# Potential roles for vitamins E and C in combination in modulating exhaustive swimming and high altitude-associated lung injury in rats

*Fahaid H. Al-Hashem,* MBBS, PhD.


## ABSTRACT

**الأهداف:** تقييم دور فيتامين ج وهـ في حماية الرئة من الإصابات المحتملة من جهد السباحة العنيف وجهد المرتفعات العالية.

**الطريقة:** أجريت هذه الدراسة خلال الفترة من يناير إلى مارس 2011م وذلك على فئران تجارب وُلدت وعاشت إما في منطقه منخفضة العلو عن مستوى البحر (الرياض)، أو في منطقه مرتفعة العلو عن سطح البحر (أبها). لقد تم تقسيم فئران التجارب في كل منطقه علو إلى ثلاث مجموعات: المجموعة الأولى مجموعه الشاهد، والمجموعة الثانية مجموعه تعرضت لجهد سباحه شديد، بينما تمت معالجة المجموعة الثالثة بفيتامين ج وهـ وذلك قبل تعرضها لجهد السباحة الشديد. بعد نهاية التجارب تم قتل الفئران بالطريقة الرحيمة، ومن ثم تم تشريح الرئة وقياس مستويات القلوتوثايون، والتيبارس، وسوبر أكسيد، وكذلك الكاتليز.

**النتائج:** أشارت نتائج الدراسة إلى أن مستوى التيبارس في فئران مجموعة الشاهد قد كان مرتفع، بينما كان هناك انخفاض في مستويات القلتوثايون والسوبر أكسيد وذلك في رئة فئران المناطق العالية. لقد أدى جهد السباحة إلى ارتفاع واضح من الناحية الإحصائية في مستوى التيبارس، وانخفاض في مستويات السوبر أكسيد والكاتليز وذلك في كلي المنطقتين، بينما انخفض مستوى القلوتوثايون في المناطق المرتفعة فقط. وأدى علاج الحيوانات بمركب فيتامين ج وفيتامين هـ قبل جهد السباحة إلى عودة المعايير السابقة إلى وضعها الطبيعي.

**خاتمة:** أظهرت هذه الدراسة بأن مركبات فيتامين ج وهـ يمكن أن تحمي الرئة من الإصابات المحتملة الناتجة عن جهد الرياضة والمرتفعات العالية.

**Objectives:** To evaluate and compare the potential role of vitamins E and C in protecting against acute swimming induced lung damage at different altitudes.

**Methods:** The study was carried out between January and March 2011. Eighteen male rats were bred and reared at either high altitude in Abha city or low altitude in Riyadh city, KSA. The rats were divided into 3 groups: 1) non-stress control, 2) forced swimming stressed, and 3) vitamin E and C pretreated stressed. At the end of the procedure, lung tissue levels of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were determined.

**Results:** In control rats, the baseline levels of TBARS were significantly increased and the baseline levels of both GSH and SOD were significantly decreased in the lungs of animals at high altitude compared with those at low altitude. Acute forced swimming resulted a significant increase in TBARS levels and a significant decrease in activities of SOD and CAT in the lungs in both altitude areas, and resulted in a significant decrease in GSH levels at high altitude rats only as compared with the resting state. Supplementation of vitamins E and C in combination effectively ameliorated all the parameters measured at both altitudes.

**Conclusion:** Our novel observations suggest that supplementation of vitamins E and C could be beneficial against exhaustive swimming- and high altitude-associated lung injury.


*Saudi Med J 2012; Vol. 33 (4): 367-374*

*From Department of Physiology, College of Medicine, King Khalid University, Abha, Kingdom of Saudi Arabia.*

*Received 5th December 2011. Accepted 12th March 2012.*

*Address correspondence and reprint request to: Dr. Fahaid H. Al-Hashem, Department of Physiology, College of Medicine, King Khalid University, Abha 61421, PO Box 641, Kingdom of Saudi Arabia. Tel. +966 (7) 2417879. Fax. +966 (7) 2418364. E-mail: fahaid999@yahoo.com*

