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Profiling of Oxidative Stress in Patients with Inborn Errors of Metabolism

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Abstract

Free radical formation resulting in oxidative stress is a hallmark of mitochondrial dysfunction. Indeed, oxidative stress has been demonstrated to be an underlying pathophysiologic process in various inborn errors of metabolism. Metabolic profiling of oxidative stress may provide a non-specific measure of disease activity that may further enable physicians to monitor disease. In the present study, we investigated two markers of oxidative damage in urinary samples from IEM subjects and controls: F-2 isoprostanes, a measure of lipid peroxidation and di-tyrosine, a measure of protein oxidation. We also determined urinary antioxidant activity in these samples. Subsets of IEM patients showed significantly higher levels of the damage markers isoprostanes and di-tyrosine. Of note, patients with cobalamin disorders (*i.e.*, CblB and CblC) consistently had the highest levels of oxidative damage markers. Lower urine antioxidant capacity was seen in all subject categories, particularly cobalamin disorders and propionic acidemia. Longitudinal studies in subjects with MSUD showed good concordance between markers of oxidative damage and acute decompensation. Overall, quantifying oxidative stress offers a unique perspective to IEM. These measures may provide a means of addressing mitochondrial function in IEM and aid in the development of therapeutic targets and clinical monitoring in this diverse set of disorders.

Keywords

oxidative stress; free radicals; reactive oxygen species; reactive nitrogen species; inborn errors of metabolism; homocystinuria; maple syrup urine disease; cobalamin disorders; propionic acidemia; ornithine transcarbamylase deficiency

1. Introduction

Oxidative stress is a physiologic state that reflects exposure to free radicals that are not neutralized by conventional antioxidant defenses such as the glutathione (GSH) and superoxide dismutase (MnSOD) systems. Free radicals occur chiefly as reactive oxygen species (ROS) or reactive nitrogen species (RNS) that are derived from incomplete reduction of oxygen to H_2O in the respiratory chain. Examples include superoxide, hydrogen peroxide, and hydroxyl radicals, with the superoxide anion being the main precursor of other ROS. These molecules can damage critical cellular components, including DNA, proteins and lipid membranes. The major sites of ROS production are from complexes I and III of the mitochondrial respiratory chain[1,2]. In selected IEM, excess ROS

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generation may be due to two discrete, but not mutually exclusive, mechanisms: (1) mitochondrial dysfunction that manifests clinically as lactic acidosis 3,4, a potential source of free radicals via the Fenton reaction5,6; and (2) inhibition of respiration by intoxicants which evoke free radical formation *in vitro* and *in vivo*[7–18]. A number of pathological conditions including atherosclerosis, Alzheimer disease, and asthma, are now associated with elevated levels of oxidative stress in a variety of tissue types[19].

Oxidative stress has been proposed as an important pathogenic feature of various inborn errors of metabolism (IEM), including organic acidurias, phenylketonuria, maple syrup urine disease, tyrosinemia, urea cycle defects and homocystinuria[20–30]. Decreased activity of complexes I, II and/or III have been reported in IEM animal models, consistent with the central role of respiratory chain dysfunction in the generation of ROS8,31[–37]. The published literature on oxidative stress in IEM to date represents a collection of human and animal studies. We attempted to validate previous findings and to expand the human list of inborn errors in which oxidative stress may play a pathophysiologic role. Urinary oxidative stress profiling was performed in several of the more relatively common IEM: homocystinuria (Hcy), maple syrup urine disease (MSUD), cobalamin C disorder (CblC), propionic acidemia (PA), and ornithine transcarbamylase deficiency (OTC). In addition, a longitudinal analysis of oxidative stress parameters and biochemical markers was performed in two disorders with clear biochemical markers of acute decompensation: OTC and MSUD. To our knowledge, this is the first study to correlate longitudinal data on oxidative stress markers and disease specific biochemical and clinical parameters. In addition, we documented grossly abnormal levels of oxidative stress markers in CblC. This study serves to expand the list of human subjects with inborn errors with documented oxidative stress and provides preliminary evidence with regard to the utility of oxidative stress monitoring in these disorders.

2. Materials and methods

2.1 Sample collection and subjects

Subjects were recruited and informed consent was obtained according to the guidelines set forth by the Institutional Review Board at the Mount Sinai School of Medicine (MSSM). Patients with IEM were recruited from the Program for Inherited Metabolic Diseases at MSSM. Urine samples were collected during clinic visits. Data regarding disease type, age, and various disease specific biochemical parameters were collected. Three subjects with OTC and three subjects with MSUD were followed longitudinally for a period of ~80–200 days. Non-IEM control subjects were recruited from the Pediatric Emergency Department at MSSM. This sample collection was chosen for the diversity of the population as well as the wide range of presenting complaints including subjects who are generally healthy with non-inflammatory conditions. In addition, normal urine samples were collected from healthy volunteers. Single urine samples were collected and clinical data regarding demographics and ICD-9 coded diagnoses was recorded. Urine for all subjects was aliquoted and stored at −80°C until analysis. All urine samples were analyzed within 6 months of collection and pre-analytic stability was confirmed by repeating 25% of samples at multiple timepoints.

2.2 Urine 15-F2t-isoprostane ELISA

Isoprostanes are a byproduct of arachidonic acid metabolism and are reliable indicators of lipid peroxidation. Urine from subjects was thawed and isoprostane levels were determined using a competitive ELISA based assay (Oxford Biomed, EA85). Briefly, urine samples were mixed with dilution buffer. In this assay, the 15-F2t-isoprostane competes with 15-F2t-isoprostane conjugated to horseradish peroxidase (HRP) for binding to a polyclonal antibody coated on the microplate. When substrate is added, the concentration of 15-F2t-isoprostane

may be determined by the intensity of color development as measured at 630nm. Results were expressed as nanograms of isoprostanes per mg urine creatinine.

2.3 Urine di-tyrosine autofluoresence determination

To determine levels of protein oxidation, di-tyrosine was measured by autofluoresence. For di-Tyr fluorescence determination, 50 µl of thawed urine was added to 950 µl 6 mol/l urea in 20 mmol/l sodium phosphate buffer pH 7.4. After 30 min, the samples were read using a fluorometer (excitation 315 nm, emission 410 nm). Results were expressed as fluorescence units per mg urine creatinine.[38]

2.4 Urine antioxidant capacity assay

The urine antioxidant capacity was determined using a chemical assay (Antioxidant Assay Kit, Cayman Chemical, 709001). This assay measures the inhibition of 2,2'-azino-di-3-ethylbenzthiazoline sulfonate oxidation by metmyoglobin by urine as a surrogate for the sum of antioxidants present. The extent of inhibition is quantified relative by generating a standard curve using Trolox, a water-soluble tocopherol analogue, allowing urinary antioxidant status to be expressed as millimolar Trolox equivalents.

