

Blood glutathione decreases in chronic diseases

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Previously a high blood glutathione level was correlated with long life span in the mouse and rat and in healthy elderly human beings. This raised the question of whether low glutathione levels occur in unhealthy subjects. To this end, 74 consecutive patients newly admitted to the hospital, with ages ranging from 21 to 89 years and diagnosed with chronic diseases, were studied along with 32 healthy control subjects. Blood samples were analyzed for reduced (GSH) and oxidized (GSSG) glutathione with a high-performance liquid chromatography–dual electrochemical method. The data were integrated with the clinical diagnoses and statistically analyzed. Marked total glutathione decreases from the control levels occurred in over 36% of the patients with chronic diseases including cancer and genitourinary, gastrointestinal, cardiovascular, and musculoskeletal diseases ($P < .001$). The deficit was due to low GSH concentrations and not to GSSG, which was the same as that in the control subjects. The conclusion is that a decrease in GSH is a risk factor for chronic diseases that may be used to monitor the severity and progress of the diseases. Future work is necessary to elucidate the mechanism of action. (J Lab Clin Med 2000;135:402-5)

Abbreviations: GSH = reduced glutathione; GSSG = oxidized glutathione; HPLC = high-performance liquid chromatography; MPA = metaphosphoric acid; RBC = red blood cell

Glutathione occurs ubiquitously in virtually all animal, plant, and microbial cells. It has many metabolic roles such as oxidation-reduction reactions, amino acid and protein structure, prostaglandin synthesis, and toxicology. Finally, blood glutathione exists in reduced, oxidized, free, and protein-bound forms that are inter-related and associated with a variety of biomedical conditions.¹⁻³

Glutathione decreases have been associated with a variety of diseases such as sepsis,⁴ adult respiratory distress syndrome,⁵ diabetes mellitus,^{6,7} liver disease,⁸

AIDS,⁹ and cataracts.¹⁰ The results, however, were often controversial, depending on whether whole blood or plasma was analyzed, which analytic method was used, and the heterogeneity of patients. Thus, different findings often cannot be compared. Usually the plasma glutathione level was studied, but plasma contains only 0.5% of the blood content, whereas erythrocytes contain 99.5%. For this reason we have regarded erythrocytic GSH as an indicator of overall GSH status.

Our research on aging has focused on biomedical changes in mosquito, mouse, and rat models and in elderly human subjects. In these cases, GSH decreases were demonstrated in the major tissues of the senescent organisms.¹¹⁻¹³

An advantage of studies in aging mammals is that decreases in blood GSH reflect similar decreases in less-accessible tissues and thus serve as an indicator of overall GSH status.^{12,13} Another advantage is that correction of the decrease by GSH enhancement after the administration of thiazolidine also increases the life span by about 38%.¹⁴ More recently our investigations of human populations demonstrated that health and longevity are correlated with blood GSH levels.^{15,16}

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Table I. Characteristics of control subjects and patients

	Controls		Patients		P value*
Age (y)	67.6 ± 6.41	(32)	62.6 ± 2.12	(74)	.346
Hospital duration (days)	—	—	8.85 ± 1.34	(66)	—
Red cell count (10 ⁶ cell/μL)	4.47 ± 0.092	(32)	4.05 ± 0.079	(74)	.002
Hemoglobin (g/dL)	14.3 ± 1.023	(31)	11.9 ± 0.229	(74)	.002
Hematocrit (%)	38.9 ± 0.469	(31)	35.9 ± 0.682	(74)	.008
GSH + GSSG (μeq/10 ¹⁰ RBCs)	2.30 ± 0.069	(32)	1.73 ± 0.036	(74)	<.001
GSH (μmol/10 ¹⁰ RBCs)	2.07 ± 0.067	(32)	1.52 ± 0.035	(74)	<.001
GSSG (μeq/10 ¹⁰ RBCs)	0.224 ± 0.018	(32)	0.207 ± 0.015	(74)	.492

Values are expressed as mean ± SEM (with number of subjects in parentheses).

*As compared with control group.

Table II. Blood glutathione content in chronic diseases

Disease category	μeq GSH/10 ¹⁰ RBCs	N	P value*
Control group	2.30 ± 0.070	32	—
Genitourinary	1.83 ± 0.100	10	.0013
Gastrointestinal	1.75 ± 0.073	18	<.001
Cancer	1.71 ± 0.051	17	<.001
Cardiovascular	1.71 ± 0.010	10	<.001
Musculoskeletal	1.66 ± 0.212	6	.0013
Lung	1.94 ± 0.155	4	.086
Gallbladder	1.87 ± 0.076	3	.070
Diabetes	1.31, 1.18	2	—
Kidney	1.37, 1.31	2	—
Endocrine	1.92	1	—
Blood	1.64	1	—
Total patients	1.73 ± 0.036	74	<.001

Values are expressed as mean ± SEM except for individual values for diabetes, kidney, blood, and endocrine categories.

