Classifying oxidative stress by F2-isoprostane levels across human diseases: A meta-analysis

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ABSTRACT

The notion that oxidative stress plays a role in virtually every human disease and environmental exposure has become ingrained in everyday knowledge. However, mounting evidence regarding the lack of specificity of biomarkers traditionally used as indicators of oxidative stress in human disease and exposures now necessitates re-evaluation. To prioritize these re-evaluations, published literature was comprehensively analyzed in a meta-analysis to quantitatively classify the levels of systemic oxidative damage across human disease and in response to environmental exposures.

In this meta-analysis, the F₂-isoprostane, 8-isopGF₂α, was specifically chosen as the representative marker of oxidative damage. To combine published values across measurement methods and specimens, the standardized mean differences (Hedges’ g) in 8-isopGF₂α levels between affected and control populations were calculated.

The meta-analysis resulted in a classification of oxidative damage levels as measured by 8-isopGF₂α across 50 human health outcomes and exposures from 242 distinct publications. Relatively small increases in 8-isopGF₂α levels (g < 0.8) were found in the following conditions: hypertension (g = 0.4), metabolic syndrome (g = 0.5), asthma (g = 0.4), and tobacco smoking (g = 0.7). In contrast, large increases in 8-isopGF₂α levels were observed in pathologies of the kidney, e.g., chronic renal insufficiency (g = 1.9), obstructive sleep apnoea (g = 1.1), and pre-eclampsia (g = 1.1), as well as respiratory tract disorders, e.g., cystic fibrosis (g = 2.3).

In conclusion, we have established a quantitative classification of the level of 8-isopGF₂α generation in different human pathologies and exposures based on a comprehensive meta-analysis of published data. This analysis provides knowledge on the true involvement of oxidative damage across human health outcomes as well as utilizes past research to prioritize those conditions requiring further scrutiny on the mechanisms of biomarker generation.

1. Introduction

The role of oxidative damage in human disease, non-physical stress, xenobiotic exposure, and aging has been extensively investigated in nearly 22,000 publications as of 2015. Despite this massive amount of research, the importance of increased oxidative damage to pathologies and toxicities is critically debated. This is especially true due to the increasing evidence for potential ambiguity in the specificity of biomarkers traditionally used as indicators of oxidative stress in human disease and exposures now necessitates re-evaluation. To prioritize these re-evaluations, published literature was comprehensively analyzed in a meta-analysis to quantitatively classify the levels of systemic oxidative damage across human disease and in response to environmental exposures.

However, mounting evidence shows that 8-isopGF₂α can be formed not only through oxidative stress but also simultaneously by the prostaglandin endoperoxide synthase enzymes which are induced during inflammation [1,2]. The problem with the specificity of this biomarker now necessitates additional work to determine the true mechanism by which the increases in the F₂-isoprostane levels occur. To prioritize the efforts to confirm the true mechanism of F₂-isoprostane generation for each condition, a comprehensive meta-analysis of past research is needed.

To perform the meta-analysis, quantitative information on F₂-isoprostane concentration in human specimens, specifically 8-isopGF₂α, was collected from over two hundred publications. These data were used to calculate the standardized mean difference in 8-isopGF₂α (Hedges’ g) between groups (affected and control or exposed and...
unexposed). The Hedges’ g was used to rank the evidence for involvement of oxidative stress across the diseases and exposures studied [10].

This first comprehensive and quantitative classification of the published evidence provides an unbiased review of the occurrence of oxidative damage as measured by 8-iso-PGF$_{2\alpha}$ in association with adverse health outcomes and chemical exposures. In addition, the magnitude of Hedges’ g for each condition provides a clue to the potential importance of oxidative damage and prioritizes those conditions in which the F$_2$-isoprostane generation mechanism should be re-evaluated first.

