

*Full Length Research Paper*

# **Co-administration of Vitamins E and C protects against stress-induced hepatorenal oxidative damage and effectively improves lipid profile at both low and high altitude**

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The aim of this study was to evaluate the effect of co-administration of vitamins E and C on exhaustive exercise induced-stress in regards to hepatorenal function in rats native to low altitude (LA) and high altitude (HA). In both LA and HA areas, native wistar rats of each area were divided into three groups of 6 rats each, which include stress-free control, forced swimming-induced experimental stress and experimental stress plus vitamins E and C treatment. Lipid profile and Liver and kidney functions were assessed in both groups. HA and LA rats exhibit similar baseline levels of liver and kidney function as well as lipid metabolism profiles. However, HA rats showed decreased levels of antioxidant markers with an increased level of lipid peroxidation. Exhaustive swimming exercise induced a significant increase in the liver and kidney function of rats at both altitudes accompanied with a decrease in antioxidants levels. However, the magnitude of change observed in HA rats was more profound. Also at LA, forced swimming exercise resulted in a significant increase in serum total cholesterol (TChol), triacylglycerides (TAG) and high-density lipoprotein cholesterol (HDL). However, in HA rats, forced swimming exercise caused a significant decrease in serum TChol and low-density lipoprotein (LDL), except for HDL levels which were significantly elevated. Pre- and co-administration of vitamins E and C counteracted the induction of liver and/or kidney function by exhaustive exercise, and lowered TChol and LDL levels in rats at either altitude. In conclusion, at native high altitude: kidney and liver function essentially remained stable; response to stress included more profound oxidative damage to liver and kidney tissues as well as augmented deterioration in lipid metabolism compared to low altitude; and combined administration of vitamins E and C protected against observed oxidative stress damage to liver and kidney tissues and preserved lipid metabolism. At low altitude, combined administration of vitamin E and C protected against stress-induced oxidative damage to the liver and kidney and did preserve normal lipid metabolism, except for HDL. These novel findings reveal the pathophysiological changes in the liver function, kidney function and lipid metabolism occurring at high altitude specifically under stress, and demonstrate the efficacy of combined supplementation of vitamins E and C to normalize these changes.

**Key words:** Exercise, oxidative stress, vitamin E, vitamin C, altitude, rats.

## INTRODUCTION

Numerous factors cause stress, including exhaustive exercise and high altitude-associated hypoxia. It is well known that forced exercise stress induces a variety of physiological and biochemical changes, which are dependent not only on the exercise duration and intensity but also on the physical fitness and training level of the individual. Therefore, exhaustion significantly compromises the benefits of physical exercise. It is suggested that exercise exhaustion, especially when it occurs sporadically, may lead to structural damage or altered inflammatory response within the muscles and other organs of the body, primarily due to the generation of reactive oxygen species (ROS) (Mackinnon, 2000). Under an exhaustive condition, cell's utilization of oxygen increases 10 to 15- folds (Finaud and Filare, 2006) and free radicals may be produced in excess of the body's natural defense, leading to oxidative stress in different tissues (Ji, 1996; Finaud and Filare, 2006).

Similar to anaerobic physical exercise, exposure to living at high altitude is considered a stressor. Environments at various high altitudes are characterized by lower partial pressure of oxygen ( $PO_2$ ). Since the reduced  $PO_2$  of inspired air will typically result in a concomitant reduction in the oxygen ( $O_2$ ) saturation of arterial blood resulting in tissue hypoxia and consequently pathophysiological changes. Oxidative stress is present during work at moderate altitude as well as high altitude and causes tissue damage (Schmidt et al., 2002; Joanny et al., 2001). In recent decades, the effect of different types of stress on lipid metabolism has attracted substantial research attention. In response to physical stress following a prolonged and intermittent exercise, plasma triacylglycerol levels decrease while plasma levels of high-density lipoprotein (HDL) and cholesterol increase (Sasaki et al., 1988; Miyashita et al., 2006). It has also been reported that lipid metabolism is altered in humans on exposure to high altitude (Ferezou et al., 1988; Young et al., 1989; Savourey et al., 1998). Despite the existence of both animal and human studies on the effect of acute physical exercise or high altitude on biochemical changes and of oxidative stress and lipid metabolism, most of these studies were carried out individually; usually they were done on non native animals or humans. Furthermore, most of these studies generated contradictory results possibly due to the different protocols of exercise intensity used and/or inappropriate geographic areas or protocol chosen (such as exposing the animals to simulated hypobaric chambers or high-altitude laboratories). To our knowledge, no study in the literature has investigated the effect of exhaustive swimming exercise in rats native to

