# Role of endothelin-1 in exposure to high altitude: Acute Mountain Sickness and Endothelin-1 (ACME-1) study

# Role of Endothelin-1 in Exposure to High Altitude: Acute Mountain Sickness and Endothelin-1 (ACME-1) Study

Pietro Amedeo Modesti, Simone Vanni, Marco Morabito, Alessandra Modesti, Matilde Marchetta, Tania Gamberi, Francesco Sofi, Giulio Savia, Giuseppe Mancia, Gian Franco Gensini and Gianfranco Parati

*Circulation* 2006;114;1410-1416; originally published online Sep 18, 2006;
DOI: 10.1161/CIRCULATIONAHA.105.605527
Circulation is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 72514
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

# Role of Endothelin-1 in Exposure to High Altitude
## Acute Mountain Sickness and Endothelin-1 (ACME-1) Study

Pietro Amedeo Modesti, MD, PhD; Simone Vanni, MD, PhD; Marco Morabito, PhD; Alessandra Modesti, BS, PhD; Matilde Marchetta, BS; Tania Gamberi, BS, PhD; Francesco Sofi, MD, PhD; Giulio Savia, MD; Giuseppe Mancia, MD; Gian Franco Gensini, MD; Gianfranco Parati, MD, PhD

***Background***—The degree of pulmonary hypertension in healthy subjects exposed to acute hypobaric hypoxia at high altitude was found to be related to increased plasma endothelin (ET)-1. The aim of the present study was to investigate the effects of ET-1 antagonism on pulmonary hypertension, renal water, and sodium balance under acute and prolonged exposure to high-altitude–associated hypoxia.

***Methods and Results***—In a double-blind fashion, healthy volunteers were randomly assigned to receive bosentan (62.5 mg for 1 day and 125 mg for the following 2 days; n=10) or placebo (n=10) at sea level and after rapid ascent to high altitude (4559 m). At sea level, bosentan did not induce any significant changes in hemodynamic or renal parameters. At altitude, bosentan induced a significant reduction of systolic pulmonary artery pressure (21±7 versus 31±7 mm Hg, $P<0.03$) and a mild increase in arterial oxygen saturation versus placebo after just 1 day of treatment. However, both urinary volume and free water clearance ($H_2OCl$/glomerular filtration rate) were significantly reduced versus placebo after 2 days of ET-1 antagonism (1100±200 versus 1610±590 mL; −6.7±3.5 versus −1.8±4.8 mL/min, $P<0.05$ versus placebo for both). Sodium clearance and segmental tubular function were not significantly affected by bosentan administration.

***Conclusions***—The present results indicate that the early beneficial effect of ET-1 antagonism on pulmonary blood pressure is followed by an impairment in volume adaptation. These findings must be considered for the prevention and treatment of acute mountain sickness. **(*Circulation*. 2006;114:1410-1416.)**

**Key Words:** endothelin ■ altitude ■ hypoxia ■ lung ■ oxygen ■ pulmonary heart disease ■ pressure

Hypoxia during ascent to high altitude is responsible for an increase in pulmonary artery pressure.[1,2] The increased venular tone with increased capillary pressure that follows rapid ascent to high altitude without adequate acclimatization[3] may contribute to force liquid across the endothelial barrier into the interstitial space, which may contribute to respiratory problems in this condition. Because tens of thousands of tourists travel to a variety of high-altitude destinations around the world on a yearly basis, the search for new strategies to prevent and treat high-altitude–induced pulmonary hypertension is now considered a priority.[4] The acute increase in pulmonary pressure in mountaineers was found to be closely related to an increased plasma concentration of endothelin (ET)-1.[3,5] Because of the reported efficacy of ET-1 antagonism in patients with pulmonary hypertension, the use of ET-1 receptor blockade at high altitudes might be considered.[6] A second relevant component of adaptation to altitude is an increase in urinary volume, associated with a reduced renal sensitivity to arginine vasopressin (AVP).[7] Recent studies revealed that the lack of increase in urinary volume and free water clearance has a relevant prognostic value for the onset of acute mountain sickness (AMS) in mountaineers reaching a high altitude (4880 m).[8] ET-1 is produced in the kidney, where it functionally antagonizes AVP.[9,10] Although exposure of healthy subjects to normobaric hypoxia was found to enhance ET-1 urinary excretion,[11] the functional role of ET-1 in renal adaptation at high altitudes is unknown.

**Editoral p 1350**
**Clinical Perspective p 1416**

We therefore designed the present placebo-controlled study to investigate the effects of bosentan, a mixed ET receptor A and B (ETA/ETB) antagonist, on pulmonary hemodynamic and renal function during the 4 days after arrival at the research laboratory on Monte Rosa, the Regina Margherita hut (Italian Alps, altitude 4559 m).

Received December 1, 2005; revision received June 23, 2006; accepted July 1, 2006.