2.5 Urine creatinine determination

Urine creatinine determination was performed by a picric acid method (Oxford Biomed CR01). Experiments were carried out according to the manufacturer's instructions. Urinary creatinine reacts with picric acid under alkaline conditions to produce an orange color. Color change was determined by absorption spectroscopy at 492 nm wavelength.

2.6 Statistical analyses

Descriptive statistics were determined for all data. Due to small numbers, categorical data were analyzed by Fisher exact test to achieve a more precise p-value. In addition, differences in median age were determined using a Mann-Whitney U test. Differences in mean levels of individual analytes were determined using a student's test ($p<0.05$).

3. Results

3.1 Subject characteristics

Subjects with inborn errors of protein metabolism were recruited from the Program for Inherited Metabolic Disease at our center. Control subjects were collected from the Pediatric Emergency Department and volunteers at our center. The subject characteristics are shown in Table 1. A total of 41 IEM subjects and 60 control subjects were studied. Control subjects were not significantly older (IEM age 13 years, control age 15 years, p=0.11). The number of males and females (IEM: 22 males, 20 females; Controls: 27 males, 33 females; p=0.55) were also similar in each group. For IEM patients, a spectrum of relatively common inborn errors of metabolism were assayed: homocystinuria (8 subjects), maple syrup urine disease (9 subjects), cobalamin disorders (9 subjects), propionic acidemia (8 subjects) and ornithine transcarbamylase deficiency (8 subjects). For control subjects, ICD-9 codes were used to categorize samples by organ system with the exception of trauma (11 samples, 18%), which was considered a separate category. The distribution of samples for other complaints was: cardiac (n = 1, 2%) neurologic (n = 1, 2%), gastrointestinal (n = 10, 17%), gynecological (n = 5, 8%), genitourinary (n = 5, 8%), respiratory (n = 5, 8%), and psychiatric (n = 1, 2%). Infectious (n = 7, 12%) samples were also included due to the production of oxidative stress as part of the inflammatory process. The category of Normal (13 samples, 22%) constituted healthy volunteers. One subject without an ICD 9 diagnosis was also collected from the Emergency Department (n = 1, 2%). A number of inflammatory conditions for which

elevated levels of oxidative stress has been described were represented in the various categories.

3.2 Urine isoprostane levels in IEM and control subjects

Isoprostanes result from the peroxidation of arachidonic acid. These by-products have a short half-life and are eliminated primarily in the urine. In addition to ease of collection, urine offers the additional benefit that urinary isoprostanes are resistant to further oxidation. Urine was collected during clinic visits for IEM patients and during Emergency Department visits for controls. Some IEM subjects showed isoprostane levels in the normal range. However, overall mean isoprostane levels in IEM urine samples (8.5 ± 5.9 ng/mg creatinine) were about 2.5× greater than those in control samples (3.3 ± 1.7 ng/mg creatinine, $p<0.001$, Figure 1a). The highest levels of isoprostanes seen in normal controls were for conditions that were presumed to be inflammatory or infectious in nature (*e.g.*, asthma, acute gastroenteritis). These levels on average were slightly higher (4.5 ng/mg creatinine, $p<0.02$) than mean control values. Genitourinary complaints such as urinary tract infections and hematuria had a slightly lower level of urine isoprostanes (3.0 ng/mg creatinine, $p=0.77$) than controls as a whole. Considered individually, all IEM studied had elevated mean isoprostane levels compared to controls (Table 2): Hcy, 5.1 ng/mg creatinine ($p<0.02$), MSUD, 5.9 ng/mg creatinine ($p<0.0009$), CblC, 12.8 ng/mg creatinine ($p<0.0001$), PA, 8.9 ng/mg creatinine ($p<0.0001$), OTC, 9.2 ng/mg creatinine ($p<0.0001$). Twelve samples (29%) had isoprostane levels greater than 10, indicating high levels of lipid peroxidation. Half of these samples were from CblC subjects.

3.3 Urine di-tyrosine levels in IEM and control subjects

Di-tyrosine is formed by the oxidation of adjacent protein tyrosine residues leading to the formation of a highly stable inter-phenolic bond that does not undergo further metabolism. Figure 1B shows urine di-tyrosine levels for IEM and control subjects. Infectious or inflammatory conditions (3.3×10^4 versus 2.1×10^4 FU/creatinine mg, $p<0.18$) and genitourinary complaints (2.6×10^4 versus 2.4×10^4 FU/creatinine, $p<0.89$) were not significantly increased. A single control subject had elevations in di-tyrosine levels outside the normal range ($>20.0 \times 10^4$ FU/creatinine mg). This subject was seen in the ED for an asthma exacerbation. The mean di-tyrosine level in IEM samples was increased approximately four-fold (8.0×10^4 FU/mg creatinine) relative to control samples (2.0×10^4 FU/mg creatinine, $p<0.0001$). Individually, mean di-tyrosine levels for each IEM subtype were significantly elevated above mean levels for control subjects (Table 2): Hcy 6.0×10^4 FU/ mg creatinine ($p<0.0005$), MSUD 3.7×10^4 FU/ mg creatinine ($p<0.05$), CblC 17.1×10^4 FU/ mg creatinine ($p<0.0001$), PA 6.7×10^4 FU/ mg creatinine ($p<0.0001$), and OTC 4.4×10^4 FU/ mg creatinine ($p<0.01$). Overall, the highest levels of di-tyrosine were seen in CblC. A single CblC subject had a di-tyosine level that was $> 60.0 \times 10^4$ FU/creatinine mg (Figure 1B).

3.4 Urine antioxidant capacity (AOx) in IEM and control subjects

The urine antioxidant capacity assay measures the ability of any fluid to prevent an oxidation reaction involving metmyoglobin and hydrogen peroxide. Results are expressed as Trolox mM equivalents. Urine antioxidant capacity is shown in Figure 1C. The range of urinary AOx for both IEM and control subjects was wide (~0–10 Trolox mM equivalents) but mean AOx levels were lower for IEM subjects (3.2 ± 2.9 Trolox mM equivalents) than for controls (5.5 ± 2.6 Trolox mM equivalents, $p<0.0001$). For individual disorders, subjects with Hcy (3.1 Trolox mM equivalents, $p<0.02$), CblC (2.1 Trolox mM equivalents, $p<0.0009$), and PA (2.2 Trolox mM equivalents, $p<0.002$) had lower mean AOx levels than controls. Controls with inflammatory or infectious diagnoses did not have lower AOx levels (5.6 versus 5.8 Trolox mM equivalents, $p<0.80$) when compared to all other diagnoses.