high altitude. Hence, the present study aimed at determining the stressful effect of exhaustive swimming exercise on liver function, kidney function and lipid metabolism in Wistar rats from the same lineage and genetic pool born and bred at low (600 m) or high (2800 m) altitudes for six months. Further, we tested the hypothesis that co-administration of vitamins E and C under these conditions exhibit a protective effect.

## MATERIALS AND METHODS

This study was carried out in two geographic areas of different levels (low and high) of altitude within the Kingdom of Saudi Arabia between January and March 2011. The low-altitude area is in Riyadh (600 m above sea levels), the center and capital of Saudi Arabia, where one of the study's locations (King Saud University at Riyadh) is located. The high-altitude area is in Abha region (2200 m above sea level), while the other site of the study locations (the College of Pharmacy of King Saud University at Riyadh) is located. The basic geographical and environmental data for these two areas were collected from the national meteorological organizations and are presented in Table 1.

### Experimental animals

A total number of 36 male Wistar rats were used in this study, and were divided into 2 major groups each of 18 rats classified as follows:

- (a) Low altitude native rats (LA rats), which were bred and maintained in the animal house at King Saud University in Riyadh city, College of Pharmacy.
- (b) High altitude native rats (HA rats) which had same age and same weight were bred and maintained in the animal house at King Khalid University in Abha city.

These two major groups were further divided in each area into subgroups as shown in the experimental procedure. All rats were from the same lineage and were born in each area (they were from the 10<sup>th</sup> generation and the parents lived in each area for 6 months prior). The rats used in this experiment were selected randomly from those bred in each area weighing 60 g. All rats were housed under the same laboratory conditions and fed with the same diet to reach a weight of 250 g. Both groups were maintained in similar polypropylene cages of standard dimensions at a temperature of  $25 \pm 1^\circ\text{C}$ , had a standard 12 h day/night cycle and were housed in groups of 6 rats per cage (50 × 26 × 16 cm). All procedures were approved by the Ethical Committee in the Medical School at the King Khalid University (Abha, 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.

### Experimental groups

In both low and high- altitude area, rats ( $n = 18$ ) were divided into two groups as, non stress control group ( $n = 6$ ) and exercised stress group ( $n = 12$ ). The control group was treated with internet protocol (IP) administration of normal saline and not exposed acute forced exhaustive swimming stress exercise. The stress group was

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**Table 1.** General geographic information of Riyadh (low altitude) and Abha (high altitude) in Saudi Arabia.

Data	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	2200
Barometric pressure (mm Hg)	711	550
Atmospheric O <sub>2</sub> tension (mm Hg)	145	110
Relative humidity (%)	15-50	20-30
Summer temperature (shade) (°C)	24-45	16-28
Winter temperature (shade) (°C)	10-25	5-15

divided into two groups each of 6 rats and they were classified as:

a) Stress group + Vitamins, which were given a single intraperitoneal dose of 25 mg/kg of vitamin E (Lee et al., 2009) and 20 mg/kg of vitamin C orally (Owu et al., 2006) for 1 h before the beginning of the experimental procedure.

b) Stress group, which served as control for the previous group (a) and were given normal saline by the same routes used in the previous group. All rats in all the groups were housed under the same conditions and they were handled and treated in similar way.

