Increased systemic low-grade inflammation in high altitude native rats mediated by adrenergic receptors

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ABSTRACT

الأهداف: يهدف هذا البحث إلى دراسة العلاقة بين حدوث الالتهابات كنتيجة لنقص الأكسجين الناتج عن العيش بصفة دائمة في المرتفعات العالية.

الطريقة: تمت هذه الدراسة خلال الفترة من بداية يناير إلى منتصف فبراير سنة 2013م وذلك على خمسين جرذ ذكور عاشوا وولدوا إما في منطقة منخفضه عن سطح البحر (الرياض) أو في منطقة مرتفعة عن سطح البحر (أبها). أنجزت الدراسة خلال مرحلتين. في المرحلة الأولى تم أخذ عينه دم وريدي وقياس كلا من: مستويات مؤشرات حدوث الالتهابات في الدم، المغنيزيوم، مستويات الدهون وكذلك مستويات بروكسيدات الدهون. في المرحلة الثانية تم قياس المؤشرات السابقة عند جرذان المناطق المرتفعة بعد تلقي الجرذان عقار المثبطات الأدرينالينة لمستقبلات ألفا (برازوسين) ومستقبلات بيتا (بربرنولول).

النتائج: دلت النتائج على ارتفاع مؤشرات حدوث الالتهابات السايتوكاينيزيه عند جرذان المناطق المرتفعة وكذلك ارتفاع الدهون في الدم مع انخفاض في مستويات المغنيزيوم وذلك عند مقارنتها بجرذان المناطق المنخفضة. ارتفاع المؤشرات السابقة عند جرذان المناطق المرتفعة انخفضت إما كليا أو جزئياً بعد تلقيها عقار مثبطات الأدرينالين لمستقبلات ألفا أو بيتا على التوالي.

النتيجة: العيش بصفة دائمة في المناطق المرتفعة قد يؤدي لزياده حدوث الالتهابات عن طرق تحفيز الجهاز العصبي الودي من خلال مستقبلات ألفا ويمكن أن يعزى ذلك إلى نقص المغنيزوم في الدم.

Objectives: To compare the serum levels of inflammatory mediators in high altitude (HA) native rats, and to search for the possible underlying mechanism(s).

Methods: The study was carried out between January and April 2013. Fifty male rats from the same genetic pool were bred at either a HA or low altitude (LA) area. The study was carried out in 2 stages. In the first stage, serum levels of inflammatory markers, adhesive molecules, lipid profiles, catecholamines, magnesium (Mg^{+2}), and lipid peroxidation were compared between theses 2 groups. In the second stages, inflammatory response and lipid peroxidation were analyzed in HA native rats after treatment with either α (Prazosin) or ß (propranolol) adrenergic blockage.

Results: The HA native rats showed significant increases in the serum levels of inflammatory cytokines, lipid profiles, as well as a significant increase in the urinary norepinephrine with a concomitant decrease in the serum levels of Mg^{+2} and increased lipid peroxidation. Blockage of the ß and α adrenergic receptors of the HA rats caused partial or complete decreases in both inflammatory and oxidative stress mediators.

Conclusion: Living under HA conditions results in an increased systemic inflammatory reaction; an effect that is mediated through the sympathetic nervous system mainly via α-adrenergic receptors and could be attributed to low Mg^{+2} levels.

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Evidence from human, animal, and culture studies indicate that hypoxic stressors can cause alterations in immune and inflammatory responses.[1-5] On the other hand, the sympathetic nervous system (SNS), via catecholamines, is a primary factor responsible for elevation in some inflammatory substances such as interlukin-6 (IL-6) levels over a short time exposure to high altitude (HA).[6,7] Despite these findings, there is a lack of study on the levels and the effect of catecholamines and their stimulatory role in the inflammatory response either in animals or humans native to HA. Interestingly, some previous studies showed that there was an association between chronic inflammatory stressor response and low serum magnesium (Mg^{+2}) levels.[8] However, such an effect has not been measured in native HA dwellers either in animals or in humans. Moreover, the effect of hypoxic stress as one of the causes of hyperlipidemia has attracted substantial research attention in humans exposed acutely to HA, or in those people who are native to HA areas.[9] More recently, we have reported similar results in rats native to HA.[10] It is well accepted that low-density lipoprotein (LDL) is extremely susceptible to be oxidized to oxidative LDL (OxLDL) under various conditions of increased levels of oxidative stress, and hence OxLDL is considered a major cause of tissue damage, and an inducer of inflammation.[11] Thus, the hypoxia resulting from HA may produce reactive oxygen species (ROS), which could increase the serum levels of OxLD. Studies regarding this hypothesis are limited. However, there is a lack of studies that monitored the inflammatory responses in native animals or people under natural hypoxic conditions provided by HA areas. Also, there are limited studies regarding the involvement of catecholamines and Mg^{+2} levels in this inflammatory response in those natives. Hence, in this study, we examined and compared serum levels of the main mediators of the inflammatory cascade in relation to lipid peroxidation, SNS, hypothalamic-pituitary-adrenal (HPA) axis functions, and serum Mg^{+2} levels in native rats from same genetic pool, which bred and lived for many generations at either low or high altitudes.