From Clinica Medica Generale e Cardiologia (P.A.M., S.V., M. Morabito, A.M., M. Marchetta, T.G., F.S., G.F.G.), University of Florence, Florence, Italy; Don Carlo Gnocchi Foundation (P.A.M., G.F.G.), Onlus IRCCS, Florence, Italy; and Department of Clinical Medicine, Prevention and Applied Biotechnologies (G.S., G.M., G.P.), University of Milano-Bicocca and Istituto Auxologico Italiano, Milan, Italy.

Correspondence to Professor Pietro Amedeo Modesti, MD, PhD, Clinica Medica Generale e Cardiologia, University of Florence, Viale Morgagni 85, 50134 Florence, Italy. E-mail pamodesti@unifi.it



*Circulation* **is available at http://www.circulationaha.org** **DOI: 10.1161/CIRCULATIONAHA.105.605527**

# Methods

## Subjects and Study Design

Twenty healthy adult volunteers (17 men and 3 women, 32 to 53 years old) with past experience in mountain hiking, nonacclimatized and without specific training, were recruited by word of mouth. Smoking and medication use were exclusion criteria for study participation.

Two to 4 weeks before ascension, all subjects underwent baseline investigations at low altitude (50 m above sea level; barometric pressure 772 mm Hg). At least 4 days before the study, subjects were instructed to restrict their normal diet to avoid salty and spicy meals, as well as the addition of salt to cooked foods. Subjects were also instructed to avoid strenuous physical activity and alcohol- and caffeine-containing drinks and to have at least 6 hours of sleep per night. Furthermore, they were instructed to fast for 8 hours before the experiment and to use passive transportation to the laboratory to minimize physical activity during the study days.

On the first experimental day, the subjects underwent laboratory investigations with echocardiographic measurements. Subjects were then randomized to receive bosentan (Actelion, Ltd, Basel, Switzerland; 62.5 mg twice daily on day 1 and then 125 mg twice daily on days 2 and 3, n=10) or matching placebo (n=10),[12] being blinded to the assigned treatment. Carbolithium (600 mg/d) was also administered to all subjects to investigate proximal tubule reabsorption. Echocardiography, blood sampling, and urine collection were then repeated by the same physicians on the following 3 consecutive days.

The same protocol of drug administration and measurements was then performed at altitude. All subjects ascended in less than 24 hours from Alagna Valsesia (Vercelli, Italy; altitude 1130 m) to a research laboratory on Monte Rosa, the Regina Margherita hut (altitude 4559 m; average barometric pressure 435 mm Hg), with an overnight stay at the Gnifetti hut (altitude 3611 m). After a first night at 4559 m, the subjects underwent laboratory investigations with echocardiographic measurements. Subjects received bosentan or matching placebo and carbolithium following the same protocol for drug administration and data collection as at sea level. At altitude, all subjects ate the same standard diet provided by the Regina Margherita hut staff, with the recommendation to avoid the addition of salt to cooked foods. Food and water intake were ad libitum. All other instructions were identical to those at sea level.

Ascent was directly supervised by investigators. The investigators continuously monitored the subjects for evidence of high-altitude pulmonary edema (HAPE; defined as severe cough, dyspnea at rest, cyanosis, and rales) or high-altitude cerebral edema (defined as ataxia, altered consciousness, uncontrolled physical movements, nausea, vomiting, or loss of consciousness). Immediate evacuation and treatment with dexamethasone and oxygen were available for such an occurrence. A standardized questionnaire for the clinical assessment of AMS (Lake Louise score) was completed daily.[13]

All subjects were requested to give informed written consent to participate in the study, in strict compliance with the Helsinki Declaration. Experimental protocol and informed consent forms were approved by the ethics committee of the Azienda Ospedaliera Careggi (protocol 211/04).

## Doppler Echocardiography

Mean and systolic pulmonary artery pressure were calculated daily by color Doppler echocardiography (CARIS PLUS, ESAOTE Biomedica, Florence, Italy) based on the time to peak velocity of the pulmonary artery Doppler velocity curve in short-axis view and on the velocity of the tricuspid regurgitant jet and the respiratory variation in inferior vena cava size, as an estimate of right atrial pressure.[14] To calculate the transtricuspid pressure gradient, a modified Bernoulli equation was used in which transtricuspid pressure equals 4 times the square of the tricuspid jet velocity.

The recordings were stored on VHS videotape for analysis by an investigator who was unaware of the study procedures. All reported values represent the mean of >2 measurements.

## General Procedures and Collection of Biological Samples

Systemic blood pressure, heart rate (ECG), and hemoglobin oxygen saturation (pulse oximeter attached to a fingertip) were measured at 8 AM. Then, plasma and urine samples were collected. Blood samples (≈25 mL/d) were drawn from subjects in the supine position from an antecubital vein for assay of ET-1, glucose, urea, creatinine, Na, K, Li, and osmolality. The samples were immediately put on ice and then centrifuged at 3000 rpm for 10 minutes. Simultaneously, urine samples for assay of ET-1, creatinine, Na, K, Cl, Li, and osmolality were separated from the daily urine collection (≈40 mL). Plasma, serum, and urine samples were then snap-frozen in liquid nitrogen and stored at −20°C until their return to the laboratory.

