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Inhibited Thyroid Function, Impaired Lipid Metabolism and Increased Tissue Oxidative Stress in Rats Native to High Altitude

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Objective: To evaluate the effect of high altitude on markers of thyroid function, serum lipid profile and tissues oxidative stress in male Wistar rats native tohigh altitude (HA) with male rats native to low altitude (LA).

Design: Randomized experimental animal study.

Setting: Physiology laboratory, Medical School of King Khalid University.

Method: Male rats agedsix months, weighing 250 gm were bred and maintained at low altitude (LA, 600 m above sea level, n=6) or high altitude (HA, 2800 m, n=6), under the same laboratory conditions and fed the same diet. Blood samples were obtained for thyroid hormones and lipid profile analysis. Livers, kidneys, lungs and testes were collected and used for determination levels of thiobarbituric acid reactive substances (TBARS),reduced glutathione (GSH),Superoxide Dismutase (SOD) and Catalase Activity (CAT).

Result: HA rats had significantly (P<0.05) lower serum T3 (17.14%), T4 (13.75%), TSH (39.29%) and total cholesterol (15.84%) and LDL (60.90%).There were no significant differences in TAG or HDL. All tissues from HA rats showed significant decreases in SOD and CAT activities compared to LA rats. These rats showed significantly higher oxidative stress in the lungs and the liver, but lower oxidative stress in the kidney and no difference in the testes.

Conclusion: Living at high altitude environment results in impaired thyroid function and lipid metabolism and causes increased tissue oxidative stress.

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Several studies have dealt withthe biochemical, physiological and metabolic changes resulting from exposure to hypoxia in both animals and humans[1]. Short or Chronic high-altitude exposure had been reported to cause cellular disturbances in a variety of organs and tissues, including the respiratory,the cardiovascular and the endocrine systems[2-4].Circulating level of thyroid hormones could be changed by a number of physiological and pathological conditions, which can alter the deiodination pathway[4].

Environmental conditions had been reported to have a significant influence on thyroid function[5]. Studies of short-term exposure to high altitude on thyroid activity in humans and animals had reported reduction in thyroid activity[6,7].We have previously reported a significant decrease in serum TSH and significant increase in the level of serum cortisol, Triiodothyronine (T3) and Thyroxin(T4) in rats exposed to hypobaric hypoxia for a period of 45 days[5].

Fasting blood glucose has been observed to increase during acclimatizing to 4000 m and to fall after[8]. In other studies, native people living at high altitude have been observed to have significantly lower fasting blood glucose than do sea level subjects[9]. It has also been reported that lipid metabolism is altered in humans during exposure to high altitude[10]. Furthermore, It has been established that exposure to high altitude often result in oxidative damage to macromolecules[2]. Many studies had found an increased production of indicators of oxidative stress in breath, blood, urine and tissue of laboratory rats in response to short or long-term hypoxia[11]. Similar results have been found in humans exposed to hypoxia[12].

The aim of the study is to determine the effect of high altitude on selected biochemical, endocrine and different tissues oxidativestress parametersin high altitude native rats and compare it with low altitude native rats from same genetic pool.

METHOD

The study was performed at high altitude (Abha region, 2800-3150 m above sea level) and at low altitude(Riyadh, 600 m above sea level).Essential geographical information for each city is presented in table 1.

Table 1: Demographic Data 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	2200-2800
Barometric pressure (mm Hg)	711	550-590
Atmospheric O_2 tension (mm Hg)	145	110-120

Relative humidity (%)	15-50	20-30
Summer temperature (shade) (°C)	24-45	16-28
Winter temperature (shade) (°C)	10-25	5-15

Twelveadult male Wistar rats from the same gene pool weighting exactly 250 gm and aged six months were used.The sample was divided to two groups; each one hadsix rats.
a) Low altitude native rats (LA rats) were bred and maintained in the animal house at King Saud University in Riyadh.
b) High altitude native rats (HA rats) had the same age and same weight and were bred and maintained in the animal house at King Khalid University in Abha. All rats werehoused under the same laboratory conditions, fed the same diet.