Methods.

Chemicals. Colorimetric determination kits of total serum cholesterol (TC), triglyceride (TG), LDL, and high density lipoprotein-cholesterol (HDL) were purchased from Human Company, Wiesbaden, Germany, while the OxLDL determination kit was supplied from Uscn Life Science Inc Houston, Texas, USA. High-sensitivity C-reactive protein (hsCRP) ELISA determination kit was purchased from ASSAYPRO, Saint Charles, MO, USA. Serum IL-6 and intracellular adhesive molecule (ICAM-1) ELISA determination kits were purchased from Ray Biotech Inc, Norcross GA, USA. The ELISA kit for determination of vascular cell adhesive molecule (VCAM-1), tumor necrosis factor alpha (TNF-α), and malondialdehyde (MDA) were purchased from TSZ scientific, Lexington, MA, USA. The ELISA kits for determination of norepinephrine, dopamine, and serotonin were purchased from Abnova, Walnut, CA, USA.

Areas of the study. This study was conducted with 2 separate groups of male Wistar rats that were from the same gene pool, and from the same breeding colony, which was transferred from a low altitude (LA) area to a HA area and allowed to bred there. Group A was bred and maintained at LA (LA group; in King Saud University, College of Pharmacy, Riyadh, Saudi Arabia), and group B was bred and maintained in HA (HA group at King Khalid University, College of Medicine, Physiology Department, Abha, Saudi Arabia). After 3 generations of breeding in each area, rats from each bred colony of each area were used in the experimental procedure in both group A and B. The experiment was started in both groups once they reached 80 days old, and the animals included in the study weighted between 240-250 g. During breeding and experimental procedures, animals in both areas were housed at a controlled ambient temperature of 25 ± 2°C and 50±10% relative humidity, with 12-h light/12-h dark cycles and were fed similar standard food. Abha city is located in the Aseer mountains, in the southwest of Saudi Arabia, and has an altitude of 2800 m above sea level. On the other hand, Riyadh is located in the center of Saudi Arabia at an altitude of 600 m above sea level. Environmental data on these areas are shown in **Table 1**.

Experimental design. Ten rats bred and maintained at an LA area, and 40 rats bred and maintained at a HA were used in the experimental procedures. All rat studies were performed between January and April 2013 according to protocols approved by the Ethical Committee of the Medical School at 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.[12] The experiments in the current study were carried out in 2 stages: Stage 1: In this stage, serum levels of inflammatory markers, cell adhesive molecules, lipid profiles, catecholamines, Mg^{+2} , and MDA were analyzed and compared between HA and LA rats; hence, 2 groups of rats (n=10, each) were studied and classified as: I. Group A: LA native rats; and II.

Group B: HA native rats. Stage 2: This stage was carried out to investigate the role of increased norepinephrine on the inflammatory response seen in HA rats based on the results obtained in stage 1. Hence, 3 groups of HA native rats (n=10 of matched age, gender (all rats were male), and weights as those used in stage 1) were subdivided into 3 groups as follows: Group 1: HA native rats treated with one ml normal saline intraperitoneally on a daily basis for 10 consecutive days, and which served as controls for groups 2 and 3. Group 2: HA native rats treated with ß-adrenergic receptor antagonist (propranolol, 40 mg/kg) orally on a daily basis for 10 consecutive days. Group 3: HA native rats treated with α-adrenergic receptor antagonist (prazosin, 4 mg/kg) intraperitoneally on a daily basis for 10 consecutive days. The dose selection and the route of administration were based on previous studies, which proved complete blockage of both receptors in rats.[13,14]