2. Materials and methods

2.1. Data collection and inclusion criteria

An electronic search for the term “biomarkers of oxidative stress” was performed in the Thomas Reuters Web of Knowledge database. This initial search was then refined by searching specifically for the F$_2$-isoprostanes and, more specifically, 8-iso-PGF$_{2\alpha}$. Results from multiple acronyms and abbreviations were combined as multiple names and abbreviations are common for these biomarkers, especially in earlier publications. The following terms were included: F$_2$-isoprostane, 8-isoprostane, 8-iso-PGF$_{2\alpha}$, 8-epi-PGF$_{2\alpha}$, 15-F$_2$t-isoprostane, iPf2a-III, and isoprostane. The selection of studies from this set for inclusion into the meta-analysis was limited to those reporting the mean and standard deviation of free or total 8-iso-PGF$_{2\alpha}$. Numeric data were gathered directly from tables or, when presented in graphs only, were inferred by digitizing the figure with Plot Digitizer for Windows by Joseph A. Huwaldt. Numeric data collected included mean, geometric mean, mean, standard deviation (SD), standard error (SE), interquartile range (IQR), 95% confidence interval, and number of participants (n). Geometric mean and mean were used without modification. The measures of variation in the mean were converted to standard deviation prior to calculation of Hedges’ g. Standard error was assumed to be SD = SE*√n. Interquartile range was assumed to be SD = IQR/1.35. The 95% confidence interval was assumed to be SD = (upper limit-lower limit)/t value. Except for serum, all biological specimens were included in the analysis. Serum is not an appropriate specimen for F$_2$-isoprostane measurement because during the clotting process 8-iso-PGF$_{2\alpha}$ is generated ex vivo by prostaglandin endoperoxide synthase [11]. No restrictions were placed on measurement methodology. Additional publications were found through the reference sections of already included publications. Studies comparing the effect of interventions or reviews were excluded. Conditions for which only a single publication could be found were also excluded from the final analysis. Ultimately, literature searches resulted in a total of 2730 unique publications on F$_2$-isoprostanes in humans (Fig. 1). The above stated criteria excluded 2455 out of the 2730 publications. In addition, 33 publications for free 8-iso-PGF$_{2\alpha}$ were excluded since there was only one report for the studied condition (e.g., amyotrophic lateral sclerosis, epilepsy, hyperthyroidism, and others). This left 209 publications reporting free 8-iso-PGF$_{2\alpha}$ levels and 33 reporting total 8-iso-PGF$_{2\alpha}$ levels to be included in the meta-analysis. The raw data from each publication can be found in Mendeley dataset doi:10.17632/g42e55594f.1.

2.2. Meta-analysis and sensitivity analyses

Extracted mean or geometric mean, standard deviation, and number of participants were used to compute the standardized mean difference (Hedges’ g) and 95% confidence intervals using R version 3.2.2 with the software package “meta” [12,13]. Studies reporting different grades or severities of disease were combined to form a single estimate per the method of Borenstein et al. [14]. The fixed-effects model was used for the meta-analysis and applied to each subgroup (i.e., disease or exposure) with inverse variance weighting of individual studies [15,16]. The DerSimonian-Laird estimator was used for each subgroup as a measure of heterogeneity [17]. Sensitivity analyses to assess the influence of the specimens were performed using the “leave-one-out” approach [18]. For this, all data in the meta-analysis was combined in a random-effects model and used as the control. Subsequently, all data with one type of specimen (e.g., plasma) were removed, the random-effects model of the remaining data was calculated, and the differences were compared to the control using a t-test. Publication bias was investigated using a funnel plot, which was subsequently tested for asymmetry using a linear regression analysis [19].

3. Results

3.1. Free 8-iso-PGF$_{2\alpha}$

Free 8-iso-PGF$_{2\alpha}$ has been the most commonly measured F$_2$-isoprostane to date. When data from all non-diseased individuals in the meta-analysis were combined, a typical concentration for free 8-iso-PGF$_{2\alpha}$ emerged (Fig. 2). Across types of specimens, urine has the highest average concentration of free 8-iso-PGF$_{2\alpha}$, 1200 ± 600 pg/mL (1.3 ± 0.8 ng/mg creatinine). On average, ~100-fold less free 8-iso-PGF$_{2\alpha}$ is detected in plasma (45.1 ± 18.4 pg/mL) and exhaled breath condensate (EBC) (30.9 ± 17.2 pg/mL). In addition to these specimens, 8-iso-PGF$_{2\alpha}$ is detected in amniotic fluid, bronchiolar alveolar lavage fluid, cerebral spinal fluid, and sputum.

