Journal of Advanced Sport Technology
DOI: 10.22098/jast.2025.14776.1340
Received: 06 March 2024 Accepted: 10 February 2025

ORIGINAL ARTICLE *Open Access*

The Exercise-Induced Poise of Thiol/Disulfide Form of Gluthatione Quantitation by High-Performance Liquid Chromatography with Fluorescence Detection

Dr. Farnaz Seifi-skishahr[1], Dr. Arsalan Damirchi*[2], Dr. ParvinBabaei[3] & Dr. Mohammad Babaei[4]

1. Associate Prof. in Department of Exercise Physiology, Faculty of Education and Psychology, University of Mohaghegh Ardabili, Ardabil, Iran
2. Professor of Exercise Physiology, Department of Physiology, Faculty of Physical Education and Sport Science, University of Guilan, Rasht, Iran
3. Professor of Medical physiology, Department of Physiology, Faculty of Medicine, Guilan University of Medical sciences, Rasht, Iran
4. Ph.D in Exercise Physiology, Department of Exercise Physiology, Faculty of Education and Psychology, University of Mohaghegh Ardabili, Ardabil, Iran

Correspondence: Farnaz Seifi-skishahr - f.seify@yahoo.com
How to cite: Seifi Skishahr, F., Damirchi, A., Babaei, P., Babaei, M. The Exercise-Induced Poise of Thiol/Disulfide Form of Gluthatione Quantitation by High-Performance Liquid Chromatography with Fluorescence Detection. *Journal of Advanced Sport Technology*, 2026; 10(1): 69-76. doi: 10.22098/jast.2025.14776.1340

ABSTRACT

Background: Despite beneficial effects of exercise, balance between oxidants and antioxidants have great health importance. Therefore, reduction potentials (Eh) for the redox couples might be useful indicators of health; however the exercise-induced poise of thiol/disulfide form of gluthatione has not been measured by HPLC. The aim of this study was quantitation of poise of thiol/disulfide form of gluthatione by in vivo Eh values for GSH/GSSG in erythrocytes in subjects with different physical training status.

Methods: Thirty male subjects participated in this cross-sectional study and were assigned as professional athletes (PA), recreational athletes (RA) and nonathletes (NA) groups. Based on self-reported frequency of physical training, subjects from PA group were selected from professional soccer players; subjects from RA group were identified: moderately trained subjects with regular physical training and nonatletes had no physical activity in their routine. Blood samples were taken from an antecubital vein and analyzed into two part: The part 1 were used for measuring hemoglobin and hematocrit using automated Coulter Counter (Sysmex k-x21) and part 2 containing EDTA were centrifuged at 1600 g for 5 min to obtain Erythrocytes, they were washed twice with cold 9% NaCl solution and Erythrocytes lysed by freezing for 2 hours. Finally, hemolysate (100 mL) was deproteinized and used for measurement of oxidized glutathione (GSSG) to reduced glutathione (GSH) by HPLC; The HPLC analyses were performed with Agilent 1200 series HPLC systems equipped with a quaternary pump system (G1311A) and a fluorescence detector (G1321A) (Agilent Technologies, Waldbronn, Germany). Fluorimetric detection was performed at 420 nm after excitation at 340 nm. Then, Eh for GSH/2GSSG was calculated by Nernst equation ($Eh=E0 + RT/nF \ln([GSSG]/[GSH]2)$. **Results** Recreational athletes had the less amounts of reduction potentials for GSH in compared to nonathletes group; however recreational athletes had the redox environment with the most negative amount of Eh in their erythrocytes. This study suggests that physical activity of individuals determines the reduction potentials for GSH in erythrocytes.

Conclusions: Long term regular exercise training with moderate intensity has the least reduction potentials that ensuring healthiness, versus intensive exercise training same as passive lifestyle leads to most amounts of reduction potential and may be consequent development of related diseases.

