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Original article:

EXHAUSTIVE EXERCISE AND VITAMINS C AND E MODULATE THYROID HORMONE LEVELS AT LOW AND HIGH ALTITUDES

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ABSTRACT

Thyroid hormones play an important role in cell growth and differentiation and regulation of oxygen consumption and thermogenesis. The effect of altitude and vitamin supplementation on thyroid hormone levels in animals or humans performing acute exhaustive exercise have not been investigated before**.** Therefore, we thought to test whether exhaustive exercise-induced stress with antioxidant supplementation was capable of modulating the level of thyroid hormones at different altitudes. Serum levels of T4 (Thyroxin), T3 (Triiodothyronine), and TSH (Thyroid Stimulating Hormone) were measured in rats (N=36) born and bred in low altitude (600 m above sea level) and high altitude (2200 m above sea level) following forced swimming with or without vitamins C and E (25 mg/kg) pre-treatments. Thyroid levels were significantly decreased in resting rats at high altitude compared to low altitude, and swimming exercise moderately increased T3 and TSH at both high and low altitudes, whereas T4 was markedly increased (62 %) at low altitude compared to a moderate high altitude increase (28 %). Co-administration of vitamins C and E augmented the observed forced swimming-induced thyroid release. However, the conversion of T4 to T3 was reduced in both altitude areas following swimming exercise and vitamin pre-treatment had no effect. We conclude that acute stress induced thyroidal hormones in rats, which was augmented by antioxidant drugs in both high and low altitude areas. These findings may play an important role in the human pathophysiology of thyroid gland at different altitudes.

Keywords: Exhaustive exercise, vitamins, altitude, thyroid hormones

INTRODUCTION

Endocrinal changes occur in response to different types of stress in order to cope with the demands of a homeostatic challenge (Miller and O’Callaghan, 2002). Thyroid hormones, which are known to be influenced by stress (Miller and O’Callaghan, 2002) act on multiple metabolic processes affecting the concentration and activity of numerous enzymes, the metabolism of substrates, vitamins and minerals, and the response of target tissues to several hormones (Yen, 2001). Physical exercise is used as a model to study physiological responses to stress in both humans and animals (Mackinnon, 2000). However, conflicting results on the effect of exercise inducing stress on the level of serum thyroid hormones were reported, which seems to depend on the characteristics of the exercise, including duration and intensity, and on the physiological condition of the animals, such as fitness and training level (Bernet and Wartofsky, 2000; Mastorakos and Pavlatou, 2005).

Previous studies on stress induced by high altitude hypoxia has monitored thyroid activities under basal and exercise conditions in humans and animals using different experimental conditions, including the methods of hypoxia induction such as artificial and natural methods (Al-Hashem, 2010; Bernet and Wartofsky, 2000; Surks, 1966). In addition, the effects of dietary supplements of vitamins C and E on thyroid hormones such as T3, T4, and TSH levels were also measured in animals (Sahin et al., 2001). To date, however, there is no single study that monitored thyroid hormones activities in *native* animals under natural hypoxic condition provided by high altitude areas, with and without antioxidant vitamins dietary supplementation under basal condition and swimming-induced acute stress.

In this study, we examined the effect of acute stress on the level of thyroid hormones in high and low altitudes in the presence and absence of vitamin C and E using native rats bred and maintained for many generations. Both swimming-induced stress and antioxidants augmented thyroid hormones in low and high altitudes.

METHODS

Reagents

Vitamins C and E were purchased from BDH chemicals, England. T3 and T4 ELISA kits were obtained from GenWay Biotech (San Diego, USA). TSH ELISA kit was from BioVendor Laboratory Medicine (Brno, Czech Republic).

Areas of the study

The study was carried out in areas of high and low altitude in different regions in 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 2200 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 about 600 m above sea level.