The meta-analysis included a total of 50 conditions ranging from diseases, to exposure to xenobiotics, to pregnancy and exercise. A Forest plot of all the data graphically illustrates the calculated Hedges’ g for all 50 conditions (Fig. 3). All studies compared a non-affected population to an affected population of similar age. Studies of pre-eclampsia had a comparison group consisting of pregnant women without complications. To generate the ranking, we selected and

![Fig. 1. Flow diagram showing the process for inclusion and exclusion of publications into the meta-analysis. N in parentheses represents the number of publications in each step, whereas the red totals represent the sum of all individuals measured for free or total 8-iso-PGF$_{2\alpha}$.

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1 http://plotdigitizer.sourceforge.net.
ordered all conditions based on their Hedges’ g value (Fig. 4). A small effect is considered to be a Hedges’ g value smaller than 0.8 [20].

When grouped together in categories, conditions having a relatively small increase in free 8-iso-PGF$_{2\alpha}$ levels ($g < 0.8$) included: neurodevelopmental disorders ($g = 0.16 \pm 0.10$), cancer ($g = 0.35 \pm 0.15$), cardiovascular diseases ($g = 0.41 \pm 0.30$), tobacco smoking (current smoker is $g = 0.67 \pm 0.20$, former smoker is $g = 0.29 \pm 0.15$), metabolic diseases ($g = 0.68 \pm 0.30$), and autoimmune disorders ($g = 0.70 \pm 0.30$). In contrast, larger quantitative effects were observed in pregnancy ($g = 0.88 \pm 0.22$), digestive system diseases ($g = 0.99 \pm 0.22$), exposure to environmental contaminants (e.g., asbestos, occupational exposure, and silicosis; $g = 1.00 \pm 0.35$), infectious diseases ($g = 1.03 \pm 0.30$), respiratory tract disorders ($g = 1.10 \pm 0.40$), congenital diseases
and urogenital diseases (g = 1.85 ± 0.20).

### 3.2. Total 8-iso-PGF$_2\alpha$

Total 8-iso-PGF$_2\alpha$ represents an aggregate of both free 8-iso-PGF$_2\alpha$ and 8-iso-PGF$_2\alpha$ esterified to phospholipids. Esterified 8-iso-PGF$_2\alpha$ is typically measured as free after liberation by base hydrolysis or upon treatment with a phospholipase. Total 8-iso-PGF$_2\alpha$ in plasma is ~10 times the concentration relative to the free 8-iso-PGF$_2\alpha$ (Fig. 5). Fewer publications were found for total 8-iso-PGF$_2\alpha$. These involved