## Analytical Methods and Calculations

Plasma and urine were assayed for electrolytes (Na, K, and Li) with an ion-sensitive electrode (Instrumentation Beckmann Astra, Brea, Calif) and for creatinine with the Jaffe reaction (Instrumentation Beckmann Astra).[9] Glomerular filtration rate (GFR) was estimated by the clearance of endogenous creatinine. Free water clearance ($H_2OCl$) was calculated as:

$$H_2OCl = \left(1 - \frac{U_{osm}}{P_{osm}}\right) \times V \quad (1)$$

where $U_{osm}$ (mOsm/L $H_2O$) and $P_{osm}$ (mOsm/L $H_2O$) are the simultaneous urinary and plasma osmolalities, respectively, and V (mL/min) is the simultaneous rate of urinary volume flow.

Osmolar clearance (OsmCl), sodium clearance (NaCl), lithium clearance (LiCl), and creatinine clearance (CrCl) were calculated with standard formula:

$$\text{clearance} = U \times \frac{V}{P} \quad (2)$$

where U and P are the simultaneous urinary and plasma concentrations, respectively, and V (mL/min) is the simultaneous rate of urinary volume flow. The LiCl is a measure of fluid delivery from the proximal tubule to the loop of Henle. Fractional proximal and distal sodium reabsorption rates were calculated as:

$$\text{Fractional proximal sodium reabsorption rate} = \frac{GFR - LiCl}{GFR} \quad (3)$$

and

$$\text{Fractional distal sodium reabsorption rate} = \frac{LiCl - NaCl}{LiCl} \quad (4)$$

## ET-1 Assay

ET-1 was extracted from both plasma and urine samples by Sep-Pak Cartridge C18 PLUS columns (catalog No. 023635, Waters Corporation, Milford, Mass) and assayed by radioimmunoassay with polyclonal rabbit anti-ET-1 serum (catalog No. S2024, Bachem-Peninsula, San Carlos, Calif), as described previously.[9,15–17] The minimum detectable ET-1 amount was 0.1 pg.

## Statistical Analysis

The sample size was calculated assuming an increase in pulmonary arterial pressure of 60±18% at 4559 m versus low-altitude values. A sample size of 20 patients with equal allocation to bosentan or placebo offered 80% power to detect a 50% reduction of the pulmonary pressure increase with bosentan compared with placebo. The error level was set at 5%.

Twenty-five subjects were contacted. Three subjects did not accept our invitation, and 2 subjects were deemed ineligible because of heavy smoking and arterial hypertension that required drug treatment. The study at 4559 m was not completed by request of 1 subject in the placebo group after the examination on day 2.

The statistical analysis was performed with the SPSS software package (SPSS Inc, Chicago, Ill). A 2-way repeated-measures

**TABLE 1. Lake Louise Score and Systemic and Pulmonary Hemodynamics in Subjects Assigned to Bosentan or Placebo-Treated Group at Sea Level and High Altitude**

| | | High Altitude | | | |
|---|---|---|---|---|---|
| | Sea Level | Day 0 | Day 1 | Day 2 | Day 3 |
| Lake Louise score | | | | | |
| Placebo | 0 | 6.4±3.1 | 2.2±2.0 | 1.2±0.9 | 1±0.8 |
| Bosentan | 0 | 7.7±3.2 | 2.7±1.6 | 1.1±0.7 | 0.6±0.8 |
| Systolic blood pressure, mm Hg | | | | | |
| Placebo | 118±9 | 125±15 | 126±28 | 126±20 | 131±23 |
| Bosentan | 116±8 | 125±14 | 131±19 | 128±10 | 128±12 |
| Diastolic blood pressure, mm Hg | | | | | |
| Placebo | 70±6 | 82±9 | 82±6 | 82±18 | 81±13 |
| Bosentan | 69±7 | 79±11 | 77±12 | 81±12 | 83±10 |
| Heart rate, bpm | | | | | |
| Placebo | 66±3 | 84±7 | 80±7 | 77±6 | 76±7 |
| Bosentan | 67±3 | 86±7 | 81±5 | 81±10 | 80±5 |
| Arterial oxygen saturation, % | | | | | |
| Placebo | 100±1 | 78±6 | 78±6 | 82±6 | 82±4 |
| Bosentan | 100±1 | 75±7 | 81±4* | 81±6 | 81±8 |
| Systolic pulmonary artery pressure, mm Hg | | | | | |
| Placebo | 16±2 | 34±6 | 31±7 | 27±7 | 21±6 |
| Bosentan | 15±2 | 34±9 | 21±7* | 19±7* | 19±8 |

*$P<0.05$ vs placebo.

