Chronic exposure of rats to native high altitude increases in blood pressure via activation of the renin-angiotensin-aldosterone system

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ABSTRACT

الأهداف: دراسة تأثير العيش في المناطق المرتفعة على ضغط الدم، ومعرفه الميكانيكية المسببة لذلك.

الطريقة: أُجريت هذه الدراسة خلال الفترة من فبراير إلى ابريل 2011م، وشملت الدراسة 20 فأر من فئران الوستيرن البيضاء ولدت وعاشت في منطقه منخفضة عن سطح البحر وذلك في مختبرات جامعة الملك سعود بالرياض، المملكة العربية السعودية. وبعد ذلك قمنا بتقسيم الفئران إلى مجموعتين تحوي كل مجموعه على 10 فئران، حيث ظلت المجموعة الأولى في الرياض بينما تم نقل المجموعة الثانية إلى منطقه عالية عن سطح البحر وذلك في مختبرات كلية الطب بجامعة الملك خالد في مدينه أبها، المملكة العربية السعودية. وبعد 90 يوم من نقل فئران المجموعة الثانية تم قياس ما يلي لدى المجموعتين وعمل مقارنة فيما بينهما: ضغط الدم ، والهمياتوكريت، ومعدل الرينين في البلازما، والدسترون، ونورأبنيفرن، وفيسوبرسن. كذلك تم حساب معدل الصوديوم والباتوسيوم في البول. وأخيرا قمنا باستخدام تفاعل سلسلة البلمرة لمعرفه كمية الرينين في الكلى.

النتائج: دلت نتائج الدراسة على ارتفاع ضغط الدم بنوعيه الانقباضي والانبساطي عند فئران المرتفعات العالية، كما كانت هناك زيادة في الرينين، والإلدستورين، ونورابنيفرين، وفيسوبريسن. بالمقابل فقد كانت كمية الصوديوم في البول أقل مقارنة بفئران المرتفعات المنخفضة. كما دلت دراسة البيولوجيا الجزيئية على ارتفاع معدل الرينين في كلى فئران المرتفعات العالية.

خاتمة: أظهرت هذه الدراسة أن التعرض المزمن للمرتفعات العالية قد يؤدي لارتفاع ضغط الدم عن طريق تحفيز نظام الرنين–أَنْجِيوتَنْسين–أَلْدوستيرون.

Objectives: To study the effect of chronic exposure to native high altitude (HA) on blood pressure, and to investigate the underlying mechanism of action.

Methods: This study was carried out between February and April 2011. A total of 20 male rats were divided into 2 groups (n=10 rats). The low altitude (LA) group were rats born and lived in an LA environment at King Saud University, College of Pharmacy, Riyadh, Kingdom of Saudi Arabia (KSA), and the HA group were rats born in the same LA area, then acclimatized to HA area in Physiology Department, King Khalid University, College of Medicine, Abha, KSA for 90 days. At the end of day 90, hematocrit, plasma renin activity, aldosterone, norepinephrine and vasopressin levels were determined in both groups. Invasive arterial blood pressure was also measured, and fractional excretion of sodium (FENa), and potassium (FE_K) were calculated. The quantitative real time-polymerase chain reaction of renin was carried out in the kidneys of both rat groups.

Results: When compared to LA native rats, HA rats exhibited a significant increase in systolic and diastolic blood pressure with a significant increase in renin plasma activity as well as an increase in the levels of aldosterone, norepinephrine, and vasopressin. Furthermore, HA rats showed a significant increase in renin expression in their kidneys, as well as decreased FENa.

Conclusion: Data shows that prolonged exposure to HA results in elevated blood pressure precipitated by the activation of the renin-angiotensin-aldosterone system.

