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## Letter to the Editor

**G.B. Quan et al., Inhibition of high glucose-induced erythrocyte phosphatidylserine exposure by leupeptin and disaccharides, Cryobiology 56 (1) (2008) 53–61<sup>\*</sup>**
*To the editor*

We address some critical issues regarding the measurement of lipid peroxidation in red blood cells (RBCs) as described recently by Quan et al. [5]. In their paper, "Inhibition of high glucose-induced erythrocyte phosphatidylserine exposure by leupeptin and disaccharides", Quan et al. suggested that the exposure of RBC phosphatidylserine (PS) induced by high glucose is not correlated with oxidative stress, and that trehalose can prevent lipid peroxidation during glucose loading. These assumptions were based on the measurements of malondialdehydes (MDAs), which are commonly used as indicators of membrane lipid peroxidation.

We suspect that the method used by Quan et al. for the measurement of lipid peroxidation in RBCs suffers from inaccuracies, which may lead to wrong conclusions. Our concern is based on our experience with that assay and our recent study, which clearly shows that the process of loading trehalose into RBCs is accompanied with hemoglobin (Hb) oxidation and membrane lipid peroxidation.

The MDA assay is based on the reaction between MDAs with thiobarbituric acid (TBA), which requires a low pH and heat. The resulting chromophore is named thiobarbituric acid reactive substances (TBARS), which can be quantified spectrophotometrically at 532 nm. This method has been criticized for its lack of consistency and specificity [4], mainly due to the fact that TBA reacts with molecules (glycoproteins, hemoglobin, carbohydrates) other than MDAs.

The measurement of TBARS in RBCs is particularly problematic due to hemoglobin interference and the presence of molecular iron, which can accelerate sample oxidation. As a result, the majority of the commercially available assay kits are not suitable for RBCs. Our awareness of these problems encouraged us to validate and modify this assay for measuring TBARS in fresh and desiccated RBCs [3].

When evaluating TBARS in RBCs, the following issues should be considered:

1. Terminology: Since TBA reacts with various molecules other than MDAs, it is more accurate to use the term "TBARS" than "MDAs". Reporting MDAs is appropriate when using more specific and sophisticated techniques, such as high performance liquid chromatography (HPLC).

2. Comparison between samples: Different samples contain a different number of cells, which significantly affects the TBARS value. Therefore, an accurate comparison between samples requires the normalization of the TBARS values to a reference. In the case of RBCs, hemoglobin concentration (g/L) is commonly used in the literature and TBARS are reported as "TBARS (nmol/g Hb)". Alternatively,

TBARS values can be normalized to membrane protein content, or membrane phosphate. Quan et al. reported MDAs in nmol/ml. This manner ignores the differences in cell number of different samples and leads to errors.

3. Correction for interfering substances: Hemoglobin and other substances can significantly interfere with this assay. The interference appears as a peak at 453 nm. The magnitude of this peak can significantly interfere with the 532 nm peak leading to false positive results. A method to correct for errors generated by the 453 nm peak in RBCs was developed by Gilbert et al. [2]. This method accounts for the interference at 453 nm peak by subtracting 20% of its value from the absolute TBARS value.

4. Correction for interferences caused by carbohydrates: monosaccharides and disaccharides, such as sucrose and trehalose, also contribute to the magnitude of the 453 nm peak and intensify its interference. A modified TBARS assay for sugar-rich plant tissue extracts was developed by Du and Bramlage [1]. We propose a correction by adding the tested sugar into the MDA standard curve and the reagent blank. Ignoring this correction when subtracting 20% of the 453 nm peak (see Section 3) may lead to false negative results and the conclusion that some sugars have antioxidant effects.

The consequences of oxidative injury during the process of sugar-loading into RBCs are not well understood and are yet to be determined. Better understanding requires reliable and validated methods for measuring oxidative injuries in RBCs. We hope that our comments will contribute to the quality of the methodology used in future studies.

## References

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Tamir Kanas,  
Jason P. Acker<sup>\*</sup>

Department of Laboratory Medicine and Pathology,  
University of Alberta, Edmonton, Alta., Canada T6G 2R8  
Research and Development, Canadian Blood Services,  
Edmonton, AB, Canada  
E-mail address: [jacker@ualberta.ca](mailto:jacker@ualberta.ca) (J.P. Acker)

<sup>\*</sup> The author has declined to provide a response.

<sup>\*</sup> Corresponding author. Fax: +1 780 702 2501.