ANOVA was used to compare differences between low and high altitude and the 2 treatment groups. A random-effects linear regression model, which takes into account the lack of independence of a subject's measurements across time in the placebo group, was used. This intrasubject correlation was modeled by a random intercept effect, and parameters were estimated by the maximum likelihood method. Coefficient estimate ($\beta$) and standard error were used for data presentation. A probability value below 0.05 was set as the level of minimum statistical significance.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

## Results

### Clinical Effects of High Altitude

On the first day at Regina Margherita hut, before the beginning of treatment (day 0), all subjects scored 3 or more points on the Lake Louise assessment (6.4±3.1 and 7.7±3.2 in the placebo and bosentan-treated group, respectively). However, the symptoms thereafter subsided, and the Lake Louise score decreased in both the placebo and bosentan-treated groups, remaining less than 2 on day 2 and day 3 in the individual subjects (Table 1). Bosentan administration did not affect either the Lake Louise score or systemic hemodynamics (Table 1). Neither side effects nor adverse events were observed in treated subjects either at altitude or at sea level. A significant increase in both plasma concentration and urinary ET-1 daily excretion was found in both groups versus sea level (Figure 1).

### ET-1 Antagonism and Pulmonary Adaptation to Altitude

On arrival at Regina Margherita hut (day 0), subjects given placebo showed a decrease in arterial oxygen saturation to 78±6% ($P<0.05$ versus sea level) with a simultaneous increase in systolic pulmonary artery pressure from 16±2 to 34±6 mm Hg ($P<0.05$; Figure 2). The reduced arterial oxygen saturation and the increased systolic pulmonary artery pressure were maintained for 2 days, with a significant increase and reduction, respectively, in these parameters at the third day at altitude only (82±6% and 27±7 mm Hg; both $P<0.05$ versus day 0; Figure 2). Arterial oxygen saturation was inversely related to systolic pulmonary artery pressure ($r=-0.654$, $P<0.001$; Figure 3), which was also directly related to ET-1 plasma concentration ($r=0.582$, $P<0.001$; Figure 3). These relationships were confirmed even after taking into account multiple observations per subject, with

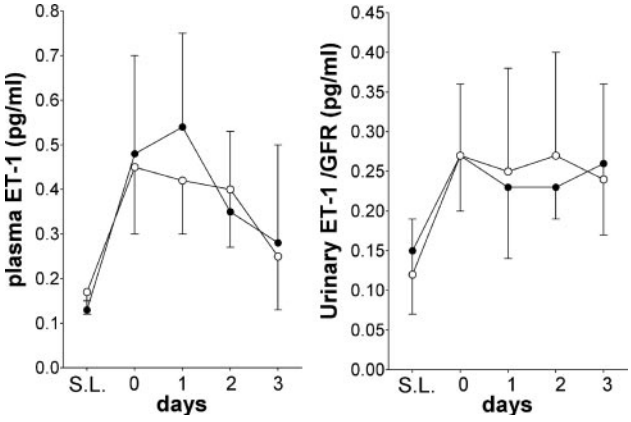


**Figure 1.** Plasma concentration and urinary ET-1 daily excretion at sea level (S.L.) and during exposure to high altitude (days 0, 1, 2, and 3) in bosentan- (●) or placebo-treated (○) group.

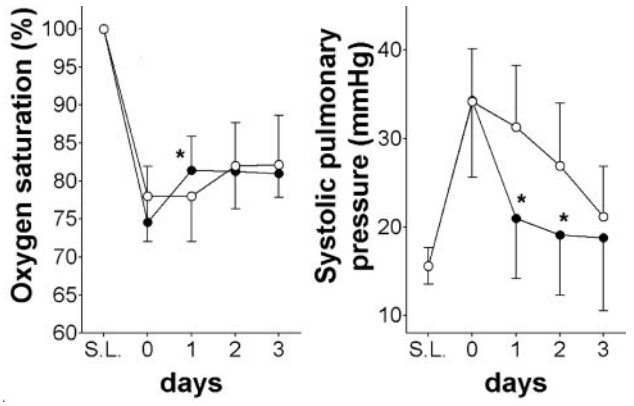


**Figure 2.** Arterial oxygen saturation (left) and systolic pulmonary artery pressure (right) at sea level (S.L.) and during exposure to high altitude (days 0, 1, 2, and 3) in bosentan- (●) or placebo-treated (○) group. *$P<0.05$ vs placebo.

the random-effects linear regression model, in the placebo group ($\beta=81.7\pm7.72$, $P<0.0001$ for oxygen saturation; $\beta=4.32\pm0.30$, $P<0.001$ for plasma ET-1).