**Disclosure**. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

Stress has been shown to induce a variety of biochemical and physiological changes in the body. To study the stress caused by exhaustive exercise, forced swimming in small laboratory animals has been a widely used tool.[1] It has been suggested that exhaustion from forced swimming may lead to structural damage and inflammatory responses within muscles and other organs of the body, primarily due to the overproduction of reactive oxygen species (ROS).[1] Under normal conditions, cells have enzymes and antioxidants to combat the harmful effects of ROS. However, in stressful conditions such as exhaustive physical exercise, oxygen flux to active skeletal muscles increases, causing enhanced production of ROS and free radicals.[2] This excess of pro-oxidants induces a state of oxidative stress, which has been implicated in a variety of different disease states, as well as the aging process.[3] Comparable to anaerobic exercise, exposure to and living in high altitudes is also considered a stress. High altitude environments are characterized by low partial pressures of oxygen ($PO_2$) relative to lower altitude environments at similar latitudes. This reduced concentration of oxygen in the air leads to decreased oxygen levels in the blood, which can result in generalized or tissue hypoxia and severe physiological stress. In short or long term hypoxia, changes indicative of increased oxidative stress have been observed in blood, urine, and other tissue samples of laboratory rats.[4] In hypoxic conditions, humans may experience similar stress.[5]

Different organs and tissues may have different susceptibilities to oxidative stress because of metabolic differences.[6] Moreover, ROS production may elicit different responses depending on the type of organ tissue and its level of endogenous antioxidants.[7] The lungs and pulmonary vasculature are potentially at high risk for injury mediated by ROS-derived free radicals, because lung tissue contains a high number of polyunsaturated fatty acids, which are substrates for lipid peroxidation. As a result, lungs are more susceptible to oxidative stress-induced injury than any other organs in the body.[7] In normal individuals, the level of lipid peroxidation in the lungs is very low due to the powerful antioxidant system, which includes both enzymatic and non-enzymatic defenses. However, under certain conditions, such as exhaustive exercise or hypoxia, the antioxidant reserve in the lungs can be overwhelmed and depleted, resulting in peroxidation of membrane lipids and tissue injury.[7]

Despite the existence of both animal and human studies on the effect of acute physical exercise or high altitude on biochemical changes and on oxidative stress and lipid metabolism, most of these studies were carried out separately or individually and generated contradictory results, possibly due to the different protocols of exercise intensity used and/or inappropriate geographic areas chosen. Through our search in literature, we were unable to find any study in the literature that investigated exhaustive swimming exercise in rats native to low or high altitude. Hence, the present study aimed: 1) to measure and compare the parameters of oxidative stress in the lungs of wistar rats from the same strain and same genetic pool native to low (600 m) and high (2270 m) altitudes before and after exhaustive swimming exercise; 2) to evaluate the protective effect of combined vitamins E and C pre administration against stressful lung damage which may be produced from both exhaustive exercise and high altitude.

## Methods.

*Selection of geographic areas.* The study was carried out between January and March 2011 in areas of high and low altitude in different regions of the Kingdom of Saudi Arabia. The high altitude area was in Abha city, which is located in the Aseer Mountains and has an altitude of 2270 m above sea level. The selected low altitude area was Riyadh, the capital of Saudi Arabia, which is located in the center of Saudi Arabia that rises around 600 m above sea level. Environmental data on these areas are shown in **Table 1**.

*Drugs.* Vitamins C and E were purchased from BDH Chemicals Ltd, Poole, Dorset, UK.

*Animals.* Adult male Wistar rats (N=36) weighing approximately 250 g each and aged 6 months were used for the experimental procedure. Eighteen rats bred and maintained in the animal house at the College of Pharmacy, King Saud University in Riyadh city were used for the low altitude native rats experiments. In parallel, the same number of rats were used for the high altitude native rats experiments that were bred and maintained in the animal house at King Khalid University in Abha city. All rats were from the same lineage and were born in each area (they were from the tenth generation and the parents lived in each area for 6 months prior). All rats were housed under the same laboratory conditions and fed the same diet. All rat studies were performed during winter time according to protocols overseen by the Ethical Committee in the Department of Physiology at the King Khalid University Medical School, Abha, Kingdom of Saudi Arabia and were performed in agreement with the Principles of Laboratory Animal Care, advocated by the National Society of Medical Research and the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health.[8]

**Table 1 -** General geographic and meteorological information of Riyadh (low altitude) and Abha (high altitude), Kingdom of Saudi Arabia.

| Variable | Riyadh | Abha |
|---|---|---|
| Coordinates (latitudes) | 24.64083; 24° 38' 27 N | 18.21639; 18° 12' 59 N |
| Coordinates (longitude) | 46.77278; 46° 46' 22 E | 42.50528; 042° 30' 19 E |
| Altitude (meters) | 600 | 2270 |
| Barometric pressure (mm Hg) | 711 | 590 |
| Atmospheric $O_2$ tension (mm Hg) | 145 | 120 |
| Relative humidity (%) | 15-50 | 20-30 |
| Summer temperature (shade) (°C) | 24-45 | 16-28 |
| Winter temperature (shade) (°C) | 10-25 | 5-15 |

