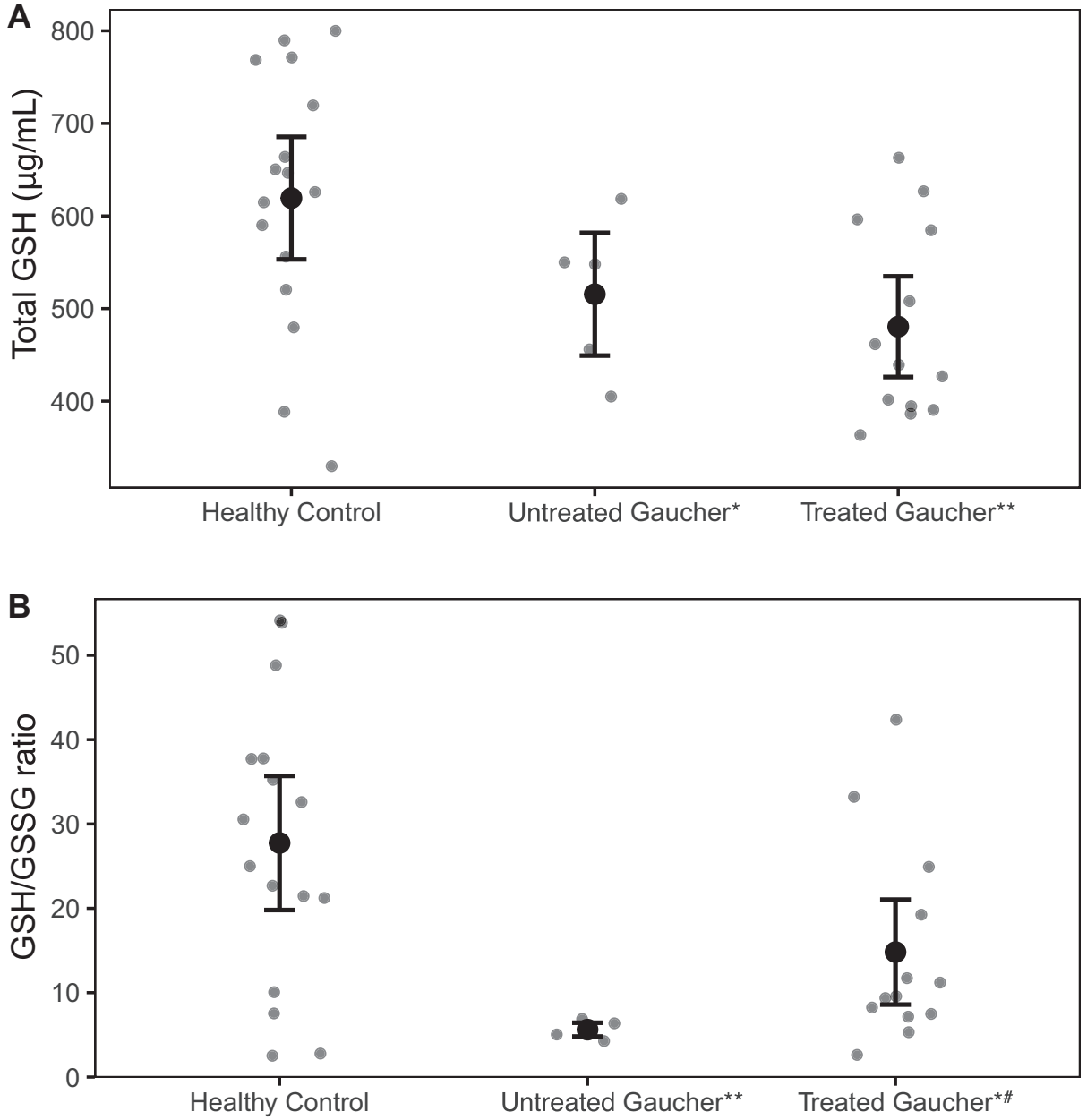

Fig. 1. Total GSH levels and GSH/GSSG redox ratio in red blood cell lysates from treated or untreated participants with GD1 and healthy controls determined using LC-MS/MS method. Values are expressed as mean (represented by the dark closed dots) and 95% confidence intervals by group. Each small dot shows the average of values obtained at three different time points from each participant. *$p < 0.05$ and ** $p \leq 0.001$ in comparison to healthy controls; $^{\#}p < 0.01$ between untreated and treated GD1.

