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PULMONARY ARTERY PRESSURE LIMITS EXERCISE CAPACITY AT HIGH ALTITUDE

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ABSTRACT

Altitude exposure is associated with decreased exercise capacity and increased pulmonary vascular resistance (PVR).

Echocardiographic measurements of pulmonary hemodynamics and a cardiopulmonary exercise test were performed in 13 healthy subjects at sea level, in normoxia and during acute hypoxic breathing (one hour, 12 % of oxygen in nitrogen), and in 22 healthy subjects after acclimatization to the altitude of 5050 m. The measurements were obtained after randomisation in double blind to the intake of placebo or the endothelin A receptor blocker sitaxsentan 100 mg/day during 7 days. Blood and urine were sampled for renal function measurements.

Normobaric as well as hypobaric hypoxia increased PVR and decreased maximum workload and oxygen uptake (VO_2max). Sitaxsentan decreased PVR in acute hypoxia and in chronic hypoxia (both $P<0.001$), and partly restored VO_2max, by 30 % in acute hypoxia ($P<0.001$) and 10 % in chronic hypoxia ($P<0.05$). Sitaxsentan-induced changes in PVR and VO_2max were correlated ($P=0.01$). Hypoxia decreased glomerular filtration rate and free water clearance, and increased fractional sodium excretion. These indices of renal function were unaffected by sitaxsentan intake.

Selective endothelin A receptor blockade with sitaxsentan improves mild pulmonary hypertension and restores exercise capacity without adverse effect on renal function in hypoxic normal subjects.

Key words: Pulmonary vascular resistance, hypoxia, maximal oxygen uptake, sitaxentan, renal function

Abstract word count: 248

INTRODUCTION

High altitude exposure is associated with a decreased aerobic exercise capacity, in relation to both a decrease in arterial oxygen content and a limitation in maximal cardiac output (1). With acclimatization, the arterial oxygen content may be restored to sea level values because of an increased hemoglobin concentration, but maximal cardiac output remains depressed (2). The decrease in maximal cardiac output at altitude has been tentatively explained by the combined effects of decreased blood volume, hypocapnia, increased viscosity of the blood, autonomic nervous system changes, or depressed myocardial function, but is probably to be accounted for by an altered coupling of convectional and diffusional oxygen transport systems (3). An additional factor might be a limitation in right ventricular flow output secondary to hypoxic pulmonary hypertension. An improvement in maximal workload and maximal oxygen uptake (VO_2max) together with a decrease in pulmonary artery pressure (PAP) has indeed been reported after the intake of sildenafil in hypoxic healthy volunteers (4,5). More recently, there has been report of decreased PAP and improved VO_2max in subjects susceptible to high altitude pulmonary edema taking dexamethasone before altitude exposure (6). However, improved exercise capacity in these studies could not unequivocally be ascribed to associated inhibition of hypoxic pulmonary vasoconstriction, because of additional effects including a variable improvement in arterial O_2 content (4-7).

We recently reported an improved VO_2max specifically related to a decreased PAP by the administration of the non-selective endothelin receptor antagonist bosentan in acutely hypoxic normal subjects (8). In the present study, we tested the hypothesis that inhibition of hypoxic pulmonary vasoconstriction by the selective endothelin A receptor antagonist sitaxsentan (9) would allow for a persistent improvement in aerobic exercise in normal

subjects at high altitudes. We also investigated the effects of sitaxsentan on renal function, as a decreased free water clearance has been reported after the intake of bosentan at high altitudes (10).

METHODS

Subjects

A total of 30 healthy subjects, 15 men and 15 women aged from 23 to 59 years, (mean 35 years), with a height of 173 ± 2 cm (mean ± SE) and a weight of 66 ± 2 kg, gave an informed consent to the study which was approved by the Ethical Committee of the Erasme University Hospital (Brussels).

Protocol design

Heart rate (HR), blood pressure (BP) and transcutaneous O_2 saturation (SpO_2) were measured in all the subjects. Clinical examinations were performed at sea level and at high altitude.

Blood and urine were sampled in 17 subjects at sea level and 22 subjects at high altitude for renal function measurements.

At sea level, twenty-two of the subjects underwent a Doppler echocardiography at rest to estimate pulmonary artery pressures, cardiac output, and right ventricular function, and an incremental cycle ergometer cardiopulmonary exercise test (CPET) with measurements of ventilatory variables, workload, BP, heart rate and SpO_2 to measure aerobic exercise capacity.

The normobaric normoxic and hypoxic measurements were repeated in 13 subjects after a one-week treatment with sitaxsentan, 100 mg/day, or a placebo, either in normoxia, or after one hour breathing a fraction of inspired O_2 (FIO_2) of 12 % from a pre-mixed tank of O_2

in nitrogen. The subjects were equipped with tightly fitted face masks to breathe either room air or the low O_2 mixture. The FIO_2 of 0.12 corresponds approximately to the inspired partial pressure of O_2 at the altitude of 4500 m. Sea level measurements were performed according to a prospective randomized controlled cross-over study design. Thus 13 subjects had four echocardiographies and CPET at sea level, each in one of the following conditions: normoxia-placebo, normoxia-sitaxsentan, hypoxia-placebo, hypoxia-sitaxsentan.

In hypobaric hypoxic condition, twenty-two subjects had two echocardiographies and CPET at high altitude, respectively at baseline and one week later, after intake of either a placebo or sitaxsentan.

The sampling of blood and urine for renal function measurements took place at 8 am only in normoxia at sea level in 17 subjects, and at the same hour at high altitude in 22 subjects. The urine collections covered the previous 24 hours at sea level, and 2 hours at altitude. The subjects were instructed to observe their usual diets and schedules, and to refrain from coffee consumption and cigarette smoking.