In bosentan-treated subjects, systolic pulmonary artery pressure at altitude was significantly reduced compared with the placebo group even after just 1 day of treatment (day 1: $21\pm7$ versus $31\pm7$ mm Hg, $P<0.03$). A simultaneous mild increase in arterial oxygen saturation on day 1 was observed ($P<0.05$ versus day 0; Figure 2). On day 2, arterial oxygen saturation was comparable in the 2 groups ($82\pm6\%$ versus $81\pm6\%$; Figure 2).

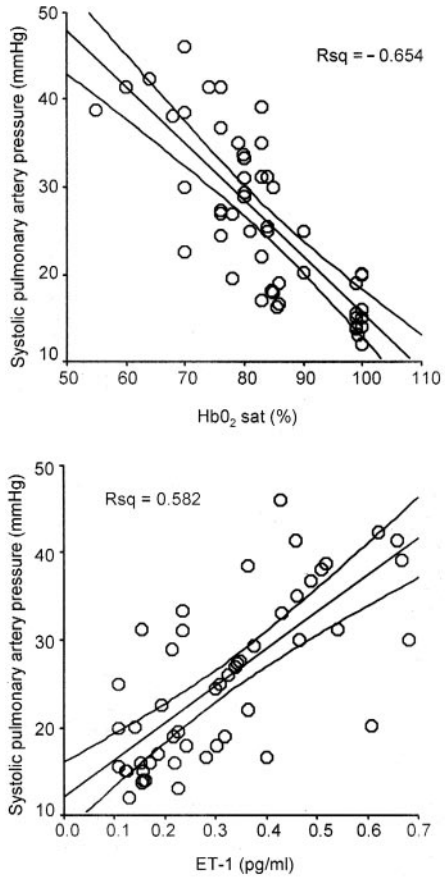


**Figure 3.** Relationship between arterial oxygen saturation (top) or ET-1 plasma concentration (bottom) and systolic pulmonary artery pressure.

### Renal Effects of ET-1 Antagonism

At altitude, 24-hour urinary volume (in milliliters) in the placebo group increased by 40% and 59% versus sea level on day 0 and day 1, respectively, then progressively decreased and normalized on day 3 (Figure 4). On day 1, the enhanced urine output was associated with a significant 10-fold change in free water clearance versus sea level (Figure 4), with no significant increases in sodium clearance (Figure 4) or lithium clearance (Table 2). Likewise, the investigation of fractional sodium reabsorption revealed no changes in absolute and fractional proximal and distal sodium reabsorption rates (Table 2). No significant changes in body weight compared with sea level were found.

In bosentan-treated subjects, 24-hour urine output was significantly lower than in the placebo group on days 1 and 2 (Figure 4). The lower urinary volume was associated with a significant reduction in free water clearance in the bosentan group compared with day 0 and with the placebo group ($P<0.01$ at day 2; Figure 4). Conversely, sodium clearance (Figure 4), lithium clearance, and absolute and fractional proximal and distal sodium reabsorption rates did not differ between groups (Table 2).

## Discussion

The present findings demonstrate for the first time that the use of a mixed ETA/ETB antagonist (bosentan) at high altitudes effectively controls the hypoxia-induced increase in pulmonary blood pressure but also causes a reduction in free water clearance. Besides the physiological implications regarding the role of ET-1 at both the pulmonary and the renal district in normal subjects, these findings appear to be of interest because they might contribute to the definition of a therapeutic window for the use of bosentan at high altitudes.

### Pulmonary Effects of ET-1 Antagonism

The present findings clarify the role of ET-1 in pulmonary hypertension at high altitudes in humans. In experimental animals, exposure to acute hypoxia (48 hours of hypoxia at 10% $O_2$, 1 atm) resulted in significant overexpression in the lung of ET-1 peptide (1.5-fold) and of genes for ET-1 and ETA receptor (4.1-fold and 2.3-fold, respectively), with unchanged mRNA levels for the ETB receptor subtype.[18] Overexpression of both ETA and ETB receptor genes was observed in lung of rats exposed to chronic hypoxia (10% $O_2$, 1 atm, 4 weeks).[19] In rats exposed to normobaric hypoxia (90 minutes, 10% $O_2$), the ETA receptor antagonist BQ-123 markedly attenuated the increase in pulmonary artery pressure.[20] Similar results were obtained after administration of BMS-182874 (a selective ETA antagonist) in pigs subjected to acute, intermittent 15-minute periods of hypoxia ($Fio_2=0.1$).[21] Each of these experimental studies suggests that ET-1 synthesized in lung in response to hypoxia acts locally on ETA receptors to acutely increase pulmonary pressure. In humans, indirect evidence was provided that indicated that the increase in pulmonary pressure after expo-