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The renin (angiotensinogenase) is the key regulator of the renin-angiotensin-aldosterone system, which is critically involved in salt, volume, and blood pressure homeostasis of the body.[1] Renin is mainly produced and released into circulation by juxtaglomerular granular cells, located in the walls of renal afferent arterioles at the entrance of the glomerular capillary network.[1] It has been known for a long time that the synthesis and secretion of renin are stimulated by the sympathetic nerves and prostaglandins while inhibited via negative feedback including elevated angiotensin II, high blood pressure, salt, and volume overload.[2] Hypoxia is defined as the lack of oxygen at the tissue level. Different clinical and experimental studies demonstrated that different biochemical, molecular and physiological pathways are involved in the cell tolerance to acute and chronic hypoxia. The effect of hypoxia on renin secretion and gene expression has been studied extensively, but the literature showed controversial results. Acute hypoxia of 6-hour duration was found to cause a marked stimulation of renin secretion and gene expression which was thought to contribute to the progression of chronic renal failure and to the development of hypertension in Sleep-apnea syndrome.[3] On the other hand, in another study, chronic hypoxia exposure suppressed renal renin gene expression in rats where the author's postulated that this effect was mediated by endothelins overexpression.[4] At the molecular level, tissue hypoxia was found to significantly stimulate renin secretion and gene expression *in vivo*, but not *in vitro*. These effects were found to be mediated by circulating catecholamines but neither by renal denervation nor by direct action on juxtaglomerular granular cells.[4] Nevertheless, Neylon et al[5] hypothesized that renin secretion is increased by the hypoxia-induced fall in renal perfusion pressure rather than by an increase in renal sympathetic activity. These findings prompted us to investigate the effect of exposure to chronic hypoxia (that is, living at high altitude) on blood pressure and the underlying renin-angiotensin system (RAS).

Methods.

Areas of the study. This study was conducted in 2 groups of rats; one that lived at low altitude (LA group; in King Saud University, College of Pharmacy, Riyadh, Kingdom of Saudi Arabia [KSA]), and the other acclimatized to high altitude (HA group) for 90 days (King Khalid University, College of Medicine, Physiology Department, Abha, KSA) prior to conducting any analysis. Abha city is located in Aseer Mountains, Southwest of KSA, and sits on an altitude of 2800-3150m (9186'-10335') above sea level. On the other hand, Riyadh, is located in the center of KSA that rises 600 m (1969') above sea level. Environmental data on these areas are shown in **Table 1**.

Experimental design. A total of 20 Adult Wistar male rats weighing between 350 g, and aged 3 months old were selected for the experimental procedure. Rats were bred (that is, tenth generation) at the animal house in King Saud University, Riyadh (LA area). Rats were divided into 2 groups 10 rats each. Blood pressure measurements were performed on LA Group rats on arrival to Abha followed by collection of blood and urine analyses and kidney tissue collection, while HA Group rats were first acclimatized at the animal house at King Khalid University Medical School in Abha for 90 days then underwent blood pressure measurement, blood and urine samples collection followed by kidney tissue collection. All rats were housed during the period of study under the same laboratory conditions, and fed the same diet with the exception of high altitude in the case of HA Group. Both groups were maintained in similar polypropylene cages of standard dimensions at a temperature of 25 ± 1°C, had a standard 12 hour day/night cycle and were housed in groups of 5 rats per cage (50×26×16 cm). All procedures were approved by the Ethical Committee of Physiology at the King Khalid University Medical School (Abha, KSA), 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.[6]

Collection of urine and plasma samples. Urine and blood samples were collected from LA Group on arrival to Abha (HA land) coming from Riyadh (LA Land). Samples from the HA Group commenced at day 88 of acclimatization to HA Land. In either case, 1.5 ml blood using cardiac puncture was collected into Eppendorff tubes with a final EDTA concentration in the blood sample of 0.64 mM. 0.1ml of collected blood was used to determine hematocrit as described previously.[7] Remaining blood was centrifuged at 5000 rpm for 10min at room temperature. Plasma was then collected, aliquoted then stored at -80°C for further analysis. Rats were returned to their cages, fed normal diet and supplied with water for 2 days after then, rats were placed in metabolic cages for a 24-hour urine collection followed by invasive blood pressure measurement.

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Table 1 - General geographic and meteorological information of Riyadh and Abha, Saudi Arabia.