The normobaric normoxic and hypoxic measurements were performed in Brussels, at sea level altitude. The hypobaric hypoxic measurements were obtained at the Pyramid International Laboratory Observatory at 5050 m, in the Khumbu area of the Sagamartha National Park. This setting is reached after a one week hike at progressively increased altitudes following an airplane transportation from Kathmandu, at approximately 800 m, to Lukla, at 2800 m. This hike is easy going, and usually allows for a satisfactory acclimatization. Once at the Pyramid hut, the subjects rested or walked about in the surroundings, avoiding strenuous exercise activities. Meals were served by the local staff, and intake of water or hot lemon was allowed *ad libitum*. Altitude measurements were obtained at baseline, and repeated after one week sitaxsentan 100 mg or a placebo given in a prospective

randomized, controlled double-blind fashion. Sitaxsentan was taken once daily for 7 days to achieve steady state of plasma levels (9).

Clinical measurements

Blood pressure was measured by sphygmomanometry, with mean pressure calculated as diastolic pressure + 1/3 pulse pressure. A three lead ECG was used to measure HR. SpO_2 was measured by ear lobe pulse oximetry (Konica Minolta Pulsox-3i; Konica Minolta Sensing, Osaka, Japan). Attention was paid to local temperature and quality of the signal, especially during exercise, as it is known that accuracy and precision of pulse oximetry at exercise may be decreased by local perfusion (11) Hemoglobin concentration was measured at sea level and at 5050 m in 17 of the subjects. The presence of acute mountain sickness in hypoxia was assessed by use of the Lake Louise consensus scoring system (12).

Echocardiography

The Doppler echocardiographic measurements were performed with a Vivid 7 ultrasound system at sea level and its Vivid I portable version at altitude (GE Ultrasound, Norway). Cardiac output (Q) was estimated from left ventricular outflow tract cross sectional area and pulsed Doppler velocity-time integral measurements (13). Systolic PAP (sPAP) was estimated from a trans-tricuspid gradient calculated from the maximum velocity of continuous Doppler tricuspid regurgitation, added to a fixed value of 5 mmHg attributed to right atrial pressure (14). Pulmonary vascular resistance (PVR) was calculated as mPAP/Q with mPAP calculated as 0.61 x sPAP + 2 (15). Systolic right ventricular function was estimated by M-mode measurement of the tricuspid annular plane systolic displacement (TAPSE) (16). A composite index of right ventricular function was calculated as by the ratio of the sum of

isovolumic contraction and relaxation times to the ejection time, as previously proposed by Tei et al (17). The echocardiographic recordings were read blinded and in duplicate.

Cycle ergometer cardiopulmonary exercise test

The CPET was performed in an erect position on an electronically braked cycle ergometer (Monark, Ergomedic 818 E, Vansbro, Sweden) with breath-by-breath measurements, through a tightly fitted facial mask, of ventilation (V_E), O_2 uptake (VO_2), and CO_2 output (VCO_2) using a Cardiopulonary Exercise System (Oxycon Mobile, Jaeger, Hoechberg, Germany). The work-rate was increased by 15-30 W/min (according to previously known exercise capacity and predicted decrease by approximately 35 % at high altitude such as for the test to last for 10-12 min) until exhaustion (18). Maximal VO_2 was defined as the VO_2 measured during the last 20 s of peak exercise. The respiratory exchange ratio (RER) was calculated as VCO_2/VO_2, and O_2 pulse as VO_2/HR. The ventilatory equivalents for CO_2 (V_E/VCO_2) were calculated by dividing V_E by VCO_2. The anaerobic threshold was estimated by the V-slope method (18).

Renal function

Blood samples and urine were immediately put on ice, and entered into the routine hospital laboratory at sea level. At altitude, blood was centrifuged, and plasma and urine samples frozen and stored at – 20 °C. Plasma and urine were assayed for electrolytes with an ion-sensitive electrode (Instrumentation Beckmann Astra, Brea, CA). Uric acid was measured by the uricase method and creatinine by the Jaffé reaction (Instrumentation Beckmann Astra, Brea, CA). Plasma and urine uric acid and osmolalities were also measured. Glomerular filtration rate (GFR) was estimated from the clearance of endogenous creatinine (Ccr). Free water clearance (CH_2O) was calculated by the difference between urine volume flow and

osmolar clearance. Fractional excretions of uric acid and sodium were calculated by the ratios of uric acid and sodium to creatinine clearances.

Statistics

Results are presented as mean ± SE. The statistical analysis consisted in a repeated measures analysis of variance. When the F ratio of the analysis of variance reached a $P < 0.05$ critical value, paired or unpaired Student's t test were applied to compare specific situations (19). Correlations were calculated by linear regression analysis.

RESULTS

Effects of acute normobaric hypoxia on CPET and hemodynamics

Exposure to acute normobaric hypoxia was well tolerated, excepted for transient mild headache in some subjects, so that the Lake Louise score remained at 2 ± 1. Hypoxia decreased SpO_2 and increased Q, HR, sPAP, mPAP and PVR, while BP remained unchanged (Table 1). Hypoxia affected CPET measurements by decreased maximum values of VO_2, workload, V_E, HR and O_2 pulse, decreased VO_2 at the anaerobic threshold, decreased SpO_2 at maximum exercise and increased V_E/VCO_2 at the anaerobic threshold (Table 2). Maximum RER was not different in hypoxia and in normoxia.