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| Study                              | Experimental Mean | Total Mean | Control Mean | Total Mean | Standardised mean difference | SMD | 95% CI |
|------------------------------------|-------------------|------------|--------------|------------|------------------------------|-----|--------|
| Alcoholic liver disease 1          |                   |            |              |            |                              |     |        |
| 24h Urine | D. B. Hill (1999)       | 9.60       | 1.70         | 10          | 1.0 [0.6; 1.3]             |     |        |
| Spot Urine | P. W. Pemberton (2005)   | 1.65       | 0.26         | 48          | 1.5 [1.0; 2.1]             |     |        |
| Plasma | P. W. Pemberton (2005)   | 156.50     | 18.80        | 48          | 1.6 [1.0; 2.1]             |     |        |
| Fixed effect model                  |                   |            |              |            | 1.5 [1.1; 1.9]             |     |        |
| Heterogeneity, p = 0.48             |                   |            |              |            | 1.5 [1.1; 1.9]             |     |        |
| **Test for effect in subgroup:** z = 0.06 (p < 0.01) |                   |            |              |            |                              |     |        |
| Allergic rhinitis 2                 |                   |            |              |            |                              |     |        |
| EBC | K. Tanou (2009)           | 10.50      | 0.30         | 30          | 0.4 [0.1; 0.7]             |     |        |
| EBC | T. Miksonen (2009)        | 13.60      | 0.90         | 14          | 1.4 [0.6; 2.2]             |     |        |
| Fixed effect model                  | 44               | 44          |              |            | 0.7 [0.2; 1.2]             |     |        |
| Heterogeneity, p = 0.05             |                   |            |              |            | 0.7 [0.2; 1.2]             |     |        |
| **Test for effect in subgroup:** z = 3.06 (p < 0.01) |                   |            |              |            |                              |     |        |
| Asthma 6                            |                   |            |              |            |                              |     |        |
| EBC | S. Battaglia (2005)       | 2.16       | 1.60         | 16          | 1.1 [1.6; 1.8]             |     |        |
| EBC | I. G. Filscher (2012)     | 0.61       | 0.80         | 22          | 1.5 [1.8; 2.0]             |     |        |
| EBC | H. O. Kosle (2012)        | 9.32       | 14.70        | 10          | 1.0 [1.0; 1.2]             |     |        |
| EBC | S. Radovanovic (2003)     | 21.02      | 22.00        | 10          | 0.1 [0.0; 0.6]             |     |        |
| EBC | H. O. Kosle (2012)        | 33.50      | 11.90        | 15          | 0.8 [0.3; 1.3]             |     |        |
| EBC | S. Radovanovic (2003)     | 106.10     | 88.70        | 10          | 0.8 [0.1; 1.6]             |     |        |
| Spot Urine | J. D. Peters (2011)      | 95.00      | 37.36        | 29          | 3.0 [0.8; 5.1]             |     |        |
| Plasma | D. Matsuo (2011)          | 58.00      | 46.90        | 29          | 1.3 [0.7; 1.9]             |     |        |
| EBC | D. Matsuo (2011)          | 89.90      | 47.00        | 46          | 1.5 [1.1; 1.9]             |     |        |
| EBC | D. Matsuo (2011)          | 71.00      | 24.00        | 10          | 1.7 [0.8; 2.6]             |     |        |
| EBC | D. Matsuo (2011)          | 83.50      | 37.60        | 29          | 2.7 [2.0; 3.4]             |     |        |
| Spot Urine | K. S. Sylos (2009)       | 0.30       | 4.02         | 26          | 0.0 [0.0; 0.0]             |     |        |
| Fixed effect model                  | 360              | 214         |              |            | 1.1 [0.8; 1.3]             |     |        |
| Heterogeneity, p = 0.01             |                   |            |              |            | 1.1 [0.8; 1.3]             |     |        |
| **Test for effect in subgroup:** z = 11.13 (p < 0.01) |                   |            |              |            |                              |     |        |
| Autism Disorder 7                   |                   |            |              |            |                              |     |        |
| Spot Urine | A. Ghazan (2013)         | 3.04       | 2.40         | 20          | 0.8 [0.2; 1.6]             |     |        |
| Plasma | G. A. Mustafa (2010)      | 174.50     | 70.50        | 44          | 1.1 [0.8; 1.5]             |     |        |
| Spot Urine | X. Ming (2005)           | 32.92      | 33          | 29          | 3.0 [2.2; 3.7]             |     |        |
| Plasma | A. E. Ansari (2018)       | 101.60     | 24.95        | 16          | 7.4 [5.7; 9.1]             |     |        |
| Fixed effect model                  | 127              | 109         |              |            | 1.6 [1.3; 1.9]             |     |        |
| Heterogeneity, p = 0.01             |                   |            |              |            | 1.6 [1.3; 1.9]             |     |        |
| **Test for effect in subgroup:** z = 9.88 (p < 0.01) |                   |            |              |            |                              |     |        |

For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

Fig. 3. Forest plots of all calculated standardized mean differences for free 8-iso-PGF$_2\alpha$ subdivided by condition. The standardized mean difference is Hedges' g. Fixed-effects model results are plotted in the blue diamonds. These are calculated for each subgroup with inverse variance weighting of individual studies. DerSimonian-Laird estimators are used for the heterogeneity calculation. The test for effect in subgroup statistically determines whether the effect size is greater than 0. Number in the affected and control groups represents the number of people tested. Data for this figure was extracted from references [32–265].
conditions. The meta-analysis results for total 8-iso-PGF2α are presented graphically in a Forest plot (Fig. 6). Due to the small number of conditions, it is hard to create a ranking order for categories like the one created for free 8-iso-PGF2α. However, a comparison can be made between the free and total 8-iso-PGF2α for each condition (Fig. 7). Interestingly, in some conditions total 8-iso-PGF2α showed a greater response than free 8-iso-PGF2α. These conditions included tobacco smoking (g=1.3 vs. 0.7) and coronary artery disease (g=1.1 vs. 0.3). In other conditions such as pre-eclampsia (g=1.9 vs. 1.2), the free 8-iso-PGF2α showed a greater response than the total 8-iso-PGF2α.
3.3. Sensitivity analyses