#### Stress protocol

At the end of the treatment , experimental procedure begins at 9:00 a.m. All rats in the stress groups (a and b) were exposed to acute forced exhaustive swimming stress each for a duration of 2.5 h. They were forced to swim in glass tanks (length 100 cm, width 40 cm and depth 60 cm) containing tap water maintained at temperature of 32°C. The depth of water in the tank was 30 cm.

#### Blood and tissue sampling

After overnight fasting, rats were anaesthetized using ether. Blood samples were immediately taken from the heart and placed in plain tubes to clot at room temperature. Following centrifugation at 4000 rpm for 10 min, serum was collected and stored at -20°C until analyses. Immediately after blood collection, animals were killed by decapitation. Livers and kidneys were quickly collected, washed in ice-cold isotonic saline and blotted individually on ash free filter paper. The tissues were then homogenized separately in 0.1 mol Tris-HCl buffer, pH 7.4 using a Potter-Elvehjem 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 min in a cold centrifuge and the supernatant was collected and stored at -20°C until analyses.

#### Biochemical analysis

##### Serum biochemical analysis

Serum samples were analyzed for total cholesterol (TChol), triacylglycerol (TAG), low-density lipoprotein (LDL), high-density lipoproteins (HDL), creatine kinase (CK), urea, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and alkaline phosphatase ( ALP). All analyses were performed with commercially kits.

##### Thiobarbituric acid reactive substances (TBARS) assay

Lipid peroxidation in the homogenates of the liver and kidney

tissues was assessed with the thiobarbituric acid reactive substances (TBARS) assay as described previously (Okhawa et al., 1979).

In brief, a reaction mixture containing 0.1 ml of tissue homogenate, 0.2 ml of sodium dodecyl sulfate (SDS), 1.5 ml of acetic acid and 1.5 ml of aqueous solution of TBARS was prepared. After pH adjustment to 3.5 with 1 M sodium hydroxide (NaOH), the mixture was heated at 95°C for 1 h in a water bath. Upon cooling, 1 ml of distilled water and 5 ml of a mixture of n-butanol and pyridine (15:1) were added. Following a vigorous shaking on a vortex, the mixture was centrifuged at 3000 rpm for 10 min. The absorbance of the upper organic layer was read at 532 nm. The values were expressed as mmol/100 g of tissues.

##### Endogenous antioxidant activity assessment

In the liver and kidney homogenates, superoxide dismutase (SOD) and reduced glutathione (GSH) levels, expressed as mmol/L, were measured by using commercial kits from Randox Laboratories Ltd, UK, while catalase activity (CAT) was determined by using a commercial kit from BioVision Inc., USA, as instructed by the manufacturer. CAT activity was expressed as U/ml.

One unit of CAT was the amount of catalase that decomposed 1.0 µmol of H<sub>2</sub>O<sub>2</sub> per min at pH 4.5 at 25°C. SOD activity was expressed as U/mg tissue and one unit of SOD was defined as a 50% inhibition of the rate reduction of 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride (INT) under the conditions of the assay.

##### Statistical analysis

Data, expressed as mean ± SD were analyzed with one way ANOVA using 16.0 (Statistical Package for Statistical Science (SPSS) version 16.0 (Chicago, IL, USA) followed by Tukey's analysis to compare all parameters. Parameters were compared between the groups of each area and between the similar groups of different areas. Differences were considered significant when p-value < 0.05.

## RESULTS

### Base-line levels of liver and kidney function and lipid metabolism profiles at low and high altitudes

The serum levels of the function markers of the liver and kidney in the blood were all similar (P>0.05) between the low (column 2 in Table 2) and high (column 5 in Table 1) altitude environments except that ALT decreased in the

**Table 2.** Serum levels of key hepatic enzymes, urea and creatine kinase (CK) in (Control) Group, (Stress) Group and (Stress + V) Group in rats at low and high altitudes.