Effects of chronic hypobaric hypoxia on CPET and hemodynamics

Exposure to the altitude of 5050 m was well tolerated. Hemoglobin concentration measured in 17 subjects increased from 14.2 ± 0.2 g/dl at sea level to 14.9 ± 0.4 g/dl at high altitude ($P<0.05$). Mild headache and fatigue were invariably present, causing the Lake

Louise score to increase to a diagnosis of mild acute mountain sickness (Table 3). However, none of the subjects felt that these symptoms were serious enough to affect their exercise capacity. Chronic hypobaric hypoxia as compared to acute normobaric hypoxia was associated with similar effects on hemodynamics and oxygenation, with however higher SpO_2 and BP ($P<0.01$), and slightly lower Q (Tables 1 and 3). The TAPSE was unchanged, at 31 ± 2 mm at sea level and 29 ± 1 mm at altitude (P NS), but the Tei index increased from 0.21 ± 0.05 at sea level to 0.27 ± 0.03 at high altitude ($P < 0.05$).

The CPET measurements were similarly affected in acute and in chronic hypoxia, with however more important decreases of the maximum values of VO_2, workload and HR, as well as of VO_2 at the anaerobic threshold, while maximum O_2 pulse was not different, and $V_E$max and V_E/VCO_2 at the anaerobic threshold were increased. The RER and SpO_2 were not different at maximum exercise capacity.

Effects of sitaxsentan on CPET and hemodynamics

At sea level, sitaxsentan slightly decreased BP in normoxia, decreased sPAP, mPAP, PVR and Tei index in hypoxia (Table 1), and improved VO_2max and maximum workload in hypoxia without other effect on CPET variables (Table 2).

At high altitude, sitaxsentan improved the Lake Louise score, decreased BP, sPAP, mPAP and PVR, increased Q, and prevented the decrease in TAPSE and increase in Tei index that occurred with 1 week of placebo intake (Table 3). Sitaxsentan affected CPET variables by increases in maximum values of workload, VO_2, O_2 pulse and VO_2 at the anaerobic threshold, while only O_2 pulse increased after one week of placebo intake (Table 4).

Sitaxsentan limited hypoxia-induced decrease in VO_2max, by an average of 30 % in acute hypoxia and 10 % in chronic hypoxia ($P<0.001$ and $P<0.05$ respectively) (Figure 1).

There was a significant inverse correlation between changes in VO_2max and resting sPAP in acute normobaric hypoxia as well as at high altitude (Figure 2).

Effects of sitaxsentan and of altitude on renal function

Altitude exposure was associated with decreased Ccr, decreased CH_2O that was out of proportion of decreased Ccr (leading to more negative CH_20/Ccr ratio) decreased fractional excretion of uric acid, and increased fractional excretion of sodium together with an increased urinary $\log_{10}$ Na/K, while urine flow rate was unchanged (Table 5).

Sitaxsentan had no effect on these renal function variables, neither at sea level nor at high altitude.

DISCUSSION

The present study suggests that moderate hypoxic pulmonary hypertension in healthy subjects is partly mediated by endothelin A receptor signaling, and that this contributes to decreased aerobic exercise capacity. The results also show that selective endothelin A receptor blockade does not affect renal function in healthy subjects at sea level or at high altitudes.

Circulating endothelin-1 has been previously shown to be increased at high altitudes, in relation to increased pulmonary artery pressures and decreased arterial blood oxygenation (10,20). Endothelin-1 appears to play a role in hypoxia-induced pulmonary hypertension. An increased expression of endothelin-1 and the endothelin A receptor has be reported in rats exposed to a FIO_2 of 0.1 during 48 hours (21), with addition of an over-expression of the endothelin B receptor after increase of the duration of the hypoxic exposure to 4 weeks (22).

Both selective endothelin A and non selective endothelin A and B receptor blockers prevent and reverse hypoxic pulmonary hypertension in experimental animals (23,24). In healthy humans at high altitude, sPAP is increased, and this has been shown to be partly reversed by the administration of the non selective endothelin receptor antagonist bosentan (10). A partial inhibition of normobaric hypoxic pulmonary vasoconstriction by the preventive intake of bosentan has been reported in healthy subjects (8). In the present study, the effects of preventive sitaxsentan on normobaric hypoxic vasoconstriction were strikingly similar, suggesting little if any participation of endothelin B receptor-mediated release of vasodilating mediators or endothelin clearance (9). The pulmonary vasodilating effects of sitaxsentan persisted during chronic exposure to hypobaric hypoxia, with partial reversal of hypoxia-induced increase in sPAP and normalized PVR. Altogether, these observations suggest a major contribution of endothelin A receptor signaling in both acute hypoxic vasoconstriction and more chronic hypoxic pulmonary hypertension in normal subjects acclimatized to high altitude.

Altitude exposure was associated with the expected decrease in aerobic exercise capacity (1,2). The mechanisms of decreased exercise capacity associated with hypoxic exposure in healthy subjects are complex, with cardiovascular disturbances added to more obvious decrease in arterial O_2 content accounting for decreased O_2 delivery to exercising muscles (1-3). Recent observations of improved exercise capacity in hypoxia by pharmacologic interventions to decrease PVR, such as sildenafil (4,5,7), dexamethasone (6), and bosentan (8), have raised the possibility of a participation of increased right ventricular afterload to limit O_2 delivery to the tissues. This interpretation is supported by significant inverse correlations between changes in VO_2max and sPAP, and better preservation of right ventricular function with unchanged TAPSE and Tei index after sitaxsentan intake in the

present study. Both TAPSE and Tei index have been shown to be altered in pulmonary hypertension, in proportion to disease severity (17,25). Previous studies have shown maintained TAPSE and increased Tei index in lowlanders acclimatized to high altitudes, but comparably lower TAPSE and higher Tei index in high altitude dwellers (26). It is of interest that the improvement in VO_2max by sitaxsentan intake was less pronounced in chronic as compared to acute hypoxic conditions, while PVR was higher, and still reversible. Our data offer no explanation for this observation, probably related to respiratory, cardiovascular or muscular adaptations over time.