Animals

Adult male Wistar rats (N=36) weighting 250 ± 5 g each and aged 8 weeks were used for the experimental procedure. 18 rats bred and maintained in the animal house at College of Pharmacy, King Saud University in Riyadh city were used for the low altitude native rats (LA rats) experiments. In parallel, the same number of rats were used for the high altitude native rats (HA 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 10^{th} 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, 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 design

Rats were divided equally into three 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 (Lee et al., 2009) and 20 mg/kg of Vitamin C orally (Owu et al., 2006) for 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 group (A and B) rats were exposed to acute forced exhaustive swimming stress for a duration of 2.5 h 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.

Immediately after the end of the experimental procedure, blood samples were taken at the same time directly from the heart and placed in plain tubes where they were allowed to clot at room temperature. Samples were centrifuged at 4000 rpm for 10 min to obtain serum. Serum was used for determining the levels of Thyroid Stimulating Hormone (TSH), free Triiodothyronine (T_3) and free Thyroxin (T_4) and using specific commercially available ELISA kits according to the manufacture's instructions.

Statistical analysis

Results are expressed as mean (SD). Data for all the test groups were analyzed statistically with SPSS 11.0 (Statistical Package for Statistical Science, SPSS, Chicago, IL, USA) using one way ANOVA test. A p-value < 0.05 was considered significant.

RESULTS

High altitudes suppress thyroid hormones in resting animals

To assess the effect of high altitudes in Saudi Arabia on the level of thyroid hormones in *native* male wistar rats which were bred and maintained in LA and HA regions. Serum T3, T4 and TSH were measured in resting animals. High altitudes significantly inhibited T3 ($P<0.05$), T4 ($P< 0.01$) and TSH ($P< 0.01$) levels under basal conditions when compared with the blood concentration of thyroid hormones at low altitudes (Figure 1). The degree of inhibition in T3 and T4 were comparable; about 17 % for T3 and 15 % for T4, whereas, a marked reduction in TSH level (37 %) was observed. To determine whether altitudes could affect the conversion rate of T4 to T3 under basal conditions, the ratio of T3/T4 (percent) between these two groups were measured and found no significant changes (LA, 1.12 % and HA, 1.08 %; Figure 3).

Exhaustive exercise induces thyroidal hormones

Conflicting data was reported on the effect of acute stress such as swimming on the levels of thyroid hormones at different altitudes. We therefore assessed the levels of T3, T4 and TSH under exhaustive exercise conditions at different altitudes in Saudi Arabia. Rats exposed to acute forced swimming exercise for 2.5 hours (stress A) resulted in a significant increase ($P< 0.05$) in the levels of T3, T4 and TSH in both low and high altitudes compared to non stressed control groups (Figure 2). The percentage increase in the level of these hormones were 18.6 %, 61.8 % and 33.3 % in T3, T4 and TSH, respectively in low altitude rats forced to swim while the increase in the levels of these hormones were 12.98 %, 27.97 % and 29.4 % in T3, T4 and TSH, respectively in the high altitude native rats forced to swim. The extent of exhaustive exercise induced thyroid release was significantly higher in LA compared to HA. Forced swimming reduced T3/T4 ratio in rats at both altitudes compared to controls (Figure 3), and the reduction rate was higher in LA than HA. Forced swimming also caused a significant increase in the level of serum cortisol (data not shown) that acts as a biomarker in human and animals under stress conditions.

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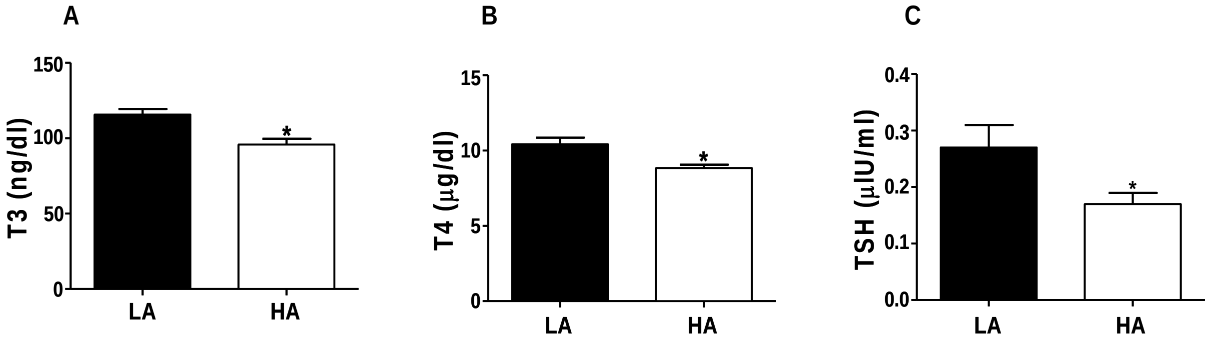