Parameter	Low altitude			High altitude		
	Control	Stress	Stress + V	Control	Stress	Stress + V
ALT (U/L)	35.0 ± 2.4	43.0 ± 5.7 <sup>b</sup>	31.1 ± 5.1 <sup>c</sup>	27.97 ± 3.68 <sup>a</sup>	43.86 <sup>b</sup>	30.12 ± 4.10 <sup>c</sup>
ALP (U/L)	196.2 ± 25.8	192.4 ± 19.4	174.8 ± 18.5 <sup>b</sup>	192.6 ± 97.6	200.4 ± 5.41	196.4 ± 8.44
GGT (U/L)	23.8 ± 2.7	23.6 ± 2.7	23.3 ± 1.8	25.96 ± 1.83	34.30 ± 1.24 <sup>b,d</sup>	31.94 ± 1.88 <sup>cb</sup>
AST (U/L)	64.2 ± 2.4	75.4 ± 2.5 <sup>b</sup>	75.8 ± 2.6 <sup>b</sup>	63.27 ± 1.92	83.48 ± 2.68 <sup>b,d</sup>	83.54 ± 3.24 <sup>b</sup>
CK (U/L)	224.0 ± 24.8	454.6 ± 40.8 <sup>b</sup>	193 ± 22.1 <sup>c</sup>	229.7 ± 50.34	522 ± 34.77 <sup>b,d</sup>	325.8 ± 17.1 <sup>b,c</sup>
Urea (mg/dl)	52.1 ± 4.9	72.4 ± 11.2 <sup>b</sup>	56.1 ± 2.7 <sup>c</sup>	54.0 ± 4.88	65.40 ± 3.36 <sup>b</sup>	54.40 ± 6.69 <sup>c</sup>

Control: Rats were stress-free; Stress: rats exposed to forced swimming-induced stress; Stress + V: rats exposed to forced swimming-induced stress along with co-administered vitamins E and C. <sup>a</sup>: Significant difference when compared with the control rats at low altitude; <sup>b</sup>: significant difference from the control rats within each altitude; <sup>c</sup>: significant difference when compared with the forced swimming-induced stressful rats within each altitude; <sup>d</sup>: Significantly different when compared to same group in LA area.

**Table 3.** Levels of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and the activities of superoxide dismutase and catalase in the liver homogenate of control group, stress group and Stress + V group in rat at low and high altitudes.

Parameter	Low altitude			High altitude		
	Control	Stress	Stress + V	Control	Stress	Stress + V
TBARS (mM/100 g)	0.34 ± 0.02	0.44 ± 0.17 <sup>b</sup>	0.319 ± 0.15 <sup>b,c</sup>	0.46 ± 0.02 <sup>a</sup>	0.63 ± 0.02 <sup>b</sup>	0.599 ± 0.08 <sup>c</sup>
GSH (mmol/L)	87.60 ± 2.58	39.36 ± 2.88 <sup>b</sup>	65.58 ± 2.88 <sup>b,c</sup>	45.64 ± 3.20 <sup>a</sup>	23.99 ± 1.21 <sup>b,d</sup>	32.322 ± 2.69 <sup>b,c</sup>
SOD (U/mg)	9.83 ± 0.50	7.89 ± 0.14 <sup>b</sup>	10.16 ± 0.30 <sup>c</sup>	8.06 ± 0.22 <sup>a</sup>	5.99 ± 0.82 <sup>b,d</sup>	6.40 ± 0.73 <sup>b</sup>
CAT (U/ml)	3.42 ± 0.22	2.66 ± 0.34 <sup>b</sup>	3.77 ± 0.38 <sup>c</sup>	2.85 ± 0.32	2.04 ± 0.453 <sup>b,d</sup>	2.10 ± 0.63 <sup>b</sup>

Control: Rats were stress-free; stress: rats exposed to forced swimming-induced stress; stress + V: rats exposed to forced swimming-induced stress along with co-administered vitamins E and C. <sup>a</sup>: Significant difference when compared with the control rats at low altitude; <sup>b</sup>: significant difference from the control rats within each altitude; <sup>c</sup>: significant difference when compared with the forced swimming-induced stressful rats within each altitude; <sup>d</sup>: Significantly different when compared to same group in LA area.