***Experimental design.*** In each of the altitude areas, rats were divided equally into 3 groups, (each n=6); control group (non-stressed and untreated); stress group A (received normal saline); and stress group B, received a single intra-peritoneal dose of 25 mg/kg of vitamin E and 20 mg/kg of Vitamin C orally,[9,10] one hour before the beginning of the experimental procedure. All rats were housed under the same conditions and handled and treated in a similar manner. Stress groups (A and B) rats were exposed to acute forced exhaustive swimming stress for a duration of 2.5 hours in glass tanks (length 100 cm, width 40 cm, depth 60 cm) containing tap water maintained at a temperature of 32°C. The depth of water in the tank was 30 cm.[11]

***Tissue specimen collection and homogenate preparation.*** The animals were humanely sacrificed by decapitation at the end of the experiment. The lungs were immediately collected, washed in ice-cold, isotonic saline and blotted individually on ash-free filter paper. The tissue specimens were then homogenized separately in 0.1 M Tris-HCL buffer of pH 7.4, using a Potter-Elvejham homogenizer at 4°C with a diluting factor of 4. The crude tissue homogenate was then centrifuged at a speed of 9000 rpm for 15 minutes in a refrigerated centrifuge, the supernatant was collected and stored at -20°C before analysis.

***Thiobarbituric acid reactive substances assay.*** Lipid peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS), was assayed by the method described by Ohkawa et al.[12] In brief, a reaction mixture containing 0.1 mL of tissue homogenate, 0.2 mL of sodium dodecyl sulfate, 1.5 mL of acetic acid with pH of 3.5 (20% acetic acid was pre-adjusted with one sodium hydroxide to desired pH) and 1.5 mL of aqueous solution of TBA was prepared. The mixture was made up to 4 mL with distilled water and heated at 95°C for one hour in a hot water bath. After cooling, one mL of distilled water and 5 mL of a mixture of n-butanol and pyridine (15:1) were added and the mixture was shaken vigorously on a vortex mixer. The tubes were then centrifuged at 3000 rpm for 10 minutes. The absorbance of the upper organic layer was read at 532 nm. The values were expressed as mM/100 g of tissues.

***Endogenous antioxidant activity assessment.*** Superoxide dismutase (SOD) and reduced glutathione (GSH) levels in the lung tissue homogenate were measured using commercials kits from Randox Laboratories Ltd, London, UK. The GSH was expressed in mmol/L while SOD activity was expressed in U/mg of tissue. One unit of SOD was defined as the amount which caused 50% inhibition of the rate of reduction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) under the conditions of the assy. Catalase activity (CAT) was determined using the commercial kit K773-100 from Biovision Inc, New York, USA. The CAT was measured as U/mL with one unit of CAT being the amount that decomposes 1.0 μmol of hydrogen hydroxide per min at a pH of 4.5 and 25°C.

***Calculations.*** 1. Percent change from baseline reading (control group) for any parameter measured was calculated for each group as follows: % change= (mean value of stress non treated group - mean value of control group) / mean value of control group.

2. Percent improvement in each measured parameter with vitamin administration was calculated as follows: % improvement = (mean value of vitamin pretreated stress group - mean value of stress group)/mean value of stress non treated group - mean value of control group.

***Statistical analysis.*** At different stages, the data were compiled and fed in to a computer. The Statistical Package for the Social Sciences, version 10 was used for standard statistical analysis. Data are given as the mean±SD. Student's t-test was used to determine the difference between groups, and $p<0.05$ denoted statistical significance.

**Results.** *Altitude-dependent antioxidant activity and lipid metabolism.* In non-stress conditions, the animals in the high altitude area had significantly higher baseline levels of TBARS (a lipid peroxidation indicator) ($p$=0.001) and significantly lower baseline levels of GSH and SOD ($p$=0.001) in pulmonary tissues than those in the low altitude area (**Figures 1-3**). The CAT activity in the high altitude control group was slightly reduced in comparison to the low altitude group, but the difference was not significant ($p$=0.142) (**Figure 4**).

