

# Assessment of glutathione in adult smokers and non smokers

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## ABSTRACT

*Studies concerning the effects of smoking or exercise on the anti oxidant defense status or glutathione activities are still indicating discrepant results. However a comprehensive picture regarding the collective effects of exercise and smoking on oxidative injury is not clear. **Aim of the study:** This study was conducted aiming to investigate the response of reduced glutathione (GSH) as an antioxidant to an acute exercise of two different intensities in adult smokers and non smokers. **Methods:** The study was done on 60 young male subjects with mean age  $21 \pm 1.36$  yrs old. They were classified equally into 2 groups, smokers and non smokers, then subjects in each group were sub divided into 2 equal subgroups (group included in moderate exercise while other involved vigorous exercise). Blood GSH was measured before and immediately after exercise. **Results:** there was significantly increase GSH level in both groups after moderate exercise while after vigorous exercise GSH level was reduced, as it was highly significant reduction in smokers in comparison to which of non smokers. **Conclusion:** Moderate exercise is beneficial and recommended while vigorous exercise is harmful especially for smoker subjects.*

## INTRODUCTION

Smoking is the largest preventable cause of death and disability in developing countries<sup>13</sup>. Smoking increases the risk of coronary heart disease and heart attack up to five times the normal risk because it reduce the quantities of antioxidants in the bloodstream, which help to protect the heart<sup>11</sup>.

Although cigarette smoking is a widely recognized health hazard and a major cause of mortality, people continue to consume cigarettes on a regular basis. Approximately one-third of the world's population 15 years or older smokes cigarettes daily (i.e., active smokers)<sup>17</sup>.

Nicotine is a highly toxic alkaloid that is both a ganglionic stimulant and depressant. Acute CVS response to nicotine observed in normal smokers include increase in systolic and diastolic pressure, heart rate, force of

myocardial contraction, myocardial oxygen consumption, coronary artery blood flow, myocardial excitability and peripheral vaso constriction<sup>14</sup>.

Smoking reduces physical endurance and impedes the improvement in physical performance in smokers compared with non-smokers. It also increases fatigue during and after exercise because of nicotine in tobacco smoke, which increases heart rate and blood pressure, impairs ventilatory functions, and constricts ventilatory pathways and blood vessels<sup>3</sup>.

The Antioxidant Defense System (ADS) includes enzymes and antioxidants to prevent the start of oxidative damage and/or control its spread. Essential antioxidants are either endogenous (internally synthesized) or exogenous (consumed). Glutathione (GSH) is our body A.I.D. (A-antioxidant, I- immune system, D- detoxifier). It is produced normally by the body in response to today's

environment such as pollution in the air we breath, water we drink, chemicals & pesticides in the food we eat, that cause damages in our cells & system<sup>19</sup>.

Higher glutathione levels influence red blood cell count, hematocrit, and hemoglobin. Conversely when red blood cells, hematocrit, and hemoglobin are reduced during anemia, low glutathione is implicated; the addition of GSH has been shown to resolve the above mentioned low blood markers<sup>2</sup>.

Cigarette smoke is a common oxidant stress factor and may adversely affect the antioxidant capacity in humans and aggravate age related disorders.. The same values after exposure to cigarette smoke were changed markedly with advancing age. These results indicate that GSH metabolism may be impaired by chronic cigarette smoke exposure<sup>20</sup>.

### **Glutathione concentration in healthy subjects**

In healthy subjects, mixed results have been reported; GSH concentrations vary from 0.684 to 2.525Mmo/L in blood, and from 0.002 to 0.1136 Mmol/L in plasma<sup>9</sup>. No significant difference was apparent between glutathione concentrations determined in the male population and those in the female, neither in blood nor in plasma<sup>5</sup>.

Elevations in blood oxidative stress markers after acute exercise had been identified indicating that oxidative stress is not limited to the cellular compartment. Furthermore, very high intensity exercise appears to exaggerate the blood oxidative stress response<sup>15</sup>.

Acute exercise increases oxidant levels and oxidative stress in untrained subjects, but long-term exercise may counter this effect by increasing the activity of antioxidant enzymes and reducing oxidant production. These

defenses may be critical for preventing chronic oxidative damage to muscle during exercise and even at rest<sup>12</sup>.

Other endurance-based exercise studies have reported similar results that reported an increase in oxidized glutathione after prolonged submaximal exercise with a concomitant decrease in reduced glutathione<sup>7</sup>.

## **METHODOLOGY AND PROCEDURES**

### **Methodology**

This study was conducted in Kasr Elaini Hospital. The goal of this study is to assess the response of reduced glutathione (GSH) as an antioxidant to an acute exercise of two different intensities in adult smokers and non smokers.