Figure 1: High altitude suppresses baseline levels of thyroid hormones. Values are given as Mean ± SD for groups of six rats each. Analysis by one way ANOVA. Values are statistically significant at *p<0.05. LA: Low altitude. HA: High altitude

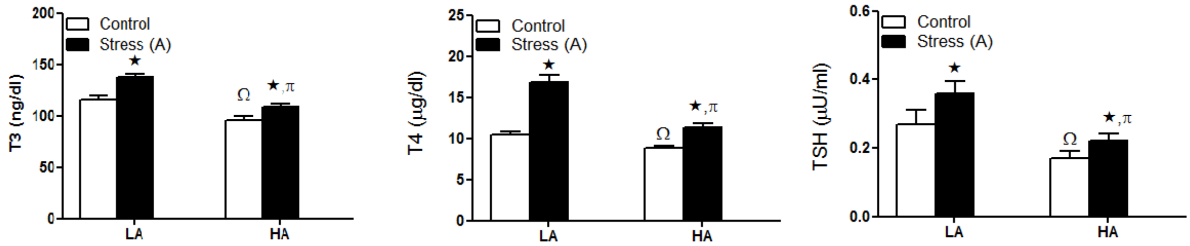

Figure 2: Exhaustive exercise induces thyroidal hormones at both low and high altitudes. Values are given as Mean ± SD for groups of six rats each. Analysis by one way ANOVA. Values are statistically significant at p<0.05. LA: Low altitude. HA: High altitude. _Control:_ Resting rats. _Stress (A):_ Rats under swimming excercise stress. *: Significantly different when compared to its area specific control group. Ω: Significantly different when compared to LA control group. π: significantly different when compared to stress group (A) at LA

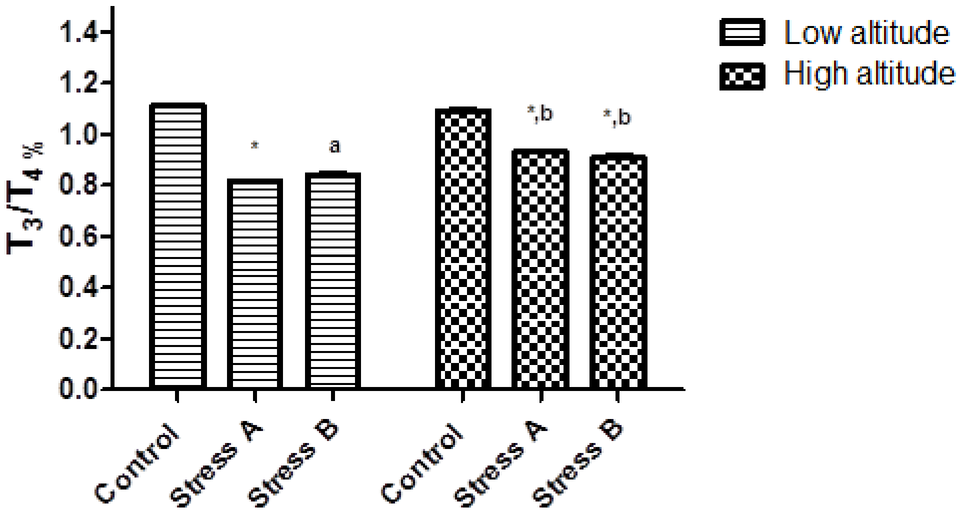

Figure 3: T3/T4 ratio in all experimental groups at low and high altitude. Analysis by one way ANOVA. Values are given as Mean ± SD for groups of six rats each. Values are statistically significant at p<0.05. _Control:_ Resting rats. _Stress (A):_ Rats under swimming excercise stress. _Stress (B):_ Rats under swimming exercise stress and treated previously with combined vitamins E and C. *: significantly different when compared to its control group, a: significantly different when compared to same area stress (A). b: significantly different when compared to low altitude similar group