*Stress-induced changes in lipid peroxidation and antioxidant activities at both altitudes.* At low altitude, the exhaustive exercise stress resulted in a significant elevation in the levels of TBARS (0.65±0.071 versus 0.401±0.018 mM/100 g, $p$=0.001) and a significant decrease in the activities of SOD (6.63±0.52 versus 10.45±0.63 U/mg, $p$=0.001) and CAT (3.99±0.40 versus 4.64±0.32 U/ml, $p$=0.004) with no effect on the levels of GSH ($p$=0.569) in the lung, as compared with the non-stress control (**Figures 1-4**). At high altitude, the levels of TBARS were significantly elevated (0.472±0.018 to 0.77±0.020 mM/100 g, $p$=0.001) but the levels of GSH (32.33±2.69 versus 45.64±3.21 mmol/L) and activities of SOD (2.10±0.27 versus 6.10±0.38 U/mg) and CAT (2.83±0 .83 versus 4.13±0.19 U/ml) were significantly decreased ($p$=0.001) in the lung in the stressed animals as compared with the control animals (**Figures 1-4**). The stress-induced percent changes in levels of TBARS and GSH and the activities of SOD and CAT for animals in the low and high altitude areas were presented in **Table 2** ($p$=0.01).

*Vitamin supplementation-conferred protection against stress-induced lung damage.* At low altitude, co-supplementation of vitamins E and C to rats prior to exhaustive swimming did not significantly affect GSH levels ($p$=0.435, **Figure 2**). However, it significantly decreased TBARS levels (from 0.65±0.071 to 0.37±0.022 mM/100 g) and increased the activities of SOD ($p$=0.040) (from 6.63±0.52 to 10.28±0.24 U/mg) and CAT ($p$=0.001) (from 3.99±0.40 to 4.63±0.16 U/mL) in the lung as compared with non-supplementation of vitamins ($p$=0.001, **Figures 1, 3, 4**). The percent improvement in the levels of TBARS was 113.8% and activities of SOD was 95.1% and CAT was 98.5% with vitamin treatment (**Table 2**).

At high altitude, vitamin E and C co-administration prior to exercise stress resulted in a significant reduction ($p$=0.001) in the level of TBARS (from 0.77±0.020 to 0.425±0.019 mM/100 g) and significant enhancements ($p$=0.001) in GSH levels (from 32.33±2.69 to 43.99±1.21 mmol/L) and SOD (from 2.10±0.27 to 6.63±0.147 U/mg) and CAT activities (from 2.83±0.83 to 4.18±0.29 U/mL) as compared to the non-treated

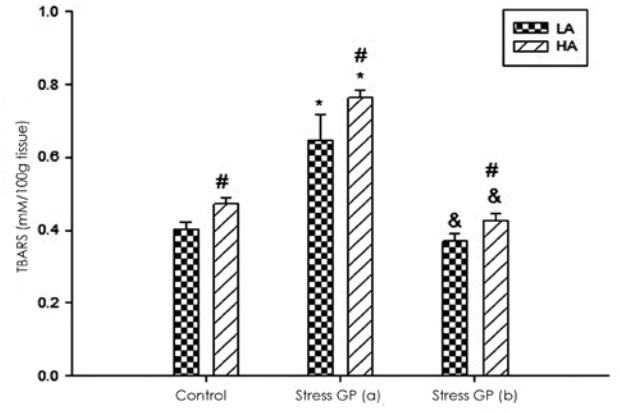


**Figure 1 -** Pulmonary levels of thiobarbituric acid reactive substances (TBARS) in the indicated groups of rats at low altitude (LA) and high altitudes (HA). Control: resting state; stress group A [GP(a)]: rats under forced swimming exercise; and stress group B [(GP(b)]: rats received vitamin supplementation prior to forced swimming exercise. *significantly different when compared to its control group; &significantly different when compared to the stress group within the same altitude area; #Significantly different when the comparison was made between the 2 altitude areas. Values were considered significantly different at $p$-value <0.01.

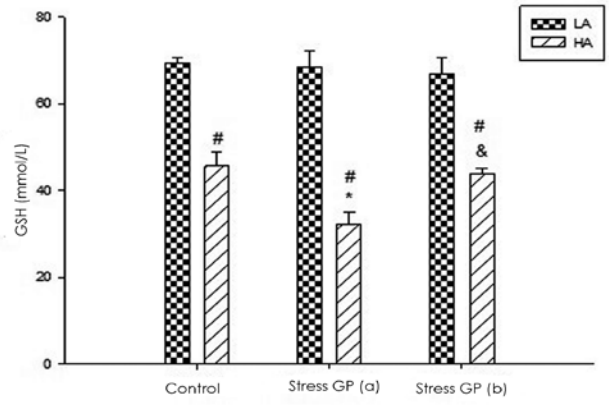


**Figure 2 -** Pulmonary levels of reduced glutathione (GSH) in the indicated groups of rats at low altitude (LA) and high altitudes (HA). Control: resting state; Stress group A [GP (a)]: rats under forced swimming exercise; and Stress group B [GP (b)]: rats received vitamin supplementation prior to forced swimming exercise. Values are expressed as Mean ± SD. *,&significantly different when compared to its control group. &significantly different when compared to the stress group within the same altitude area. #Significantly different when the comparison was made between 2 altitude areas. Values were considered significantly different at $p$-value <0.01.

