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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 haemodynamics and a cardiopulmonary exercise test were performed in 13 healthy subjects at sea level, in normoxia and during acute hypoxic breathing (1 h, 12% oxygen in nitrogen), and in 22 healthy subjects after acclimatisation to an altitude of 5,050 m. The measurements were obtained after randomisation, double-blinded to the intake of placebo or the endothelin A receptor blocker sitaxsentan (100 mg·day^{-1} for 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 ($V'_{O_2,max}$). Sitaxsentan decreased PVR in acute and chronic hypoxia (both $p<0.001$), and partly restored $V'_{O_2,max}$, by 30 % in acute hypoxia ($p<0.001$) and 10% in chronic hypoxia ($p<0.05$). Sitaxsentan-induced changes in PVR and $V'_{O_2,max}$ 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 effects on renal function in hypoxic normal subjects.

KEYWORDS: Hypoxia, maximal oxygen uptake, pulmonary vascular resistance, renal function, sitaxentan

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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 acclimatisation, the arterial oxygen content may be restored to sea-level values because of an increased haemoglobin concentration, but maximal cardiac output remains low [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 reduced myocardial function, but is probably 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 ($V'_{O_2,max}$) together with a decrease in pulmonary artery pressure (P_{pa}) has indeed been reported after the intake of sildenafil in hypoxic healthy volunteers [4, 5]. More recently, there has been report of decreased P_{pa} and improved $V'_{O_2,max}$ in subjects susceptible to high-altitude pulmonary oedema 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 $V'_{O_2,max}$ specifically related to a decreased P_{pa} by the administration of the nonselective 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].

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METHODS

Subjects

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

Protocol design

Measurements of heart rate (HR), blood pressure (BP) and transcutaneous arterial O_2 saturation measured by pulse oximetry (S_{p,O_2}) were taken in all 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, 22 of the subjects underwent Doppler echocardiography at rest to estimate P_{pa}, cardiac output and right ventricular function, and an incremental cycle ergometer cardiopulmonary exercise test (CPET) with measurements of ventilatory variables, workload, BP, HR and S_{p,O_2} to measure aerobic exercise capacity.

The normobaric normoxic and hypoxic measurements were repeated in 13 subjects after a 1-week treatment with sitaxsentan (100 mg·day^{-1}) or a placebo, either in normoxia, or after 1 h breathing a fraction of inspired O_2 (F_{I,O_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 F_{I,O_2} of 0.12 corresponded approximately to the inspired partial pressure of O_2 at an altitude of 4,500 m. Sea-level measurements were performed according to a prospective, randomised, controlled cross-over study design. Thus, 13 subjects had four echocardiograms and CPET at sea level, each in one of the following conditions: normoxia–placebo, normoxia–sitaxsentan, hypoxia–placebo, hypoxia–sitaxsentan.

Under the hypobaric hypoxic condition, 22 subjects had two echocardiograms and CPET at high altitude at baseline and 1 week later, after intake of either a placebo or sitaxsentan.

The sampling of blood and urine for renal function measurements took place at 08:00 h 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 h at sea level, and 2 h 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 5,050 m, in the Khumbu area of the Sagamartha National Park (Nepal). This is reached after a 1-week hike at progressively increased altitudes following aeroplane transportation from Kathmandu (Nepal), at ~800 m, to Lukla (Nepal), at 2,800 m. The hike is easy-going and usually allows for a satisfactory acclimatisation. Once at the Pyramid hut, the subjects rested or walked about in the surrounding area, 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 1 week of sitaxsentan (100 mg) or a placebo given in a prospective, randomised, controlled, double-blind fashion. Sitaxsentan was taken once daily for 7 days to achieve steady state of plasma levels [9].

Clinical measurements

BP was measured by sphygmomanometry, with mean pressure calculated as diastolic pressure plus one-third of the pulse pressure. A three-lead ECG was used to measure HR. S_{p,O_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]. Haemoglobin concentration was measured at sea level and at 5,050 m in 17 of the subjects. The presence of acute mountain sickness in hypoxia was assessed by the 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, Horten, Norway). Cardiac output (Q) was estimated from left ventricular outflow tract cross-sectional area and pulsed Doppler velocity–time integral measurements [13]. Systolic P_{pa} was estimated from a transtricuspid 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 mean P_{pa} ($\bar{P}_{pa}$)/Q, with $\bar{P}_{pa}$ calculated as 0.61 × systolic P_{pa} + 2 [15]. Systolic right ventricular function was estimated by M-mode measurement of the tricuspid annular plane systolic excursion (TAPSE) [16]. A composite index of right ventricular function was calculated as 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 blind and in duplicate.