**Table 4.** Levels of thiobarbituric acid reactive substances (TBARS), reduced glutathione (GSH) and the activities of superoxide dismutase and catalase in the kidney homogenate of control group, stress group and Stress + V group in rat at low and high altitudes.

Parameter	Low altitude			High altitude		
	Control	Stress	Stress + V	Control	Stress	Stress + V
TBARS (mM/100 g)	0.18 ± 0.01	0.18 ± 0.01	0.179 ± 0.01	0.15 ± 0.004 <sup>a</sup>	0.22 ± 0.01 <sup>b</sup>	0.18 ± 0.02 <sup>c</sup>
GSH (mmol/L)	28.48 ± 2.8	33.42 ± 3.77 <sup>b</sup>	31.19 ± 2.88	59.14 ± 4.99 <sup>a</sup>	52.82 ± 3.93 <sup>b</sup>	56.62 ± 2.24
SOD (U/mg)	5.94 ± 0.77	5.87 ± 0.43	5.97 ± 0.21	4.46 ± 0.24 <sup>a</sup>	3.25 ± 0.17 <sup>b</sup>	5.28 ± 0.19 <sup>b</sup>
CAT (U/ml)	5.19 ± 0.22	4.95 ± 0.16	4.99 ± 0.14	4.60 ± 0.31 <sup>a</sup>	4.05 ± 0.28 <sup>b</sup>	4.54 ± 0.30 <sup>b</sup>

Control: Rats were stress-free; stress: rats exposed to forced swimming-induced stress; stress + V: rats exposed to forced swimming-induced stress along with co-administered vitamins E and C. <sup>a</sup>: Significant difference when compared with the control rats at low altitude; <sup>b</sup>: significant difference from the control rats within each altitude; <sup>c</sup>: significant difference when compared with the forced swimming-induced stressful rats within each altitude; <sup>d</sup>: Significantly different when compared to same group in LA area.

high altitude area.

In contrast, in the tissue homogenates, all the liver and kidney function parameters evaluated, except for CAT, were significantly different ( $P < 0.05$ ) between the low (column 2 in Tables 3 and 4) and high (Column 5 in Tables 3 and 4). In regard to lipid metabolism, the levels of total cholesterol and LDL in the blood were significantly higher ( $P < 0.05$ ) in rats native to the high altitude than those at the low altitude, while the serum levels of TAG and HDL were similar ( $P > 0.05$ ) in both altitudes (Columns

2 and 5 in Table 5).

### Stressful effect of exhaustive exercise

At low altitude, as compared with the non-stress control, forced swimming resulted in:

(1) A significant ( $P < 0.05$ ) increase in levels of ALT, AST, CK and urea in the serum.

**Table 5.** Serum levels of total triglyceride (TAG), total cholesterol (TChol), high density lipoproteins (HDL) and low density lipoproteins (LDL) in control group, stress group and stress + V group in rat at low and high altitudes.

Parameter	Low altitude			High altitude		
	Control	Stress	Stress + V	Control	Stress	Stress + V
TChol (mg/dl)	64.0 ± 3.6	80.2 ± 9.4 <sup>b</sup>	70.2 ± 3.07 <sup>b,c</sup>	74.1 ± 1.9 <sup>a</sup>	68.86 ± 3.6 <sup>b</sup>	62.4 ± 4.6 <sup>b,c</sup>
TAG (mg/dl)	108.9 ± 16.9	132.8 ± 19.3 <sup>b</sup>	121.0 ± 12.3	107.6 ± 13.6	107.4 ± 12.4	102.2 ± 4.0
LDL (mg/dl)	23.7 ± 4.9	25.2 ± 5.3	11.7 ± 4.3 <sup>b,c</sup>	38.1 ± 4.8 <sup>a</sup>	25.4 ± 7.8 <sup>b</sup>	9.7 ± 3.4 <sup>b,c</sup>
HDL (mg/dl)	18.5 ± 4.0	28.9 ± 3.7 <sup>b</sup>	30.0 ± 4.9 <sup>c</sup>	14.4 ± 6.3	21.748 ± 3.04 <sup>b</sup>	33.2 ± 5.2 <sup>b,c</sup>

Control: Rats were stress-free; stress: rats exposed to forced swimming-induced stress; stress + V: rats exposed to forced swimming-induced stress along with co-administered vitamins E and C. <sup>a</sup>: Significant difference when compared with the control rats at low altitude; <sup>b</sup>: significant difference from the control rats within each altitude; <sup>c</sup>: significant difference when compared with the forced swimming-induced stressful rats within each altitude.