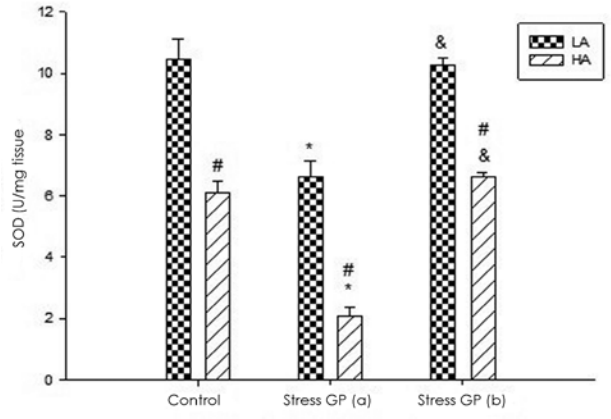


**Figure 3 -** Pulmonary activities of superoxide dismutase (SOD) in the indicated groups of rats at low altitude (LA) and high altitudes (HA). Control: resting state; Stress group A [GP (a)]: rats under forced swimming exercise; and Stress group B [GP (b)]: rats received vitamin supplementation prior to forced swimming exercise. *significantly different when compared to its control group; &significantly different when compared to the stress group within the same altitude area; #Significantly different when the comparison was made between 2 altitude areas. Values are expressed as mean±SD. Values were considered significantly different at *p*-value <0.01.

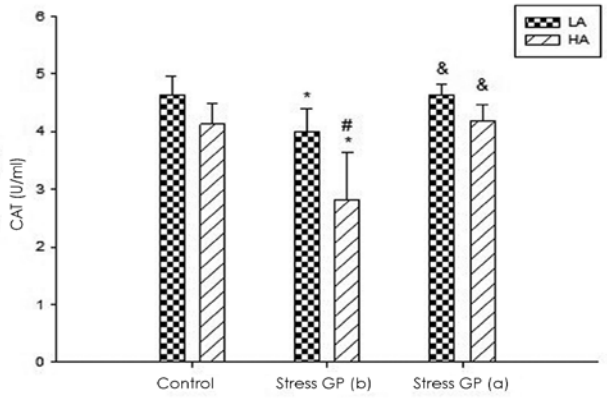


**Figure 4 -** Pulmonary activities of catalase (CAT) in the indicated groups of rats at low altitude (LA) and high altitude (HA). Control: resting state; Stress group A [GP (a)]: rats under forced swimming exercise; and Stress group B [GP (b)]: rats received vitamin supplementation prior to forced swimming exercise. *significantly different when compared to its control group; &significantly different when compared to the stress group within the same altitude area; #Significantly different when the comparison was made between 2 altitude areas. Values are expressed as mean±SD. Values were considered significantly different at *p*-value <0.01.

**Table 2 -** Percent of changes and percent of improvement of TBARS, reduced GSH, SOD, and CAT measured at both low and high altitudes. The definition and calculation of both percent of changes and percent of improvement were described in the text.

| Variable | Low altitude | | High altitude | |
|---|---|---|---|---|
| | % change | % improvement | % change | % improvement |
| TBARS | + 60.6 | 113.8 | + 61.6 | 116.04 |
| Reduced glutathione | -1.5 | - | -29.12 | 87.9 |
| Superoxide dismutase | -36.7 | 95.1 | -65.6 | 112.5 |
| Catalase | -14.0 | 98.5 | -31.5 | 103.8 |

TBARS - thiobarbituric acid reactive substances. Percent change from baseline reading (control group) for any parameter measured was calculated for each group as follows: % change = (mean value of stress non treated group - mean value of control group) / mean value of control group.Percent improvement in each measured parameter with vitamin administration was calculated as follows: % improvement = (mean value of vitamin pretreated stress group - mean value of stress group)/mean value of stress non treated group - mean value of control group.

exercise group (**Figures 1-4**). The percent of improvement was 116.04% for TBARS, 87.9% for GSH, 112.5% for SOD and 103.8% for CAT (**Table 2**).

At both altitudes, the levels of TBARS and GSH and activities of SOD and CAT in animals that were treated with vitamins E and C prior to exhaustive exercise stress were all close to the baseline readings in the control animals ($p$=0.002, **Figures 1-4**).