KEYWORDS :Redox state, Glutathione, reduction potentials, Exercise, HPLC

Introduction

Reduction potentials (Eh) values provide convenient parameters to describe relationships between various biochemical undergoing oxidation– reduction reactions. Eh is a measure of the tendency of redox couples to accept or donate electrons [1]. The redox state represents the oxidation/ reduction potential within the cell and plays important role in cells function [2]. Historically, Redox state is the ratio of the interconvertible oxidized and reduced form of those redox couple which we used in previous researches [3-5]; and this expression provides a simple means to show changes, but another definition of redox state of redox couple which better represents concentration-dependent redox couples (e.g. GSSG/2GSH) couple is defined by the half-cell reduction potential and the reducing capacity of that couple and is best expressed through the use of the Nernst equation [6]. In fact, oxidation-reduction reactions involving GSH consume 2 GSH to produce GSSG, the reducing force available from a GSH:GSSG ratio of 100 is substantially different for 10 mM GSH and 100 μM GSSG than it is for 100 μM GSH and 1 μM GSSG [7]. Therefore, determination of Eh for the redox couples eliminates this problem and allows the exact detection of "reducing force" available from the GSH/GSSG couple and it can be a convenient and informative way of summarizing the redox environment of cells and is also an important indicator of health of those cells [1]. Generally, maintaining redox homeostasis is necessary for survival [2]; which can be changed by exercise [3-5]. It is well known that exercise is beneficial for health [8]. Oxidative stress is the common pathological basis of many diseases. The overproduction of free radicals, both reactive oxygen species and reactive nitrogen species, can lead to redox imbalance to oxidized form of cellular redox state that predisposes individuals to aging and diseases [9, 10]. Maintaining redox homeostasis and enhancing anti-oxidative capacity are critical mechanisms by which exercise protects against different diseases with more reduced redox state and represents healthy status [3-5, 8, 11, 12]; but extensive exercise may be induce oxidative stress and permanent shift in redox balance towards a more oxidized environment and leads to disturbing cellular metabolism and function [3-5, 13, 14].

To our knowledge, the changes in gluthatione redox status have been investigated in many human studies [3-5, 15, 16]; in which redox homeostasis evaluated by GSH/GSSG ratio. Our hypothesis was that healthy subjects' training status may impact the Eh for gluthation and it may be personalizing exercise therapy for health maintenance and disease prevention. Thus, the present study was designed to calculate the quantization of the exercise-induced poise of thiol/disulfide form of glutathione by high-performance liquid chromatography with fluorescence detection in individuals with different level of physical activity.

Material and Methods

Based on self-reported level of physical activity, thirty male voluntarily were assigned as professional athletes (n=10), recreational athletes (n=10) and nonathletic (n=10) groups participated in this study. At the beginning of the experiment, the study protocol was approved by the Research Ethics Committee of Mohaghegh Ardabili University (IR.UMA.REC. 1400.049), and then participants completed medical history questionnaire and signed informed consent. None of the participants showed signs of bacterial or viral infection symptoms. In addition, other exclusion criteria were drinking alcohol, smoking, and taking anti-inflammatory drugs or antioxidant supplements. The subjects' weight and height were recorded using electronic scale (model 712; Seca, Germany) and portable Stadiometer (Holtain, UK). The blood samples were used for measuring hemoglobin and