Collection of urine, serum samples, and biochemical analysis. For both groups of rats in stage one, and after a 24-hour adaptation period of housing the rats in Nalgene metabolic cages with free access to water and food (these cages offer a big space for rats to move about freely without stress due to restraint), urine samples were collected into tubes containing 20 µL of 2.5 mol/L HCl. Then, the samples were filtered with 0.2 µm millipore filters and stored at -78°C to measure the levels of dopamine, serotonin, and norepinephrine (only 8 samples of 10 were available for analysis in each group). Two days later, and after overnight fasting, all rats were anesthetized with light diethyl ether. Blood was collected from the rat's eye directly into hematocrit tubes to measure hematocrit value (Hct %), and another 2 ml blood was collected into plain tubes and centrifuged at 5000 rpm for 10 minutes at room temperature. Serum was then collected, and stored at -80°C for further biochemical measurements including TC, TG, LDL, HDL, OxLDL, cortisol, Mg^{+2}, MDA, and inflammatory markers and adhesive molecules including IL-6, TNF-α hsCRP, ICAM-1, and VCAM-1. The atherogenic index (AI) was calculated according to Tan et al[15] using this formula: AI=log (TG/HDL). A similar procedure was carried out for rats of stage 2, but serum only was collected at both baseline (before drug treatment), and after 10 days of either normal saline, or interventional drugs to measure the levels of the circulatory levels of IL-6, TNF-α, hsCRP, VCAM, and ICAM.

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

Variables	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	2800
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

Statistical analysis. Unpaired student's t-test with Welch's correction was used for all comparisons between LA and HA rats (stage 1). Two-way ANOVA with post hoc comparisons (Tukey's t-test) were used to compare all parameters analyzed between all groups used in stage 2 of the study. In both cases, GraphPad prism (version 6, La Jolla, CA, USA) was used for analysis and graphing. Differences were considered significant when $p \leq 0.05$.

Results. ***Basal levels of hematocrit, cortisol, and malondialdehyde.*** Data on hematocrit (Hct) values, serum levels of cortisol, and MDA are presented in **Figure 1**. The Hct and MDA values were significantly higher in rats native to HA compared with those native to LA, as Hct increased by 18% ($p<0.001$) while MDA increased by 466% ($p<0.01$) (**Figures 1A and 1C**). However, the baseline serum levels of cortisol were not significantly different between the HA and LA groups ($p=0.97$) (**Figure 1B**).

Basal levels of serum lipids, atherogenic index, and magnesium. The differences in serum lipids including TG, TC, LDL, HDL, and OxLDL, and changes in the AI between HA and LA native rats are depicted in **Figure 2**. In rats native to HA, serum level of TC increased by 40% (p=0.002), LDL by 68% ($p<0.01$), OxLDL by 50% ($p<0.01$) and AI by 26% ($p=0.002$) compared with those native to LA. However, serum levels of TG and HDL were not significantly different between the 2 groups). As shown in **Figure 3**, the Mg^{+2} levels in HA native rats were significantly decreased by 124% ($p<0.0001$) compared with the LA native rats.

Baseline levels of inflammatory markers/mediators and adhesion molecules. High altitude native rats showed marked and significant elevations in serum levels of all inflammatory mediators/markers including IL-6 (538%), TNF-α (109%), and hsCRP (46.6%), as well as adhesive molecules including VCAM-1 (182%), and ICAM-1 (242%) (**Figure 4**).

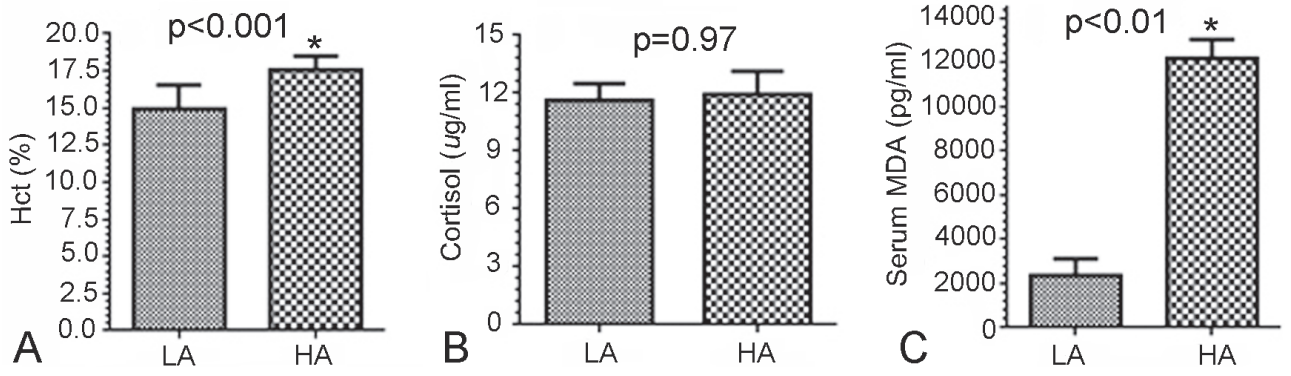

Figure 1 - Percentage levels of: **A**) hematocrit (Hct); **B**) cortisol; and **C**) malondialdehyde (MDA) in the serum of low altitude (LA) and high altitude (HA) native rats. Statistical analysis was performed using student's t-test. Data were expressed as mean ± standard deviation for a group of 10 rats and statistical significance was assigned at $p<0.05$ levels. *significantly different when compared with LA native rats.