After overnight fasting, both groups of rats were anesthetized using diethyl ether. Blood was collected by cardiac puncture.Serum was used for determining the level of Thyroid Stimulating Hormone (TSH), free Triiodothyronine (T3) and free Thyroxin (T4), TotalCholesterol (CHOL), Triglycerides (TG), High Density Lipoproteins (HDL), Glucose, Urea, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma Glutamyltransferase(GGT) and Alkaline Phosphatase (ALP). Immediately after blood collection,the animals were killed by decapitation. Lungs, livers and kidneys were quickly removed, washed in ice-cold isotonic saline, homogenized in 0.1 M Tris-HCl buffer, pH 7.4, centrifuged and the supernatant was kept at -20°C for estimation of thiobarbituric acid reactive substances (TBARS), reduced Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase Activity (CAT).

Lipid peroxidation, as evidenced by the formation of thiobarbituric acid reactive substances (TBARS), was assayed by the method described previously by Ohkawa et al[13]. SOD activity in the tissue homogenates was measured by using commercial kits (Randox laboratories Ltd, UK). CAT activity in the homogenates was determined by using a commercial kit (Biovision K773-100).

Results are expressed as the mean value ± SD. Statistical analysis was performed using SPSS software, version 16, and differences between groups were assessed using student's paired t-tests. Values of $p < 0.05$ were considered significantly different. Graphs were created by graphpad prism version 5.

RESULT

There was a significant increase in the levels of T3, T4 and TSH in the serum of high altitude native rats compared to low altitude native rats, see table 2. The T3/T4 ratio was not significantly different between the high or low altitude native rats.

Table 2: Serum Levels of T3, T4 and TSH in Low and High Altitude Rats

Parameter	Low Altitude	High Altitude	Percent of Difference
T3 (ng/dl)	115.64 ± 3.83	95.82 ± 3.89*	↓ 17.14%
T4 (μg/dl)	10.25 ± 0.44	8.84 ± 0.22*	↓ 13.76%
TSH (μIU/ml)	0.28 ± 0.04	0.17 ± 0.20*	↓ 39.29%
T3/T4 ratio (%)	1.128	1.084	

Values are given as Mean ± SD for groups of six rats each. Values are statistically significant at $p < 0.05$

Lipids profile showed significantly higher cholesterol and LDL levels, but no difference in the levels of triglycerides or HDL in the serum of high altitude native rats compared to low altitude native rats, see table 3.

Table 3: Serum Levels of Total Triglyceride (TAG), Total Cholesterol (Tchol), High Density Lipoproteins (HDL) and Low Density Lipoproteins (LDL) in Low and High Altitude Rats

Parameter	Low Altitude	High Altitude	Percent of Difference
TAG (mg/dl)	108.96 ± 16.90	107.64 ± 13.61	↓ 1.21%
TChol (mg/dl)	64.00 ± 3.61	74.14 ± 1.96*	↑ 15.84%
HDL (mg/dl)	18.50 ± 4.09	14.46 ± 6.34	↓ 20.81%
LDL (mg/dl)	23.71 ± 4.912	38.15 ± 4.86*	↑ +60.9%

Values are given as Mean ± SD for groups of six rats each. Values are statistically significant at $p < 0.05$

TBARS was significantly higher in the livers and lungs of the high altitude native rats and significantly lower in their kidneys compared to the low altitude native rats, see figure 1. The TBARS levels were not significantly different in the testes of rats in both groups. Moreover, high altitude native rats showed significantly lower activities of both SOD and CAT in their livers, kidneys, testes and lungs compared with low altitude native rats, see figures 2 and 3. High altitude rats showed significantly lower levels of reduced glutathione (GSH) in their livers and lungs and showed higher levels in their kidneys, see figure 4. The levels of GSH were not significantly different in the testes of rats of both groups.