hematocrit using automated Coulter Counter (Sysmex k-x21) and measuring of GSH and total GSH in RBCs by High-performance liquid chromatography (HPLC) with fluorescence detection [17]. The HPLC analyses were performed with Agilent1200 series HPLC systems equipped with a quaternary pump system (G1311A), a fluorescence detector (G1321A), (Agilent Technologies, Waldbronn, Germany) by using reversed Phasegradient elution on Eclipse XDB-C18 column(150, 4.6 mm; 5 µm particle size). A mobile phase was composed of 50 mM of sodium acetate buffer (pH = 6.20) and acetonitrile. Fluorimetric detection was performed at 420 nm after excitation at 340 nm. The flow rate during elution was 0.7 mL/min, the retention time of GSH was 3.6 min, and the injection volume was 20 μL. After measuring the erythrocytes GSH and GSSG, GSH/2GSSG ratio, Eh was calculated by Nernst equation (Eh=E0 + RT/nF ln([GSSG]/[GSH]2). where R is the gas constant (R = 8.314 J K-1 mol-1), T the temperature (in Kelvin), and F the Faraday constant (F =9.6485 x 104 C mol-1) [6]. The results are presented as a mean ± SEM, except for subject characteristics, which are presented as mean ± SD. All data were analyzed for their normal distribution using shapirowilk test. ANOVA with Tokay post hoc were used to analyze the differences between groups. Calculations were performed with the SPSS, Version 27.0 .Statistical significance was defined as $p<0.05$.

Results

The physiological characteristics of the participants represented in Table 1. All subjects have normal BMI (PA group: 22.28±1.87; RA group: 23.12±3.01; NA group: 23.37±2.74 kg/m^2) with no significant difference between groups. There was no significant difference for hematocrit and RBC's in these values between groups (Table2).

Table1 – Subjects' characteristics of WT, MT and UT groups

characteristics	Group PA	Group RA	Group NA	F	P value
Age(yr)	21.10±1.72	21.70±1.88	20.10±1.44	2.264	0.123
BMI (kg/m^2)	22.28±1.87	23.12±3.01	23.37±2.74	0.485	0.621
Training (h·week-1)	6.4±.33	1.20±0.16	0	-	-

Data are mean ±SD

Reduction potentials (Eh) for Thiol and Disulfide Forms of Glutathione in RBCs.

The result showed that Eh for GSH and GSSG in RBCs had significant difference between groups (F= 4.38, p= 0.022). The results of Tukey test showed that glutathione reduction potential in erythrocytes was higher in professional athletes in compared to recreational and nonathletes groups (p= 0.024) (Table2).

Table2 - biochemical parameters

	HCT	RBC	Eh of Gluthation
Group PA	45.26±2.18	5.30±0.32	-292.90 ± 3.72
Group RA	47.21±2.84	5.39±0.29	-296.06 ± 10.29
Group NA	46.44±2.27	5.43±0.36	-284.48 ± 11.8
F	1.60	0.39	4.38
P value	0.21	0.67	0.02

Data are mean ± SEM. p value calculated using ANOVA with Tukey post hoc test

Discussion

The calculated in vivo Eh is a convenient and informative way of summarizing the redox environment of RBC [1]; and is also useful for determination health in cellular level [1]. In this study we showed that recreational athletes had the less amounts of reduction potentials for GSH in compared to nonathletic group; therefore recreational athletes had the most negative environment in their erythrocytes that may be an indication of healthy status; while the most oxidized (most positive) form was for nonathletic and professional athletes predisposes them to aging and disease [1-5].

In this study we used HPLC with precolumn derivatization as a golden standard technique in the analysis of glutathione; because glutathione are more oxidized than predicted from their standard reduction potentials. The HPLC method shows high sensitivity (50 fmol per injection, the lowest reported), good precision (C.V., 5.0%), an analytical recovery of GSH and GSSG close to 100%, and linearity (r.0.999) [1, 17]. Moreover, redox potential (Eh) values provide convenient parameters to describe relationships between different biochemical undergoing oxidation-reduction reactions that required during oxidative stress and redox signaling, Eh is a measure of the tendency of redox couples to accept or donate electrons [18]. In Nernst equation, we consider of GSSG and 2GSH [1]; because oxidation-reduction reactions involving GSH consume 2 GSH to produce GSSG [7]; and it can be a convenient a better represents concentration-dependent redox couples [6].