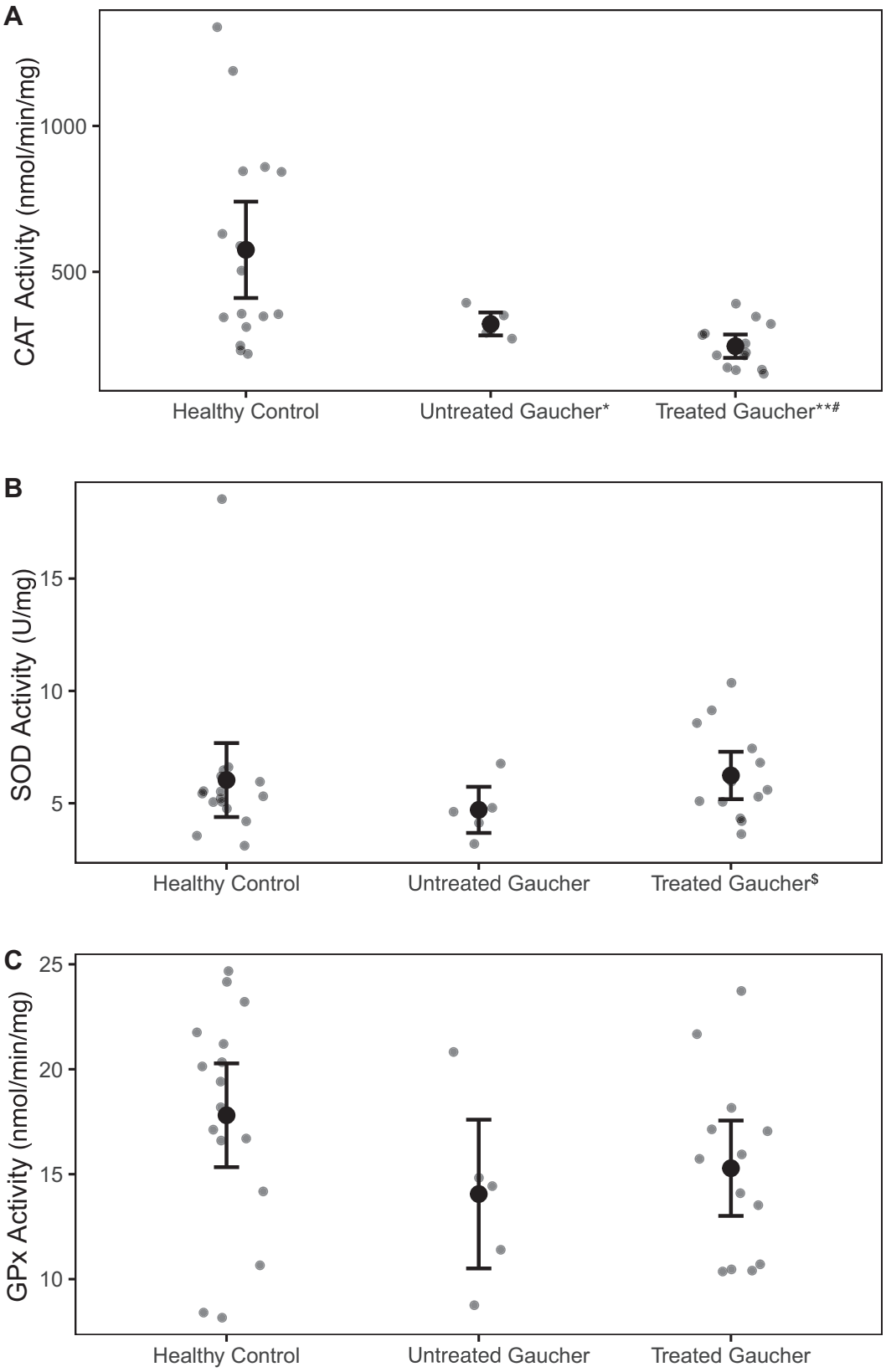

Fig. 2. Antioxidant enzymatic activity in untreated and treated GD1 and healthy controls. Catalase (CAT; panel A), superoxide dismutase (SOD; panel B) and glutathione peroxidase (GPx; panel C) activity in red blood cell lysates were determined colorimetrically. Values are expressed as mean (represented by the dark closed dots) and 95% confidence intervals by group. Each small dot represents the average of values obtained at three different time points from each participant. $^{*}p < 0.01$ and $^{**}\ p \leq 0.001$ in comparison to healthy controls; $^{\$}p < 0.05$ and $^{\#}p < 0.01$ between untreated and treated GD1.

significant alterations in SOD or protein carbonylation markers.

3.4. Current GD1 therapies are beneficial but do not normalize patient oxidative stress measures to the healthy control range

Compared to healthy controls, treated patients continued to show lower GSH ($p = 0.001$; Fig. 1a) and catalase activity ($p < 0.001$; Fig. 2a), with corresponding increase in lipid peroxidation ($p < 0.001$; Fig. 3a), suggesting current therapies do not fully resolve systemic oxidative stress (Table 3). To better understand the effect of therapy in normalizing underlying oxidative stress, we compared oxidative stress values between participants with GD1 who were stable on therapy with those who were untreated. Interestingly, treated participants had significantly higher GSH redox status ($p = 0.004$; Fig. 1b) and SOD ($p = 0.042$; Fig. 2b), suggesting improvement in intracellular antioxidants (Table 3). Similarly, treated participants showed significant reduction in lipid peroxidation ($p = 0.026$; Fig. 3a), consistent with alleviating some of the oxidative stress present. However, these values do not return to the healthy control range. Moreover, catalase activity was observed to be significantly lower in the treated group ($p = 0.008$; Fig. 2a); while the activity of GPx (Fig. 2c), total GSH levels and protein carbonylation (Fig. 3b) measures did not show significant differences between treated and untreated groups in our cohort.

4. Discussion

Lysosomal dysfunction has been identified in numerous pathological conditions. The lysosomal membrane and organelle function are highly susceptible to oxidative stress [26], a homeostatic aberration that has also been linked to GD intracellular pathophysiology [6,9]. Fibroblasts obtained from GD patients showed higher basal levels of ROS and were more susceptible to cell death than control cells [9]. Abnormal glucosylceramide accumulation in GD macrophages has been related to inflammation and