CPET

The CPET was performed in an erect position on an electronically braked cycle ergometer (Monark Ergomedic 818 E; Monark, Vansbro, Sweden) with breath-by-breath measurements, through a tightly fitted face mask, of minute ventilation (V'_E), O_2 uptake (V'_{O_2}), and CO_2 output (V'_{CO_2}) using a cardiopulonary exercise system (Oxycon Mobile®; Jaeger, Hoechberg, Germany). The work rate was increased by 15–30 W·min^{-1} (according to previously known exercise capacity and predicted decrease by ~35% at high altitude so that the test would last 10–12 min) until exhaustion [18]. Maximal V'_{O_2} ($V'_{O_2,max}$) was defined as the V'_{O_2} measured during the last 20 s of peak exercise. The respiratory exchange ratio (RER) was calculated as V'_{CO_2}/ V'_{O_2}, and O_2 pulse as V'_{O_2}/HR. The ventilatory equivalents for CO_2 (V'_E/V'_{CO_2}) were calculated by dividing V'_E by V'_{CO_2}. The anaerobic threshold was estimated by the V-slope method [18].

Renal function

Blood samples and urine were immediately put on ice and sent to the routine hospital laboratory, located at sea level. At altitude, blood was centrifuged, and plasma and urine samples

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

Variables	Normoxia		Acute hypoxia#	
	Placebo	Sitaxsentan	Placebo	Sitaxsentan
LL score			2±0.5	4±1.5
S_{p,O_2} %	98±0.3	98±0.2	72±2¶	73±3¶
BP mmHg	87±2	82±2+	83±2	81±2
HR beats·min^{-1}	57±3	60±4	69±5§	69±5§
Q L·min^{-1}	4.7±0.3	5.0±0.4	6.2±0.5§	6.6±0.6§
Systolic P_{pa} mmHg	23±1	23±2	37±2¶	28±2§,ƒ
$\bar{P}_{pa}$ mmHg	16±1	16±1	25±2¶	19±1§,ƒ
PVR mmHg·L^{-1}·min^{-1}	3.5±0.2	3.4±0.3	4.2±0.3§	3.1±0.3ƒ
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

Data are presented as mean± SE. LL: Lake Louise; S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry; BP: blood pressure; HR: Heart rate; Q: cardiac output; P_{pa}: pulmonary artery pressure; $\bar{P}_{pa}$: mean P_{pa}; PVR: pulmonary vascular resistance; TAPSE: tricuspid annular plane systolic excursion. #: hypoxia is defined as fraction of inspired oxygen (F_{I,O_2}) 0.12. ¶: p<0.001, hypoxia *versus* normoxia with same drug; +: p<0.05, hypoxia *versus* normoxia with same drug; §: p<0.05, sitaxsentan *versus* placebo at same F_{I,O_2}; ƒ: p<0.001, sitaxsentan *versus* placebo at same F_{I,O_2}.

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

Variables	Normoxia		Acute hypoxia#	
	Placebo	Sitaxsentan	Placebo	Sitaxsentan
Workload max W	280±18	288±18	179±14¶	196±16+,¶
$V'_{O_2,max}$ mL·kg^{-1}·min^{-1}	47±2	48±2	32±2¶	35±2§,¶
V'_E max L·min^{-1}	114±7	118±7	95±7ƒ	96±7ƒ
RER max	1.21±0.02	1.21±0.02	1.26±0.03	1.26±0.02
HR max beats·min^{-1}	177±6	181±3	166±6ƒ	168±6ƒ
O_2 pulse mL·beat^{-1}	18±1	18±1	13±1¶	14±1¶
V'_{O_2} at AT mL·kg^{-1}·min^{-1}	35±2	35±2	22±2¶	23±3¶
V'_E/V'_{CO_2} at AT	30±1	30±1	33±2¶	33±1¶
Exercise S_{p,O_2} %	92±1	92±1	78±2¶	79±1¶

Data is presented as mean± SE. max: maximum; $V'_{O_2,max}$: maximum oxygen uptake; V'_E: minute ventilation; RER: respiratory exchange ratio; HR: heart rate; AT: anaerobic threshold; V'_{CO_2}: CO_2 output; S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry. #: hypoxia is defined as fraction of inspired oxygen (F_{I,O_2}) 0.12. ¶: p<0.001, hypoxia *versus* normoxia with same drug; +; p<0.001, sitaxsentan *versus* placebo at same F_{I,O_2}; §: p<0.01, sitaxsentan *versus* placebo at same F_{I,O_2}; ƒ: p<0.01, hypoxia *versus* normoxia with same drug.

