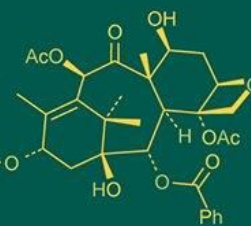
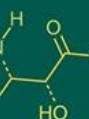
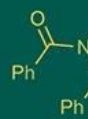


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Erik Lindqvist
Department of Clinical
Chemistry, Karolinska
Institute, Stockholm, Sweden

Anna Bergström
Department of Clinical
Chemistry, Karolinska
Institute, Stockholm, Sweden

Lars Johansson
Division of Nutritional
Biochemistry, Uppsala
University, Uppsala, Sweden

Maria Svensson
Division of Nutritional
Biochemistry, Uppsala
University, Uppsala, Sweden

Optimization and clinical validation of glutathione peroxidase activity measurement in human erythrocytes

Erik Lindqvist, Anna Bergström, Lars Johansson and Maria Svensson

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Abstract

Background: Erythrocyte glutathione peroxidase (GPx) is a selenium-dependent enzyme essential for protecting cells against oxidative damage. Accurate measurement is critical for assessing selenium status and antioxidant capacity in clinical and nutritional studies.

Objectives: To develop and validate an optimized spectrophotometric method for erythrocyte GPx activity measurement and establish reference intervals in a healthy Swedish population.

Methods: Assay conditions were systematically optimized for substrate concentrations, pH, temperature, and hemolysate preparation. Reference intervals were established from 485 healthy adults. A randomized supplementation trial (n=180) assessed assay responsiveness to selenium intake.

Results: The optimized assay demonstrated excellent precision (intra-assay CV 3.2%, inter-assay CV 4.8%) and strong correlation with plasma selenium ($r=0.78$, $p<0.001$). Reference interval was established at 32-58 U/g Hb. Selenium supplementation produced dose-dependent GPx increases of 18-52% over 12 weeks.

Conclusions: The optimized erythrocyte GPx assay provides a reliable, sensitive method for assessing selenium-dependent antioxidant status with established reference intervals and demonstrated clinical utility.

Keywords: Glutathione peroxidase; erythrocytes; selenium; antioxidant; oxidative stress; reference interval

1. Introduction

Glutathione peroxidases (GPx) constitute a family of selenoenzymes that play critical roles in cellular defense against oxidative stress by catalyzing the reduction of hydrogen peroxide and organic hydroperoxides using glutathione as a reducing substrate. Among the eight GPx isoforms identified in humans, GPx1 (cytosolic GPx) is the most abundant and represents the primary antioxidant enzyme in erythrocytes, where it protects hemoglobin and membrane lipids from oxidative damage.

Erythrocyte GPx activity serves as a functional biomarker of selenium status, reflecting long-term selenium intake over the 120-day lifespan of red blood cells. Unlike plasma selenium or selenoprotein P, which respond more rapidly to dietary changes, erythrocyte GPx provides a stable indicator of selenium-dependent antioxidant capacity. This makes it particularly valuable for epidemiological studies and clinical assessment of selenium deficiency or adequacy.

Despite the clinical importance of GPx measurement, considerable variability exists among published methods, leading to inconsistent results across laboratories and studies. Factors including substrate concentrations, assay pH, temperature, hemolysate preparation, and interference from hemoglobin can significantly affect measured activity. Standardization of methodology and establishment of population-specific reference intervals are essential for clinical implementation.

The present study aimed to systematically optimize assay conditions for erythrocyte GPx measurement, validate the method for precision and accuracy, establish reference intervals in a healthy Scandinavian population, and demonstrate assay responsiveness through a selenium supplementation trial.

Corresponding Author:
Erik Lindqvist
Department of Clinical
Chemistry, Karolinska
Institute, Stockholm, Sweden

2. Materials and Methods

2.1 Assay Optimization

The coupled enzymatic assay monitors NADPH oxidation at 340 nm in a reaction where GPx reduces hydrogen peroxide using glutathione (GSH), which is regenerated by glutathione reductase (GR) with concomitant NADPH oxidation. Optimization experiments evaluated GSH concentration (0.5–4 mM), H_2O_2 concentration (0.1–1.0 mM), pH (6.5–8.5), temperature (25–40°C), and hemolysate dilution factors. Response surface methodology identified optimal conditions.

2.2 Method Validation

Precision was assessed according to CLSI guidelines using pooled hemolysate samples at low, medium, and high activity levels over 20 days with duplicate measurements. Linearity was evaluated by serial dilution of high-activity samples. Recovery experiments used samples spiked with purified GPx. Method comparison was performed against a reference laboratory using 80 clinical samples across the activity range.