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Vitamins C and E augment exhaustive exercise-induced thyroid hormone release

To determine whether supplement of antioxidant could augment the observed swim-induced thyroid hormones, vitamins C and E were given one hour before the exhaustive exercise session to both low and high altitude native rats (Figure 4). It resulted in a further significant increase in serum levels of T3, T4 and TSH (stress B) when compared to their area specific non stressed control group or non-vitamin treated group forced to swim (Figure 5). The percentage increases in T3, T4 and TSH levels from their control baseline levels were (30.9 %, 80.2 % and 51.8 %) respectively, and were (28.4 %, 52.5 % and 70.5 %) respectively in the high altitude native rats which received the vitamins and were forced to swim. The maximum increase in the levels of T3 and T4 occurred in the low altitude native rats which received the vitamins and were forced to swim, whereas the maximum increases in the levels of TSH occurred in the high altitude native rats which received the vitamins and were forced to swim. Vitamin supplement had no effect on T3/T4 ratio (Figure 3).

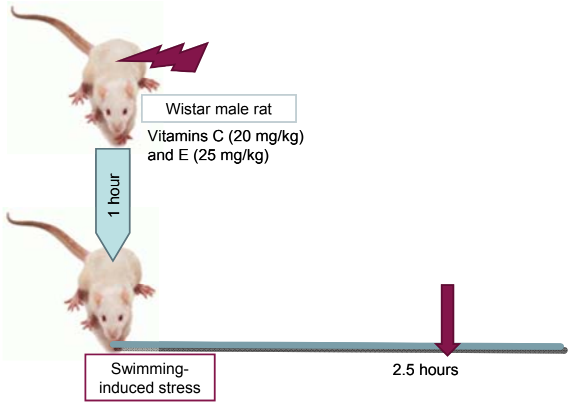

Figure 4: Suggested model that investigates the effect of vitamins E and C on stress induced thyroid hormonal changes in rats in both low and high altitudes

DISCUSSION

These studies are the first to investigate: (1) the effect of high altitude induced hypoxia on the thyroid hormones in *native* rats bred and maintained for many generations at a high altitude area and (2) the effect of acute swimming induced stress with antioxidant supplement on the level of thyroid hormones in low and high altitudes. The main finding of our study was that co-administration of vitamins C and E augmented exhaustive exercise induced thyroid hormones in rats. This conclusion was supported by the data indicating that the maximum T3, T4 and TSH levels were obtained when these vitamins were given to rats before being exposed to acute stress through exhaustive swimming (Figures 2 and 3).

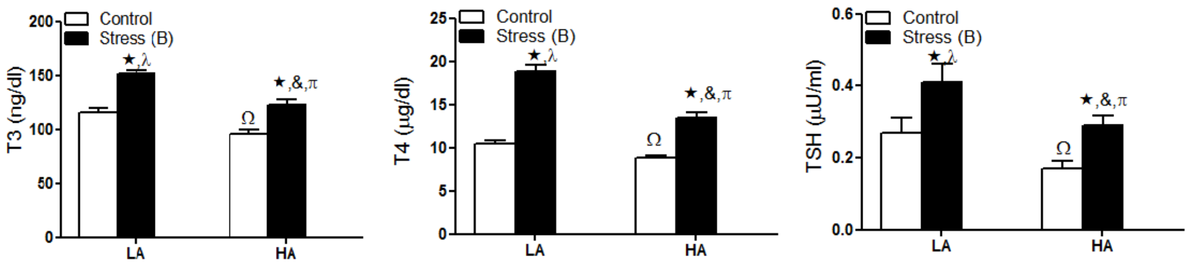

Figure 5: Vitamin C and E augment exhaustive exercise - induced thyroidal hormones. Values are given as Mean ± SD for groups of six rats each. Analysis by one way ANOVA. Values are statistically significant at p<0.05. **LA:** Low altitude. **HA:** High altitude. <u>*Control:*</u> Resting rats. <u>*Stress (B):*</u> Rats under swimming exercise stress and treated previously with combined vitamins E and C. *: Significantly different when compared to its area specific control group. Ω: Significantly different when compared to LA control group. π: significantly different when compared to stress group (B) at LA. λ: Significantly different when compared to stress group (A) at LA. &: Significantly different when compared to stress group (A) at HA.