were frozen and stored at -20°C. Plasma and urine were assayed for electrolytes with an ion-sensitive electrode (Instrumentation Beckmann Astra, Brea, CA, USA). Uric acid was measured by the uricase method and creatinine by the Jaffé reaction (Instrumentation Beckmann Astra). Plasma and urine uric acid, and osmolalities were also measured. Glomerular filtration rate (GFR) was estimated from the clearance of endogenous creatinine (C_{Cr}). Free water clearance (C_{H_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 of a repeated measures ANOVA. When the F ratio of the ANOVA reached a critical value of p<0.05 critical value, paired or unpaired t-tests were applied to compare specific situations [19]. Correlations were calculated by linear regression analysis.

RESULTS

Effects of acute normobaric hypoxia on CPET and haemodynamics

Exposure to acute normobaric hypoxia was well tolerated, except for transient mild headache in some subjects, such that the Lake Louise score remained at 2±1. Hypoxia decreased S_{p,O_2} and increased Q, HR, systolic P_{pa}, $\bar{P}_{pa}$ and PVR, while BP remained unchanged (table 1). Hypoxia affected CPET measurements by decreasing $V'_{O_2,max}$, workload, V'_E, HR and O_2 pulse, decreasing V'_{O_2} at the anaerobic threshold, decreasing S_{p,O_2} at maximum exercise and increasing V'_E/V'_{CO_2} at the anaerobic threshold (table 2). Maximum RER was not different in hypoxia compared to normoxia.

Effects of chronic hypobaric hypoxia on CPET and haemodynamics

Exposure to the altitude of 5,050 m was well tolerated. Haemoglobin concentration measured in 17 subjects increased from 14.2±0.2 g·dL^{-1} at sea level to 14.9±0.4 g·dL^{-1} 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 haemodynamics and oxygenation, with, however, higher S_{p,O_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>0.05), 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 chronic hypoxia, with however more important decreases of the maximum values of V'_{O_2}, workload and HR, as well as of V'_{O_2} at the anaerobic threshold, while maximum O_2 pulse was not different, and maximum V'_E and V'_E/V'_{CO_2} at the anaerobic threshold were increased. The RER and S_{p,O_2} were not different at maximum exercise capacity.

Effects of sitaxsentan on CPET and haemodynamics

At sea level, sitaxsentan slightly decreased BP in normoxia, decreased systolic P_{pa}, $\bar{P}_{pa}$, PVR and Tei index in hypoxia

TABLE 3 Effects of sitaxsentan on haemodynamics and oxygen saturation in 22 subjects at high altitude

Variables	Placebo group#		Sitaxsentan group#	
	Baseline	Placebo	Baseline	Sitaxsentan
LL score	7±2	8±2	8±2	5±1*
S_{p,O_2} %	83±1	86±1	80±1	83±1
BP mmHg	98±3	99±3	102±2	95±3*
HR beats·min^{-1}	64±5	64±4	71±4	74±4
Q L·min^{-1}	5.6±0.4	5.7±0.4	5.4±0.2	6.3±0.4*
Systolic P_{pa} mmHg	38±2	36±3	37±1	31±2*
$\bar{P}_{pa}$ mmHg	25±1	24±2	24±1	21±1*
PVR mmHg·L^{-1}·min^{-1}	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

Data are presented as mean± SE. LL: Lake Louise; S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry; BP: blood pressure; HR: heart rate; Q: cardiac output; P_{pa}: pulmonary artery pressure; $\bar{P}_{pa}$: mean P_{pa}; PVR: pulmonary vascular resistance; TAPSE: tricuspid annular plane systolic excursion. #: n=11; *: p<0.05, sitaxsentan or placebo intake compared with baseline; **: p<0.01, sitaxsentan or placebo intake compared with baseline; ***: p<0.001, sitaxsentan or placebo intake compared with baseline.

(table 1), and improved $V'_{O_2,max}$ and maximum workload in hypoxia without other effects on CPET variables (table 2).

At high altitude, sitaxsentan improved the Lake Louise score, decreased BP, systolic P_{pa}, $\bar{P}_{pa}$ 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).