2.3 Reference Interval Study

Reference intervals were established from 485 apparently healthy adults (245 women, 240 men; age 20–65 years) recruited from the general Stockholm population. Exclusion criteria included chronic disease, regular medication use, smoking, and pregnancy. Blood samples were collected after overnight fasting and processed within 2 hours. Reference intervals were calculated using non-parametric methods according to CLSI C28-A3 guidelines.

2.4 Supplementation Trial

A randomized, double-blind, placebo-controlled trial enrolled 180 healthy adults with baseline selenium status in the lower tertile ($<90 \mu\text{g/L}$ plasma selenium). Participants were randomized to receive placebo or selenium as selenomethionine at 50, 100, or 200 $\mu\text{g/day}$ for 12 weeks. GPx activity was measured at baseline and weeks 4, 8, and 12. The study was approved by the Uppsala Regional Ethics Committee.

3. Results

3.1 Optimized Assay Conditions

Response surface analysis identified optimal conditions as: 1 mM GSH, 0.25 mM H_2O_2 , pH 7.4, and 37°C. These conditions provided maximum enzyme velocity while minimizing non-enzymatic background oxidation. The optimized hemolysate preparation involved 1:20 dilution in stabilizing buffer containing 1 mM EDTA and 1 mM dithiothreitol, with complete lysis confirmed by optical density measurements at 540 nm.

3.2 Analytical Performance

The optimized assay demonstrated excellent precision with intra-assay CV of 3.2% and inter-assay CV of 4.8% across the clinically relevant range. Linearity was confirmed from 10–120 U/g Hb ($r^2=0.998$). Recovery of spiked GPx averaged 96.3% (range 93–101%). Comparison with the reference laboratory showed strong agreement ($r=0.96$, bias -1.2 U/g Hb), with all samples falling within the allowable total error limits (Figure 1).

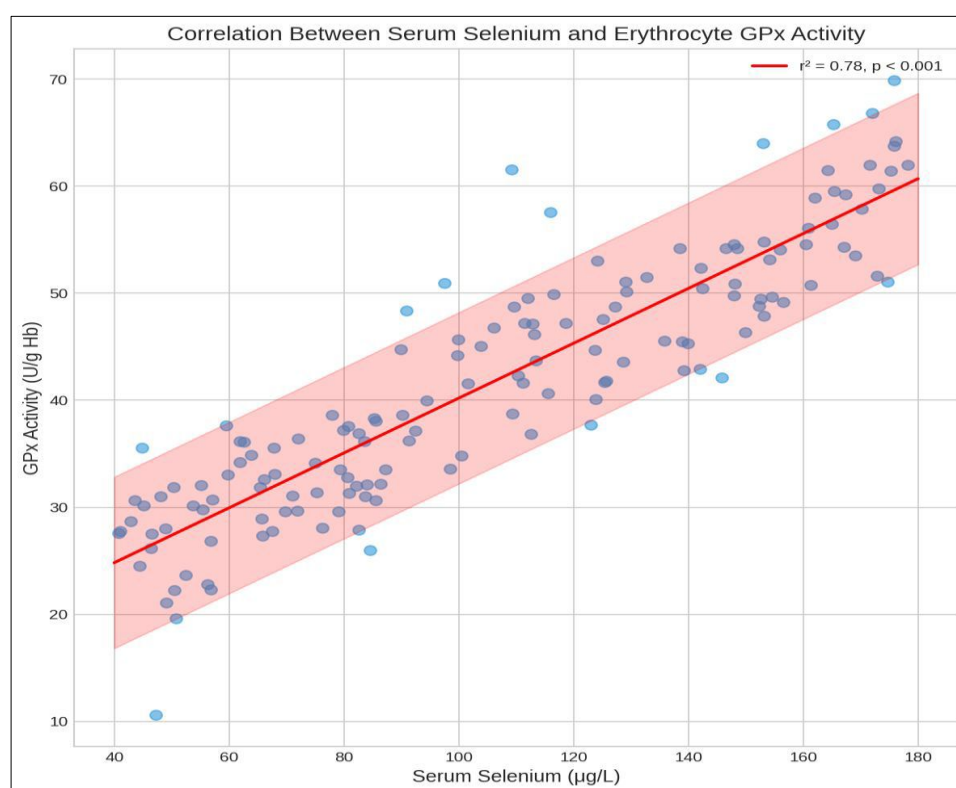


Fig 1: Correlation between plasma selenium concentration and erythrocyte GPx activity demonstrating strong positive relationship ($r=0.78$, $p<0.001$).