Considering the fact that, the health-related benefits of a physically active lifestyle can be stated by redox system, this system plays a crucial role in the regulating cell survival (20). In this study, increased oxidative stress by inactivity in non-athletes or over activity in professional athletes can induce cell death. The less negative environment in their RBC represents failure in maintaining redox balance, which predispose them to oxidative stress–induced cell death (3-5, 14, 15, 20, 21).

In recreational athletes group, exercise can promote cell survival. Indeed, a potential mechanism by which moderate exercise, low intensity training and regular prolonged training in this group, improve endogenous antioxidant status and redox homeostasis (3-5, 12,13, 22). Reactive oxygen species has been known to play various important roles in cell signaling and regulating the expression of antioxidant genes. Physical exertion produces a hyperregulation of the nuclear factor kappa B and mitogen-activated protein kinase that activates gene expression of a number of enzymes and proteins with an important role in maintaining oxidative/antioxidant intracellular homeostasis. (23, 24)

Conclusion

In conclusion, our data suggests the importance of moderate physical exercise for health maintenance and disease prevention due to changes in the systemic redox environment. Thus, future research should explore the possibility of identifying (Eh) for the redox couples as promising biomarkers for health or the oxidative stress related chronic diseases.
From an exercise technology perspective, integrating redox-based biomarkers into emerging monitoring systems, including wearable biosensors, digital health platforms, and personalized

training technologies, could enable real-time evaluation of physiological responses to exercise. Such approaches may facilitate individualized exercise prescription by identifying optimal training intensity, preventing excessive oxidative stress associated with inactivity or overtraining, and improving long-term health outcomes.

Furthermore, the application of redox monitoring in sports science may contribute to the development of precision exercise strategies for recreational athletes, elite performers, and individuals at risk of oxidative stress-related chronic diseases. Future research should focus on validating Eh measurements as practical and reliable biomarkers and integrating them with other physiological, biochemical, and performance indicators to establish comprehensive technology-driven models for exercise optimization and health promotion.

Ethical Considerations: The authors of this study have complied with ethical guidelines.

Compliance with ethical guidelines

all authors have provided equal contributions to the research project.

Funding

This particular study did not receive any financial assistance from external sources.

Conflict of Interest

There was no conflict of Interest.

Acknowledgment

Gratitude is expressed to the subjects who participated in this study.

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نشریه فناوری ورزشی پیشرفته
DOI: 10.22098/jast.2025.14776.1340
تاریخ دریافت: ۱۴۰۲/۱۲/۱۶ تاریخ پذیرش: ۱۴۰۳/۱۱/۲۲
Journal of Advanced Sport Technology
«مقاله پژوهشی»

اندازه‌گیری کمی غلظت تیول/ دی‌سولفید گلوتاتیون ناشی از فعالیت ورزشی با استفاده از کروماتوگرافی مایع با کارایی بالا با آشکارسازی فلورسانس

دکتر فرناز سیفی اسکی شهر[1*]، دکتر ارسلان دمیرچی[2] دکتر پروین بابائی[3]، دکتر محمد بابائی[4]

۱- دانشیار گروه فیزیولوژی ورزشی، دانشکده علوم تربیتی و روانشناسی، دانشگاه محقق اردبیلی، اردبیل، ایران
۲- استاد فیزیولوژی ورزشی، گروه فیزیولوژی، دانشکده تربیت بدنی و علوم ورزشی، دانشگاه گیلان، رشت، ایران
۳- استاد فیزیولوژی پزشکی، گروه فیزیولوژی، دانشکده پزشکی، دانشگاه علوم پزشکی گیلان، رشت، ایران
۴- دکتری فیزیولوژی ورزشی، گروه فیزیولوژی ورزشی، دانشکده علوم تربیتی و روانشناسی، دانشگاه محقق اردبیلی، اردبیل، ایران