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

Variables	Placebo group#		Sitaxsentan group#	
	Baseline	Placebo	Baseline	Sitaxsentan
Workload max W	148±12	151±13	152±10	167±13*
$V'_{O_2,max}$ mL·kg^{-1}·min^{-1}	27±2	27±2	27±1	29±1*
V'_E max L·min^{-1}	118±13	115±10	128±11	132±14
RER max	1.13±0.03	1.12±0.02	1.22±0.03	1.15±0.03**
HR max beats·min^{-1}	142±7	135±8	159±6	153±6
O_2 pulse mL·beat^{-1}	12±1	13±1*	12±1	13±1*
V'_{O_2} at AT L·min^{-1}	18±3	19±2	20±1	21±1*
V'_E/V'_{CO_2} at AT	50±3	52±2	47±2	48±1
Exercise S_{p,O_2} %	78±2	78±2	77±2	77±2

Data is presented as mean± SE. max: maximum; $V'_{O_2,max}$: maximum oxygen uptake; V'_E: minute ventilation; RER: respiratory exchange ratio; HR: heart rate; AT: anaerobic threshold; V'_{CO_2}: CO_2 output; S_{p,O_2}: arterial oxygen saturation measured by pulse oximetry. #: n=11; *: p<0.05, sitaxsentan or placebo intake compared with baseline; **: p<0.01, sitaxsentan or placebo intake compared with baseline.

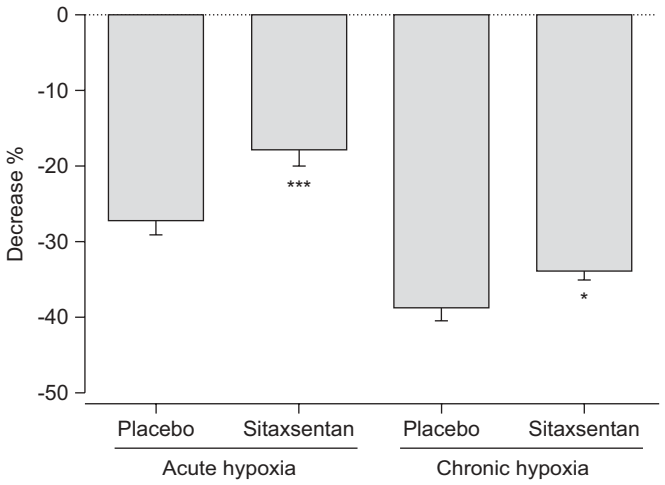

FIGURE 1. Mean±SE decrease in maximum oxygen uptake ($V'_{O_2,max}$) induced by acute hypoxia and chronic hypoxia compared to sea-level value with and without sitaxsentan treatment. Sitaxsentan restored the hypoxia-induced decrease in $V'_{O_2,max}$ by approximately one-third in acute hypoxic conditions and by 12% during a stay at high altitude. *: p<0.05; ***: p<0.001.

Sitaxsentan limited hypoxia-induced decrease in $V'_{O_2,max}$, by an average of 30% in acute hypoxia and 10% in chronic hypoxia (p<0.001 and p<0.05, respectively) (fig. 1). There was a significant inverse correlation between changes in $V'_{O_2,max}$ and resting systolic P_{pa} in acute normobaric hypoxia as well as at high altitude (fig. 2).

Effects of sitaxsentan and altitude on renal function

Altitude exposure was associated with decreased C_{cr}, decreased C_{H_2O} that was out of proportion with the C_{cr} decrease (leading to more negative C_{H_2O}/C_{cr} ratio), decreased fractional excretion of uric acid, and increased fractional excretion of sodium together with an increased urinary log10 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 signalling, 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 P_{pa} 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 been reported in rats exposed to a F_{I,O_2} of 0.1 for 48 h [21], with addition of an overexpression of the endothelin B receptor after increase of the duration of the hypoxic exposure to 4 weeks [22]. Both selective endothelin A

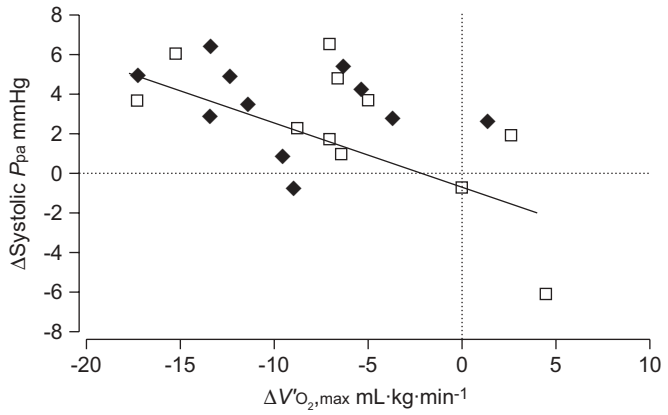

FIGURE 2. Correlation between systolic pulmonary artery pressure (P_{pa}) and maximum O_2 uptake ($V'_{O_2,max}$) in acute (◆) and chronic (□) hypoxia. $r^2=0.33$, $p<0.01$.