We considered other possible explanations for sitaxsentan-induced improvement in VO_2max in the present study. Arterial oxygenation was unchanged at rest or at exercise. This argues against an improvement in pulmonary gas exchange, as was observed with the administrations of sildenafil (4,5,7) or dexamethasone (6). Maximum ventilation and ventilatory equivalents for CO_2 were unchanged. This excludes a significant change in chemosensitivity, in keeping with previous report of unchanged chemosensitivity by bosentan intake in healthy subjects (27). Maximum RER was unchanged, which argues against a central effect which has been suggested as a potential mechanism of exercise capacity limitation in hypoxia (28). Sitaxsentan slightly decreased blood pressure and increased cardiac output, indicating a systemic vasodilating effect. This could have affected the distribution of systemic perfusion, which has been reported to be altered in hypoxia (2). Selective endothelin antagonist-mediated increase in nitric oxide availability (9) could improve mitochondrial capacity by a protein kinase G-mediated pathway, thereby improving myocardial and skeletal O_2 uptake (29,30). Thus, sitaxsentan could also have affected the matching of convectional and diffusional oxygen transport systems, which have been modeled to occur at a lower Q in hypoxia (3). In the present study, there were renal function changes

suggestive of decreased effective plasma volume, but this was unaffected by sitaxsentan, which indirectly argues against systemic changes in plasma volume to account for improved O_2 delivery.

In the present study, renal function was evaluated by Ccr to estimate GFR, CH_2O/Ccr to estimate the effects of antidiuretic hormone (ADH) on collecting duct handling of water, FE Na and $\log_{10}$ Na/K to evaluate the effects of aldosterone on collecting duct handling of sodium and FE urate as an index of effective plasma volume (31). High altitude exposure has been repeatedly shown to be associated with increased diuresis and natriuresis, and secondary hemoconcentration with decreased effective plasma volume (32,33). These effects are essentially related to decreased ADH and aldosterone signaling at the collecting ducts (32,33). Healthy volunteers rapidly brought to the altitude of 4559 m typically presented with increased diuresis, CH2O/GFR and FE Na, in keeping with decreased renal tubular actions of both ADH and aldosterone (10). However, these effects may vary over time, and are modulated by exercise and/or acute mountain sickness symptomatology (33,34). In the present study, renal function tests were suggestive of relative hypovolemia, as assessed by decreases in both Ccr and FE urate, decreased CH_2O/Ccr suggestive of increased effects of ADH and increased FE Na and $\log_{10}$ Na/K suggestive of decreased effects of aldosterone. The diuresis was unchanged compared to sea level, in keeping with time taken for acclimatization and achieve new steady-state. Persistently decreased aldosterone signaling is in keeping with previous observations (33). Increased ADH signaling in the present study may be either time-related or associate to hiking activities and mild high altitude sickness.

Endothelin-1 is locally produced by the kidney, and exerts endothelin A receptor-mediated vasoconstrictive effects together with endothelin B receptor-mediated inhibition of

both ADH and aldosterone at the collecting duct (35,36). In healthy subjects exposed to 4559 m, bosentan decreased free water clearance corrected for GFR, with no significant effect on GFR or fractional excretion of sodium, strongly suggesting predominant effects of endothelin-1 on the renal handling of water (10). In the present study sitaxsentan had no effect on indices of renal handling of water or sodium, in keeping with the notion that the renal tubular effects of endothelin-1 are essentially B receptor-mediated (10,35,36). Sitaxsentan intake was not associated with the increased GFR that could be expected on the basis of endothelin A receptor control of renal vascular resistance and renal vasodilating effects of endothelin B receptor-mediated nitric oxide and prostacyclin release (35,36). However, there might have been a trend, as assessed by a 10 % increase in Ccr that did not reach significance.

Limitations of the present study are in non invasive echocardiographic estimations of pulmonary artery pressures and cardiac output. However, we previously reported satisfactory inter-observer and intra-observer variabilities on these measurements (26). A good agreement between invasive and non invasive measurements of sPAP has been previously reported at high altitude (37). A more important limitation is in the absence of echocardiographic measurements at exercise. This was attempted at, but did not produce sufficient quality signals, in spite of encouragements to the volunteers to increase fluid intake before the examination such as to avoid effects of dehydration. Thus our results only indirectly support the notion that increased sPAP limited VO_2 max by a decrease in maximal cardiac output. Iit must be stressed that increased sPAP would only partly contribute to hypoxia-related decrease in aerobic exercise capacity, as supported by r^2 of the correlation between changes in sPAP and VO_2max of only 0.33, suggesting that one third of VO_2max changes would be possibly explained by changes in sPAP. Furthermore, remote high altitude conditions prevented measurements of hematocrit and hemoglobin in all the subjects, before and after intake of

sitaxsentan, so that changes in arterial O_2 content associated to the intake of the drug could not be excluded. It may be added that, in the absence of arterial blood gas analysis, uncertainty remains about the validity of pulse oximetry estimations of arterial blood O_2 saturations. Finally, there were no direct measurements of renal function or circulating hormones.

Sitaxsentan intake was associated with an unexpected decrease in maximum RER in hypoxia. We have no explanation for this effect which was already observed with the intake of bosentan (8). Also, the intake of sitaxsentan was associated with an improved acute mountain sickness score. This may be possibly related to cytoprotective effects of endothelin A receptor blockade reported in hypoxic astrocytes (38) or improved cerebral hemodynamics and oxygenation (39).