3.5 Longitudinal study of oxidative stress and biochemical markers in IEM subjects

Oxidative stress markers and biochemical parameters commonly used for clinical monitoring, plasma ammonia and glutamine levels, were studied longitudinally in three subjects with the most common urea cycle disorder, ornithine transcarbamylase deficiency (OTC). Urine was collected for ~120–200 days during clinic visits. The results of this longitudinal study are presented in Figure 2. Overall, the fluctuations observed in the three oxidative stress markers were non-random. Good concordance of lipid peroxidation (isoprostane, blue), protein oxidation (di-tyrosine, red) and antioxidant capacity (middle panel, green) markers was seen (top panel). Of interest, standard biochemical markers for monitoring urea cycle disorders, with the exception of a decompensation requiring hospitalization (bottom right), plasma ammonia (purple) and glutamine (orange) showed poor concordance.

A similar analysis was performed for three subjects with maple syrup urine disease (MSUD) using leucine (bottom row, purple) as a biochemical marker of metabolic decompensation. For subjects who were generally in good metabolic control (leucine < 500 uM; Fig. 3, middle and right rows), both isoprotanes (blue) and di-tyrosine (red) showed good concordance with changes in leucine levels over time. Less concordance was seen with antioxidant markers (middle, green). Di-tyrosine levels were elevated during episodes of hyperleucinemia or, in one case, three days prior to its onset (Fig. 3, right column). Of note, isoprostane and di-tyrosine peaks showing concordance with metabolic decompensation were above the normal control range. The relationship with antioxidant activity was less evident (data not shown).

3.6 F-2 isoprostanes and glycine in disorders of proprionate and methylmalonate metabolism

Propionic acidemia (PA) and methylmalonic aciduria (MMA) are two related organic acidemias in series along the same metabolic pathway. In these disorders, glycine is used as a surrogate marker of metabolic control due to the inhibitory effects on the glycine cleavage system in PA and MMA. Pooled PA and MMA samples (n=33) were stratified by clinical status using plasma glycine levels as surrogate for metabolic control (Figure 4). For subjects in poor metabolic control (n=13, glycine >800 μM), the mean isoprostane level was elevated almost two-fold above that of subjects in good metabolic control (p=0.03).

4. Discussion

The pathophysiology of many IEM involves three inter-related and often overlapping factors: 1) toxicity secondary to the accumulation of a noxious metabolite; 2) deficiency of an essential metabolic intermediate; and 3) impaired mitochondrial function. Oxidative stress often results from mitochondrial dysfunction and has been proposed as a prime cause of the cellular injury that occurs in many IEM[39]. In this study, we quantified urinary markers of oxidative stress in various IEM to test their potential utility as clinically relevant biomarkers. A variety of approaches (*i.e.*, TBARS, malondialdehyde, glutathione levels, di-tyrosine, isoprostanes) have been employed to measure oxidative stress i*n vivo*. In a multi-investigator NIH sponsored study, the Biomarkers of Oxidative Stress Study (BOSS), F2-isoprostanes were proposed to be the best available biomarkers of oxidative stress *in vivo*[40]. These highly stable compounds are formed by the non-enzymatic peroxidation of arachidonic acid to the reduced F2-isoprostanes. Unlike plasma, urinary F2-isoprostanes do not undergo further oxidation, making this marker a stable indicator of whole body oxidative stress41. Although individual studies of have reported fold differences between plasma and tissue F2-isoprostanes (*e.g.*, plasma and liver42), studies correlating plasma, urine and tissue levels are lacking. Despite the utility of this marker, we chose to analyze two additional

analytes to obtain a fuller assessment of overall oxidative/reductive potency in our sample set including a measure of protein peroxidation and urinary antioxidant capacity[43]. The incorporation of both pro- and antioxidant factors is in keeping with Helmut Sies original concept of oxidative stress[44].

Taken as a whole, elevated markers of oxidative damage and depleted antioxidant defenses were seen in most of the IEM diagnostic groups we assessed (Figure 1). In MSUD, accumulating keto-acids have been shown to induce oxidative stress[8,29,30,37,45,46] and inhibit energy metabolism[8] in various models. Previous studies on MSUD patients under treatment found elevated levels of lipid peroxidation and decreased antioxidant capacity[27,28]. Subjects with MSUD described in this study had elevated markers of lipid peroxidation (Figure 1A). The mean antioxidant capacity was lower than that of controls (Figure 1C) but this difference was not statistically significant. The discrepancies in our findings may reflect the overall clinical status of the subjects analyzed in each population, as the variation we observed over time was most pronounced in clinically non-compliant patients. Alternatively, variation across the different study populations in dietary intake of antioxidants might account for the observed difference.

The highest individual levels of oxidative damage were seen in subjects with CblC disease, PA and OTC. Decreased antioxidant capacity was also marked in propionic acidemia and CblC. Oxidative stress in disorders of propionate metabolism[15,47–49] and hyperammonemia[25,50–54] has been studied for the most part in cell culture and animal disease models. To date, quantification of oxidative stress in CblC disorder has not been reported. This is particularly interesting with regard to the neurologic phenotype seen in CblC patients[55]. Despite good biochemical control of homocysteine and methylmalonic acid levels, a subset of patients experience neurological deterioration. Oxidative stress has been implicated in the pathophysiology and progression of neurologic disorders such as Alzheimer disease, Parkinson disease and amyotrophic lateral sclerosis[56]. Further studies on oxidative stress in CblC may provide insight into the neurologic phenotype seen particularly with regard to the role of oxidative stress in retinal degeneration in CblC[57].

The samples used for these analyses were collected without respect to clinical state and most were collected during routine clinic visits. These data show that for certain IEM, elevated levels of oxidative stress were sometimes present under conditions of good biochemical control based on the analytes currently used to monitor treatment efficacy. Similar findings have been described for MSUD, where elevated levels of lipid peroxidation and decreased antioxidant capacity were found in patients with good biochemical control[27]. With regard to the strength of correlations between oxidative stress and biochemical markers, the same study of MSUD found no correlation between markers of lipid peroxidation, antioxidant status and branched chain amino acids[27]. However, the analysis was limited to a single time-point and did not assess markers of protein oxidation. In our longitudinal analyses, patients in good metabolic control showed concordance between leucine and oxidative damage markers isoprostanes and di-tyrosine (Figure 3) during acute decompensations. We note that in well-controlled MSUD patients (Fig. 3, middle and right columns), elevations of plasma leucine were temporally associated with elevations in di-tyrosine, raising the possibility that this marker might have predictive value. Similarly, in disorders of proprionate and methylmalonate metabolism, samples collected from subjects with plasma glycine levels >800 μM had higher levels of isoprostanes than samples from subjects with lower glycine levels (Figure 4). The concordance between both measures may reflect the concurrent inhibition of the glycine cleavage pathway as well as respiratory chain function by elevated organic acid levels. Indeed propionic and methylmalonic acids have been shown to inhibit respiratory chain activity leading to oxidative stress *in vitro* and *in vivo*[7,15,49].