*As compared with control group.

These findings were the basis of our current hypothesis that normal blood GSH concentrations occur in healthy adults and decreased GSH levels are found in the elderly and in those with chronic diseases. To test the disease link, newly admitted hospital patients were quantified for blood GSH and GSSG, diagnosed for disease, and compared with the control subjects.

METHODS

The subjects were patients with chronic diseases newly admitted to a metropolitan Louisville hospital during a 3-day period. There were 74 patients, including 28 men and 46 women, with an age range of 21 to 89 years. The final diagnoses by the different attending physicians were frequently made several days after admission and obtained from the case histories. The control group (N = 32) comprised healthy subjects free of chronic diseases in the age range of 30 to 98 years who were on the hospital staff, were students, or were from the community. All participants signed informed consent forms approved by our institutional review board.

Sample collection and processing were essential for opti-

mal results. Most important was chilling of the freshly drawn sample immediately in an ice bath and acid denaturation of enzymes. In addition, the acidic condition stabilizes thiols by preventing autoxidation. Failure to quickly follow these processing steps will result in low GSH and Cys values.

Coded blood samples were obtained by venipuncture from control subjects and patients, chilled immediately on ice, and deproteinized within an hour by addition of 1.0 vol of blood to 4.0 vol of 5% (wt/vol) MPA. In addition, MPA stabilizes GSH and GSSG by inactivating GSSG reductase that gives low GSSG values, as shown by repeated analysis of a sample for up to several hours.¹⁷ MPA was chosen as the protein precipitant, because it does not oxidize blood GSH and does not lead to hydrogen peroxidation like perchloric and trichloroacetic acids. Recoveries of added authentic GSH and GSSG to samples provided >99% validation for this sample preparation.

After centrifugation, the acid extracts were quantified for GSH and GSSG by HPLC–dual electrochemical detection as described previously in detail.^{18,19} Detection limits were 50 pmol for GSH and 25 pmol for GSSG. Whole blood values expressed per 10¹⁰ RBCs are the same as for isolated red cells,

which contain 99.5% of the total GSH.²⁰ Concentrations for reduced plus oxidized forms are expressed as GSH equivalents per 10^{10} RBCs.

An aliquot of each sample was also analyzed for RBC count, hematocrit, and hemoglobin with standard tests. Then the laboratory and clinical findings were decoded, integrated, and statistically evaluated.

Comparisons of patient and control groups were performed by using analysis of variance and *t* test.^{21,22} Data are presented as mean \pm SEM, and a value of $P = .05$ was considered significant.

RESULTS

Characteristics of the participants are summarized in Table I. The mean age of the patients was 62.6 years and that of the controls was 67.6 years, and these were not significantly different ($P = .34$).¹¹⁻¹³ There was a distinct difference between GSH and GSSG. Total GSH equivalents (GSH plus GSSG) were significantly decreased in the patients ($P < .001$). On the other hand, the GSSG concentrations were not different for patients and control subjects ($P = .49$).

The blood GSH levels versus the ages of male and female patients are plotted in Fig 1. The shaded area represents the 95% confidence limits of healthy control subjects in this and earlier studies¹⁵ with a lower limit of $1.43 \mu\text{mol}/10^{10}$ RBCs. There were 27 patients with values lower than this limit who thus were considered to have GSH deficiency.

The GSH levels expressed per 10^{10} RBCs dropped significantly, yet the RBC count, hemoglobin concentration, and hematocrit were lower in the patients. These data indicate that the GSH decrease was not caused by a fall in RBCs, because it would have resulted in higher, not lower, GSH values.

The disease categories and their GSH concentrations were compared with the control group and listed in descending order of GSH level (Table II). The concentrations in the top five disease categories—genitourinary, gastrointestinal, cancer, cardiovascular, and musculoskeletal—were significantly lower than those in the control subjects ($P < .002$). As a group, the patients showed lower concentrations than did the control subjects ($P < .001$). Also, GSH decreases occurred in 2 patients with diabetes mellitus and in 2 patients with kidney disease.