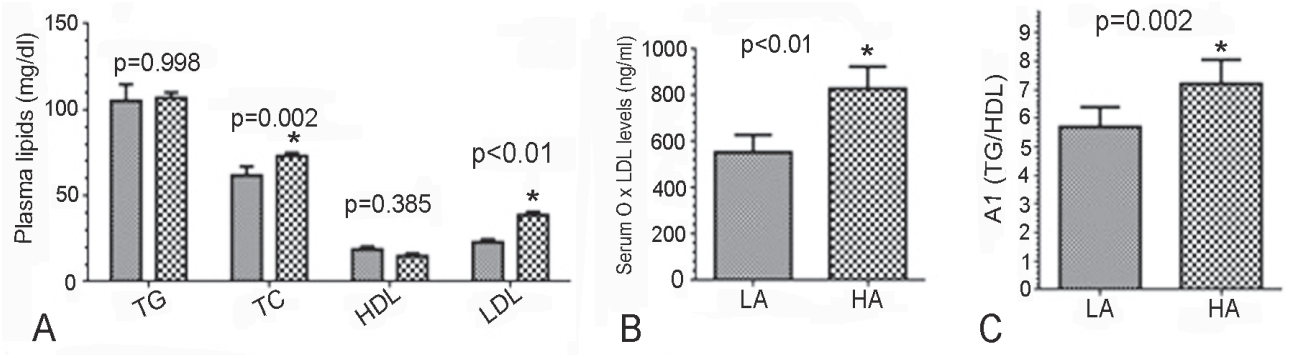

Figure 2 - Levels of total triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL), and low density lipoprotein cholesterol (LDL) in: **A**) oxidized LDL; **B**) oxidative LDL (OxLDL), and **C**) atherogenic index (AI) in the serum of low altitude (LA) and high altitude (HA) native rats. Statistical analysis was performed using student's t-test. Data were expressed as mean ± standard deviation for a group of 10 rats and statistical significance was assigned at $p<0.05$ levels. *significantly different when compared with LA native rats.

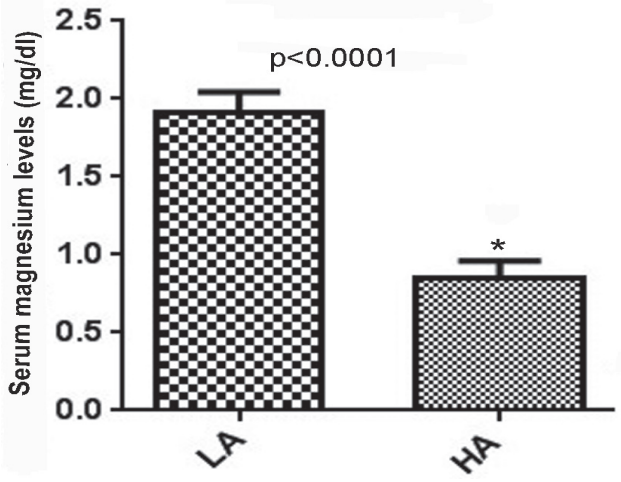

Figure 3 - Magnesium levels in the serum of low altitude (LA) and high altitude (HA) native rats. Statistical analysis was performed using student's t-test. Data were expressed as mean ± standard deviation for a group of 10 rats and statistical significance was assigned at $p<0.05$ levels. *significantly different when compared with LA native rats.

Baseline levels of serotonin, dopamine, and norepinephrine. As shown in **Figure 5**, HA native rats showed a significant decrease in the levels of urinary serotonin (52.8%), with a concomitant increase in the urinary levels of both dopamine (248.5%), and norepinephrine (56%).