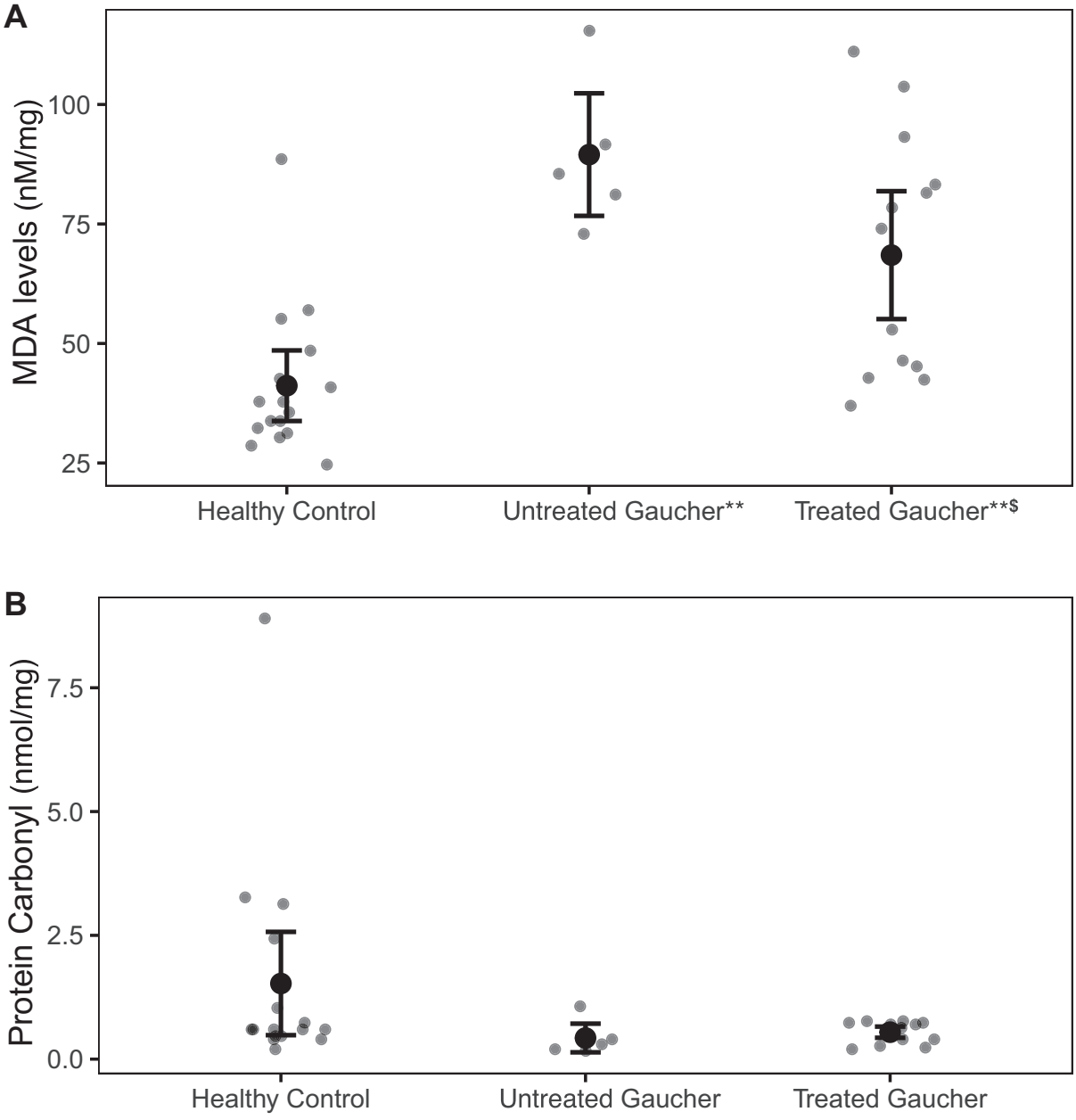

Fig. 3. Lipid peroxidation (measures as MDA-TBA adduct) and protein carbonylation in plasma from untreated and treated GD1 participants and healthy control. Values are expressed as mean (represented by the dark closed dots) and 95% confidence intervals by group. Each small dot indicates the individual mean values obtained after assessing three independent blood samples from each participant. ** $p \leq 0.001$ in comparison to healthy controls; $^{\$}p < 0.05$ between untreated and treated GD1.

Table 3
Significant differences in oxidative stress measures in participants with Gaucher disease irrespective of treatment status. Differences in mean outcomes with 95% confidence intervals and p-values are given. Estimated using generalized estimating equations with exchangeable working correlation and robust standard errors.

Comparison	Outcome	Difference (95% CI)	P-value
Untreated–control	Total GSH	−103.8 (−197.5, −10.1)	0.030
	GSH/GSSG	−22.1 (−30.1, −14.1)	< 0.001
	CAT activity	−254.0 (−423.7, −84.3)	0.003
	SOD activity	−1.33 (−3.27, 0.61)	0.180
	GPx activity	−3.8 (−8.1, 0.6)	0.089
	MDA levels	48.4 (33.6, 63.1)	< 0.001
	Protein carbonyl	−1.10 (−2.18, −0.02)	0.046
Treated–control	Total GSH	−138.9 (−224.5, −53.2)	0.001
	GSH/GSSG	−12.9 (−23.0, −2.8)	0.012
	CAT activity	−330.3 (−500.2, −160.4)	< 0.001
	SOD activity	0.20 (−1.75, 2.16)	0.838
	GPx activity	−2.5 (−5.9, 0.8)	0.141
	MDA levels	27.3 (12.0, 42.6)	< 0.001
	Protein carbonyl	−0.98 (−2.03, 0.07)	0.066
Treated–untreated	Total GSH	−35.1 (−120.8, 50.7)	0.423
	GSH/GSSG	9.2 (2.9, 15.5)	0.004
	CAT activity	−76.3 (−132.4, −20.2)	0.008
	SOD activity	1.53 (0.06, 3.00)	0.042
	GPx activity	1.2 (−3.0, 5.4)	0.567
	MDA levels	−21.0 (−39.6, −2.5)	0.026
	Protein carbonyl	0.12 (−0.19, 0.43)	0.460