In the meta-analysis results presented above, all 8-iso-PGF\(_{2\alpha}\) results were combined and not separately analyzed based on specimen type or analytical method. There is significant controversy in the literature on the applicability of the different 8-iso-PGF\(_{2\alpha}\) measurements and whether different methods or specimens measure different things and should thus not be compared directly [21–24]. To evaluate the influence of potential bias introduced by specimen and analytical method on the outcome of the meta-analysis (Hedges’ \(g\)), a sensitivity analysis was performed. Even though there was no perfect agreement in the exact amount of 8-iso-
αPGF2α measured in each specimen (Figs. 2 and 5), no statistically significant differences in the standardized mean differences (Hedges' g) between cases and control were observed in the specimen sensitivity analysis (Supplementary material Fig. 1A). This leads us to conclude that the analyzed specimens provide comparable results with no evidence for bias and, thus, the meta-analysis does not need to be stratified by specimen. Similarly, no statistically different responses in the Hedges' g are observed when different methodologies are used (Supplementary material Fig. 1B); therefore, all results regardless of method or specimen can be compared for the purposes of this meta-analysis. Publication bias was evaluated in 5 conditions (current tobacco smoking, cancer, pre-eclampsia, etc.).
asthma, and chronic obstructive pulmonary disease) as the minimum recommended amount of independent publications is ten. This analysis found no statistically significant asymmetry in the funnel plot (p < 0.05) of all conditions except asthma and chronic obstructive pulmonary disease (Supplementary material Fig. 2). However, this asymmetry is highly dependent on a single publication in both conditions; therefore, to conclude publication bias is occurring would be an overstatement.

4. Discussion

Based on the results from this meta-analysis, there is a general
increase in the levels of both free and total 8-iso-PGF$_2\alpha$ associated with a variety of conditions and environmental exposures. The increases are the largest in pathologies involving the kidneys and lungs as well as in pregnant vs. non-pregnant and non-complicated pregnancy vs. pre-eclampsia. Interestingly, conditions long touted as having high levels of oxidative damage, such as tobacco smoking and cardiovascular disease, were near the bottom of the ranking resulting from this meta-analysis.

Out of the total 64 F$_2$-isoprostane regio- and stereoisomers, this meta-analysis focuses on a single isomer (8-iso-PGF$_2\alpha$, also known as iPF$_2\alpha$-III; 8-epi PGF$_2\alpha$; 8-isoprostane; or 15-F$_2$t-isoprostane). This isomer has been used as a proxy for a change in general F$_2$-isoprostane levels. Other isomers are occasionally measured, such as 5-iPF$_2\alpha$ or 8,12-iso-iPF$_2\alpha$, as well as metabolites of 8-iso-PGF$_2\alpha$, but there are limited publications; therefore, a comprehensive meta-analysis including these isomers or analyzing them separately cannot be performed currently.

There is significant controversy in the literature on the ability to...
compare 8-iso-PGF$_{2\alpha}$ levels measured with different methods and cleanup procedures [21–23]. It should be noted, that with proper sample cleanup, both analytical methods result in statistically indistinguishable quantities of 8-iso-PGF$_{2\alpha}$ measured in aliquots from the same sample [24]. Despite not being able to account for variations in the sample cleanup procedure due to poor method description, in this meta-analysis, we found no statistically significant differences in the quantity of free and total 8-iso-PGF$_{2\alpha}$ when comparing populations analyzed with different analytical methods (Figs. 2 and 5). The population comparison shows that, in a large collection, measurement
variability due to antibody cross-reactivity, poor sample cleanup, peak overlap, and other analytical differences does not significantly contribute to different quantities of 8-iso-PGF$_{2\alpha}$ measured. Therefore, since the sample size in this analysis is so large, we can reasonably compare results across studies and conditions. More important than exact overlap, and other analytical differences does not significantly contribute to different quantities of 8-iso-PGF$_{2\alpha}$ measured. Therefore, since the sample size in this analysis is so large, we can reasonably compare results across studies and conditions. More important than exact agreement is the fact that the effect size (Hedges’ $g$) between healthy and diseased populations is not statistically significantly changed depending on which analytical method was used (Supplementary material Fig. 1). Since each analytical method globally provides comparable results in effect size, even in the absence of exact quantitative agreement, we are justified in combining all data and need not stratify the analysis based on analytical method.