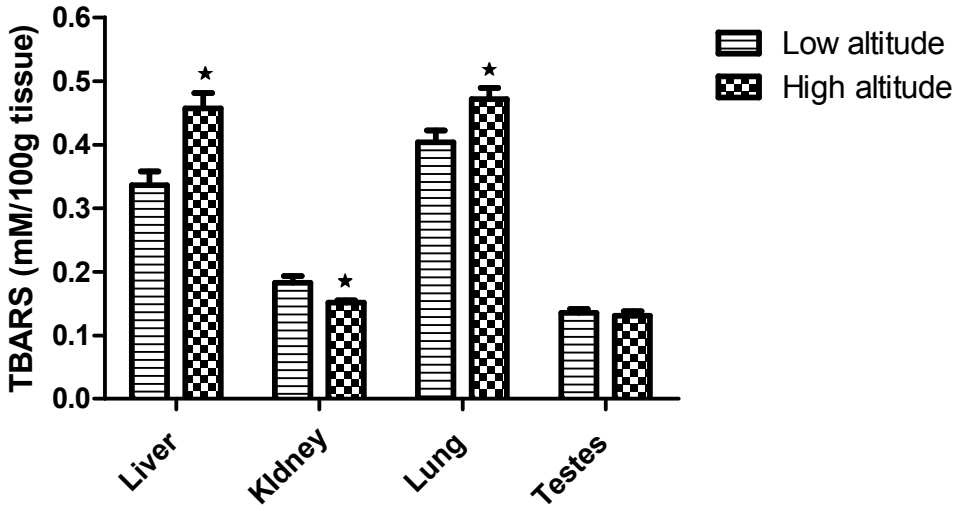

Figure 1: Comparison of Levels of Thiobarbituric Acid Reactive Substances (TBARS) in Four Rat Tissues at Low and High Altitudes (*Values are given as Mean ± SD for groups of six rats each. Values are statistically significant* at $p < 0.05$, in all the figures)