The concept of lowering oxidative stress to promote health and reduce the burden of disease is one with a long history but proof of efficacy requires further investigation. Several studies have suggested a role for antioxidant therapy in different types of IEM [18,27,30,47,51,58–64]. To date, the main category of disease where antioxidant therapy is regularly employed is mitochondrial disease and multiple acyl Co-A dehydrogenase deficiency. However, the efficacy of these interventions remains unproven[65]. Attempts at lowering oxidative stress and preventing deleterious outcomes using tocopherol, ascorbate, melatonin and glutathione have been successful in a variety of animal models including organic acidemias[39], PKU[60], tyrosinemia type I[64] and MSUD[37]. Addressing respiratory chain dysfunction specifically with newer mitochondrial-targeted antioxidants[66] may enhance the effectiveness of this approach.

4.1 Conclusions

Management of many IEM is complicated by the fact that the currently available biomarkers may not reliably reflect the patient's current clinical status or disease progression and have limited prognostic value. Quantifying oxidative stress may provide a means of addressing mitochondrial function in IEM and aid in the development of therapeutic targets and clinical monitoring in this diverse set of disorders.

References

1. Han D, et al. Mitochondrial respiratory chain-dependent generation of superoxide anion and its release into the intermembrane space. Topology of superoxide production from different sites in the mitochondrial electron transport chain. Biochem J 2001;353:411–416. [PubMed: 11139407]
2. St-Pierre J, Buckingham JA, Roebuck SJ, Brand MD. Topology of superoxide production from different sites in the mitochondrial electron transport chain. J Biol Chem 2002;277:44784–44790. [PubMed: 12237311]
3. Scriver, CR. The Online Metabolic and Molecular Bases of Inherited Disease. 8th edn. Scriver, CR., editor. Vol. Vol. 2006. McGraw-Hill Professional; 2006.
4. Das AM. Regulation of the mitochondrial ATP-synthase in health and disease. Mol Genet Metab 2003;79:71–82. [PubMed: 12809636]
5. Ali MA, Konishi T. Enhancement of hydroxyl radical generation in the Fenton reaction by alpha-hydroxy acid. Biochem Mol Biol Int 1998;46:137–145. [PubMed: 9784848]
6. Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. Drugs Aging 2001;18:685–716. [PubMed: 11599635]
7. Brusque AM, et al. Inhibition of the mitochondrial respiratory chain complex activities in rat cerebral cortex by methylmalonic acid. Neurochem Int 2002;40:593–601. [PubMed: 11900854]
8. Sgaravatti AM, et al. Inhibition of brain energy metabolism by the alpha-keto acids accumulating in maple syrup urine disease. Biochim Biophys Acta 2003;1639:232–238. [PubMed: 14636955]
9. Silva AR, et al. Inhibition of rat brain lipid synthesis in vitro by 4-hydroxybutyric acid. Metab Brain Dis 1999;14:157–164. [PubMed: 10646691]
10. Silva CG, et al. Inhibition of energy production in vitro by glutaric acid in cerebral cortex of young rats. Metab Brain Dis 2000;15:123–131. [PubMed: 11092579]
11. Ullrich K, et al. Glutaric aciduria type I: pathomechanisms of neurodegeneration. J Inherit Metab Dis 1999;22:392–403. [PubMed: 10407776]
12. Wajner M, Coelho JC. Neurological dysfunction in methylmalonic acidaemia is probably related to the inhibitory effect of methylmalonate on brain energy production. J Inherit Metab Dis 1997;20:761–768. [PubMed: 9427143]
13. Fighera MR, et al. Ascorbic acid and alpha-tocopherol attenuate methylmalonic acid-induced convulsions. Neuroreport 1999;10:2039–2043. [PubMed: 10424671]
14. Fighera MR, et al. GM1 ganglioside attenuates convulsions and thiobarbituric acid reactive substances production induced by the intrastriatal injection of methylmalonic acid. Int J Biochem Cell Biol 2003;35:465–473. [PubMed: 12565708]

15. Fontella FU, et al. Propionic and L-methylmalonic acids induce oxidative stress in brain of young rats. Neuroreport 2000;11:541–544. [PubMed: 10718311]
16. Latini A, et al. 3-Hydroxyglutaric acid induces oxidative stress and decreases the antioxidant defenses in cerebral cortex of young rats. Brain Res 2002;956:367–373. [PubMed: 12445707]
17. de Oliveira Marques F, et al. Glutaric acid induces oxidative stress in brain of young rats. Brain Res 2003;964:153–158. [PubMed: 12573524]
18. Pettenuzzo LF, et al. Ascorbic acid prevents water maze behavioral deficits caused by early postnatal methylmalonic acid administration in the rat. Brain Res 2003;976:234–242. [PubMed: 12763258]
19. Montuschi P, Barnes PJ, Roberts LJ 2nd. Isoprostanes: markers and mediators of oxidative stress. Faseb J 2004;18:1791–1800. [PubMed: 15576482]
20. Bird S, Miller NJ, Collins JE, Rice-Evans CA. Plasma antioxidant capacity in two cases of tyrosinaemia type 1: one case treated with NTBC. J Inherit Metab Dis 1995;18:123–126. [PubMed: 7564225]
21. Ercal N, Aykin-Burns N, Gurer-Orhan H, McDonald JD. Oxidative stress in a phenylketonuria animal model. Free Radic Biol Med 2002;32:906–911. [PubMed: 11978492]
22. Kienzle Hagen ME, et al. Experimental hyperphenylalaninemia provokes oxidative stress in rat brain. Biochim Biophys Acta 2002;1586:344–352. [PubMed: 11997085]
23. Streck EL, et al. Inhibition of rat brain Na+, K+-ATPase activity induced by homocysteine is probably mediated by oxidative stress. Neurochem Res 2001;26:1195–1200. [PubMed: 11874200]
24. Streck EL, et al. In vitro effect of homocysteine on some parameters of oxidative stress in rat hippocampus. Metab Brain Dis 2003;18:147–154. [PubMed: 12822833]
25. Wyse AT, et al. Nitric oxide synthase inhibition by L-NAME prevents the decrease of Na+,K+-ATPase activity in midbrain of rats subjected to arginine administration. Neurochem Res 2001;26:515–520. [PubMed: 11513478]
26. Wyse AT, et al. Inhibition of Na(+),K(+)-ATPase activity in hippocampus of rats subjected to acute administration of homocysteine is prevented by vitamins E and C treatment. Neurochem Res 2002;27:1685–1689. [PubMed: 12515323]
27. Barschak AG, et al. Oxidative stress in plasma from maple syrup urine disease patients during treatment. Metab Brain Dis 2008;23:71–80. [PubMed: 18026828]
28. Barschak AG, et al. Evidence that oxidative stress is increased in plasma from patients with maple syrup urine disease. Metab Brain Dis 2006;21:279–286. [PubMed: 17091400]
29. Bridi R, et al. Induction of oxidative stress in rat brain by the metabolites accumulating in maple syrup urine disease. Int J Dev Neurosci 2003;21:327–332. [PubMed: 12927581]
30. Bridi R, et al. alpha-keto acids accumulating in maple syrup urine disease stimulate lipid peroxidation and reduce antioxidant defences in cerebral cortex from young rats. Metab Brain Dis 2005;20:155–167. [PubMed: 15938133]
31. Barschak AG, et al. Inhibition of the electron transport chain and creatine kinase activity by ethylmalonic acid in human skeletal muscle. Metab Brain Dis 2006;21:11–19. [PubMed: 16773466]
32. Beretta S, et al. Partial mitochondrial complex I inhibition induces oxidative damage and perturbs glutamate transport in primary retinal cultures. Relevance to Leber Hereditary Optic Neuropathy (LHON). Neurobiol Dis 2006;24:308–317. [PubMed: 16959493]
33. Latini A, et al. 3-Hydroxyglutaric acid moderately impairs energy metabolism in brain of young rats. Neuroscience 2005;135:111–120. [PubMed: 16111821]
34. Okun JG, et al. Neurodegeneration in methylmalonic aciduria involves inhibition of complex II and the tricarboxylic acid cycle, and synergistically acting excitotoxicity. J Biol Chem 2002;277:14674–14680. [PubMed: 11847233]
35. Pettenuzzo LF, et al. Differential inhibitory effects of methylmalonic acid on respiratory chain complex activities in rat tissues. Int J Dev Neurosci 2006;24:45–52. [PubMed: 16324816]
36. Reis de Assis D, et al. Inhibition of energy metabolism in cerebral cortex of young rats by the medium-chain fatty acids accumulating in MCAD deficiency. Brain Res 2004;1030:141–151. [PubMed: 15567346]