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REFERENCES

1. Fulco CS, Rock PB, Cymerman A. Maximal and submaximal exercise performance at altitude. Aviat Space Environ Med. 1998; 69: 793-801.
2. Calbet JA, Boushel R, Radegran G, Sondergaard H, Wagner PD, Saltin B. Why is VO2 max after altitude acclimatization still reduced despite normalization of arterial O2 content? Am J Physiol Regul Integr Comp Physiol. 2003; 284: R304-316.
3. Wagner PD. Reduced maximal cardiac output at altitude. Mechanisms and significance. Respir Physiol 2000; 120: 1-11.
4. Ghofrani HA, Reichenberger F, Kohstall MG et al. Sildenafil increased exercise capacity during hypoxia at low altitudes and at mount Everest base camp: a randomized, double-blind, placebo-controlled crossover trial. Ann Intern Med. 2004; 141: 169-177.
5. Fischler M, Maggiorini M, Dorschner L et al. Dexamethasone but not Tadalafil Improves Exercise Capacity in Adults Prone to High Altitude Pulmonary Edema. Am J Respir Crit Care Med. 2009; 180: 346-352.
6. Richalet JP, Gratadour P, Robach P, et al. Sildenafil inhibits altitude-induced hypoxemia and pulmonary hypertension. Am J Respir Crit Care Med. 2005; 171: 275-281
7. Faoro V, Lamotte M, Deboeck G et al. Effects of sildenafil on hypoxic exercise capacity in normal subjects. High Alt Med Biol. 2007; 8: 155-163.
8. Faoro V, Boldingh S, Moreels M et al. Bosentan decreases pulmonary vascular resistance and improves exercise capacity in hypoxia**.** Chest 2009; 135:1215-1222
9. Barst RJ. Sitaxsentan: a selective endothelin-A receptor antagonist, for the treatment of pulmonary arterial hypertension. Expert Opin Pharmacother. 2007; 8: 95-109

10. Modesti PA, Vanni S, Morabito M et al. Role of endothelin-1 in exposure to high altitude: Acute Mountain Sickness and Endothelin-1 (ACME-1) study. Circulation. 2006; 114: 1410-1416.
11. Yamaya Y, Boogard HJ, Wagner PD, Niizerki K, Hopkins SR. Validity of pulse oximetry during maximal exercise in normoxia, hypoxia, and hyperoxia. J Appl Physiol 2002; 92: 162-168
12. Roach, RC, Bärtsch P, Hackett PH, Oelz O. The Lake Louise acute mountain sickness scoring system. In: *Hypoxia and Mountain Medicine*, edited by Sutton JR, Houston CS, and Coates G. Burlington, VT: Queen City, 1993, p. 327-330.
13. Christie J, Sheldahl LM, Tristani FE, Sagar KB, Ptacin MJ, Wann S. Determination of stroke volume and cardiac output during exercise: comparison of two-dimensional and Doppler echocardiography, Fick oximetry, and thermodilution. Circulation. 1987; 76: 539-547.
14. Yock PG, Popp RL. Noninvasive estimation of right ventricular systolic pressure by Doppler ultrasound in patients with tricuspid regurgitation. Circulation. 1984; 70: 657-662.
15. Chemla D, Castelain V, Humbert M et al. New formula for predicting mean pulmonary artery pressure using systolic pulmonary artery pressure. Chest 2004; 126: 1313-1317.
16. Ghio S, Recusani F, Klersy C et al. Prognostic usefulness of tricuspid annular planes systolic excursion in patients with congestive heart failure secondary to idiopathic or ischemic dilated cardiomyopathy. Am J Cardiol. 2000; 85: 837-842.
17. Tei C, Dujardin K, Hodge D et al. Doppler echocardiographic index for assessment of global right ventricular function. J Am Soc Echocardiogr. 1996; 9: 838-847.

18. Wasserman K, Hansen JE, Sue DY, and Whipp BJ. *Principles of Exercise Testing and Interpretation.*3rd Ed., Baltimore, MD. Lippincott Williams & Wilkins, 1999, pp. 143-164.
19. Winer BJ, Brown DR and Michels KM. Statistical principles in experimental design. 3rd Eds. Mc Grall-Hill, New York.1991; pp. 220-283.
20. Goerre S, Wenk M, Bartsch P et al. Endothelin-1 in pulmonary hypertension associated with high-altitude exposure. Circulation. 1995; 91: 359-364.
21. Li H, Elton TS, Chen YF, Oparil S. Increased endothelin receptor gene expression in hypoxic rat lung. Am J Physiol. 1994; 266: L553-L560.
22. Li H, Chen SJ, Chen YF et al. Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. J Appl Physiol. 1994; 77: 1451-1459.
23. Chen SJ, Chen IF, Meng QC, Durand J, Di Carlo VS, Oparil S. Endothelin-receptor antagonist bosentan prevents and reverses hypoxic pulmonary hypertension in rats. J Appl Physiol. 1995; 79: 2122-2131.
24. DiCarlo VS, Chen SJ, Meng QC et al. ETA-receptor antagonist prevents and reverses chronic hypoxia-induced pulmonary hypertension in rat. Am J Physiol. 1995; 269: L690-L697.
25. Forfia PR, Fisher MR, Mathai SC et al. Tricuspid annular displacement predicts survival in pulmonary hypertension. Am J Respir Crit Care Med. 2006; 174: 1034-1041.
26. Huez S, Faoro V, Guénard H, Martinot JB, Naeije R. Echocardiographic and tissue Doppler imaging of cardiac adaptation to high altitude in native highlanders versus acclimatized lowlanders. Am J Cardiol. 2009; 103: 1605-1609.
27. Gujic M, Houssiere A, Xhaet O et al. Does endothelin play a role in chemoreception during acute short term hypoxia in normal men. Chest 2007; 131: 1467-1472.