Changes in malondialdehyde and inflammatory markers/mediators after adrenergic blockage. Again, native HA rats showed significant increases ($p<0.05$) in basal serum levels of MDA, IL-6, TNF-α, and hsCRP as compared with their basal levels in LA native rats (**Figure 6**). Serum levels of all these parameters in HA native rats were not significantly different after 10 days administration of normal saline as compared to their basal levels. In contrast, administration of propanolol (ß-blocker) or prazosin (α-blocker) significantly depressed the increases in the levels of these parameters seen in the HA native rats after 10 days of drug administration (**Figure 6**). Propanolol

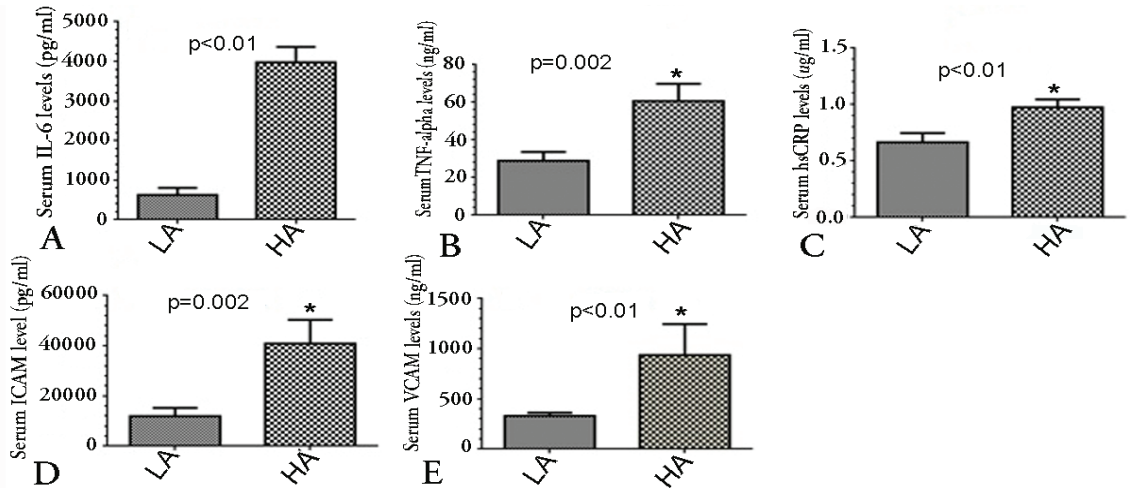

Figure 4 - Serum levels of low altitude (LA) and high altitude (HA) native rats in: **A**) interleukin (IL)-6; **B**) tumor necrosis factor- alpha; **C**) high sensitivity C-reactive protein (hsCRP); **D**) intracellular adhesion molecule (ICAM); and **E**) vascular cell adhesion molecule (VCAM). Statistical analysis was performed using student's t-test. Data were expressed as mean ± standard deviation for a group of 10 rats and statistical significance was assigned at $p<0.05$ level. *significantly different when compared with LA native rats.

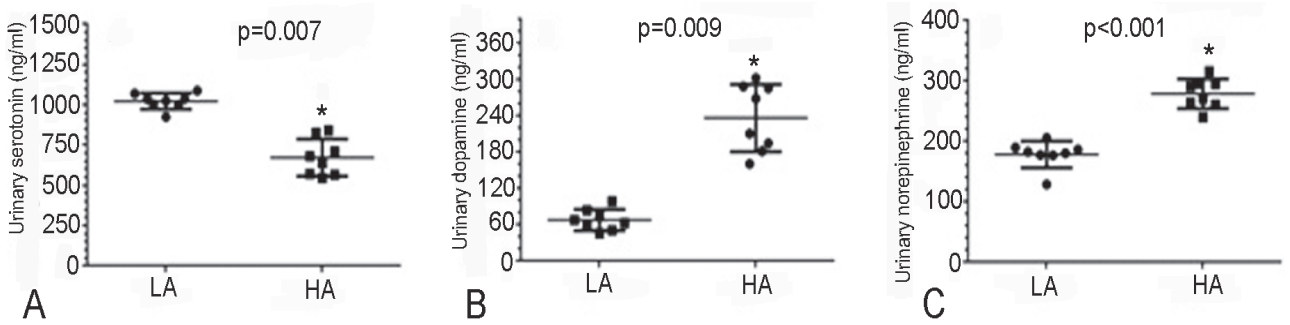

Figure 5 - Levels of urinary serotonin (**A**), dopamine (**B**), and norepinephrine (**C**) in the low altitude (LA) and high altitude (HA) native rats. Statistical analysis was performed using student's t-test. Data were expressed as mean ± standard deviation for a group of 8 rats and statistical significance was assigned at $p<0.05$ levels. *significantly different when compared with LA native rats.