37. Ribeiro CA, et al. Inhibition of brain energy metabolism by the branched-chain amino acids accumulating in maple syrup urine disease. Neurochem Res 2008;33:114–124. [PubMed: 17680360]
38. Kirschbaum B. Correlative studies of urine fluorescence and free radical indicators. Clin Nephrol 2002;58:344–349. [PubMed: 12425484]
39. Wajner M, Latini A, Wyse AT, Dutra-Filho CS. The role of oxidative damage in the neuropathology of organic acidurias: insights from animal studies. J Inherit Metab Dis 2004;27:427–448. [PubMed: 15303000]
40. Kadiiska MB, et al. Biomarkers of oxidative stress study II: are oxidation products of lipids, proteins, and DNA markers of CCl4 poisoning? Free Radic Biol Med 2005;38:698–710. [PubMed: 15721980]
41. Nourooz-Zadeh J. Key issues in F2-isoprostane analysis. Biochem Soc Trans 2008;36:1060–1065. [PubMed: 18793189]
42. Comporti M, et al. Plasma F2-isoprostanes are elevated in newborns and inversely correlated to gestational age. Free Radic Biol Med 2004;37:724–732. [PubMed: 15288129]
43. Dotan Y, Lichtenberg D, Pinchuk I. Lipid peroxidation cannot be used as a universal criterion of oxidative stress. Prog Lipid Res 2004;43:200–227. [PubMed: 15003395]
44. Sies H, Cadenas E. Oxidative stress: damage to intact cells and organs. Philos Trans R Soc Lond B Biol Sci 1985;311:617–631. [PubMed: 2869521]
45. Bridi R, et al. Evaluation of the mechanisms involved in leucine-induced oxidative damage in cerebral cortex of young rats. Free Radic Res 2005;39:71–79. [PubMed: 15875814]
46. Funchal C, et al. Morphological alterations and induction of oxidative stress in glial cells caused by the branched-chain alpha-keto acids accumulating in maple syrup urine disease. Neurochem Int 2006;49:640–650. [PubMed: 16822590]
47. Pettenuzzo LF, et al. Ascorbic acid prevents cognitive deficits caused by chronic administration of propionic acid to rats in the water maze. Pharmacol Biochem Behav 2002;73:623–629. [PubMed: 12151037]
48. Rigo FK, et al. Propionic acid induces convulsions and protein carbonylation in rats. Neurosci Lett 2006;408:151–154. [PubMed: 16997462]
49. Schwab MA, et al. Secondary mitochondrial dysfunction in propionic aciduria: a pathogenic role for endogenous mitochondrial toxins. Biochem J 2006;398:107–112. [PubMed: 16686602]
50. Cagnon L, Braissant O. Hyperammonemia-induced toxicity for the developing central nervous system. Brain Res Rev 2007;56:183–187. [PubMed: 17881060]
51. Garcia MV, Lopez-Mediavilla C, Juanes de la Pena MC, Medina JM. Antioxidant defence of the neonatal rat brain against acute hyperammonemia. Brain Res 2004;1001:159–163. [PubMed: 14972665]
52. Prestes CC, et al. Citrulline and ammonia accumulating in citrullinemia reduces antioxidant capacity of rat brain in vitro. Metab Brain Dis 2006;21:63–74. [PubMed: 16773471]
53. Singh S, Koiri RK, Trigun SK. Acute and chronic hyperammonemia modulate antioxidant enzymes differently in cerebral cortex and cerebellum. Neurochem Res 2008;33:103–113. [PubMed: 17676388]
54. Widmer R, Kaiser B, Engels M, Jung T, Grune T. Hyperammonemia causes protein oxidation and enhanced proteasomal activity in response to mitochondria-mediated oxidative stress in rat primary astrocytes. Arch Biochem Biophys 2007;464:1–11. [PubMed: 17475207]
55. Andersson HC, Marble M, Shapira E. Long-term outcome in treated combined methylmalonic acidemia and homocystinemia. Genet Med 1999;1:146–150. [PubMed: 11258350]
56. Barnham KJ, Masters CL, Bush AI. Neurodegenerative diseases and oxidative stress. Nat Rev Drug Discov 2004;3:205–214. [PubMed: 15031734]
57. Siu AW, Maldonado M, Sanchez-Hidalgo M, Tan DX, Reiter RJ. Protective effects of melatonin in experimental free radical-related ocular diseases. J Pineal Res 2006;40:101–109. [PubMed: 16441546]
58. Artuch R, et al. A longitudinal study of antioxidant status in phenylketonuric patients. Clin Biochem 2004;37:198–203. [PubMed: 14972641]

59. Colome C, et al. Lipophilic antioxidants in patients with phenylketonuria. Am J Clin Nutr 2003;77:185–188. [PubMed: 12499340]

60. Delwing D, et al. Alpha-tocopherol and ascorbic acid administration prevents the impairment of brain energy metabolism of hyperargininemic rats. Cell Mol Neurobiol 2006;26:177–189. [PubMed: 16619133]

61. Kosenko E, et al. Superoxide production and antioxidant enzymes in ammonia intoxication in rats. Free Radic Res 1997;27:637–644. [PubMed: 9455699]

62. Martinez-Cruz F, et al. Oxidative stress induced by phenylketonuria in the rat: Prevention by melatonin, vitamin E, and vitamin C. J Neurosci Res 2002;69:550–558. [PubMed: 12210848]

63. Moyano D, et al. Tocopherol in inborn errors of intermediary metabolism. Clin Chim Acta 1997;263:147–155. [PubMed: 9246419]

64. Sierra C, et al. Antioxidant status in hyperphenylalaninemia. Clin Chim Acta 1998;276:1–9. [PubMed: 9760015]

65. Chinnery P, Majamaa K, Turnbull D, Thorburn D. Treatment for mitochondrial disorders. Cochrane Database Syst Rev. 2006 CD004426.