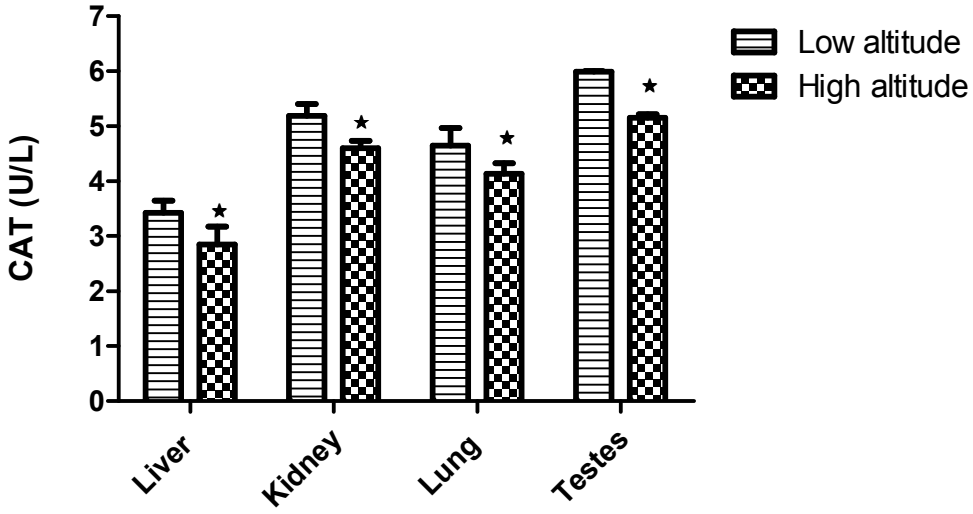

Figure 2: Catalase Activity in Tissues of Low and High Altitude Rats

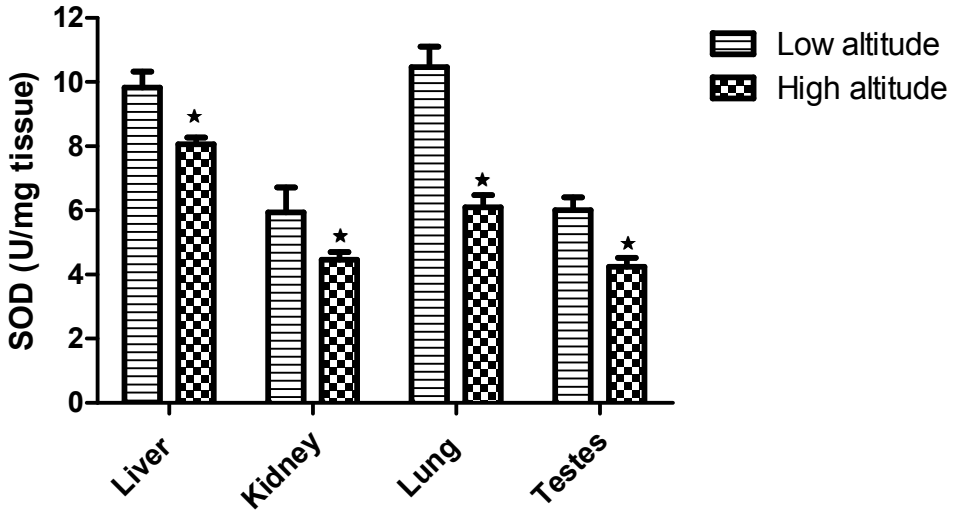

Figure 3:Superoxide Dismutase (SOD) Activity in Tissues of Low and High Altitude Rats

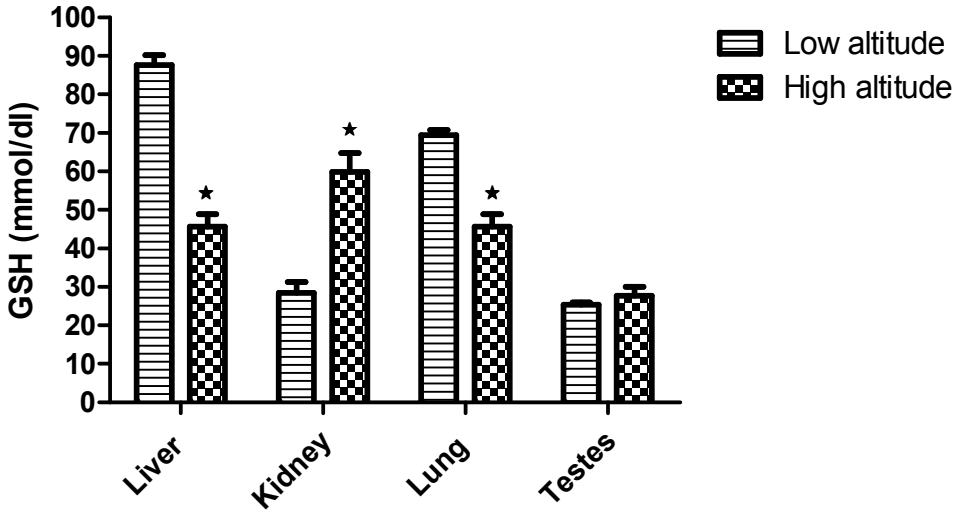

Figure 4:Reduced Glutathione (GSH) in Tissues of Low and High Altitude Rats

DISCUSSION

Relatively few studies of organ functions at different altitudes have been done because of land geographic and technical difficulties. Most studies have utilized high altitude laboratories or hypobaric chambers[14]. Our study is unique because it was performed on the same rat species living in an area of high or low altitude in the same country. All rats were housed under the same laboratory conditions and were fed the same diet; therefore, the observed hormonal and biochemical differences were not due to dietary or genetic factors or to adaptive evolutionary changes.