Geographic and meteorological information	Riyadh (LA)	Abha (HA)
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 (m) / (feet)	600/1969	(2800-3150)/(9186-10335)
Barometric pressure (mm Hg)	711	590-590
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

LA - low altitude, HA - high altitude

Analysis of urine and plasma samples. Plasma levels of renin, aldosterone and norepinephrine were determined using mouse/rat ELISA kits (Cat# E90889Ra, E90911Ra and E90907Ra, respectively, Uscn lifescience Inc. Japan). Plasma levels of vasopressin were measured using mouse/rat ELISA kit (Cat # 583951, Cayman Chemical Company, Michigan, USA). Plasma Na+ and K+ levels were determined using commercially available kits (Human Diagnostics, Wiesbaden, Germany). The concentration of Na+ and K+ in each urine specimen was determined by indirect potentiometry using the Beckman Coulter Synchron Clinical System. Urinary creatinine was determined according to Jaffe' reaction using urinary creatinine assay kit (500710, Cayman Chemical Company, Michigan, USA). All biochemical measurements were carried out according to the manufacturer's instructions.

Fractional excretion of sodium (FENa) and fractional excretion potassium (FEK) was calculated from the following standard equations:[8,9]

$$FE_{Na} = 100 \times \frac{sodium_{urinary} \times creatinine_{plasma}}{sodium_{plasma} \times creatinine_{urinary}}$$

$$FE_{k} = 100 \times \frac{potassium_{urinary} \times creatinine_{plasma}}{potassium_{plasma} \times creatinine_{urinary}}$$

Surgical procedure and measurement of arterial blood pressure. In order to measure arterial blood pressure, the following procedure was applied to LA Group on arrival while HA Group after 90 days of acclimatization to high altitude. However, urine and blood samples collection was performed first as described below prior to measuring blood pressure. Low altitude and HA rats were anesthetized by a single intraperitoneal administration of pentobarbital sodium (150 mg/kg). Anesthetized animal were rapidly transferred to a well temperature controlled table and were placed in dorsal recumbency. Individually, each rat was dissected and the right carotid artery was located and prepared for cannulation to measure the systemic arterial blood pressure (ABP). Surgical procedure and canulation of carotid artery lasted for approximately 5 min. The vagus nerve was first detached from the carotid artery. Then, a 2-line fluid filled pressure transducer (MLT0670, AD Instruments, Australia) connected to a bridge amplifier (FE117 BP Amp, ADI) was inserted. The arterial line was prefilled with heparinized saline (50 U/ml). PowerLab data acquisition system (PL3516/P PowerLab 16/35, ADI) was used to receive and record the signal detected by the bridge amplifier, which was later analyzed by Labchart pro.7.2 software (ADI). Throughout the experiment, rats' body temperature was maintained at 38°C.

RNA isolation and cDNA synthesis. Kidney tissue was collected from the LA and HA Group following the determination of blood pressure described above. Total RNA was extracted from kidney tissue homogenate using GStract™ RNA Isolation kit II (SA-40005, Maxim BioTech, Inc. San Francisco, USA) Guanidium Thiocynate method. The purity and concentration of RNA were quantified by spectrophotometry. Reverse transcription reaction was performed using oligo (dT) primers (USA). The 25 µl cDNA synthesis reaction consisted of 2.5 µl (5x) buffer with MgCl2, 2.5 µl (2.5 mM) dNTPs (Pharmacia Biotech), 1 µl (10 pmol) Oligo d (T) primer (Pharmacia Biotech), 2.5 µl RNA (2mg/ml) and 0.5 unit reverse transcriptase enzyme (Qiagene, US). The mixture was incubated at 37°C for one hour. The PCR amplification was performed in a thermal cycler (Applied Biosystems (ABI), USA) programmed at 42°C for one hour, 72°C for 10 minutes (enzyme inactivation), and the product was stored at 4°C until used.