66. Szeto HH. Mitochondria-targeted peptide antioxidants: novel neuroprotective agents. Aaps J 2006;8:E521–E531. [PubMed: 17025271]

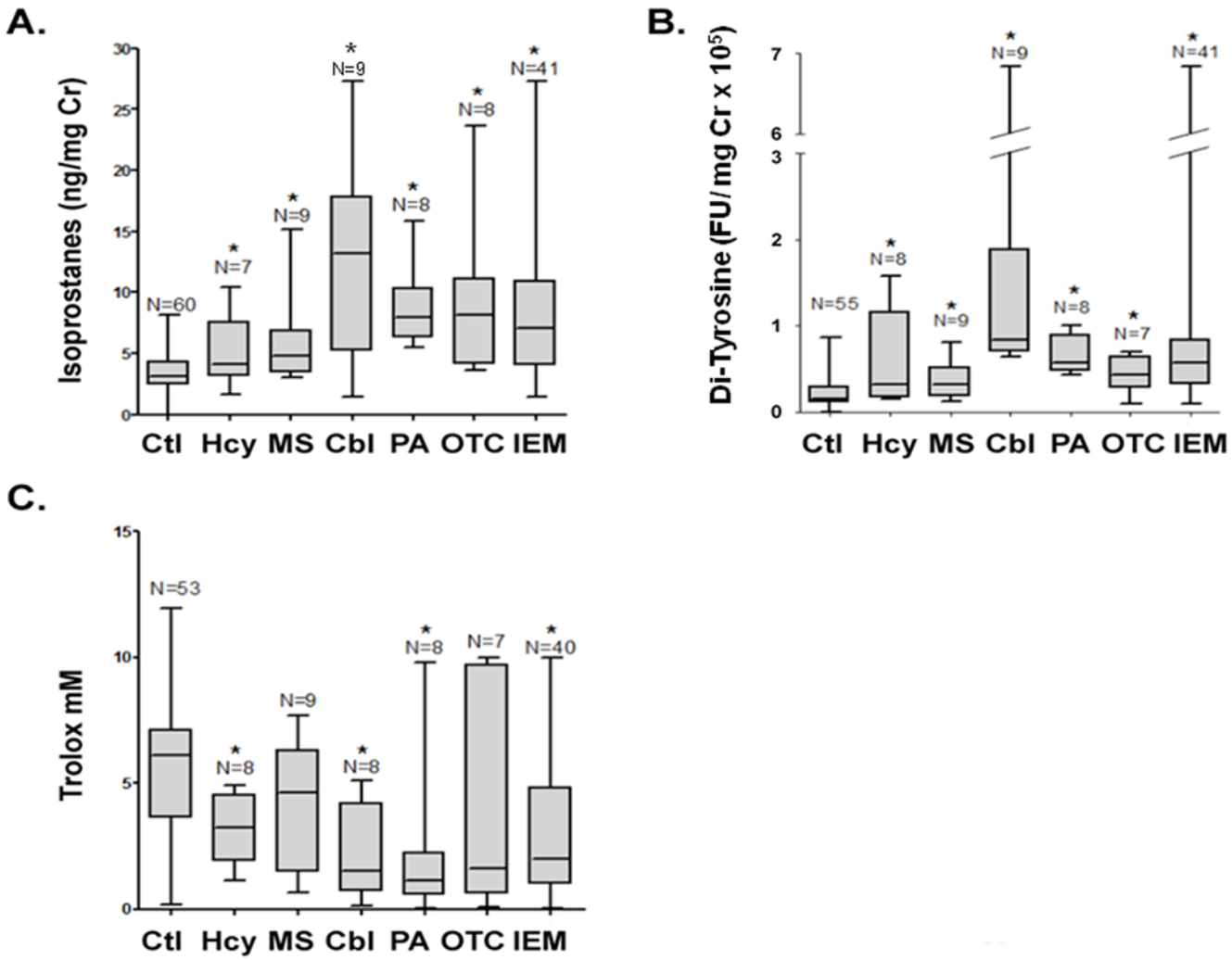

Figure 1. Oxidative Stress Markers in Inborn Errors of Metabolism

Quantification of urinary oxidative stress markers from a collection of subjects with a variety of IEM are shown. Nearly all IEM subjects were collected during well clinic visits. Analytes were A.) isoprostanes; B.) di-tyrosine levels; C.) mM Trolox equivalents of antioxidant capacity. IEM assessed are indicated below the graph: Ctl = control; Hcy = homocystinuria; MS = Maple syrup urine disease, Cbl = Cobalamin C disease; PA = propionic academia; OTC = ornithine transcarbamylase deficiency; IEM = total IEM. *$p < 0.05$, unpaired Student's t test.