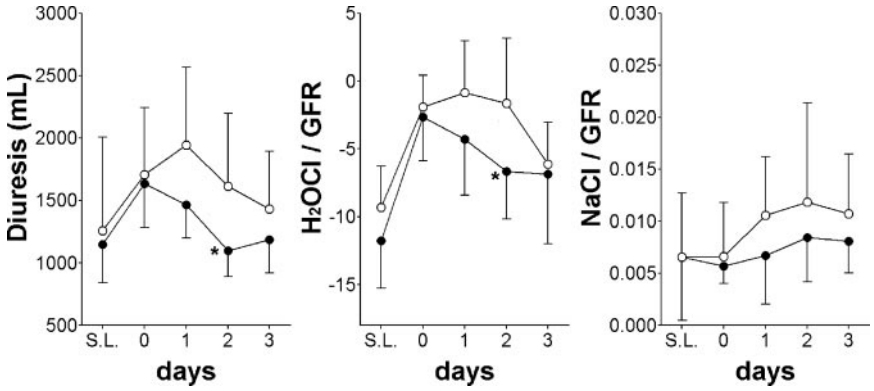


**Figure 4.** Urinary volume (left), free water clearance (middle), and renal sodium clearance (right) at sea level (S.L.) and during exposure to high altitude (days 0, 1, 2, and 3) in bosentan- (●) or placebo-treated (○) group. $H_2OCl$ indicates free water clearance; NaCl, renal sodium clearance. *$P<0.05$ vs placebo.

sure to high altitude is correlated with an increased plasma ET-1 concentration.[3,5,22] However, the pathogenetic role of ET-1 for human pulmonary hypertension at high altitudes remained to be investigated, and no studies reported the use of ET receptor blockers as a possible prophylactic strategy.

In subjects exposed to high altitude in the present study, administration of bosentan was associated not only with a reduction in pulmonary pressure but also with an increase in oxygen saturation. This is not in line with observations that pulmonary vasoconstriction (and thus increases in pulmonary pressure) in many pulmonary diseases optimizes the ratio of alveolar ventilation to regional pulmonary perfusion and that the therapeutic use of vasodilators (prostacyclin) increases ventilation/perfusion mismatch and decreases arterial oxygenation.[23]

It would be simplistic to consider that a reduction in pulmonary pressure would necessarily correspond to changes in ventilation/perfusion mismatch. This is because different drugs that cause similar reductions in pulmonary arterial pressure trigger different responses in arterial oxygenation. In particular, in the setting of high-altitude–induced pulmonary vasoconstriction, nitric oxide[24] and sildenafil[25] failed to modify oxygen saturation, whereas in the present study, ET-1 antagonism by bosentan improved oxygen saturation. This

**TABLE 2. Renal Function and Segmental Tubular Function in Subjects Assigned to Bosentan or Placebo-Treated Group at Sea Level and High Altitude**

| | | High Altitude | | | |
|---|---|---|---|---|---|
| | Sea Level | Day 0 | Day 1 | Day 2 | Day3 |
| Urinary volume, mL | | | | | |
| Placebo | 1220±610 | 1710±540 | 1940±620 | 1610±590 | 1430±470 |
| Bosentan | 1120±250 | 1640±350 | 1460±260* | 1100±200* | 1190±270 |
| Creatinine clearance, mL/min | | | | | |
| Placebo | 125±13 | 122±20 | 107±23 | 103±36 | 92±37 |
| Bosentan | 126±12 | 121±17 | 108±33 | 109±13 | 102±16 |
| Free water clearance/creatinine clearance | | | | | |
| Placebo | −9.9±3.7 | −2.1±2.3 | −0.9±3.8 | −1.8±4.8 | −6.3±3.1 |
| Bosentan | −11±4.1 | −2.7±3.1 | −4.3±4.1 | −6.7±3.5* | −6.8±5.1 |
| Sodium clearance/creatinine clearance | | | | | |
| Placebo | 0.006±0.005 | 0.007±0.005 | 0.011±0.006 | 0.012±0.010 | 0.011±0.006 |
| Bosentan | 0.005±0.002 | 0.006±0.002 | 0.007±0.005 | 0.007±0.003 | 0.008±0.003 |
| Lithium clearance/creatinine clearance | | | | | |
| Placebo | 0.14±0.04 | 0.13±0.05 | 0.10±0.02 | 0.20±0.11 | 0.18±0.05 |
| Bosentan | 0.14±0.05 | 0.15±0.05 | 0.13±0.05 | 0.17±0.09 | 0.16±0.11 |
| Fractional proximal sodium reabsorption rate [(GFR−LiCl)/GFR] | | | | | |
| Placebo | 0.86±0.05 | 0.87±0.05 | 0.90±0.02 | 0.80±0.11 | 0.82±0.05 |
| Bosentan | 0.86±0.05 | 0.85±0.05 | 0.87±0.05 | 0.83±0.09 | 0.84±0.11 |
| Fractional distal sodium reabsorption [(LiCl−NaCl)/LiCl] | | | | | |
| Placebo | 0.95±0.04 | 0.95±0.02 | 0.90±0.05 | 0.93±0.05 | 0.93±0.06 |
| Bosentan | 0.96±0.02 | 0.96±0.01 | 0.94±0.05 | 0.95±0.03 | 0.93±0.05 |

*$P<0.05$ vs placebo.

response might highlight the importance of drug effects on the pulmonary vein district, because pulmonary veins are acknowledged as a main target only for ET-1.[26] The control of venoconstriction might thus more favorably reduce pulmonary capillary pressure,[3] ultimately preventing interstitial edema and enhancing gas exchange even before the onset of HAPE. In contrast, in subjects with clinically manifest HAPE, the reduction of pulmonary arterial pressure could have therapeutic effects per se, because in addition to inhaled nitric oxide,[24] other vasodilator agents, such as nifedipine,[27] hydralazine, and phentolamine,[28] were reported to improve oxygen saturation.