GSH – glutathione; GSH/GSSH – GSH redox ratio; CAT – catalase; SOD – superoxide dismutase; GPx – glutathione peroxidase; MDA – malondialdehyde.

stress signaling pathways that might cause ROS overproduction, oxidative stress and damage [8,35]. Furthermore, oxidative stress can result from the depletion of GSH triggered by excess misfolded GCase proteins [34].

GSH is the most abundant thiol in mammalian cells and is crucial for antioxidant defense and for regulating cellular redox status. GSH depletion activates cell death pathways and is considered a marker of oxidative stress [10]. In support of the hypothesis that cellular oxidative stress is heightened in GD, our results show that both treatment naïve and ERT/SRT-treated GD1 participants exhibit significantly decreased levels of total GSH and a shift in cellular GSH/GSSG status in favor of GSSG, but more so in naïve than in treated individuals. Similar observations were reported by Moraitou et al. Patients with GD1 and GD2 showed significantly elevated MDA in erythrocytes and reduced plasma total antioxidant capacity which accounts for both enzymatic and non-enzymatic antioxidants [21]. The measurement of MDA and protein carbonyl content have been broadly used as oxidative stress parameters of lipid peroxidation and protein oxidation, respectively [1,7,32].

Few clinical studies have investigated systemic oxidative stress in patients with GD1 and results, largely based on single time point measurements, have been inconclusive. Roversi et al., examined changes in oxidative stress markers before and following ERT infusions. Ten GD patients (age 6–52 years) who were on therapy for at least 2 years had lower SOD and higher catalase activity compared to healthy volunteers [29]. However, alterations in other markers were less conclusive and untreated individuals were not evaluated. Mello et al. reported that, on average, SOD and catalase activities were higher in ten treatment naïve GD1 patients (age range 3–46 years) when compared to healthy controls [19]. However, they did not observe any differences in MDA and protein carbonyl levels. Zahran et al. reported increased plasma lipid peroxidation products and decreased concentrations of Cu and Zn as indicators of oxidative stress among 15 Egyptian GD children (mean age 6 years) compared with age-matched control subjects. Abnormalities persisted despite one year of ERT [35]. Similarly, statistically significant differences in Cu and Zn concentrations were also found in 21 treated and 12 untreated Iranian GD children (mean age 11.5 ± 8.1 years) compared with age-matched controls. However, unlike the Egyptian cohort, total antioxidant capacity (TAC) was lower in untreated than treated patients in whom TAC was statistically equivalent to the controls [22]. Despite the increase in TAC with treatment, decreased catalase activity did not normalize or increase with ERT and elevated malondialdehyde levels in the treated GD patients did not significantly decrease. Aside from the inconsistencies among the above studies, it is difficult for us to make valid comparisons with our results because our cohort is substantially older and most likely phenotypically different than patients from Brazil, Egypt and Iran [19,22,29,35]. Additionally, none of these studies report *GBA1* genotype distributions to which we can match up our study population or validate that some patients with type 3 GD were not included.