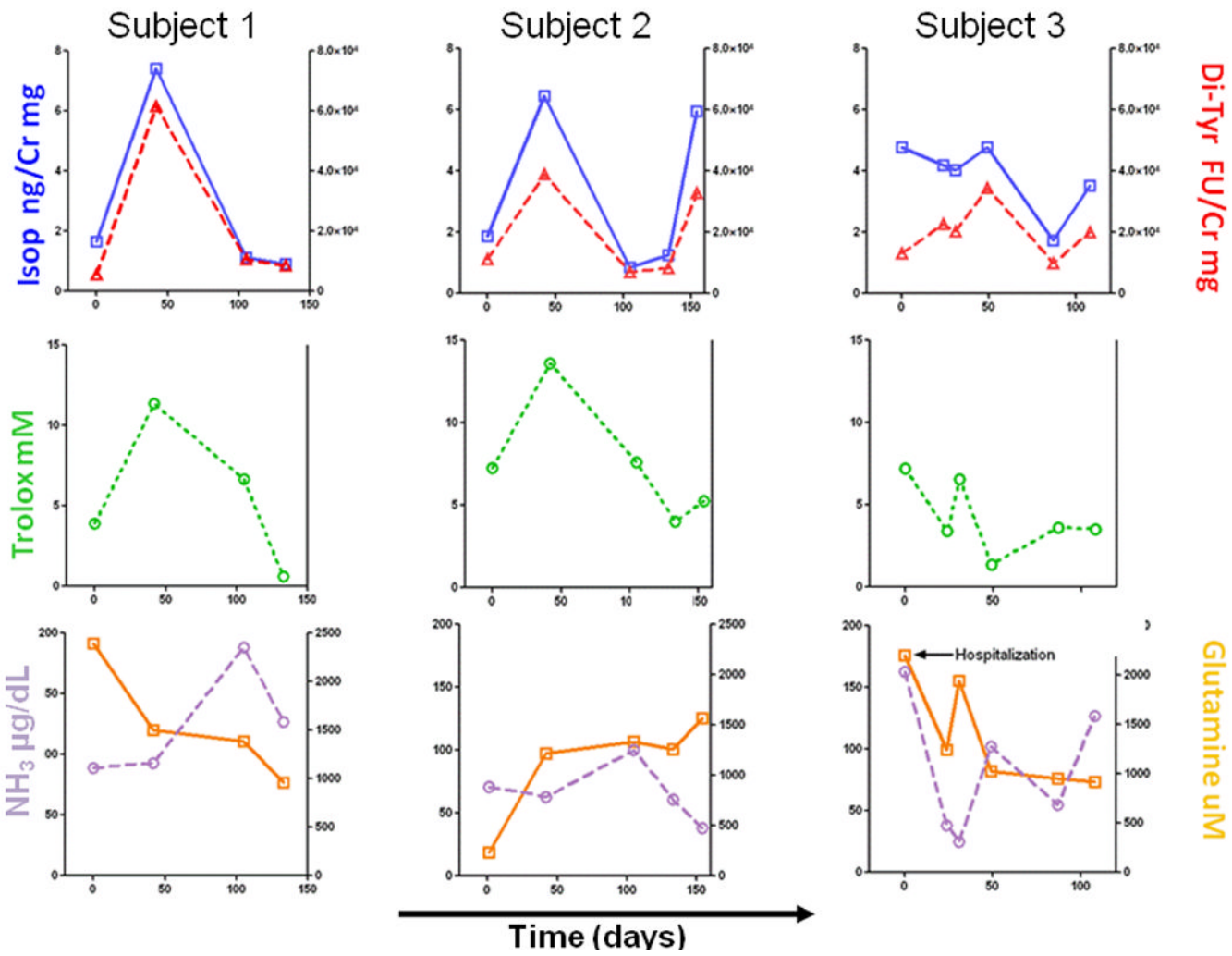

Figure 2. Correlations of ROS markers and biochemical markers of clinical status in OTC

Three OTC subjects for whom urinary ROS marker data F2-isoprostane (blue, -□-) and dityrosine (red, -Δ-) (top row), antioxidant capacity (green, -○-) and biochemical data (plasma glutamine (orange, -□-) a n d N H_3 (purple, -○-) (bottom panel) were available for direct comparison.

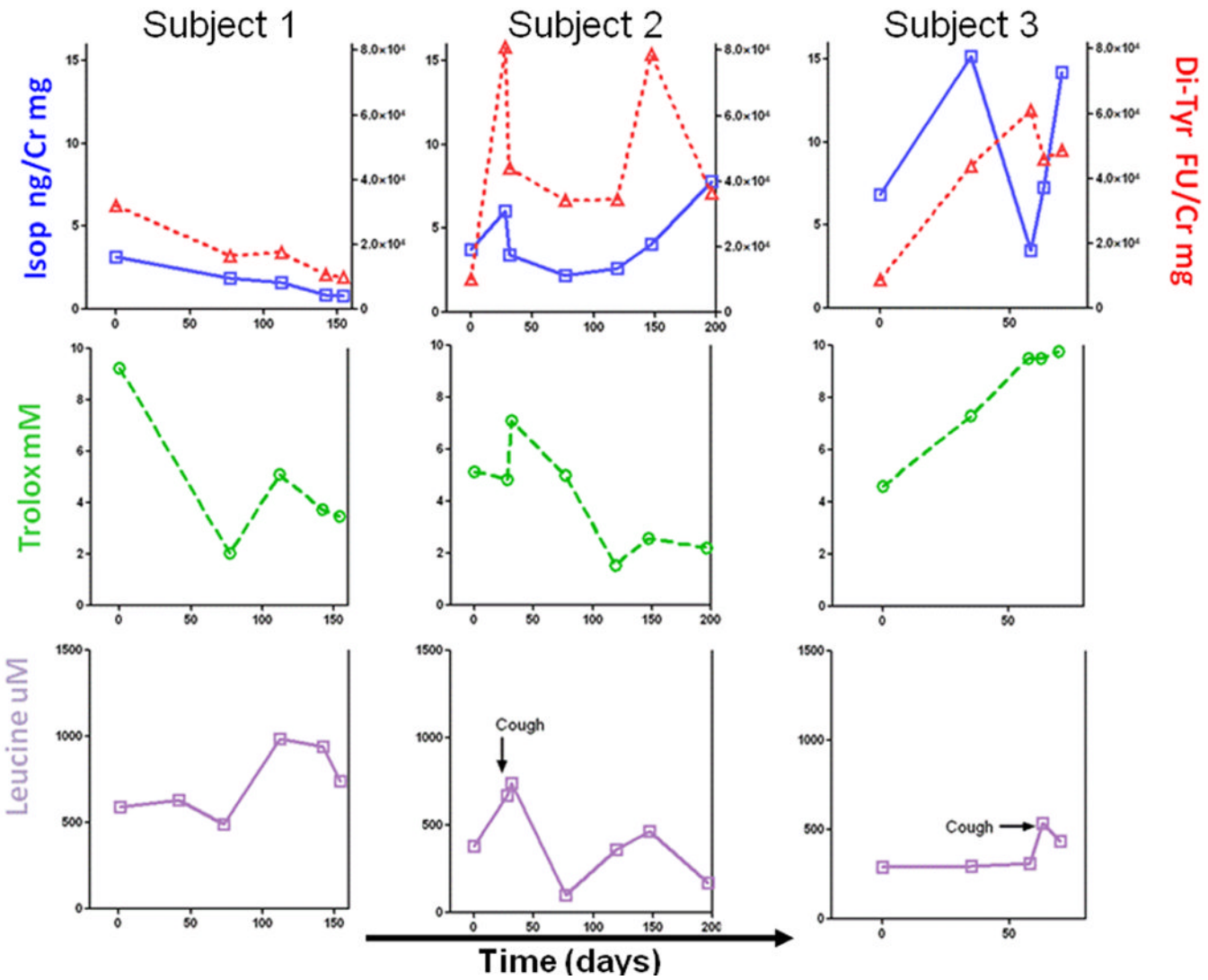

Figure 3. Correlation of ROS markers and leucine levels in MSUD

Data for F2-isoprostane (blue, -□-) and dityrosine (red, -Δ-) (top row), antioxidant capacity (green, -○-) and a standard biochemical analyte, plasma leucine (purple, -□-) (bottom row). Samples were collected during routine visits except where indicated by arrow.