treatment resulted in significant depressions in the levels of MDA by 60% (p=0.004), IL-6 by 57% ($p<0.01$), TNF-α by 30% ($p<0.01$), and hsCRP by 31% ($p=0.005$). More significant decreases were obtained by 10 days of administration of prazosin as these parameters decreased by 83% ($p=0.007$) in MDA, 92% ($p=0.004$) in IL-6, 48% ($p<0.001$) in TNF-α, and 56% ($p=0.004$) in hsCRP as compared with their basal levels in HA native rats that received normal saline. The ANOVA and Tukey's t test revealed that the levels of all of these parameters in HA native rats after 10 days treatment with propanolol were significantly higher than those obtained in the LA group, whereas prazosin administration for the same period of time resulted in non-significant changes in the levels of MDA, TNF-α, and hsCRP levels, and a significantly lower level of IL-6 as compared to corresponding values reported in the LA native rats ($p<0.0001$) (**Figure 6**).

Discussion. In general, systemic low grade inflammation is defined as 2 to 4-fold elevation in circulating levels of pro-inflammatory and anti-inflammatory cytokines, as well as numerous other markers of immune system activity.[16] Although these increases are far from levels observed during acute severe infections, systemic low grade inflammation is reported to be strongly associated with increasing age, lifestyle factors, cardiovascular diseases, type 2 diabetes, and chronic pulmonary diseases.[16] After searching the PubMed database, there was a lack of studies demonstrating that the environmental stresses of living under HA conditions are sufficient to induce systemic

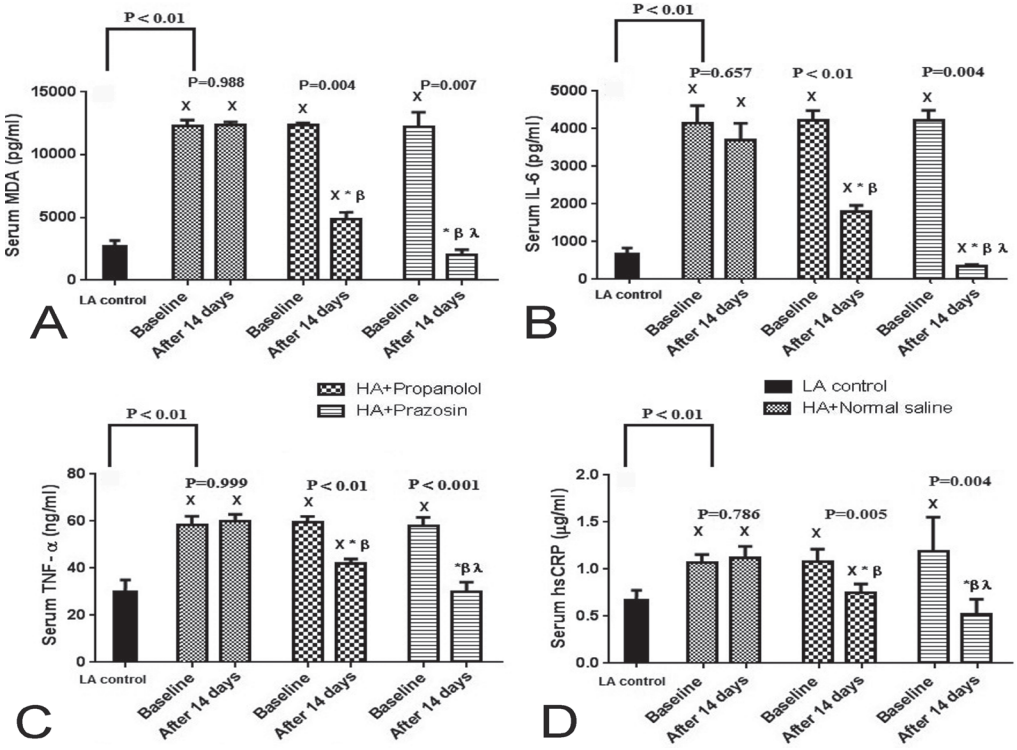

Figure 6 - Levels of malondialdehyde (MDA) (**A**), interleukin-6 (IL-6) (**B**), tumor necrosis factor-alpha (TNF-α) (**C**), and high sensitivity C-reactive protein (hsCRP) (**D**) in high altitude (HA) native rats at baseline, and after 10 days treatment with normal saline, propanolol, or prazosin in comparison with baseline levels in LA. Statistical analysis was performed by 2 way ANOVA. Data were expressed as mean ± standard deviation for a group of 6 rats and statistical significance was assigned at $p \leq 0.05$ level. ^{X} significantly different when compared with baseline level in the LA group, *significantly different when compared with its baseline level, $^{\beta}$significantly different when compared with the same period of the HA+ normal saline, $^{\lambda}$ significantly different when compared with the same period of HA+ propanolol group.

low grade inflammation and lipid peroxidation in rats, and that the mechanism behind this is due to the sustained activation of the SNS. which could be a result of low serum Mg^{+2} levels. In our study, we showed that both α and ß adrenergic receptors are involved in this HA-induced inflammatory response.