Real time PCR and quantitative estimation of renin mRNA. For qRT-PCR, a set of primers:

Sense: 5'-AGTACTATGGTGAGATCGGCATT-3'; Antisense:5'-AGATTCACAACCTCTATGACTCCTC-3') were designed from the published cDNA sequences of the rat renin 1c gene which amplified a 123bp product. The reaction was carried out using Rotor-Gene6000system (Qiagen, USA) and consisted of 12.5 µl of 2X QuantitechSYBR® Green RT Mix (Fermentas,

Germany), 1.0 µl of 25 pm/µl renin primers, 2 µl cDNA (100 ng) and 9.25 µl of RNase free water. Samples were spun well before loading in the Rotor's wells. The real time PCR program was performed as follows: initial denaturation at 95°C for 10 min.; 40 cycles of 95°C for 15 sec.; annealing at 60°C for 30 sec and extension at 72°C for 30 sec. Data acquisition performed during the extension step. This reaction was carried out using Rotor-Gene6000system (Qiagen, USA). In every PCR set run, negative controls were added where cDNA was replaced with water as a control for contamination from any exogenous sources. In addition, RT was omitted in some samples as a negative control for amplification of genomic DNA. Housekeeping gene, GDPH (Glyceraldehyde 3-phosphate dehydrogenase) was amplified under similar conditions:

Sense: 5'-ATTGATCACTATCTGGGCAA-3';

Antisense reverse primer 5'-GAGATACACTTCAATACTTTGACCT- 3'

for comparative quantification analysis using Rotor-Gene-6000 Series Software (Qiagen, USA). The PCR products were electrophoresed on 1% agarose gel and visualized by ethidium bromide staining under UV light. The fold increase in the mRNA expression in both HA and LA rat groups was calculated. Comparative quantification analysis was carried out using Rotor-Gene-6000 Series Software based on the following equation:

$$\text{Ratio target gene expression (Experimental/control)} = \frac{\text{Fold change in target gene expression (expt/control)}}{\text{Fold change in reference gene expression (expt/control)}}$$

Statistical analysis. Graphing and comparison between the HA and LA groups were analyzed using either student's t-test or Mann-Whitney U test as needed (Graphpad prism software, version 5). Statistical significance is defined at $p \leq 0.05$.

Results. All mean data were compared by using student's t-test except assessment of RAS components (**Table 2**) as Mann-Whitney U test have been used. The data shows a significant increase (28%; $p=0.002$) in hematocrit value in the group of rats acclimatized to high altitude for 90 days (from 38.65 ± 1.94 to 49.4 ± 2.34) (**Figure 1**). **Figure 2** shows the changes in arterial blood pressure in the LA and HA Group. A significant increase ($p=0.001$) in systolic (24.8%), diastolic (25%), and mean arterial pressure (24.8%) in the HA compared to LA Group rats was observed. Assessment of the components of the RAS system showed that renin plasma levels significantly ($p=0.0001$) increased by 172.9% following 90 days of acclimatization at hypoxic high altitude. A parallel significant increase of 241.2% ($p=0.0001$) was also observed in aldosterone levels, 113.8% ($p=0.001$) in vasopressin levels, and 47% ($p=0.0001$) in norepinephrine levels (**Table 2**). Further, in acclimatized rats, a significant increase of 35.7% ($p=0.008$) in serum Na+ concentration occurred combined with a significant reduction of 31.4% ($p=0.005$) in urinary Na+ concentration. However, plasma creatinine, plasma K+ and urinary K+ levels did not significantly change in the HA group ($p=0.078$), while urinary creatinine level significantly decreased by 15% ($p=0.038$) in these rats in the same corresponding time interval. Interestingly, FENa dropped significantly by 24.2% ($p=0.006$) in HA group, while no significant change was noted in FEk ($p=0.091$) (**Table 3**). Renin gene expression in the kidney tissues in either rat groups was assessed using real time PCR. **Figure 3A** shows a