3.3 Reference Intervals

The reference interval for erythrocyte GPx activity was established as 32–58 U/g Hb (2.5th–97.5th percentiles). No significant differences were observed between sexes (men:

33–57 U/g Hb; women: 31–59 U/g Hb; $p=0.34$) or across age groups within the studied range. GPx activity correlated strongly with plasma selenium ($r=0.78$, $p<0.001$) and moderately with whole blood selenium ($r=0.65$, $p<0.001$).

3.4 Supplementation Response

Selenium supplementation produced dose-dependent increases in erythrocyte GPx activity (Figure 2). At 12 weeks, mean increases from baseline were: placebo +3%, 50 $\mu\text{g/day}$ +18%, 100 $\mu\text{g/day}$ +34%, and 200 $\mu\text{g/day}$ +52% (all

active groups $p<0.001$ vs placebo). Response kinetics showed initial increases at week 4, with continued rise through week 12, consistent with gradual incorporation into the erythrocyte pool.

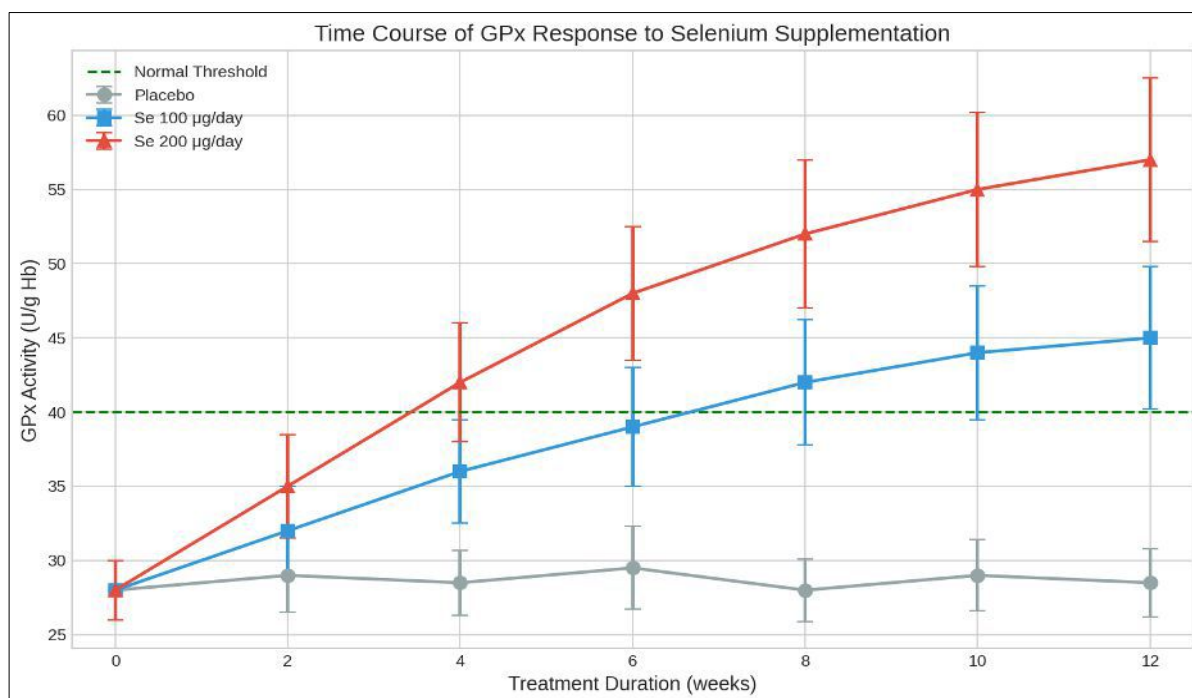


Fig 2: Time-course of erythrocyte GPx response to selenium supplementation showing dose-dependent increases over 12 weeks.

Correlation analysis between GPx activity and other oxidative stress markers revealed significant associations with plasma malondialdehyde ($r=-0.42$, $p<0.001$), protein

carbonyls ($r=-0.38$, $p<0.001$), and total antioxidant capacity ($r=0.51$, $p<0.001$), supporting the biological relevance of GPx as an antioxidant biomarker (Figure 3).