(2) A significant increase in the levels of TBARS, a significant decrease in the levels of GSH and in the activities of SOD and CAT in liver homogenate and resulted in a significant decrease in GSH levels in kidney homogenate

(3) No significant ( $P > 0.05$ ) change in the levels of ALP and GGT in the serum with no changes in TBARS levels and the activities of SOD and CAT in the kidney homogenate

(4) A significant ( $P < 0.05$ ) increase in serum levels of TChol, TAG and HDL levels (columns 2 and 3 in Tables 2, 3, 4 and 5).

At high altitude, forced swimming resulted in:

(1) A significant ( $P < 0.05$ ) increase in ALT, GTT, AST, CK and urea levels in the serum;

(2) A significant increase in TBARS levels and a significant decrease in the levels of GSH and the activities of SOD and CAT in both liver and kidney homogenates;

(3) A significant decrease in serum levels of TChol and LDL with a significant increase in HDL. TGs levels remained unchanged in this group of rats (columns 5 and 6 in Tables 2, 3 and 4).

When comparing the native rats of both low and high altitude forced to swim with each other, the ANOVA test revealed that:

(1) The significant increases in the levels of ALT, AST, CK and urea were significantly higher in high altitude group of rats but urea levels showed a higher increase in the low altitude native rats.

(2) The increase in the levels of TBARS and the decrease in the activities of SOD and CAT in liver were significantly higher in the high altitude native rats forced while the decrease in the levels of GSH was higher in the low altitude rats.

(3) The increase in HDL levels in high altitude native rats were less than its increase in the group of rats performing

the same exercise session in low altitude, but the decrease in LDL levels was higher in this group of rats.

### Protection of vitamins against exhaustive exercise stress-induced organ damage

At low altitude, supplementation with vitamins E and C significantly ( $P < 0.05$ ) decreased the exhaustive exercise induced increase in the serum levels of ALT (27.5%), CK (57.5%), urea (22.4%), TChol (12.4%), LDL (42.9%). It also significantly increased HDL levels (5.6%), GSH (66.6%), SOD (28.7%) and CAT (41.7%), while decreased TBARS levels (27.5%) in the liver homogenate (columns 3 and 4 in Tables 2, 3, 4 and 5).

At high altitude, vitamin supplementation significantly decreased exhaustive swimming-induced increase in serum levels of ALT (31.3%), GGT (6.9%), CK (37.6%), urea (16.8%), TChol (15.8%), LDL (74.6%). In contrast, vitamin supplementation significantly increased HDL (129.9%). Also, it caused a significant decrease in tissue levels of TBARS in both liver and kidney homogenates (4.9 and 18.5%, respectively). Further, vitamins significantly increased GSH levels in the liver homogenate only (34.7%) and significantly increased the activities of SOD and CAT in the kidney homogenate (62.5% and 12.1%, respectively) (columns 6 and 7 in Tables 2, 3, 4 and 5).

When comparing the low and high altitudes, native rats received the vitamins and are forced to swim with each other, the ANOVA test revealed that:

(1) The decrease in the levels of ALT and urea were significantly higher in the high altitude native rats group, while the decrease in CK levels was more profound in the low altitude native rats.

(2) The decrease in the levels of TBARS and the GSH in the liver homogenate were more profound and significantly higher in the low altitude native rats.

(3) The decrease in TChol was higher in the low altitude rats, while the increase in HDL and the decrease in LDL levels were significantly higher in the high altitude rats.