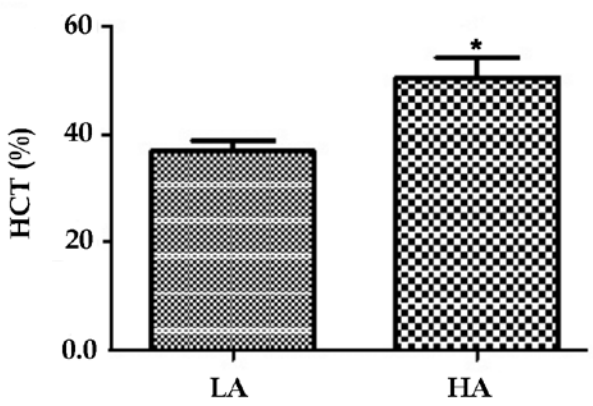
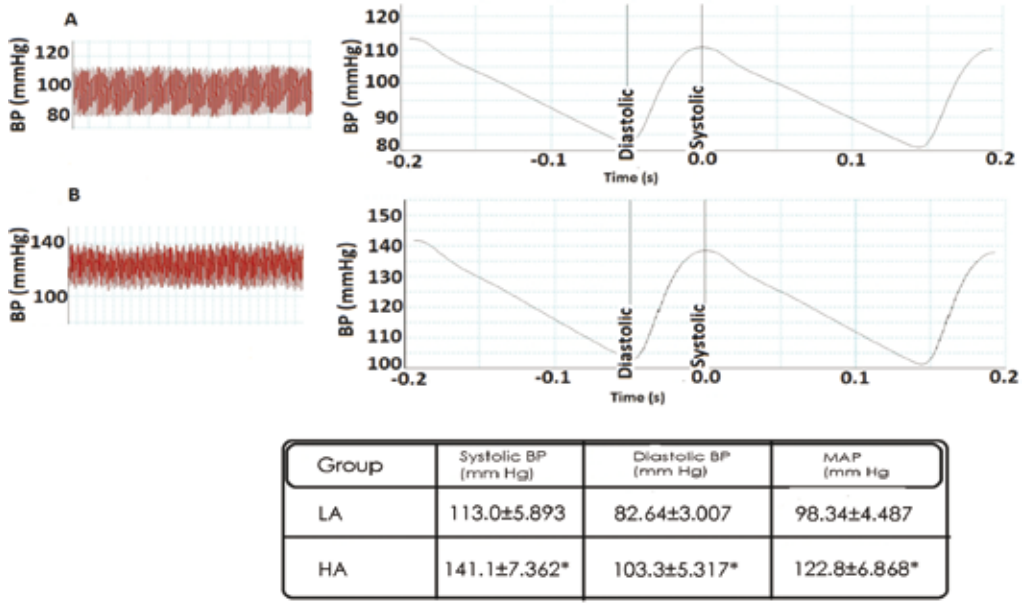


Figure 1 - Hematocrit (Hct) of blood samples of rats in low altitude (LA) and high altitude (HA) groups. Values are expressed as mean±SD (n=10) in each. Analysis by student's t-test. Values were considered significantly different at *$p<0.05$.

Table 2 - Plasma levels of renin, aldosterone, norepinephrine, and vasopressin in low altitude (LA) and high altitude (HA) groups of rats.

Parameter	LA	HA	*P*-value (Mann-Whitney U test)	% Change
Plasma renin activity (ng/ml/hr)	11.8 ±1.70	32.2 ± 3.17 *	0.0001	+172.88
Plasma aldosterone concentration (pg/ml)	159.87 ±18.78	545.5 ± 38.8*	0.0001	+241.2
Plasma vasopressin concentration (pg/ml)	3.51 ± 0.25	5.16 ± 0.81*	0.001	+47.01
Plasma norepinephrine concentration (pg/ml)	67.40 ± 8.16	144.1 ±13.8*	0.0001	+113.80

Values are expressed as mean ± SD. Ten rats in each group, analysis by Mann-Whitney U test, values were considered significantly different at *$p<0.05$.



Group	Systolic BP (mm Hg)	Diastolic BP (mm Hg)	MAP (mm Hg
LA	113.0±5.893	82.64±3.007	98.34±4.487
HA	141.1±7.362*	103.3±5.317*	122.8±6.868*

Figure 2 - A graph showing continuous trace recording of invasive arterial blood pressure (BP) from the right carotid artery of **A**) low altitude (LA) and **B**) high altitude (HA) group of rats. Left chart: represents the signals from the blood pressure (BP) bridge amplifier that were detected, filtered, amplified, and sent to an analog-to-digital converter (Power Lab data acquisition and analysis system: ADI, Australia). Right chart: Graphic analysis of the raw data on the left via by Labchartpro7 software (ADI, Australia). Table (inset): Numeric values of systolic, diastolic and mean arterial blood pressure (MAP) in LA and HA group of rats. Values are expressed as mean ± SD (N=10) in each group (*$p \leq 0.05$).