In contrast to the previous research, which examined the inflammatory response in either animals or humans after acute exposure to HA using natural or simulated hypoxic chambers, our study is considered unique because it analyzes the inflammatory reaction in relation to its possible causes in native HA rats, which were from the same lineage and bred and maintained in 2 natural areas of different altitudes. All rats in the 2 areas were housed under the same laboratory conditions and were fed the same diet; thus, the observed biochemical changes were not due to dietary factors or to adaptive evolutionary changes. It is well-recognized that the reaction of an individual to a given stressor involves the direct stimulation of pathways within the brain leading to activation of the HPA axis and the central sympathetic outflow.[16] Activation of these pathways results in the "stress response" and ultimately in the release of the key peripheral mediators of the stress response, namely, cortisol and catecholamines.[16] Cortisol has a powerful inhibitory effect on the inflammatory response by direct or indirect effects on immune cell function, adhesion molecule expression, immune cell recruitment, and inflammatory mediator and cytokine generation.[16] In our current study, the levels of circulatory cortisol were similar between both LA and HA native rats demonstrating a state of acclimatization of the rats to HA, and suggesting no role of hypoxia on HPA-induced inflammation. On the other hand, urinary levels of norepinephrine and dopamine were significantly higher while serotonin levels were significantly lower in HA native rats as compared with LA native rats, suggesting a sustained alteration in response to HA stress and occurrence of depression in the HA native rats. However, in addition to the pivotal role of catecholamine in fuel metabolism and the response to changes in energy demand during flight or fight, it is now well evident that IL-6 synthesis and secretion are under the regulation

of catecholamines. In rodents, SNS activation during stress was associated with elevated plasma IL-6 levels.[17] Furthermore, epinephrine, as a result of infusion or due to acute exposure to any acute stress has been shown to induce an acute rise in plasma IL-6[18] from different tissues via ß and α-adrenergic receptors,[19,20] and this release is enough to initiate the inflammatory cascade by increasing synthesis and secretion of other inflammatory mediators and cytokines.[19,20] However, it has been reported that lipid metabolism is altered in humans acutely exposed to HA,[21,22] and that acclimatization to HA leads to changes in serum levels of lipids as reported by Young et al.[22] In our laboratories, we have previously reported that rats native to HA environments exhibit hyperlipidemia and have higher levels of TC and LDL as opposed to those in LA.[10] Moreover, other researchers found similar findings in people native to HA areas.[9] Moreover, under the influence of oxygen free radicals, LDL can be oxidized to OxLDL.[23] Our study showed a significant increase in the serum level of OxLDL in HA native rats as compared to LA native rats. Thus, one possible mechanism is that the OxLDL could activate the inflammatory processes at the level of gene transcription such as up-regulation of TNF-α, which induces up-regulation of nuclear factor kappa-B (NF-kappa B), which is responsible for over expression of cell adhesion molecules including VCAM and ICAM, responsible for the rolling, adhesion, and extravasation of monocytes and T-lymphocytes.[23,24] Also, OxLDL up-regulates the synthesis and secretion of pro-inflammatory markers such as IL-1, IL-6, and cytokines, chemokines, and growth factors.[25] Moreover, elevated OxLDL has also been shown to trigger the release of the inflammatory mediator hsCRP via IL-6 synthesis (as a stimulator of CRP production by the liver).[25,26] Additionally, it has been reported earlier that HA exposure results in increased ROS generation, leading to enhanced oxidative damage to lipids, proteins, and DNA, and this is mainly due to the reductive stress mechanism.[22] Numerous oxidative stress-sensitive transcription factors such as NF-kappa B and activator protein 1 (AP-1) can mediate an inflammatory response caused by oxidative stress by inducing gene transcription of cytokines such as IL-1, IL-6, TNF-α, and adhesion molecules ICAM-1 and VCAM-1.[27] In the current study, the increased MDA serum levels of HA native rats as compared with LA native rats indicates increased free-radical production. Supporting this, hypoxia is well reported to increase plasma and tissue MDA levels at HA and even in in-vitro conditions by different mechanisms,[22] and our results are in accordance with earlier studies. Based on these findings, we therefore, hypothesize that the observed increase in these pro-inflammatory and cell adhesion molecules could be partially attributed to enhanced NF-kappa B levels in the tissues of HA native rats, but the mechanism remains unclear.