**Discussion.** Several previous studies have investigated the effect of different altitudes on organ functions. However, most of these studies utilized high altitude simulators or hypobaric chambers in laboratories,[13,14] rather than natural environments of different altitudes, due to geographic limitations and technical difficulties. In this study, we took advantage of the unique geographic characteristics within Saudi Arabia and created rats "native" respectively to low and high altitude environments in the same country by breeding and maintaining them at the 2 different altitudes, and compared side-by-side the stressful effect of exhaustive exercise and the protective effect of vitamins E and C on the lung function in these animals in the low and high altitude areas.

We observed that at the baseline levels, TBARS (lipids peroxidation marker) were significantly higher and levels of GSH and activities of SOD were significantly lower in pulmonary tissues at the high altitude area than in the low altitude area, indicating different basal states of lipid peroxidation and anti-oxidation in acclimated animals in the low and high altitude environments. The elevation in TBARS levels at high altitude might be due to generation of oxygen free radicals under hypoxia (reductive stress)[15] or/and due to hypoxia-induced glucocorticoid secretion.[16] The decrease in GSH levels and SOD enzyme activities in high altitudes might be due to the utilization of GSH and SOD in protection against the free radicals. In addition, free radical species themselves may directly decrease GSH levels and inhibit SOD activities by converting Cu+2 to Cu+1,[17] thus modulating the activity of the hydroxyl promoter via Haber-Weis reaction.[18] Also, it is well documented that the hypoxia stimulates the expression of the steroidogenic acute regulatory protein and enhances the secretion of glucocorticoids.[16] The activities of the antioxidant enzymes (systolic SOD and CAT) have been observed to be reduced in the brain of rats treated with glucocorticoids.[19]

We also observed that exhaustive swimming exercise resulted in a significant increase in the levels of TBARS and significant decreases in the activities of SOD and CAT in the lung in both low and high altitude areas and a significant decrease in GSH levels at high altitude but not low altitude areas. Overall, these observations suggest that exhaustive exercise swimming resulted in generation of ROS in both low and high altitude areas, but at higher levels in the hypoxic high altitude environment. It is known that large quantities of ATP are used for muscular contraction during exercise, producing ADP and AMP purine nucleotides, which are normally regenerated back to ATP through oxidative phosphorylation.[20] The combined outcome of high rates of ATP utilization and the lack of oxygen for regeneration of ATP during excessive exercise is the accumulation of xanthine and hypoxanthine and so the activation of xanthine oxidase and producing superoxide.[20] This process is most likely exacerbated in hypoxic conditions, which leads to an insufficient supply of ATP and malfunction of the ATP-dependent calcium pumps of the muscle cell and thus calcium accumulation which in turn highly activates xanthine oxidase, leading to more superoxide production.[20] This reaction would be most likely to occur in a metabolically compromised muscle when working under hypoxic conditions where ATP is rapidly depleted[20] and may even contribute to impaired physiologic function in tissues other than muscle, such as the lung.[7,20] Another factor related to exercise and hypoxia that can augment the production of free radicals is autooxidation of catecholamines.[20,21] Catecholamine levels in the blood increase with both exercise and acute or chronic hypoxia;[22] in both men and women.[23,24] Autoxidation of monoamines, such as dopamine, produces both superoxide anion (+O2-) and hydrogen peroxide ($H_2O_2$).[25] One limitation in our current study is that we did not measure arterial haemoglobin oxygen saturation ($SaO_2$%) in both rested and exercised animals at the different altitudes to sustain the hypothesis of generalized tissue hypoxia and severe physiological oxidative stress with swimming at high altitude.

Several studies have demonstrated that exercise-induced oxidation stress in various tissues and blood of experimental animals and humans might be prevented by antioxidant interventions.[26,27] In this study, we evaluated the protective effect of vitamins E and C in combination on the swimming exercise stress-induced lung damage in rats at low and low altitude areas as a combination of both vitamins has been shown to have a better antioxidant effect than either alone.[28] Oxidants generated near cellular membranes can oxidize vitamin E forming a tocopheroxyl radical. Vitamin C may reduce the vitamin E radical, thereby regenerating vitamin E.[29] This reaction forms the semi-dihydroascorbate (vitamin C radical), which in turn is reduced by a GSH.[30] Our results demonstrated that supplementation of vitamins E and C in combination to animals prior to forced swimming exercise robustly attenuated the exhaustive swimming-induced lung damage and almost brought TBARS and GSH levels and SOD and CAT activities back to the normal baseline states at both low and high altitude areas, although the effect was more profound at in high altitude area. Unfortunately, we did not measure the vitamins levels in the lung tissues or in the serum of rats after treatment, but at this stage we could suggest that the differential effects in low and high altitude areas might be due to a higher absorption of vitamins E and C in rats performing exercise under hypoxic conditions, as suggested in a previous study,[31] in which intravenous administration of alpha-tocopherol and alpha-tocopheryl acetate separately resulted in high concentrations of alpha-tocopherol in the liver and alpha-tocopheryl acetate in the lung respectively in rabbits. Recommendations for future experiments to study the oxidative stress in other tissue like liver, kidney, and brain of these rats, and measuring the levels