Table 3 - Plasma and urinary levels of sodium (Na+), potassium (K+) and creatinine in low (LA) and high altitude (HA) rats.

Parameters	LA	HA	*P*-value (student's t test)	Changes (%)
Plasma Na^+ (mmol/L)	130.0 ± 4.43	176.4 ± 4.67*	0.008	+35.69
Plasma K^+ (mmol/L)	0.70 ± 0.09	0.79 ± 0.12	0.078	+12.85
Plasma Creatinine (mg/dl)	0.351 ± 0.062	0.4 ± 0.01	0.078	+13.96
Urinary Na^+ (mmol/L/24 hours)	174.9 ± 6.34	120.0 ± 4.56*	0.005	-31.40
Urinary K^+ (mmol/L/24 hours)	15.35 ± 1.23	12.5 ± 3.1	0.064	-18.57
Urinary Creatinine (mg/dl/24 hours)	20.0 ± 2.1	17.0 ± 1.87*	0.038	-15.0
FE_{Na} (%)	2.11 ± 0.003	1.6 ± 0.098*	0.006	-24.17
FE_K (%)	38.4 ± 1.98	37.2 ± 2.34	0.091	-3.125

FE_{Na} - fractional excretion of sodium, FE_K - fractional excretion of potassium, values are expressed as mean ± SD, n=10 in each group, analysis by student's t-test, values were considered significantly different at *$p<0.05$.

representative gel of the PCR-amplified products for rat's renin cDNA from kidney tissue homogenate from both LA and HA rats. Rat's renin cDNA from kidneys tissue homogenate was amplified and quantified (**Figure 3A**). The specific primer pairs used did amplify the expected product of renin cDNA with a size of 123 bp. The negative control (-ve CR) for each cDNA synthesis reaction was absence of Reverse Transcriptase (RT). No amplified product(s) was/were seen in these PCR reactions affirming absence of contaminating genomic DNA. The PCR product amplified from HA group rats exhibited higher levels than that detected in the LA group. Upon visual inspection of the agarose gel, the PCR product amplified from HA group was higher than that detected in the LA group. Further quantitation of amplified product from kidney homogenate from the LA and HA groups using glyceraldehyde 3- phosphate dehydrogenase gene in the same tissue (GDPH) as internal control, revealed a significant difference (p=0.001) in renin transcript levels between the LA (0.24.6 ± 0.05) and HA group rats (0.65.7 ± 0.032). These results indicate that the average levels of renin gene mRNA in the kidneys of HA rats were 2.7 times that present in the LA group (**Figure 3B**). Correlation