Consequently, the factor that appears to be at study in this situation is environmentally derived. One limitation in our studyis the small sample size but our study is the first study in field that shows the effect of high altitude on lipid profile, thyroid function and tissue oxidative stress in rats native to low and high altitude.

In this study, rats living at high altitude for six months, from birth, had a significantly lower level of circulating TSH, T3 and T4 compared to low altitude rats. Other studies in both animal and human showed that chronic hypoxia suppresses the function of the hypothalamus-pituitary-thyroid axis, decreases the number of thyrotrophs, reduces the function of the thyroid gland and reduces the levels of T3 and T4[7].

Hypoxia suppresses TRH mRNA expression in the paraventricular nucleus of the rat hypothalamus both sub-acutely and chronically and this may explain the decrease in TSH seen in our study[15]. Additionally, somatostatin suppresses thyroid hormones release and it may play an important role in the present findings by inhibiting TSH and Thyroid hormone levels in high altitude[15].

The evaluation and comparison of the levels of lipid profile parameters in the present study between the low and high altitude rats was challenging, as no such clinical study has previously been undertaken or reported such comparisons in human or animals. The higher levels of total serum cholesterol and LDL cholesterol reported in the high altitude rats suggest a modulatory role of the environmental factors prevailing in the respective region. The rats used in our study share the same gene pool; however, they are under the influence of different climatic conditions. Previous studies showed increased levels of serum cholesterol and LDL cholesterol in people native to high altitude areas[16].

An inverse correlation between serum levels of cholesterol and thyroid hormone has been found 80 years ago[17]. Recent study has established that the levels of LDL receptor mRNA and protein in the liver are directly associated with serum thyroid hormone levels[17].

Thyroxin is known to decrease plasma cholesterol concentration due to increased formation of LDL receptors in the liver, resulting in increased hepatic removal of cholesterol from the circulation. The decreased levels of TSH and decrease levels of thyroid hormones in the high altitude rats shown in the present study may be responsible for the increased levels of cholesterol and LDL in those rats by the mechanism mentioned above.

Oxidative stress results when the antioxidant defenses are overwhelmed by pro-oxidants and reactive oxygen species (ROS), which are not adequately removed.Hypoxia could lead to a reductive stress, which results in increased ROS production by the mitochondrial electron transport system[2,11].

In the present study, high altitude was associated with an inhibition of the activity of SOD and CAT in the measured tissues. The decrease in these antioxidant enzyme activities in the livers and lungs might be due to their use against the ROS and their inhibition by free radical speciesproduced[18]. It is known that hydrogen peroxide can inhibit SOD activity by reducing Cu^{+2} to Cu^{+1} in SOD[18]. Furthermore, it is known that hypoxia stimulates the expression of the steroidogenic acute

regulatory protein and enhances the secretion of glucocorticoids, which may reduce the activity of the antioxidant enzymesand this may be the mechanism responsible for lower SOD and CAT activities in the tissues of high altitude rats[19,20].

The significantly lower levels of GSH and higher TBARS concentrations (as an indicator of lipid peroxidation) in the liver and lung tissues of the high altitude rats provide strong evidence that these rats were subject to higher levels of oxidative stress. The lack of difference in GSH and TBARS levels in the testes and kidneys despite significantly lower SOD and CAT activities in high altitude rats, suggests that high altitude does not induce oxidative stress in these tissues or that the SOD and CAT activities, although lower, were still sufficient to deal with any increase in ROS, in these tissues.

CONCLUSION

Impaired thyroid function and lipid metabolism parallel with increased oxidative stress in different tissues were reported in high altitude native rats. These novel findings may help to understand the physiology of animal at high altitude and may open a window for future research in understanding the pathophysiology of thyroid gland and lipid metabolism at high altitude.

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All procedures were approved by the Ethical Committee in the Department of Physiology at the King Khalid University Medical School (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.

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