From our data, elevated serum catecholamine levels but not OxLDL are implicated to be the most stimulating force for the systemic inflammatory reaction through direct up-regulation of the inflammatory cytokines, or indirectly through oxidative stress-related mechanisms, an effect that is mediated by α and ß-adrenergic receptors. Although the present study was not designed to clarify a possible relationship between catecholamines and increased oxidative stress in HA natives, our data are compatible with such a relationship as administration of α and ß-blockers ameliorated or completely abolished the increased MDA levels in HA native rats. After 10 days of treating the HA native rats with propanolol, a ß blocker, the levels of all inflammatory cytokines and MDA levels were partially ameliorated and significantly reduced as compared with HA rats, while α-adrenergic blockade with prazosin for the same time interval completely abolished the increase in these inflammatory cytokines and mediators and resulted in normal levels of MDA suggesting that OxLDL is not responsible for the HA-induced inflammatory response. Supporting our findings is the fact that norepinephrine is able to induce apoptosis of cardiomyocytes in many studies.[28,29] Furthermore, ß-adrenergic receptor-stimulated apoptosis is mediated by a ROS-dependent pathway.[30]

To search for the sustained increase of catecholamines under HA conditions, we studied the levels of serum Mg^{+2} in both LA and HA native rats. Magnesium ions are considered as the second most plentiful cations in the intracellular fluid and are essential for the activity of many enzymes.[31] In addition to these biochemical actions, Mg^{+2} salts have been shown to lower blood pressure.[32,33] Supporting this proposal, Mg^{+2} deficiency results in a high catecholamine level, altered biofunction, and induced hypertension.[34,35] In our current study, we report that prolonged living at HA results in Mg^{+2} deficiency in rats, which could explain the observed sustained increase in the levels of serum catecholamines at this HA. In agreement with these findings, a study carried out by Shimosawa et al[36] showed that Mg^{+2} blocks mainly N-type Ca+2 channels at nerve endings, and thus inhibits norepinephrine release, which decreases blood pressure independent of its direct

vasodilatory action. Furthermore, most pathological conditions associated with a low Mg^{+2} status have been characterized as having a chronic inflammatory stress component.[8] Evidence obtained in the past 25 years, mostly from animal experiments, have confirmed that severely limiting Mg^{+2} intake to less than 10% of the requirement results in an inflammatory response characterized by leukocyte and macrophage activation, release of inflammatory cytokines and acute-phase proteins, and excessive production of free radicals or oxidative stress in multiple tissues and organs.[37,38]

Human studies also indicate that a low Mg^{+2} status is associated with increased inflammatory and oxidative stress.[39] Several studies have found that Mg^{+2} intake was inversely related to elevated serum or plasma C-reactive protein (CRP). An analysis of 5,007 child participants (age range: 6-17 years) in the 1999-2002 National Health and Nutrition Examination Survey (NHANES),[39] found that children consuming less than 75% of the required daily allowance (RDA) of magnesium were 1.94 times more likely to have elevated serum CRP than children consuming enough RDA.[39] At this stage, we can draw a hypothesis from our study and the previous human and animal studies regarding the increased oxidative stress and systemic inflammation associated with low Mg^{+2}, which could be mediated through catecholamine over secretion and further research to investigate the mechanism behind low Mg^{+2} levels, such as investigation of parathyroid function as a regulator of Mg^{+2} absorption, or studying the excretion rate of magnesium is highly advisable.

In conclusion, our study shows that living under HA conditions results in an increased systemic inflammatory reaction due to sustained rise in catecholamine levels, which could up-regulate the expression of inflammatory markers and increase oxygen free radical generation possibly by up-regulation of oxidative-induced stress transcriptional factors (NF-kB and AP-1), an effect that is mediated through ß and α-adrenergic receptors and could be attributed to low Mg^{+2} levels. Unfortunately, one limitation of our study is that the tissues responsible for the adrenergic-induced surge in plasma IL-6 levels have not been identified as we did not use Mg^{+2} therapy to demonstrate such effect. Future research at the gene level is required to study the relationship between catecholamines and the up-regulation of stress transcriptional factors under hypoxic conditions.

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