28. Kayser B. Exercise begins and ends in the brain. Eur J Appl Physiol. 2003; 90: 411-419.

29. Nisoli E, Clementi E, Paolucci et al. Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. Science 2003 ; 299: 896–899.

30. Mouchaers KT, Schalij I, Versteilen AM et al. Endothelin receptor blockade combined with phosphodiesterase-5 inhibition increases right ventricular mitochondrial capacity in pulmonary arterial hypertension. Am J Physiol Heart Circ Physiol. 2009 ; 297: H200-H207.

31. Koeppen BM, Stanton BA. Renal Physiology. 2nd Ed. Mosby, St Louis, 1997.

32. Maresh CM, Kreaemer WJ, Judelson DA et al. Effects of high altitude and water deprivation on arginine vasopressin release in men. Am J Physiol Endocrinol Metab. 2004; 286: E20-E24.

33. Zacarria M, Rocco S, Noventa D, Varnier M, Opocher G. Sodium regulating hormones at high altitude: basal and post-exercise levels. J Clin Endocrinol Metab. 1998; 83: 570-574.

34. Loeppky JA, Icenogle MV, Maes D, Riboni K, Hinghofer-Szalkay H, Roach RC. Early fluid retention and severe acute mountain sickness. J Appl Physiol. 2005; 98: 591-597.

35. Kohan DE. Endothelins, renal tubule synthesis and actions. Clin Exp Pharmacol Physiol. 1996; 23: 337-344.

36. Neuhofer W, Pittrow D. Endothelin receptor selectivity in chronic kidney disease: rationale and review of recent evidence. Eur J Clin Invest. 2009; 39(S2): 50-67.

37. Allemann Y, Sartori C, Lepori M et al.. Echographic and invasive measurements of pulmonary artery pressure correlate closely at high altitude. Am J Physiol Heart Circ Physiol. 2000; 279: H2013-H2016.

38. Danielyan L, Gembizki O, Proksch B et al. The blockade of endothelin A receptor protects astrocytes against hypoxic injury: common effects of BQ-123 and erythropoietin on the rejuvenation of the astrocyte population. Eur J Cell Biol. 2005; 84: 567-579.
39. Schubert GA, Schilling L, Thomé C. Clazosentan, an endothelin receptor antagonist, prevents early hypoperfusion during the acute phase of massive experimental subarachnoid hemorrhage: a laser Doppler flowmetry study in rats. J Neurosurg. 2008; 109: 1134-1140.

LEGENDS OF THE FIGURES

Figure 1. Mean ± SE decrease in maximum O_2 uptake (VO_2max) induced by acute hypoxia and in chronic hypoxia compared to sea level value (%) with and without sitaxsentan treatment. Sitaxsentan restored the hypoxia-induced decrease in VO_2max by approximately one third in acute hypoxic conditions and by 12% during a stay at high altitude.

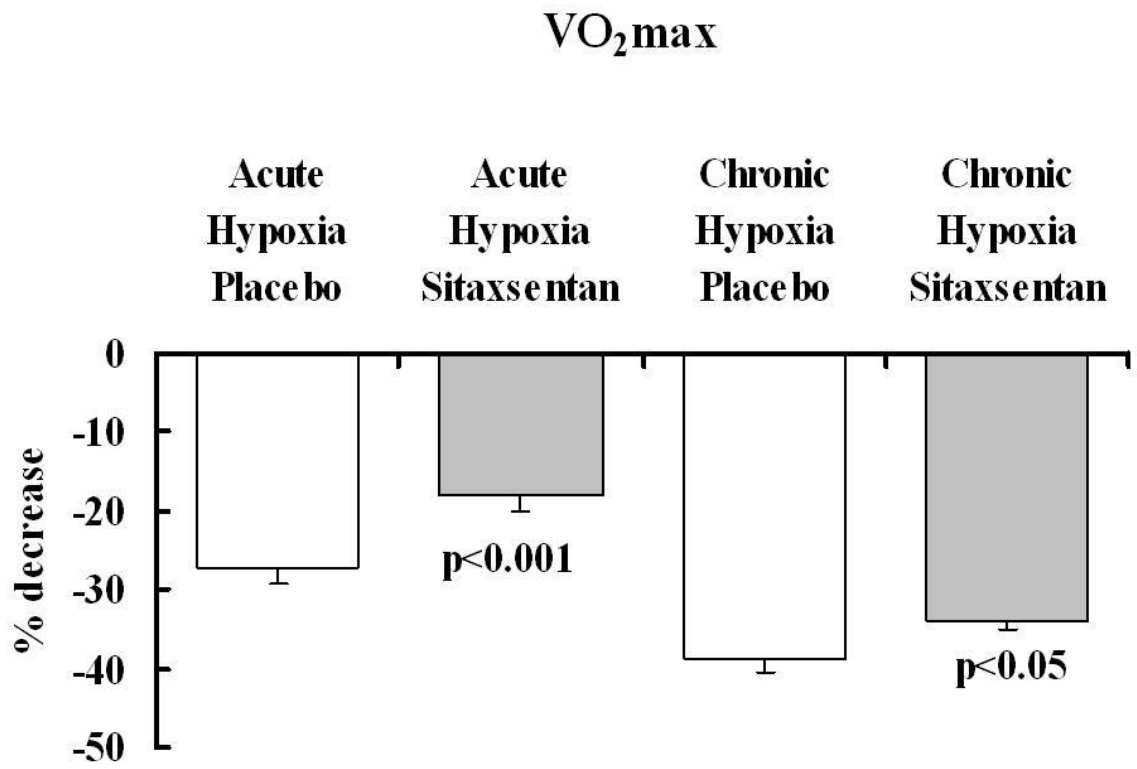

Figure 2. Correlation between systolic pulmonary artery pressure (sPAP) and maximum O_2 uptake (VO_2max) in acute and in chronic hypoxia.