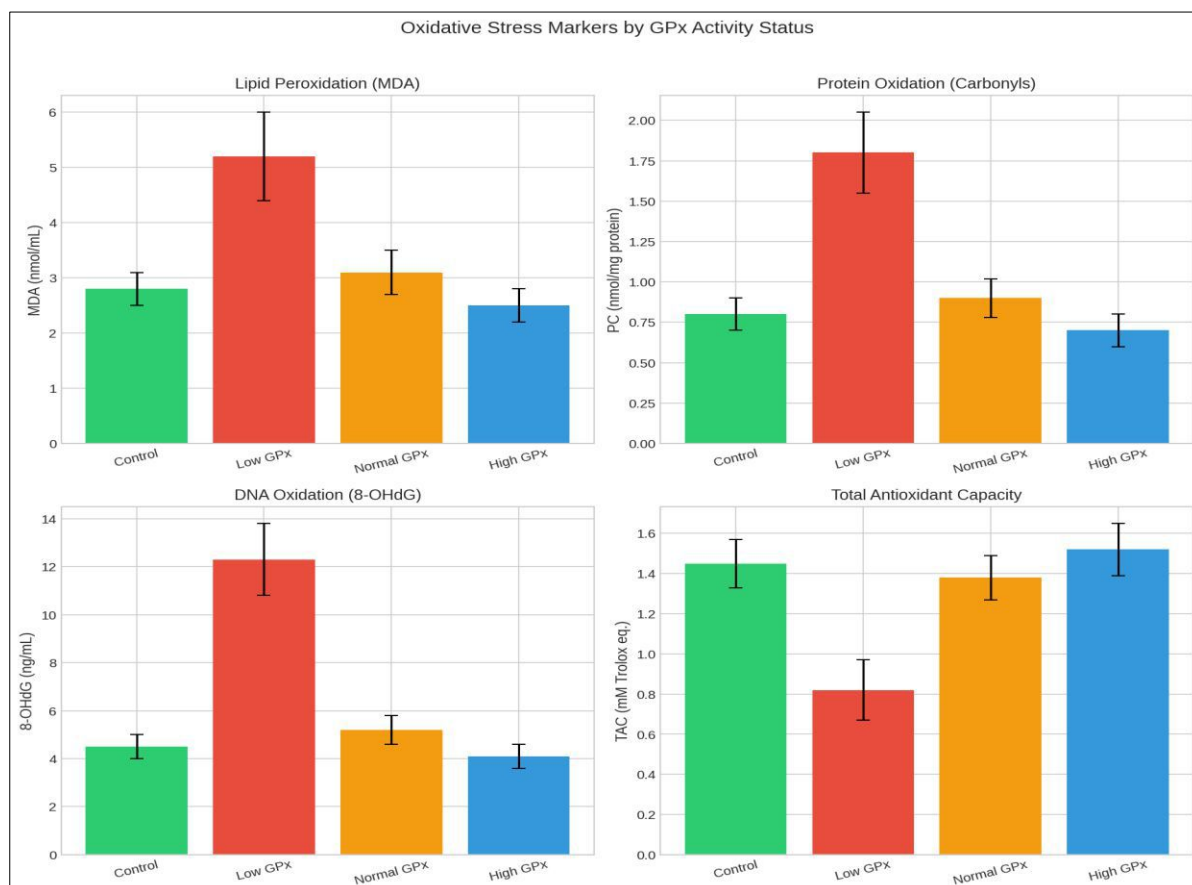


Fig 3: Relationship between erythrocyte GPx activity and oxidative stress markers.

4. Discussion

This study presents an optimized and validated method for erythrocyte GPx activity measurement with established reference intervals and demonstrated clinical utility. The optimized conditions represent a balance between maximum enzyme activity, assay stability, and practical considerations for routine laboratory implementation. The strong correlation with selenium status confirms the biological validity of the assay.

The reference interval of 32-58 U/g Hb established in our Scandinavian population is similar to values reported in other Northern European populations but somewhat lower than those from regions with higher selenium intake such as North America. This geographic variation underscores the importance of establishing population-specific reference intervals that reflect local dietary selenium exposure.

The supplementation trial demonstrated that the assay is responsive to changes in selenium status, with dose-dependent increases confirming its utility for monitoring nutritional interventions. The magnitude of response (18-52% increase) indicates that erythrocyte GPx activity provides sufficient dynamic range for detecting clinically meaningful changes in selenium-dependent antioxidant capacity.

Limitations include the exclusively Swedish study population and the need for validation in clinical populations with diseases affecting oxidative stress. Future studies should evaluate the utility of GPx monitoring in conditions such as cardiovascular disease, cancer, and inflammatory disorders where selenium status may influence outcomes.

5. Conclusion

The optimized erythrocyte GPx assay provides a reliable, precise method for assessing selenium-dependent antioxidant status with established reference intervals and demonstrated responsiveness to supplementation. Implementation of standardized methodology will improve comparability across studies and facilitate clinical application in nutrition assessment and intervention monitoring.

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