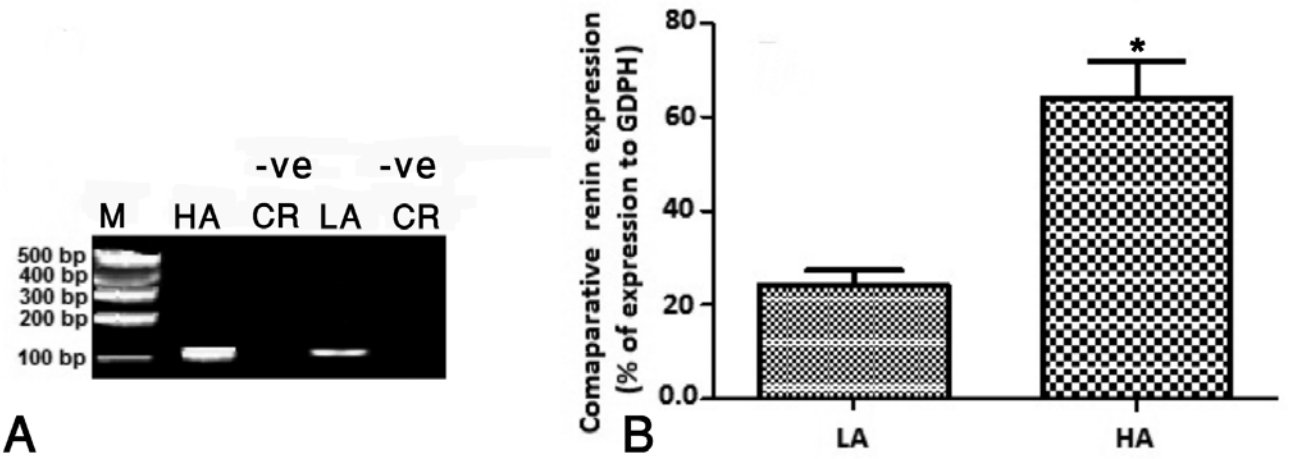


Figure 3 - A) Gel electrophoresis of the quantitative reverse transcription polymerase chain reaction (QRT-PCR) amplification products for renin mRNA using primers designed from the published cDNA sequences of the rat renin 1c gene that produced the expected 123 bp product. **B**) Renin mRNA/Glucose-6-phosphate dehydrogenase (G6PDH) mRNA expression ratio in rats kidneys of low LA and HA Group rats. LA - rats raised at low altitude, HA - rats native to high altitude, -ve CR - negative control where reverse transcriptase was removed from the RT reaction, M - marker analysis by student's t-test. Values were considered significantly different at $*p<0.05$.

analysis of renin activity and renin gene expression showed a positive correlation (r=0.837).

Discussion. In this study, all rats 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. Furthermore, as a limitation in previous researches, racial factors were eliminated as this study was carried out in rats rather than humans and all animals were born and bred in the same facility. Thus, the factor that appears to be at study in this situation is environmentally derived. The rats in the HA group showed a significant increase in systolic, diastolic and mean arterial pressures. This is congruent with the reported effect of hypoxia characteristic of high altitudes like Abha, KSA, which resides at 2800-3150 m above sea level. Also consistent with these findings is the significant elevation of hematocrit, which is suggestive of erythrocytosis in rats acclimatized rats to HA. This typically occurs to increase local oxygen delivery via production of angiogenic factors and erythropoietin,[10] which explains the increase in hematocrit value in the HA rats seen here. Our findings are consistent with earlier work at high altitude as reported by Wheatley et al,[11] Hooper and Mellor,[12] and Jefferson et al.[13]

The underlying mechanism for the observed elevated blood pressure in acclimatized rats appears to include at least 3 strategies: activation of the RAAS system, central release of vasopressin, and sympathetic induced norepinephrine release. With regard to the RAAS, renin gene expression was activated as evidenced by the 2.67-fold increase in renin mRNA compared to rats raised at LA. Further, renin activity increased in the HA rats by 172.9%. Post analysis also showed a strong positive correlation between renin gene expression in the kidney tissue and its plasma activity in the HA rats. Further, aldosterone levels also increased by 241.2% in the HA group combined with a 35.7% increase in plasma Na+ and 31.4% decrease in urinary Na+ affirming response to released aldosterone. Interestingly, kidney response to correct blood pressure via increasing blood volume is known to be a systemic long-term stable adaptive mechanism of action rather than an acute positive physiological feedback response that will return to normal. This suggests that the observed increase in blood pressure resulted from the chronic exposure to HA and is not an acute effect. The fact that our results are detected after acclimatization of rats for the duration of 90 days at high altitude supports this contention. It is also relevant to point out the observed increase in hematocrit, which has been shown to contribute to pulmonary hypertension in simulated HA in rats.[14]

The juxtaglomerular cells constitute the main source of circulating renin in the body,[15] and it has been postulated that regulation of renin release from the kidney is complex process and involved many mechanisms.[16-19] Upregulation of the renin-angiotensin system was found to be increased under sympathetic discharge to the juxtaglomerular cells.[19] The peripheral chemoreceptors located in the aortic and carotid bodies are responsible for sensing oxygen partial pressure

and they do discharge when it decrease.[19] This may draw a relationship between the increased renin serum levels and kidney expression and the increased levels of norepinephrine seen in this study. In the same line to these findings, Krämer et al,[4] reported stimulated renin secretion and gene expression in vivo under hypoxia conditions and these effects were found to be mediated by circulating catecholamines,[4] on the other hand, Neylon et al[5] hypothesized that renin secretion is increased by the hypoxia-induced fall in renal perfusion pressure rather than by an increase in renal sympathetic activity.