## DISCUSSION

Due to land geographic limitations and technical difficulties, relatively few studies have investigated the effects of two different altitudes on organ functions simultaneously. Further, the majority of studies utilize stimulated high altitude hypobaric chambers (Coates et al., 1979; Welsh et al., 1993), which is a limited enactment of living at native high altitude environments. In order to minimize the effect of confounding factors and investigate the altitude-associated effects of exhaustive exercise stress on key organ functions as well as potential roles for vitamins in a more appropriately controlled manner in an animal model, we attempted to take the advantage of the unique geographic characteristics within Saudi Arabia to create rats "native" to low altitude and others to high altitude environment in the same country by breeding and maintaining them at the two different altitudes. Testing was performed under the same laboratory and dietary conditions at both altitudes. To validate acclimatization of the animals to their environments, we measured the base-line levels in the control groups of major liver and kidney function parameters in the blood at both altitudes.

The results show that rats native to either altitudes had similar serum levels of ALT, GGT, ALP, urea and CK, indicating normal functions of the liver and kidney at both altitudes. Also, it has been reported in humans that lipid metabolism is altered at high altitude (Ferezou et al., 1988) and that acclimatization to high altitude leads to changes in serum levels of lipids as reported by Young et al. (1989). In the present study, significantly higher levels of total serum cholesterol and LDL were detected in rats at high altitude as oppose to those at low altitude, despite no significant difference was detected in key liver and kidney function parameters between rats acclimatized to the low and high altitudes. This observation is in line with several previous studies where increased levels of serum cholesterol and LDL were found to be increased in people native to high altitude areas (Chakraborti et al., 1984; Temte, 1996; Savourey et al., 1998). It has been suggested that increased levels of serum total cholesterol and LDL are primarily a result of decreased rate of lipid metabolism, possibly due to decrease mobilization of the lipids through the blood plasma to various tissues.

In addition, rats at high altitude had significantly higher levels of TBARS and decreased activities of SOD, CAT and decreased levels of GSH in the liver homogenate, but significantly decreased levels of TBARS, SOD and CAT activities and higher GSH levels in the kidney homogenate as compared with rats at low altitude. These observations suggest an increased lipid peroxidation thus higher generation of free radicals in the livers of animals at high altitude. This increase in oxidative stress in the liver of normal control animals at high altitude seems to be within the normal limits, given that the key liver parameters in the blood were not abnormal as discussed

above. It has been documented that hypoxia stimulates the expression of the steroidogenic acute regulatory protein and enhances the secretion of glucocorticoids (Raff et al., 2003). The activities of the antioxidant enzymes (systemic SOD and CAT) have been found to be reduced in the brain of rats treated with glucocorticoids (McIntosh et al., 1985). This may be an explanation of the decrease in tissue levels of SOD and CAT seen in our study.

Regular and appropriate exercise is believed to be beneficial to overall health and has a positive effect on various organs, heart and liver in particular. However, exhaustive exercise may result in organ damage in the liver and kidney as was first demonstrated by Fojt et al. (1976). The present study shows that high-intensity swimming exercise caused changes in liver and kidney functions typical of physical stress at both low and high altitudes, but with more changes observed at high altitude. Our results are in accordance with previous studies where exhaustive swimming exercise has been shown to increase plasma levels of CK, AST, GTT and blood urea nitrogen (BUN) and caused skeletal muscle, liver, and kidney damages (Bowers et al., 1987; Antunes-Neto et al., 2006). With regard to the effect of exhaustive exercise on lipid metabolism, inconsistent results have been reported (Boyden et al., 1993; Prabhakaran et al., 1999; Leon et al., 2001; Tall, 2002; Kodama et al., 2007; Boardley et al., 2007; Kelley and Kelley, 2009).