and nonselective endothelin A and B receptor blockers prevent and reverse hypoxic pulmonary hypertension in experimental animals [23, 24]. In healthy humans at high altitude, systolic P_{pa} is increased, and this has been shown to be partly reversed by the administration of the nonselective 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 systolic P_{pa} and normalised PVR. Altogether, these observations suggest a major contribution of endothelin A receptor signalling in both acute hypoxic vasoconstriction and chronic hypoxic pulmonary hypertension in normal subjects acclimatised 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 and more obvious decreases 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 the 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 $V'_{O_2,max}$ and systolic P_{pa}, 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 acclimatised to high altitudes, but comparably lower TAPSE and higher Tei index in high-altitude dwellers [26]. It is of interest that the improvement in $V'_{O_2,max}$ 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 $V'_{O_2,max}$ in the present study. Arterial oxygenation was unchanged at rest or during 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, consistent with a 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 BP 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 increases in nitric oxide availability [9] could improve mitochondrial capacity by a protein kinase G-mediated pathway, thereby improving myocardial and skeletal

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

Variables	Sea level		Altitude		
	Placebo	Sitaxsentan	Baseline	Placebo	Sitaxsentan
Subjects n	17	17	22	11	11
Diuresis mL·min^{-1}	1.1±0.1	1.2±0.2	1.1±0.2	1.6±0.3	1.8±0.3
GFR mL·min^{-1}	117±6	123±8	93±7*	97±6	109±11
C_{H_2O} mL·min^{-1}	-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

Data are presented as mean±SE. GFR: glomerular filtration rate; C_{H_2O}: free water clearance; FE: fractional excretion; Na: urinary sodium; K: urinary potassium. *: $p<0.05$, altitude *versus* sea level.

O_2 uptake [29, 30]. Thus, sitaxsentan could also have affected the matching of convectional and diffusional oxygen transport systems, which have been modelled 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 C_{cr} to estimate GFR, C_{H_2O}/C_{cr} to estimate the effects of antidiuretic hormone (ADH) on collecting duct handling of water, fractional excretion of sodium (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 haemoconcentration with decreased effective plasma volume [32, 33]. These effects are essentially related to decreased ADH and aldosterone signalling at the collecting ducts [32, 33]. Healthy volunteers rapidly brought to the altitude of 4,559 m typically presented with increased diuresis, C_{H_2O}/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 hypovolaemia, as assessed by decreases in both C_{cr} and FE urate, decreased C_{H_2O}/C_{cr} 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 acclimatisation, and achieve new steady-state. Persistently decreased aldosterone signalling is consistent with previous observations [33]. Increased ADH signalling in the present study may be either time-related or associated with 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 4,559 m, bosentan decreased C_{H_2O} corrected for GFR, with no significant effect on GFR or FE Na, 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, consistent 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 include the noninvasive echocardiographic estimations of P_{pa} and cardiac output. However, we previously reported satisfactory interobserver and intraobserver variabilities on these measurements [26]. A good agreement between invasive and non invasive measurements of systolic P_{pa} has been previously reported at high altitude [37]. A more important limitation is the absence of echocardiographic measurements during exercise. This was attempted, but did not produce sufficiently high 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 systolic P_{pa} limited VO_2 max by a decrease in maximal cardiac output. Iit must be stressed that increased systolic P_{pa} would only partly contribute to hypoxia-related decrease in aerobic exercise capacity, as supported by the r^2-value of the correlation between changes in systolic P_{pa} and $V'_{O_2,max}$ of only 0.33, suggesting that one-third of $V'_{O_2,max}$ changes could possibly be explained by changes in systolic P_{pa}. Furthermore, remote, high-altitude conditions prevented measurements of haematocrit and haemoglobin in all the subjects before and after intake of sitaxsentan, such that changes in arterial O_2 content associated with 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]. In addition, the intake of sitaxsentan was associated with an improved acute mountain sickness score. This may possibly be related to the cytoprotective effects of endothelin A receptor blockade reported in hypoxic astrocytes [38] or improved cerebral haemodynamics and oxygenation [39].

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STATEMENT OF INTEREST

Statements of interest for R. Naeije, S. Huez and V. Faoro, and for the study itself, can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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