The second key player in the RAAS is the systemic angiotensin II, which plays a pivotal role in the regulation of blood pressure and in fluid and electrolyte homeostasis.[1] Angiotensin II plays several physiological roles: it binds to angiotensin II receptor type I (AT1), which induce vasoconstriction thus increasing systemic vascular resistance and arterial pressure; acts on the adrenal cortex to release aldosterone, which in turn acts on the kidneys to increase sodium and fluid retention; stimulates the release of vasopressin (antidiuretic hormone, ADH) from the posterior pituitary, which increases fluid retention by the kidneys; stimulates thirst center in the subfornical organ within the brain; potentiates norepinephrine release via direct action on postganglionic sympathetic fibers, and inhibits norepinephrine re-uptake by nerve endings, thereby enhancing sympathetic adrenergic function and stimulates cardiac hypertrophy and vascular hypertrophy.[1] With regard to the third component of the RAAS, aldosterone, there was a significant increase in its levels increase. It is noteworthy that adrenal glomerulosa cells have been shown to be sensitive to levels of oxygen, in particular the conversion of corticosterone to aldosterone reaction.[20] In fact, Brickner et al[20] reported an inhibition of aldosterone secretion under in vitro induced-hypoxia as opposed to the increase observed here. This could be attributed to posttranslational modifications in 18-hydroxlase resulting in desensitization to O_2 levels and/or altered 18-hydroxylase gene expressions under in vivo chronic exposure to native high altitude hypoxia.

The recorded 24.1% in EFNA in HA rats could be the result of increased Na+-K+/ATPase pump density in the distal segment principal cells in response to increased aldosterone secretion,[21] or due to increased Na+ reabsorption at the proximal tubule as a result of increase angiotensin II levels.[22] Alternatively, the increase in aldosterone levels resulted in potentiating the sympathetic system and increased norepinephrine release by postganglionic neurons. The increase in norepinephrine resulted in vasoconstriction induced-drop in renal perfusion pressure leading to a decreased glomerular hydrostatic pressure and decreased filtered load of Na+ and Cl-.[23,24] Recently, an increase in Na+ blood plasma, which is known to increase vascular tone and cardiac output, has been hypothesized to activate endogenous digitalis synthesized by human brain that increases vascular tone, vascular resistance, cardiac output and sympathetic activity.[25] Interestingly, the physiological response to native high altitude also mobilized a central, as well as peripheral nervous systems responses in the form of increased vasopressin release from the posterior pituitary and the sympathetic catecholamine norepinephrine from postganglionic neurons; both of which are known to exert potent increase in blood pressure via antidiuresis and vasoconstriction, and increased cardiac output.

Hypoxia is one important factor to address when it comes to interpreting altered physiology resulting from living at high altitudes. Our hypothesis is that the observed increase in blood pressure and thereafter activation of the RAAS system were triggered by the hypoxic conditions that the HA group have lived in continuously for 90 days. At the molecular level, it was reported that hypoxia-inducible factors (HIFs) are expressed under hypoxic conditions.[26] The HIFs regulate the transcription of hundreds of genes in response to changes in oxygen pressure and it is regulated by O_2-dependent hydroxylation by prolyl hydroxylase.[26] This hydroxylase activity causes the degradation of the active alpha subunit of the HIFs under normoxic conditions. However, under hypoxic conditions the hydroxylase is inhibited since it utilizes O_2 as co-substrate,[27] resulting in the activation of HIFs. High concentrations of tricarboxylic acid cycle intermediates or chelators of Fe (II) 18 have also been shown to inhibit the hydroxylase activity.[27,28] The HIF-1 has been reported to augment renin expression under hypoxia.[26] Future investigations should compare HIF-1 expression in the HA and LA group to better understand the molecular mechanism of adaptation to HA hypoxia. Further research on human are required to better understand the physiology of blood pressure at HA.

In conclusion, this study focuses on the effect of chronic living at HA on blood pressure and investigates the underlying mechanism. Systolic, diastolic and mean arterial blood pressures, determined via invasive direct measurement, were increased significantly in rats acclimatized to HA for 90 days. The RAAS, vasopressin (that is, anti-diuretic hormone), and epinephrine were the activated physiological response to living at hypoxic HA.

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