of these vitamins in the lungs and other tissues after the same treatment route or using a prolonged treatments period after acute exhaustive swimming exercise in both altitudes is highly advisable. Biochemical and immunological responses after swimming exercise at both altitudes could be also be beneficial to sustain the effect of exercise and the protective effect of these vitamins at both altitudes.

In conclusion, exhaustive swimming exercise induced oxidative stress to the lungs of native rats in both low and high altitude regions. Supplementation of vitamins E and C in combination effectively ameliorated the major biochemical and functional parameters indicative of lung injury induced by exhaustive exercise in both altitude conditions. These novel observations suggest that vitamins E and C may be helpful in protecting against hypoxia-associated and exhaustive exercise-induced organ damage or even sudden death in high altitude environments or during excessive exercise in untrained individuals.

**Acknowledgment.** *The author would like to thank Mr. Mahmoud Al-Khateeb from the College of Medicine, King Khalid University for his contribution to the current work by helping in the biochemical measurements. Also thanks to Dr. Naser Al-Dagri and his colleagues from the College of Science, King Saud University for allowing us to perform the experiment on low altitude animals at their laboratory.*

## References

1. Özda S. Effects of short-term exercise on heart-rate blood pressure oxidative stress araoxonase activity and lipid hydroperoxide. ***African Journal of Pharmacy and Pharmacology*** 2010; 4: 658-661.
2. Furlan G, Balduino JR, Estrela HFJ, Dias MC, Cavaglieri CR, Ruggiero AC, et al. Oat rotection against the oxidative stress induced in rats by exercise. ***Journal of Food Biochemistry*** 2010; 34: 611-624.
3. Atalay M, Laaksonen DE. Diabetes, oxidative stress and physical exercise. ***J Sports Sci Med*** 2002; 1: 1-14.
4. Hoshikawa Y, Ono S, Suzuki S, Tanita T, Chida M, Song C, et al. Generation of oxidative stress contributes to the development of pulmonary hypertension induced by hypoxia. ***J Appl Physiol*** 2001; 90: 1299-1306.
5. Davison GW, Morgan RM, Hiscock N, Garcia JM, Grace F, Boisseau N, et al. Manipulation of systemic oxygen flux by acute exercise and normobaric hypoxia: implications for reactive oxygen species generation. ***Clin Sci (Lond)*** 2006; 110: 133-141.
6. Santos RX, Cardoso S, Silva S, Correia S, Carvalho C, Crisóstomo J, et al. Food deprivation promotes oxidative imbalance in rat brain. ***J Food Sci*** 2009; 74: H8-H14.
7. Domenicali M, Caraceni P, Vendemiale G, Grattagliano I, Nardo B, Dall'Agata M, et al. Food deprivation exacerbates mitochondrial oxidative stress in rat liver exposed to ischemia-reperfusion injury. ***J Nutrition*** 2001; 131: 105-110.
8. National Institutes of Health-Institute for Laboratory Animal Research. Guide for the Care and Use of Laboratory Animals. Published by the national academies press: Washington, D.C. 2010. Available from URL: http://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals_prepub.pdf.
9. Lee SP, Mar GY, Ng LT. Effects of tocotrienol-rich fraction on exercise endurance capacity and oxidative stress in forced swimming rats. ***Eur J Appl Physiol*** 2009; 107: 587-95.
10. Zaidi SM, Banu N. Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. ***Clin Chim Acta*** 2004; 340: 229-233.
11. Nagaraja HS, Jeganathan PS. Forced swimming stress induced alterations in ingestive behavior in rats. ***Indian J Physiol Pharmacol*** 2003; 47: 94-100.
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. ***Anal Biochem*** 1979; 95: 351-358.
13. Coates G, Gray G, Mansell A, Nahmias C, Powles A, Sutton J, et al. Changes in lung volume, lung density, and distribution of ventilation during hypobaric decompression. ***J Appl Physiol*** 1979; 46: 752-755.
14. Welsh CH, Wagner PD, Reeves JT, Lynch D, Cink TM, Armstrong J, et al. Operation Everest. II: Spirometric and radiographic changes in acclimatized humans at simulated high altitudes. ***Am Rev Respir Dis*** 1993; 147: 1239-1244.
15. Mayr M, Sidibe A, Zampetaki A. The paradox of hypoxic and oxidative stress in atherosclerosis. ***J Am Coll Cardiol*** 2008; 51: 1266-1267.
16. Raff H, Hong JJ, Oaks MK, Widmaier EP. Adrenocortical responses to ACTH in neonatal rats: effect of hypoxia from birth on corticosterone, StAR, and PBR. ***Am J Physiol Regul Integr Comp Physiol*** 2003; 284: R78-R85, 502-509.
17. Hodgson EK, Fridovich I. The interaction of bovine erythrocyte superoxide dismutase with hydrogen peroxide: inactivation of the enzyme. ***Biochemistry*** 1975; 14: 5294-5299.
18. Tidball JG, Wehling-Henricks M. The role of free radicals in the pathophysiology of muscular dystrophy. ***J Appl Physiol*** 2007; 102: 1677-1686.
19. McIntosh LJ, Cortopassi KM, Sapolsky RM. Glucocorticoids may alter antioxidant enzyme capacity in the brain: kainic acid studies. ***Brain Res*** 1998; 791: 215-222.
20. Hellsten Y. The role of xanthine oxidase in exercise. In: Sen CK, Packer L, Hanninen O, editors. Exercise and Oxygen Toxicity. Amsterdam: Elsevier Science; 1994. p. 211-234.
21. Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. ***Br J Nutr*** 2001; 85: S67-S74.
22. Mazzeo RS, Bender PR, Brooks GA, Butterfield GE, Groves BM, Sutton JR, et al. Arterial catecholamine responses during exercise with acute and chronic high-altitude exposure. ***Am J Physiol*** 1991; 261: E419-E424.
23. Mazzeo RS, Child A, Butterfield GE, Mawson JT, Zamudio S, Moore LG. Catecholamine response during 12 days of high-altitude exposure (4,300 m) in women. ***J Appl Physiol*** 1998; 84: 1151-1157.
24. Sandoval DA, Matt KS. Gender differences in the endocrine and metabolic responses to hypoxic exercise. ***J Appl Physiol*** 2002; 92: 504-512.
25. Rouleau JL, Pitt B, Dhalla NS, Dhalla KS, Swedberg K, Hansen MS, et al. Prognostic importance of the oxidized product of catecholamines, adrenolutin, in patients with severe heart failure. ***Am Heart J*** 2003; 145: 926-932.