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Relatively few studies of organ function at different altitudes have been done and most have utilized high altitude laboratories or hypobaric chambers (Coates et al., 1979; Welsh et al., 1993). Further, conflicting data was reported on the effect of high altitudes on the level of circulating thyroid hormones in both humans and animals under resting and stressed conditions (Gordon et al. 1943; Hornbein, 1962; Moncloa et al., 1965; Surks, 1966; Mordes et al., 1983; Bernet and Wartofsky, 2000; Huang et al., 2004). This prompts us to thoroughly investigate these important metabolic regulating hormones using native rats under different conditions in a natural hypoxic environment provided by the high altitude locations of our laboratories. Our results with the inhibition of thyroid hormones in the blood circulation of rats at high altitudes under resting conditions can be compared with data obtained by others with rodent and human experiments (Gordon et al., 1943; Surks, 1966; Bernet and Wartofsky, 2000). Hypoxia was found to suppress TRH mRNA expression in the paraventricular nucleus of the rat hypothalamus both sub-acutely and chronically (Hirooka et al., 1978) and this may explain the decrease in TSH seen in our study. Additionally, somatostatin, secreted under stress conditions, suppresses thyroid hormone release (Hirooka et al., 1978; Tapia-Arancibia et al., 1984; Du, 1998) and it may play an important role in our study in inhibiting TSH and thyroid hormone levels in a high altitude environment.

In contrast, in the work reported previously using WKY rats, hypobaric hypoxia did not influence thyroid status (Henley and Tucker, 1987). These differences are possibly due to using different hypoxic conditions and non-native rats compared to this work. Indeed, we recently reported (Al-Hashem, 2010) a decrease in TSH and increase in T4 and T3 in resting non-native rats using natural hypobaric hypoxia after 45 days. Contrasted changes in hormonal levels also reported in men exposed to hypoxia for 3-4 days at a 4350 m altitude demonstrated no change in TSH and about 20 % increase in thyroid hormones (Richalet et al., 2010). Taken together all of the studies would suggest that further work will thus be required to clarify these discrepancies in thyroid functions in man and animal at high altitudes.

Our data obtained using exhaustive swimming as a model to study the effect of acute stress on the thyroid glands (Figure 2) point to the negative effect of high altitude on the magnitude increase in the circulating thyroid hormones following stress compared to low altitudes. Nonetheless, our data was at least partially in agreement with previous studies with the effect of exercise on the level of thyroid hormones at normal altitude in rats exposed to 20 minute treadmill exercise showed an increase in T3 and T4, and a decrease in T3/T4 ratio indicating an impairment in T4 to T3 conversion (Fortunato et al., 2008), whereas a 30 min swim exercise in rats caused an increase in TSH but a decrease in both T3 and T4 (Sullo et al., 2003). On the other hand, human exercise at 70 % of maximum heart rate in normal altitude was in agreement with our findings of increased T3, T4, and TSH (Ciloglu et al., 2005).

We extended our studies to look at the effect of antioxidants such as vitamins C and E on swimming-induced stress in native rats under different altitudes. Although, there was no similar previous study to compare with, but a report on heat-induced stress on poultry supplemented with vitamin C induced T3 and T4 (Abdel-Wahab et al., 1975), it is in fact in agreement with this study.

In conclusion, data generated from our novel approach of using *native* rats to study the effect of natural hypoxia induced by high altitudes on thyroid hormones are expected to be a true resemblance of the effect of these adverse conditions on the *native* inhabitants of this area.

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