### **Subjects**

Sixty (60) volunteer sedentary young and healthy male (30 non smokers and 30 smokers) were included in the study. The physical characteristics of these subjects, expressed as mean  $\pm$  SD, were age, 21 $\pm$ 1.36 years old (ranging from 19-24); height, 171.46 $\pm$ 9.22 cm (ranging from 160-183 cm); body weight, 65.93 $\pm$ 8.7kg (ranging from 55-80 kg); and BMI, 22.37 $\pm$ 1.42 Kg/m<sup>2</sup> (ranged from 20.4-24.9 Kg/m<sup>2</sup>).

The subjects were further provided with an explanation of the purpose, nature, procedures of the study and the proper technique of the exercise.

### **A- Evaluation Equipments**

- Body weight and height scale.
- Analyzing chemicals and commercial kits (QuantiChrom TM Glutathione Assay Kit: Colorimetric Determination of Reduced Glutathione at 405nm will be used to measure level of reduced glutathione in blood samples before and after exercise.

- Polypropylene (lithium heparin) tubes and ice tank will be used to keep blood samples.

### **B- Training Equipment**

A stationary bicycle (Universal 814-E, Sweden) supplied with a digital heart rate monitor was used for exercise.

### **Procedures**

#### **Evaluation Procedures**

All subjects were assessed following eight hours cessation of smoking.

All of the subjects underwent several evaluation steps including: Resting heart rate (RHR).

#### **Laboratory Investigations**

QuantiChrom TM Glutathione Assay Kit was used. This kit provide colorimetric determination of reduced glutathione at 405nm. It is a simple, convenient, direct and automation-ready procedure designed to accurately measure reduced glutathione in biological samples.

The main advantage of this method is the specificity for glutathione and it does not require an enzyme as a reagent.

#### **Sample preparation**

Take 0.1 ml of whole blood sample in centrifuge tubes, Add 0.5 ml distilled water, Add 0.5 ml of solution R1(TCA), Vortex to mix well, Allow to stand for 5 minutes at room temperature and mix with 1.0 ml R 2 (Buffer) then Add 0.1 ml R 3 (DTNB ) and vortex to mix well.

Measure the absorbance after 5-10 minutes at 405 nm of sample (a Sample) against the blank. Linearity up to 120 mg/dL (4 mmol/L) as: Glutathione (GSH) concentration In blood Sample is calculated using the following equation: Blood GSH concentration = A Sample X 2.22 mmol/L.

### **Determination of Exercise Intensity**

Exercise intensity was determined using percentage of maximum heart rate (MHR). The use of MHR to determine exercise intensity is based on the following physiological principal: as exercise intensity increases, heart rate increase in a linear relationship.

To estimate maximal heart rate (MHR):  
(MHR. = 220-age)<sup>1</sup>.

#### **Exercise intensity was used in this study**

- Moderate exercise intensity: was calculated at 65% MHR.
- Maximum exercise intensity: was calculated at 85% MHR.

#### **Cycling exercise test**

One day before the actual study subjects came to the outpatient clinic to perform a simple exercise test to determine work load appropriate with different exercise intensities.

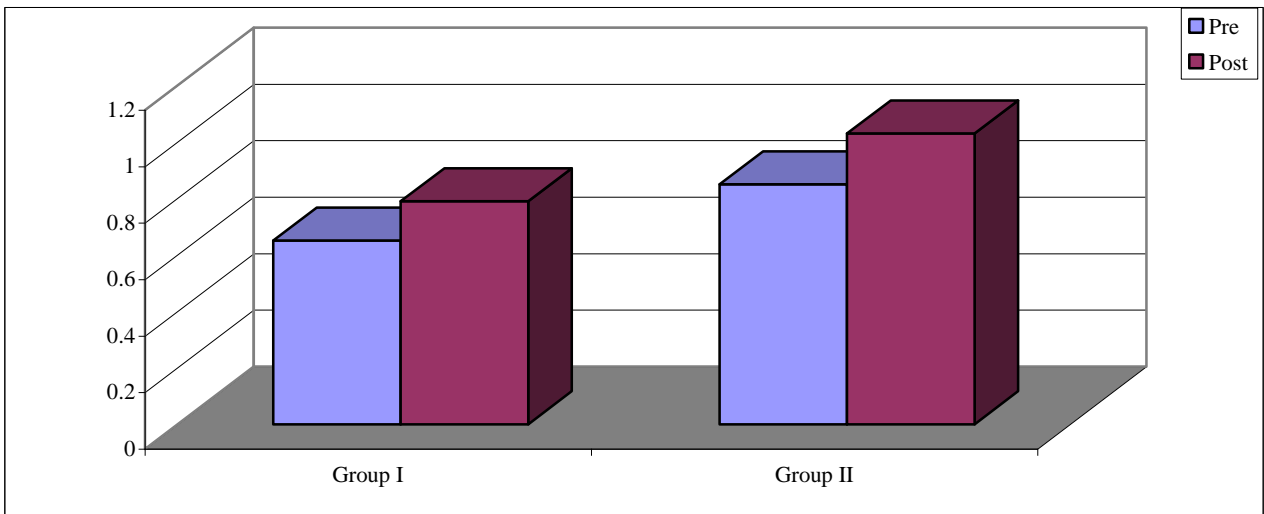
MHR at 70 W for 30 subjects (15 subjects in each group) and 85 % MHR at 100 W for the other 30 subjects (15 subjects in each group).