26. Ramel A, Wagner KH, Elmadfa I. Plasma antioxidants and lipid oxidation after submaximal resistance exercise in men. ***Eur J Nutr*** 2004; 43: 2-6.
27. Leeuwenburgh C, Heinecke JW. Oxidative stress and antioxidants in exercise. ***Curr Med Chem*** 2001; 8: 829-838.
28. Naziroğlu M, Kilinç F, Uğuz AC, Celik O, Bal R, Butterworth PJ, et al. Oral vitamin C and E combination modulates blood lipid peroxidation and antioxidant vitamin levels in maximal exercising basketball players. ***Cell Biochem Funct*** 2010; 28: 300-305.
29. Beyer RE. The role of ascorbate in antioxidant protection of biomembranes interaction with Vitamin-E and Coenzyme-Q. ***J Bioenerg Biomembr*** 1994; 26: 349-358.
30. Rokitzki L, Logemann E, Sagredos AN, Murphy M, Wetzel-R oth W, Keul J. Lipid peroxidation and antioxidative vitamins under extreme endurance stress. ***Acta Physiol Scand*** 1994; 151: 149-158.
31. Knight ME, Roberts RJ. Tissue vitamin E levels in newborn rabbits after pharmacologic dosing. Influence of dose, dosage form, and route of administration. ***Dev Pharmacol Ther*** 1985; 8: 96-106.

## Related Articles

Meng QT, Xia ZY, Luo T, Cao C, Zhao B, Wu Y, et al. Ligustrazine attenuates acute lung injury induced by blunt chest trauma. ***Saudi Med J*** 2012; 33: 139-145.

Tutuncu EE, Gurbuz Y, Sencan I, Ozturk B, Senturk GC, Kilic AU. Device-associated infection rates and bacterial resistance in the intensive care units of a Turkish referral hospital. ***Saudi Med J*** 2011; 32: 489-494.

Attar SM. Adverse effects of low dose methotrexate in rheumatoid arthritis patients. *A hospital-based study.* ***Saudi Med J*** 2010; 30: 909-915.

Sigari N, Mohammadi S. Anthracosis and anthracofibrosis. ***Saudi Med J*** 2009; 30: 1063-1066.