We believe that our methodology, a battery of oxidative stress markers at 3 different time points, although limited by small patient numbers, has yielded consistent results. The untreated participants with GD1 in our cohort did not take therapies for GD1 as they are mostly asymptomatic or do not have serious morbidities associated with GD. Even these clinically mild, never-treated patients with GD1 have abnormal catalase activity with corresponding increases in lipid peroxidation markers, consistent with elevated systemic oxidative stress. It is very difficult to currently find severely affected, treatment naïve GD1 patients in the United States. Therefore, we do not know whether oxidative stress markers increase proportionately with disease severity. However, it is well known that patients with GD1 manifestations that are deemed too mild to justify expensive and burdensome therapeutic intervention nevertheless have increased risk for late onset complications such as Parkinsonism and hematologic malignancies, especially plasma cell malignancies and B cell lymphomas [20]. Our results raise the possibility that a chronic uncorrected state of oxidative stress in GD1 patients might be a candidate risk factor for these late adverse outcomes.

Although long-term ERT/SRT appeared to decrease markers of oxidative stress and lipid damage, it is noteworthy that the abnormalities were not completely reversed or normalized even after many years of either ERT alone or after switching to SRT. This finding fits well with observations that GD biomarkers such as CHITO and glucosylsphingosine (lyso-GL1) rarely normalize even after years of ERT or SRT [23]. In fact, total GSH and catalase activities in treated patients were even more abnormal than in the untreated patients. On the other hand, the erythrocyte SOD activity in participants on treatment were on an average within normal range but significantly higher than those who were untreated. This imbalance between these antioxidant enzymes is further amplified when we calculate SOD to catalase ratio. Treated GD1 participants showed a 2.4-fold higher ratio than either healthy controls or untreated participants, indicating prooxidant status in this cohort. This is because the increased SOD converts more superoxides to hydrogen peroxide. However, due to decreased catalase and GPx enzymes, the peroxides are not adequately converted to water and removed [2,5]. This observation is similar to what is observed in Fabry disease (FD), where patients treated with ERT showed low concentration of GSH and GPx activity, but high SOD/catalase ratio in erythrocytes when compared to the healthy control group indicating higher susceptibility to oxidative stress in these patients [3].

Overall, our findings suggest current therapies for GD1 can attenuate, but not fully resolve chronic oxidative stress. We recently reported neurochemical abnormalities in patients with GD1 who were on stable therapy, and without neurological symptoms [17]. These abnormalities conceivably might be related to oxidative stress and inflammation in the brain. Thus, investigations of antioxidant therapies that reverse or limit the harmful effects of oxidative stress are justifiable with the goal of preventing or reversing late onset peripheral and CNS manifestations of GD1 that are inadequately addressed by current approved therapies. For example, it has been reported that treatment with Coenzyme Q10, a well know antioxidant, can reduce glucosylceramide accumulation, mitochondrial dysfunction and oxidative stress in chemically-induced GD macrophages model [8].

5. Conclusions

Our results show evidence of oxidative stress and lower antioxidant reserves in participants with GD1 whether treated or untreated. Our findings need to be confirmed in a larger number of patients, particularly those

who are untreated. Future studies should also aim to establish correlations between oxidative stress and surrogate biomarkers of Gaucher cell mass such as plasma chitotriosidase. Additionally, relationships between oxidative stress and bioactive molecules that are strongly suspected as involved in GD pathophysiology such as lyso-GL1, complement pathway ligands and receptors and other mediators or products of immune activation and systemic and CNS inflammation should be critically assessed. Our data also offer a rationale for evaluating the use of antioxidants as adjunctive therapies for patients with GD1.

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Ethics approval

The study protocol was approved by the University of Minnesota Human Research Protection Program and New York University Langone Medical Center and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Consent to participate

Informed consent was obtained from all patients for being included in the study.

Consent for publication

All authors have given consent to publish this manuscript.

Availability of data and material

All de-identified data will be made available upon request.
Code availability (software application or custom code)
Not applicable.

Declaration of Competing Interest

RVK and JCC has received grants from NIH, Sanofi-Genzyme, Pfizer Inc.; NJW has received grants from Sanofi-Genzyme and Takeda-Shire, personal fees from Sanofi-Genzyme, Takeda-Shire and Pfizer Inc., and non-financial support from Sanofi-Genzyme; MRT, RB, AT, KR and JRJ declare no conflict of interest.

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