### Renal Effects of ET-1 Antagonism

The present study shows that exposure to high altitude is followed by increased excretion of ET-1 in the urine. Plasma and urinary ET-1 were reported to reflect the existence of 2 separate systems.[15] Indeed, ET-1 excreted in the urine does not derive from plasma, rather being locally produced in the kidney.[9,15] The second main result of the present study is the clear demonstration that bosentan administration limited the physiological increase in free water clearance observed at high altitude. An enhanced diuresis with increased free water clearance is now considered a relevant component of adaptation to altitude.[8] This enhanced diuretic response was found to be associated with a reduction in renal sensitivity to AVP even after 2 days of exposure to high altitude,[7] although conflicting results are available with regard to changes in plasma concentration of AVP. Convincing evidence exists that ET-1 may inhibit AVP-stimulated water reabsorption in the collecting ducts. Several studies have demonstrated that ET-1 binding to the ETB receptor subtype reduces Na+-K+-ATPase activity in the inner medullary collecting duct[29] and inhibits vasopressin-stimulated cAMP accumulation and water transport on isolated cortical collecting ducts from rats.[30,31] These and other studies[32,33] indicate that ET-1 might regulate water reabsorption independently of its effects on Na+ reabsorption or renal hemodynamics. Only indirect evidence of ET-1 effects on renal water handling in humans was available until now.[9] The present study clearly indicates for the first time that the administration of a mixed ET-1 receptor (ETA/ETB) antagonist in a condition characterized by marked activation of renal ET-1 excretion did not affect sodium excretion but rather reduced free water clearance. Edema has been reported to occur in up to 20% of patients who undergo chronic bosentan treatment for pulmonary hypertension, despite demonstration of a reduction in right atrial pressure.[34] Presumably, this is related to a similar effect on renal function with a reduction in free water clearance. An increased hypoxia-induced atrial natriuretic peptide secretion was reported to provide a further compensatory mechanism to modulate the development of hypoxia-induced pulmonary hypertension and to regulate fluid balance during acute and chronic exposure to hypoxia.[35] In this condition, ET-1 produced in the kidney appears to participate in the control of volume by enhancing free water excretion, too.

According to a current theory in the field, the lack of increase in urinary volume in the bosentan group should have resulted in AMS. This did not appear to be the case in subjects in the present study; however, it must be considered that our study was neither sized or designed to investigate the effects of bosentan on AMS symptoms. Indeed, the study sample size was calculated on the basis of the hypothesized effects of the drug on pulmonary pressure. In addition, according to the study protocol, bosentan administration was started after 1 day at altitude (day 1). The effects on diuresis were observed at day 2, ie, at a time when a lower AMS score was also already recorded in the placebo group. Thus, although in theory, lack of urinary volume increase by bosentan could be expected to favor AMS symptoms, we were unable to detect such a phenomenon, probably because of the concomitant beginning of subjects' adaptation to altitude, which might have reduced the sensitivity of our assessment of changes in AMS symptoms. As an alternative explanation, the study group may have been composed of experienced mountaineers who were particularly resistant to AMS.

A relevant clinical implication of the present study might derive from the different timing of the pulmonary and renal adaptive responses. Indeed, although pulmonary hypertension was also significantly reduced after 3 days at altitude in the group treated with placebo, a persistent increase in free water clearance characterized the renal response. These different patterns appear to reflect the different patterns of plasma and renal ET-1 production. As a result of these differences, only a short period of bosentan administration at high altitude could be considered useful to control pulmonary hypertension without impairing free water clearance in the long term. In conclusion, compared with drugs that have already demonstrated their efficacy in the field (such as nifedipine, sildenafil, or hydralazine), bosentan had potentially detrimental effects on diuresis that may preclude its use as a pulmonary artery pressure–lowering agent at high altitude. In particular, if bosentan were proposed to prevent HAPE, its administration would need to be begun before the individual began to climb at high altitude, and the resulting fluid retention might not only precipitate AMS but also worsen alveolar fluid flooding.

## Acknowledgments

We are indebted to the study participants; to the Sezione di Varallo del Club Alpino Italiano for providing the facilities at the Capanna Regina Margherita; to ESAOTE for providing the echocardiographic equipment; and to Dr Raffaele Cioffi of the University G. d'Annunzio of Chieti-Pescara for his statistical support.