In the present study, acute exhaustive swimming resulted in a significant increase in TAG, TChol and HDL levels at low altitude, but a significant decrease in TChol and LDL and a significant increase in HDL at high altitude. Our observations support the concept that physical activity has salutary effects on lipid and lipoprotein metabolism (Oyelola and Rufai, 1993). It is believed that the exhaustive exercise-induced organ damage involves the generation of ROS (Mackinnon, 2000). In the present study, acute exhaustive swimming exercise resulted in generation of ROS in the liver as indicated by increases levels of TBARS, decreased levels of GSH and activities of SOD and CAT at both altitudes, thereby leading to an increase in the serum concentration of hepatic enzymes. The forced swimming-induced oxidative stress, lipid peroxidation and the resultant damages to the liver and kidney were more profound at high altitude as compared with low altitude. This might be attributed to an increase production of ROS due to a combined effect of exercise and hypoxia at high altitude (Kehrer and Land, 1994).

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 (Ji, 1999; Ramel et al., 2004). In this study, we chose a combination of vitamins E and C based on the following reasons: vitamins E ( $\alpha$ -tocopherol) and C (ascorbic acid) are antioxidants thought to have a protective effect by either reducing or preventing

oxidative damage. Due to their different subcellular localization, combined treatment with vitamins E and C has been shown to have a better antioxidant effect than either alone (Rinne et al., 2000; Rokitzki et al., 1994). Oxidants generated near cellular membranes can oxidize vitamin E forming a tocopheroxyl radical. Vitamin C may reduce vitamin E radical, thereby regenerating vitamin E (Beyer, 1994). This reaction forms the semi-dihydroascorbate (vitamin C radical), which in turn is reduced by a glutathione (GSH) (Rokitzki et al., 1994). Our results demonstrated that vitamin E and C combined supplementation attenuated exhaustive swimming-induced changes in serum levels of enzymatic markers, lipids and oxidative stress parameters. Our findings are similar to some previous studies which showed that vitamin E supplementation significantly decreased the amount of lipid peroxidation and tissues damage associated with single bouts of low- and high-intensity sub-maximal exercise (Kanter et al., 1993) and resistance exercise (MCbridge et al., 1998; MCbridge and Kramer, 1999). The protective effect of vitamins E and C supplementation was more effective in low altitude area and this may be due to less ROS generation and a more efficient uptake of vitamins at low altitude, as compared with under hypoxia at high altitude. In contrast, it seems that vitamins E and C are more efficient in lowering lipid after exhaustive exercise at high altitude than at low altitude; this may open a window for many people hoping to lose weight and for cardiovascular disease patients trying to improve their lipid profile. This improvement in lipid profile after vitamins supplementation at either low and high altitudes that underwent forced swimming exercise in the present study is supported by previous studies (Anderson et al., 1999; Kurowska et al., 2000), which showed that vitamin C and E prevent oxidation of LDL-cholesterol, decrease total and LDL-cholesterol and triglyceride, and also raise HDL-cholesterol level.

Moreover, in a recent study Okey and Harrison (2007) presented a correlation analysis whereby plasma vitamin E concentration was inversely correlated to total cholesterol and to triglycerides but positively related to HDL cholesterol. Similarly, plasma vitamin C concentrations were inversely correlated to total cholesterol but not to HDL-Cholesterol. The possible explanation for the hypocholesterolaemic effect of vitamin C and E is that vitamin C prevents LDL-cholesterol from oxidative damage and aids in degradation of cholesterol. Secondly, it has been suggested that these vitamins convert more cholesterol towards bile acid synthesis and thus reduces its level in serum (White et al., 1994).

## Conclusion

Despite some variations, the major liver and kidney function parameters were similar in rats at both low and high altitudes, suggesting that liver and kidney function

do not get altered at native high altitude characterized by hypoxia even after acclimatization. However, physiological response to stress was different at high altitude. It induced oxidative stress damage to the kidney and liver and deteriorating lipid metabolism in a greater magnitude than that observed at low altitude.

Finally, vitamin E and C supplementation was able to protect against the stress-induced oxidative damage to the liver and kidney and did preserve normal lipid metabolism at low altitude, except for HDL, and even to a greater extent at high altitude. Our findings, therefore, strongly suggest a potential protective role for vitamins E and C, when administered in combination, against vital organ damage, as well as, against abnormal lipid metabolism precipitated by exposure to stress at native high altitude.

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