DISCUSSION

The results of this study confirmed our hypothesis that blood GSH decreases in chronic diseases. This risk factor may be useful in monitoring the progress and treatment of the disease. The rationale underlying the GSH connection was based on the presence of GSH in virtually all cells and thus its possible involvement in

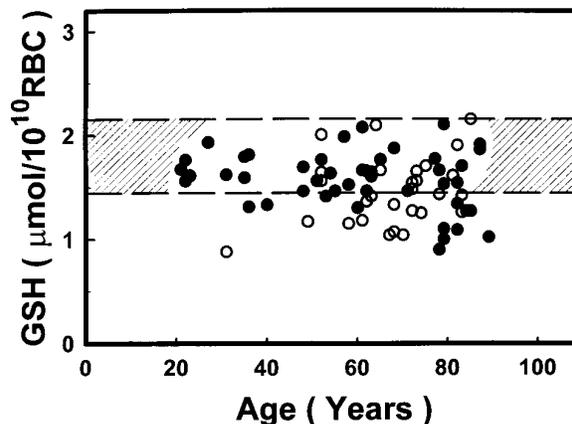


Fig 1. Glutathione levels in hospital admissions. The shaded area represents the 95% confidence limits of the healthy control group. ●, Women; ○, men.

many chronic diseases.

This relationship of glutathione, longevity, and chronic diseases suggests a common molecular mechanism that may be corrected by GSH enhancement. This was demonstrated in mosquitoes fed magnesium thiazolidine-4-carboxylate, a precursor of cysteine and GSH. The results were that the GSH concentration was maintained at a high level longer and the life span was increased 36% to 40% longer than that in the control subjects ($P < .01$).¹⁴

This enhancement effect in aging may also apply to chronic diseases that predominate in old age. Thus it is conceivable that the correction of low GSH may represent a new therapeutic intervention. Furthermore, the testing of doses and conditions can be easily determined.

This study has several unique features. First, we have a record of blood GSH levels in normal human subjects of ages ranging from 21 to 94 years old. Second, our HPLC–dual electrochemical analytic method is specific for GSH and GSSG. Third, the fragility of blood glutathione has been studied, and conditions were developed to stabilize the sample. Finally, patients newly admitted to the hospital were recruited to ensure that study subjects were acutely ill.

There are several confusing definitions of old age. Most persons refer to chronologic age and thus, arbitrary ages such as 65 years are considered old and imply poor health. Unfortunately, this criterion may be misleading, for many individuals are spry and active at that age while others are frail and sickly.

A better definition is biologic or physiologic age, which refers to functional and health status relative to 21 to 23 years at the end of growth. Thereafter, during maturity, metabolic processes decrease gradually until senescence, when more acute critical changes occur at

the end of the life span. Chronologic ages are practical but variable depending on health and disease. Thus the use of blood GSH status may be a simple quantitative index of health and disease.

Was the GSH decrease caused by aging itself? To test this, the data in Fig 1 were divided into a younger and an older age group. There was no correlation when analyzed as GSH content versus age in subjects 60 years and older ($r = 0.041$, $P < .79$) and in subjects <60 years ($r = -0.072$, $P = .73$). Thus aging was not a confounding factor in this study.

Subnormal GSH levels have been reported before in various diseases listed in the introduction, but these studies dealt with low plasma levels, not erythrocytic GSH levels. In whole blood we have shown that 99.5% of total GSH occurs in erythrocytes and <0.5% occurs in plasma. Hence our term for blood GSH is actually *erythrocytic* GSH. Measurement of whole blood saves time and effort in separating red cells and reduces exposure to oxidation artifacts.

Our quantification of blood GSH is expressed per 10^{10} erythrocytes and not in terms of blood or plasma volume, hemoglobin concentration, or hematocrit. Not only is GSH localized in red cells to keep the hemoglobin reduced, but the predominant role of erythrocytes in GSH mobility and inter-organ transport was demonstrated clearly in the liver-to-erythrocyte-to-kidney pathway of a rat with an acute surgical preparation.²³

The cause of the GSH decreases in this study is unclear and may be related to a drop in GSH synthesis, a rise in its degradation, or utilization by other linked systems. Although a GSH decrease could result in an increase in GSSG, no change in GSSG was observed. Thus the most likely possibility is a decrease in GSH synthesis.

Glutathione has been shown to be a risk factor for a variety of chronic diseases. Additional studies may determine whether further progress of a disease can be monitored by blood GSH. Also, a prospective longitudinal study could be useful in determining whether the GSH deficit might be a harbinger of mortality. Further work may focus on more precise individual data rather than group parameters of most clinical data. Thus quantitative analysis of individuals before and after treatment could be a future use for blood glutathione level. Further studies can answer these questions.

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