#### **Exercise protocol**

Sixty young males shared in this study. They were classified into equally two groups of thirty subjects (30 non smokers, 30 heavy smokers). They all undergo the same procedures except for the intensity of exercise they were assigned to:

- Each subject started with warming up exercise in the form of cycling on a stationary bicycle at a work load of 20 W for five minutes.
- Then all subjects performed aerobic cycling exercise for 20 minutes at intensity equivalent to (65% MHR at 70 W or 85 % MHR at 100 W).





**Fig. (1):** The mean values of GSH at pre and post exercise for group I & group II.

**Table 2 and Figure 2 showed that:**

For group Ia, the mean value of GSH at the beginning of exercise was (0.7±0.14 mmol /L) while after the end of exercise it was (0.65±0.14 mmo/L), with a mean difference of (0.05), and percentage of change of 7.14 %. This change was found to be significant.

For group IIa the mean value of GSH at the beginning of exercise was (0.89±0.27 mmol /L), while after the end of exercise it was (0.4±0.14 mmo/L), with a mean difference of (0.49), and percentage of change of 55.05 %. This change was found to be highly significant.

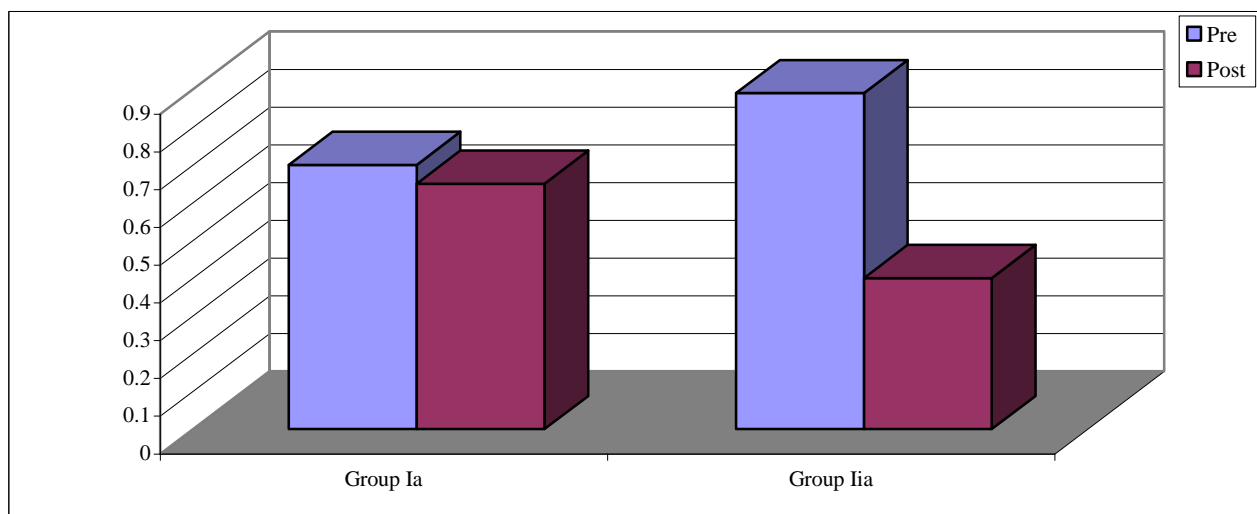
**Table (2): Shows GSH mean values of group Ia and Group IIa before and after acute exercise.**

Group	Parameters	Before Exercise	After Exercise	Mean Difference	%Change	P Value
Group IA		0.7±0.14	0.65±0.14	0.05	7.14%	0.01*
Group IIa		0.89±0.27	0.4±0.14	0.49	55.05%	0.0001*

Level of significance at P<0.05

\* = significant

\*\* = Non-significant



**Fig. (2): The mean values of GSH at pre and post exercise for group Ia & group Iia.**

## DISCUSSION

The glutathione (GSH) antioxidant system has been shown to play an important role in the maintenance of good health and disease prevention<sup>4</sup>.

Glutathione status has also been used as a marker of oxidative stress following aerobic exercise. It appears as though alterations in glutathione status are directly related to the intensity of the exercise session<sup>18</sup>.