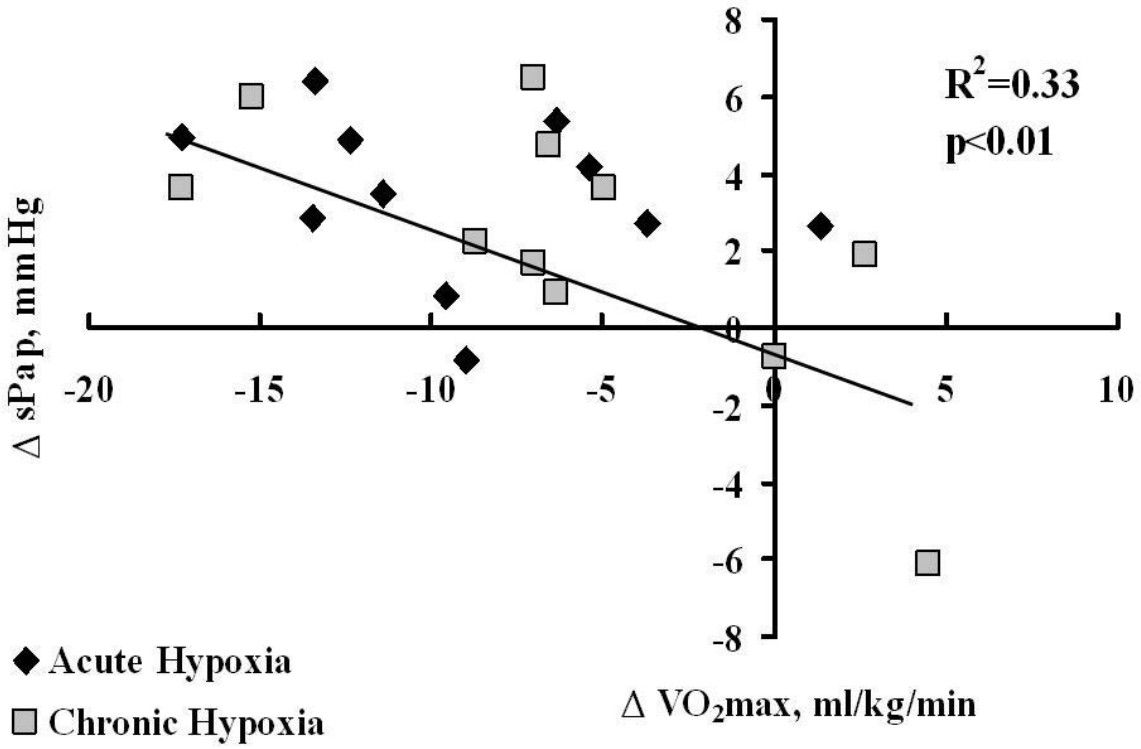

Table 1. Effects of sitaxsentan on hemodynamics and oxygen saturation in normoxia and in acute hypoxia in 13 normal subjects at sea level at rest

	Normoxia		Acute Hypoxia	
Variables	Placebo	Sitaxsentan	Placebo	Sitaxsentan
Llscore	-	-	2 ± 0.5	4 ± 1.5
SpO_2, %	98 ± 0.3	98 ± 0.2	72 ± 2***	73 ± 3***
BP, mmHg	87 ± 2	82 ± 2 §	83 ± 2	81 ± 2
HR, beats/min	57 ± 3	60 ± 4	69 ± 5*	69 ± 5*
Q, l/min	4.7 ± 0.3	5.0 ± 0.4	6.2 ± 0.5*	6.6 ± 0.6*
sPAP, mmHg	23 ± 1	23 ± 2	37 ± 2***	28 ± 2* §§§
mPAP, mmHg	16 ± 1	16 ± 1	25 ± 2***	19 ± 1* §§§
PVR, mmHg/l/min	3.5 ± 0.2	3.4 ± 0.3	4.2 ± 0.3*	3.1 ± 0.3 §§§
Tei index	0.21 ± 0.05	0.18 ± 0.03	0.30 ± 0.05	0.19 ± 0.03§
TAPSE, cm	3.1 ± 0.2	3.1 ± 0.1	3.0 ± 0.1	3.0 ± 0.1

Abbreviations: HR: Heart rate; SpO_2: pulse oximetry O_2 saturation; BP: mean systemic arterial pressure; Q: cardiac output; sPAP: systolic pulmonary artery pressure; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; TAPSE: tricuspid annular plane systolic excursion.

Hypoxia: fraction of inspired O_2 (FIO_2) 0.12

*: $P < 0.05$, *** $P < 0.001$, hypoxia vs normoxia at same drug

§: $P < 0.05$, §§§ $P < 0.001$ sitaxsentan vs placebo at same FIO_2.

Table 2. Effects of sitaxsentan on cardiopulmonary exercise variables in normoxia and in acute hypoxia in 13 normal subjects at sea level

.