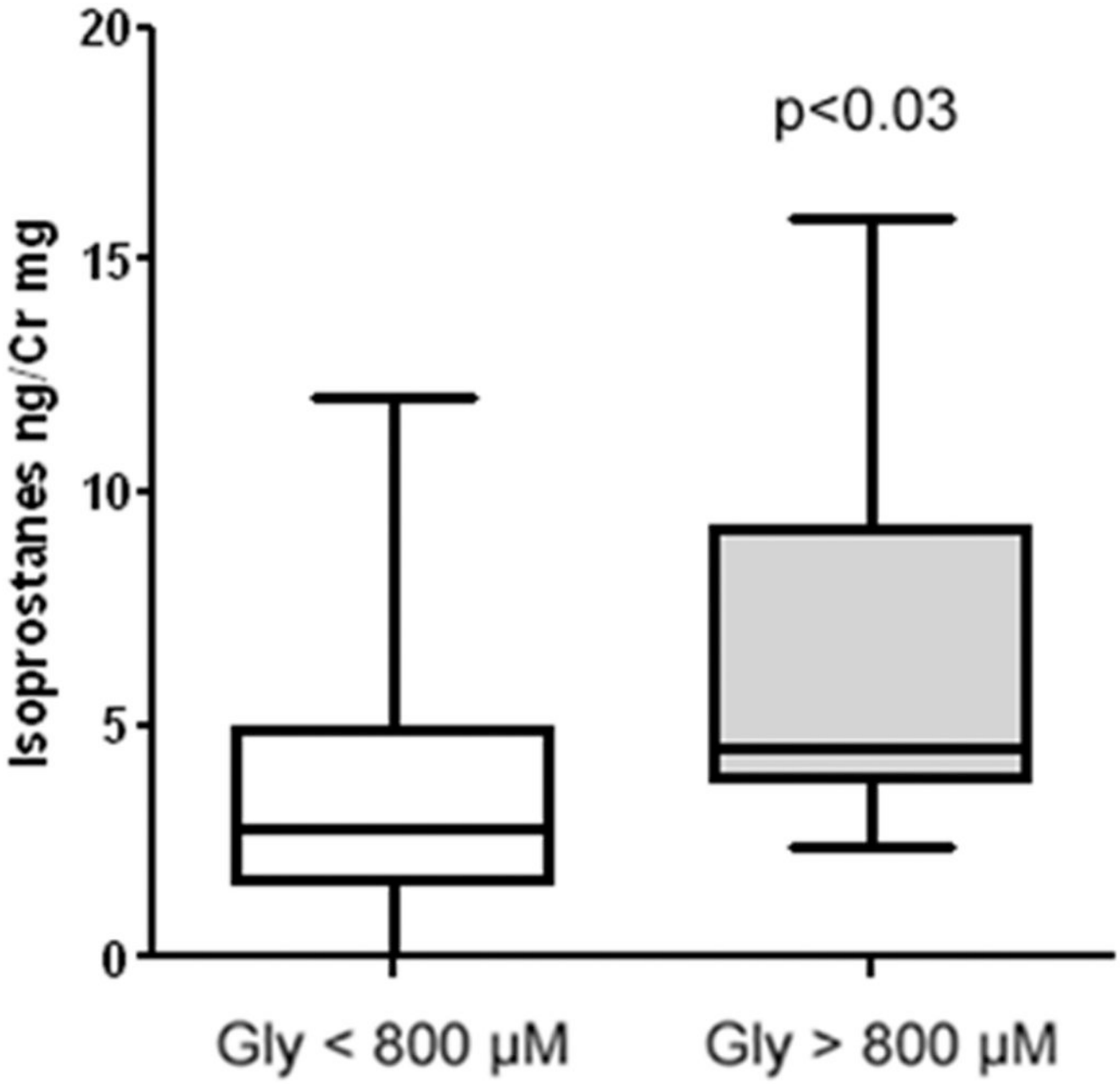

Figure 4. F2-isoprostane levels in the organic acidurias methylmalonic (MMA) and propionic acidemia (PA) as a function of metabolic control

Urine samples were collected during well visits for MMA and PA subjects. The distribution of urinary isoprostane levels is shown for pooled samples of MMA and PA divided into bins based on concurrent plasma glycine levels (> or < 800 µM), a metabolite that reflects biochemical control in these disorders. Sample sizes were n=20 for Gly<800 µM, n=13 for Gly>800 µM. All patients were collected during clinic visits, the majority of which were well visits. Significance was determined by Student's unpaired t test.

Table 1

Demographic features of the study population

Demographics	Control (n= 60)	IEM (n=41)	Significance
Male:	27	22	
Female:	33	20	$p<0.55^{*}$
Median age:	15	13	$p<0.11^{\#}$
Diagnoses:	Trauma (n=11)	Homocystinuria (n=8)	
	Infectious (n = 7)	Maple syrup urine disease (n=9)	
	Gastrointestinal (n = 10)	Cobalamin C disorder (n=9)	
	Genitourinary (n = 5)	Propionic acidemia (n=8)	
	Gynecological (n = 5)	Ornithine Transcarbamylase deficiency (n=8)	
	Respiratory (n = 5)		
	Cardiac (n = 1)		
	Neurologic (n = 1)		
	Psychiatric (n = 1)		
	Normal (n=13)		

For details of specific control diagnoses, please see section 3.1. Significance $p<0.05$ was determined using *Fisher exact test, $^{\#}$Mann-Whitney U test.

Table 2

Summary of Urine Oxidative Stress Markers in IEM Subjects

Group	Isoprostanes (ng/mg Cr)		Di-Tyrosine (FU × 10^4/ mg Cr)		AOX (mM Trolox)	
Control	3.3 ± 0.2		2.3 ± 0.3		5.5 ± 0.4	
Hcy	5.1 ± 1.1	(p<0.02)	6.0 ± 0.2	(p<0.0005)	3.1 ± 0.5	(p<0.02)
MSUD	5.9 ± 1.3	(p<0.0009)	3.7 ± 0.7	(p<0.05)	3.9 ± 0.9	(p<0.10)
Cbl	12.8 ± 2.7	(p<0.0001)	17.1 ± 0.07	(p<0.0001)	2.1 ± 0.7	(p<0.0009)
PA	8.9 ± 1.2	(p<0.0001)	6.7 ± 0.8	(p<0.0001)	2.2 ± 1.1	(p<0.002)
OTCD	9.2 ± 2.3	(p<0.0001)	4.4 ± 0.7	(p<0.01)	4.7 ± 1.8	(p<0.050)

Hcy – homocystinuria; MS – maple syrup urine disease; Cbl –cobalamin disorders; PA – propionic acidemia; OTC – ornithine transcarbamylase deficiency. For details, please see sections 3.2 – 3.4. Significance p<0.05 was determined using Students t-test.