Therefore, the aim of this study was to investigate the effect of acute exercise of two intensities (moderate and high intensity) on glutathione in smokers versus non smokers to determine the appropriate intensity of aerobic exercise that can be used by smokers aiming to increase glutathione level without subjecting them to more oxidative damage, and to approve hazards of smoking.

In the present study, it was found that reduced glutathione level in the blood was higher in heavy smokers than in non smokers.

This was consistent with other study which stated that, smoking and alcohol consumption were associated with elevated

blood GSH, perhaps as a defense against chronic oxidative stress exposure<sup>21</sup>.

It was found that, the mean GSH levels were significantly higher in non-smokers compared to smokers with COPD and those without COPD<sup>16</sup>.

However, cigarette smoking, irrespective of dose or duration, had negative effects on antioxidant status<sup>8</sup>.

According to the obtained results and statistical analysis, it's found that among the two groups (non smokers, heavy smokers) subjects who performed 30 minutes of acute exercise with moderate intensity at 65% HRM had gained better outcome and the mean value of blood glutathione had increased. This indicate that moderate exercise intensity is better for all especially for smokers as it was followed by increase in blood glutathione level.

The elevated levels of blood glutathione during recovery has the following possible explanations:

- The slowing of oxidant production with the cessation of exercise giving antioxidant defenses an opportunity to return to resting levels.

- During moderate physical exercise, energy is provided by aerobic metabolism with a low production of free radicals and a low request of antioxidant defense such as the oxidation of glutathione<sup>6</sup>.

Also it was found in the present study that all subjects who performed 30 minutes of acute exercise with high intensity at 85% HRM had shown a decrease in the mean value of blood glutathione. This indicate that high intensity exercise is harmful especially for smokers as glutathione reduction was obvious in heavy smokers however the reduction was very little in non smokers .This reduction of reduced glutathione could be used as a marker of oxidative stress, which means that smokers are more liable to oxidative damage as a result of intensive exercise.

The decreased levels of blood glutathione after intensive exercise has the following possible explanation:

- Liver synthesizes GSH from endogenous or dietary amino acids and supplies most of the circulating GSH. During high intensity exercise, hepatic GSH efflux is increased due to the stimulation of elevated blood glucagon and vasopressin levels. This ensures blood GSH homeostasis despite enhanced tissue GSH use . However, blood and muscle GSH content may be decreased eventually during exhaustive exercise when hepatic GSH reserve is diminished and GSH use exceeds GSH uptake<sup>21</sup>.

To sum up, these data indicate that GSH is actively used in blood during intensive exercise and that exercise associated oxidative stress is related to the intensity of the physical exercise.

### Conclusion

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subjects who performed 30 minutes of acute exercise with moderate intensity at 65% HRM had gained better outcome and the mean value of blood glutathione had increased. This indicate that moderate exercise intensity is better for all especially for smokers as it was followed by increase in blood glutathione level.

Also it was found in the present study that all subjects who performed 30 minutes of acute exercise with high intensity at 85 % HRM had shown a decrease in the mean value of blood glutathione. This indicate that high intensity exercise is harmful especially for smokers as glutathione reduction was obvious in heavy smokers more than non smokers.

In summary, aerobic exercise, while increasing oxidative stress levels, can also increase protection from oxidative stress by either activating the antioxidant glutathione or possibly up regulating the concentration of it. For any adaptation to occur, the muscle must be recruited at a sufficient intensity and duration.

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### الملخص العربي

#### تقييم الجلوتاثيون في الشباب المدخنين والغير مدخنين

يعتبر الجلوتاثيون من أهم مضادات الأكسدة بل هو المنظم الرئيسي لعمل معظم مضادات الأكسدة بالجسم ولا يزال تأثير التدخين والتمارين على معدل مضادات الأكسدة يحتاج إلى دراسة . لذلك كان الهدف من هذه الدراسة هو تقييم معدل الجلوتاثيون بعد أداء الشباب المدخنين والغير مدخنين لنوع من التمرينات . أجريت الدراسة على ستين شاباً تم تقسيمهم إلى مجموعتين متساويتين:مدخنين و غير مدخنين وقسمت بعد ذلك كل مجموعة إلى مجموعتين متساويتين إحداهما أدت التمرين الواحد بشدة متوسطة والأخرى أدت التمرين الواحد بشدة عالية وتم تقييم الجلوتاثيون بالدم قبل التمرين وبعده مباشرة . أظهرت النتائج أن التمرين متوسط الشدة يرفع معدل الجلوتاثيون بالدم بعد التمرين مباشرة في المجموعتين بينما التمرين العالي الشدة يقلل معدل الجلوتاثيون وكان الانخفاض واضحاً أكثر في مجموعة المدخنين بالمقارنة بغير المدخنين. لذلك ينصح بأداء التمرينات الهوائية بشدة متوسطة وخاصة المدخنين الذين لم يستطيعوا الإقلاع عن التدخين .