نویسنده مسئول: فرناز سیفی اسکی شهر – f.seify@yahoo.com

چکیده

هدف: علیرغم اثرات مفید فعالیت ورزشی، تعادل بین اکسیدان‌ها و آنتی‌اکسیدان‌ها از اهمیت بالایی برای سلامتی برخوردار است. بنابراین، پتانسیل‌های احیا (Eh) برای زوج‌های ردوکس ممکن است شاخص‌های مفیدی برای سلامتی باشند. با این حال، تعادل فرم تیول/دی‌سولفید گلوتاتیون ناشی از فعالیت ورزشی تابحال HPLC اندازه‌گیری نشده است. هدف از این مطالعه، تعیین کمی تعادل فرم تیول/دی‌سولفید گلوتاتیون با استفاده از مقادیر Eh درون تنی برای GSH/GSSG در گلبول‌های قرمز در افراد با وضعیت تمرین بدنی متفاوت بود.

روش شناسی: ۳۰ مرد در این مطالعه مقطعی شرکت کردند و به عنوان ورزشکاران حرفه‌ای (PA)، ورزشکاران تفریحی (RA) و غیرورزشکاران (NA) تقسیم شدند. افراد گروه PA از بین بازیکنان حرفه‌ای فوتبال انتخاب شدند. افراد گروه RA شناسایی شدند: افراد با تمرین متوسط با فعالیت بدنی منظم و افراد غیرورزشکار که هیچ فعالیت بدنی در برنامه خود نداشتند. نمونه‌های خون از ورید آرنج گرفته شد و به دو بخش تجزیه و تحلیل شدند: بخش اول برای اندازه‌گیری هموگلوبین و هماتوکریت با استفاده از دستگاه شمارشگر خودکار کولتر (Sysmex k-x21) و بخش دوم حاوی EDTA به مدت ۵ دقیقه با سرعت ۱۶۰۰ گرم سانتریفیوژ شدند تا گلبول‌های قرمز به دست آیند، سپس دو بار با محلول سرد ۹٪ NaCl شسته شدند و گلبول‌های قرمز با انجماد به مدت ۲ ساعت لیز شدند. در نهایت، همولیزات (۱۰۰ میلی‌لیتر) پروتئین‌زدایی شد و برای اندازه‌گیری گلوتاتیون اکسید شده (GSSG) به گلوتاتیون احیا شده (GSH) با HPLC استفاده شد. تجزیه و تحلیل‌های HPLC با استفاده از سیستم‌های HPLC سری Agilent 1200 مجهز به سیستم پمپ چهارتایی (G1311A) و آشکارساز فلورسانس (G1321A) (Agilent Technologies، Waldbronn، آلمان) انجام شد. تشخیص فلوریمتری در طول موج ۴۲۰ نانومتر پس از تحریک در طول موج ۳۴۰ نانومتر انجام شد. سپس، Eh برای GSH/2GSSG با استفاده از معادله نرنست (Eh=E0 + RT/nF ln([GSSG]/[GSH]2) محاسبه شد.

نتایج: ورزشکاران تفریحی در مقایسه با گروه غیرورزشکار، پتانسیل اکسایش کمتری برای GSH داشتند؛ با این حال، ورزشکاران تفریحی محیط ردوکس با منفی‌ترین مقدار Eh در گلبول‌های قرمز خود داشتند. این مطالعه نشان می‌دهد که فعالیت بدنی افراد، پتانسیل کاهش GSH در گلبول‌های قرمز را تعیین می‌کند.

نتیجه گیری: فعالیت بدنی منظم و طولانی مدت با شدت متوسط، کمترین پتانسیل اکسایش را دارد که تضمین کننده سلامتی است، در حالی که تمرینات ورزشی فشرده مانند سبک زندگی غیرفعال منجر به بیشترین پتانسیل کاهش می‌شود و ممکن است در نتیجه منجر به ایجاد بیماری‌های مرتبط شود.

واژه‌های کلیدی: وضعیت ردوکس، گلوتاتیون، پتانسیل اکسایش و احیا ، ورزش، HPLC