	Normoxia		Acute Hypoxia	
Variables	Placebo	Sitaxsentan	Placebo	Sitaxsentan
Workload max, W	280 ± 18	288 ± 18	179 ± 14***	196 ± 16 §§§***
VO_2max, ml/kg/min	47 ± 2	48 ± 2	32 ± 2***	35 ± 2 §§***
V_E max, l/min	114± 7	118 ± 7	95 ± 7**	96 ± 7**
RERmax	1.21 ± 0.02	1.21 ± 0.02	1.26 ± 0.03	1.26 ± 0.02
HR max, beats/min	177 ± 6	181 ± 3	166 ± 6**	168 ± 6**
O_2 pulse, ml/beat	18 ± 1	18 ± 1	13 ± 1***	14 ± 1***
VO_2 at AT, ml/kg/min	35 ± 2	35 ± 2	22 ± 2***	23 ± 3***
V_E/VCO_2 at AT	30 ± 1	30 ± 1	33 ± 2***	33 ± 1***
Exercise SpO_2, %	92 ± 1	92 ± 1	78 ± 2***	79 ± 1***

Abbreviations: Workload max: maximum workload; VO_2max: maximum O_2 uptake; V_E: ventilation; RER: respiratory exchange ratio; HR: heart rate; AT: anaerobic threshold; VCO_2: CO_2 output; SpO_2 oxygen saturation. Hypoxia: fraction of inspired O_2 (FIO_2) of 0.12

** $P < 0.01$, *** $P < 0.001$ hypoxia vs normoxia at same drug

§§ $P < 0.01$, §§§ $P < 0.001$ sitaxsentan vs placebo at same FIO_2

Table 3. Effects of sitaxsentan on hemodynamics and oxygen saturation in 22 subjects at high altitude

Chronic hypoxia	Placebo Group (n=11)		Sitaxsentan Group (n=11)	
Variables	Baseline	Placebo	Baseline	Sitaxsentan
Llscore	7 ± 2	8 ± 2	8 ± 2	5 ± 1*
SpO_2, %	83 ± 1	86 ± 1	80 ± 1	83 ± 1
BP, mmHg	98 ± 3	99 ± 3	102 ± 2	95 ± 3*
HR, bpm	64 ± 5	64 ± 4	71 ± 4	74 ± 4
Q, l/min	5.6 ± 0.4	5.7 ± 0.4	5.4 ± 0.2	6.3 ± 0.4 *
sPAP, mmHg	38 ± 2	36 ± 3	37 ± 1	31 ± 2 *
mPAP, mmHg	25 ± 1	24 ± 2	24 ± 1	21 ± 1 *
PVR, mmHg/l/min	4.6 ± 0.3	4.3 ± 0.4	4.6 ± 0.2	3.5 ± 0.2 ***
Tei index	0.28 ± 0.04	0.35 ± 0.05*	0.25 ± 0.02	0.27 ± 0.04
TAPSE, cm	3.1 ± 0.1	2.8 ± 0.1*	2.8 ± 0.1	2.9 ± 0.1

Abbreviations: HR: Heart rate; SpO_2: O_2 saturation; BP: mean systemic arterial pressure; Q: cardiac output; sPAP: systolic pulmonary artery pressure; mPAP: mean pulmonary artery pressure; PVR: pulmonary vascular resistance; TAPSE: tricuspid annular plane systolic excursion.

*: $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ sitaxsentan or placebo intake compared with baseline.

Table 4. Effects of sitaxsentan on cardiopulmonary exercise variables in 22 subjects at high altitude

Chronic hypoxia	Placebo Group (n=11)		Sitaxsentan Group (n=11)	
Variables	Baseline	Placebo	Baseline	Sitaxsentan
Workload max, W	148 ± 12	151 ± 13	152 ± 10	167 ± 13 *
VO_2max, ml/kg/min	27 ± 2	27 ± 2	27 ± 1	29 ± 1 *
V_E max, l/min	118 ± 13	115 ± 10	128 ± 11	132 ± 14
RERmax	1.13 ± 0.03	1.12 ± 0.02	1.22 ± 0.03	1.15 ± 0.03 **
HR max, beats/min	142 ± 7	135 ± 8	159 ± 6	153 ± 6
O_2 pulse, ml/beat	12 ± 1	13 ± 1*	12 ± 1	13 ± 1 *
VO_2 at AT, l/min	18 ± 3	19 ± 2	20 ± 1	21 ± 1 *
V_E/VCO_2 at AT	50 ± 3	52 ± 2	47 ± 2	48 ± 1
Exercise SpO_2, %	78 ± 2	78 ± 2	77 ± 2	77 ± 2

Abbreviations: Workload max: maximum workload; VO_2max: maximum O_2 uptake; V_E: ventilation; RER: respiratory exchange ratio; HR: heart rate; AT: anaerobic threshold; VCO_2: CO_2 output; SpO_2 oxygen saturation; Hypoxia: Altitude, 5050m

*: $P < 0.05$, ** $P < 0.01$ sitaxsentan or placebo intake compared with baseline.

Table 5. Effects of sitaxsentan and of hypobaric hypoxia on renal function in healthy volunteers

Variables	Sea level		Altitude		
	Placebo n = 17	Sitaxsentan n = 17	Baseline n = 22	Placebo n = 11	Sitaxsentan N = 11
Diuresis, ml/min	1.1 ± 0.1	1.2 ± 0.2	1.1 ± 0.2	1.6 ± 0.3	1.8 ± 0.3
GFR, ml/min	117 ± 6	123 ± 8	93 ± 7*	97 ± 6	109 ± 11
CH_2O , ml/min	- 0.9 ± 0.1	- 0.7 ± 0.1	- 1.7 ± 0.2*	- 1.5 ± 0.2	- 1.9 ± 0.2
FE urate, %	6.5 ± 0.3	6.7 ± 0.3	4.7 ± 0.7*	5.8 ± 0.6	6.1 ± 0.8
FE Na, %	0.63 ± 0.07	0.58 ± 0.05	1.0 ± 0.1*	0.9 ± 0.1	1.1 ± 0.1
Log_{10} Na/K	0.37 ± 0.04	0.39 ± 0.03	0.63 ± 0.05*	0.69 ± 0.09	0.082 ± 0.05

Abbreviations: GFR: glomerular filtration rate; CH_2O: free water clearance; FE: fractional excretion; Na: urinary sodium; K: urinary potassium

*: $P < 0.05$: altitude vs sea level