As a measure of oxidative damage, 8-iso-PGF$_{2\alpha}$ has some significant potential limitations. There is an alternate enzymatic pathway to generate 8-iso-PGF$_{2\alpha}$ catalyzed by prostaglandin endoperoxide synthase [5,25–31]. This mechanism is independent of the non-enzymatic, free radical-mediated peroxidation of arachidonic acid, which was alleged to be the only significant mechanism for 8-iso-PGF$_{2\alpha}$ formation in vivo. Therefore, even if there was an increase in 8-iso-PGF$_{2\alpha}$ concentration between cases and controls or exposed and unexposed, there is no guarantee that this is due to non-enzymatic peroxidation. A method to separate the contribution of the two pathways has been described [1] and shows that under different conditions the pathway responsible for 8-iso-PGF$_{2\alpha}$ generation is different [2]. The evidence for the role of an alternate generation pathway voids the conclusion that 8-iso-PGF$_{2\alpha}$ by itself is a biomarker of oxidative stress. This calls into question the conclusions of previous studies with this biomarker. The meta-analysis data here can serve as a priority list for reanalysis of those conditions where the largest effect has been shown in the past and to guide future research into oxidative stress.

We can further prioritize the conditions for reanalysis by looking at the difference in effect size between total and free 8-iso-PGF$_{2\alpha}$ (Fig. 7). Total 8-iso-PGF$_{2\alpha}$ is a combination of 8-iso-PGF$_{2\alpha}$ esterified to phospholipids and free 8-iso-PGF$_{2\alpha}$. Enzymatic generation of 8-iso-PGF$_{2\alpha}$ through the inflammation-induced prostaglandin-endoperoxide synthases is a confounding mechanism only for interpretation of free 8-iso-PGF$_{2\alpha}$. Total 8-iso-PGF$_{2\alpha}$ is less affected by the confounding mechanism because esterified arachidonic acid is not a substrate for prostaglandin-endoperoxide synthases. Therefore, measurement of only esterified 8-iso-PGF$_{2\alpha}$ or total 8-iso-PGF$_{2\alpha}$, which is predominantly esterified 8-iso-PGF$_{2\alpha}$ may be a more indicative marker of oxidative stress. Thus, by looking at the different responses for total and free 8-iso-PGF$_{2\alpha}$, we can infer that in conditions with a greater response of total compared to free 8-iso-PGF$_{2\alpha}$, the generation mechanism is most likely non-enzymatic oxidative damage. In the meta-analysis, this is true for two conditions, namely tobacco smoking and coronary artery disease. In the other conditions, especially in pre-eclampsia, the free 8-iso-PGF$_{2\alpha}$ gives a greater response than total, which indicates that the major source of 8-iso-PGF$_{2\alpha}$ is most likely the prostaglandin-endoperoxide synthases.

There are some limitations to the interpretation of this meta-analysis. There are several conditions, such as overweight, Raynaud’s disease, pulmonary arterial hypertension, bronchiectasis, secondary smoking, and amyotrophic lateral sclerosis, which have only two included publications describing populations with these conditions. The estimates for these conditions and others with few studies are not ideal, but hopefully, with future research, these current estimates can be confirmed. Also, certain categories, e.g., congenital diseases and infectious diseases, are comprised of only a single disease in this meta-analysis. This severely limits the broad interpretation of these categories until more conditions are included.

We present here a ranking of the conditions in which there is the most potential for lipid peroxidation to play a major role in the etiology or pathology of human diseases and exposure to environmental pollutants. The exact mechanism must now be evaluated using approaches such as the 8-iso-PGF$_{2\alpha}$/PGF$_{2\alpha}$ ratio [1,2] to evaluate the involvement of inflammation and, thus, make the best possible conclusions for future clinical study and the development of cures for those conditions.
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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.redox.2017.03.024.

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