## Disclosures

None.

## References

1. Bartsch P, Swenson ER, Maggiorini M. Update: high altitude pulmonary edema. *Adv Exp Med Biol*. 2001;502:89–106.
2. Hackett PH, Roach RC. High-altitude illness. *N Engl J Med*. 2001;345: 107–114.
3. Maggiorini M, Melot C, Pierre S, Pfeiffer F, Greve I, Sartori C, Lepori M, Hauser M, Scherrer U, Naeije R. High-altitude pulmonary edema is initially caused by an increase in capillary pressure. *Circulation*. 2001; 103:2078–2083.

4. Moore LG, Niermeyer S, Zamudio S. Human adaptation to high altitude: regional and life-cycle perspectives. *Am J Phys Anthropol*. 1998;27: 25–64.
5. Goerre S, Wenk M, Bartsch P, Luscher TF, Niroomand F, Hohenhaus E, Oelz O, Reinhart WH. Endothelin-1 in pulmonary hypertension associated with high-altitude exposure. *Circulation*. 1995;91:359–364.
6. Rubin LJ, Badesch DB, Barst RJ, Galie N, Black CM, Keogh A, Pulido T, Frost A, Roux S, Leconte I, Landzberg M, Simonneau G. Bosentan therapy for pulmonary arterial hypertension. *N Engl J Med*. 2002;346: 896–903.
7. Maresh CM, Kraemer WJ, Judelson DA, VanHeest JL, Trad L, Kulikowich JM, Goetz KL, Cymerman A, Hamilton AJ. Effects of high altitude and water deprivation on arginine vasopressin release in men. *Am J Physiol Endocrinol Metab*. 2004;286:E20–E24.
8. 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.
9. Modesti PA, Cecioni I, Migliorini A, Naldoni A, Costoli A, Vanni S, Serneri GG. Increased renal endothelin formation is associated with sodium retention and increased free water clearance. *Am J Physiol*. 1998;275:H1070–H1077.
10. Ge Y, Stricklett PK, Hughes AK, Yanagisawa M, Kohan DE. Collecting duct-specific knockout of the endothelin A receptor alters renal vasopressin responsiveness, but not sodium excretion or blood pressure. *Am J Physiol Renal Physiol*. 2005;289:F692–F698.
11. Hildebrandt W, Ottenbacher A, Schuster M, Swenson ER, Bartsch P. Diuretic effect of hypoxia, hypocapnia, and hyperpnea in humans: relation to hormones and O(2) chemosensitivity. *J Appl Physiol*. 2000; 88:599–610.
12. Rich S, McLaughlin VV. Endothelin receptor blockers in cardiovascular disease. *Circulation*. 2003;108:2184–2190.
13. Keller HR, Maggiorini M, Bartsch P, Oelz O. Simulated descent v dexamethasone in treatment of acute mountain sickness: a randomised trial. *BMJ*. 1995;310:1232–1235.
14. Currie PJ, Seward JB, Chan KL, Fyfe DA, Hagler DJ, Mair DD, Reeder GS, Nishimura RA, Tajik AJ. Continuous wave Doppler determination of right ventricular pressure: a simultaneous Doppler-catheterization study in 127 patients. *J Am Coll Cardiol*. 1985;6:750–756.
15. Neri Serneri GG, Modesti PA, Cecioni I, Biagini D, Migliorini A, Costoli A, Colella A, Naldoni A, Paoletti P. Plasma endothelin and renal endothelin are two distinct systems involved in volume homeostasis. *Am J Physiol*. 1995;268:H1829–H1837.
16. Neri Serneri GG, Cecioni I, Migliorini A, Vanni S, Galanti G, Modesti PA. Both plasma and renal endothelin-1 participate in the acute cardiovascular response to exercise. *Eur J Clin Invest*. 1997;27:761–766.
17. Vanni S, Polidori G, Cecioni I, Serni S, Carini M, Modesti PA. ET(B) receptor in renal medulla is enhanced by local sodium during low salt intake. *Hypertension*. 2002;40:179–185.
18. Li H, Elton TS, Chen YF, Oparil S. Increased endothelin receptor gene expression in hypoxic rat lung. *Am J Physiol*. 1994;266:L553–L560.
19. Li H, Chen SJ, Chen YF, Meng QC, Durand J, Oparil S, Elton TS. Enhanced endothelin-1 and endothelin receptor gene expression in chronic hypoxia. *J Appl Physiol*. 1994;77:1451–1459.
20. Oparil S, Chen SJ, Meng QC, Elton TS, Yano M, Chen YF. Endothelin-A receptor antagonist prevents acute hypoxia-induced pulmonary hypertension in the rat. *Am J Physiol*. 1995;268:L95–L100.
21. Holm P, Liska J, Franco-Cereceda A. The ETA receptor antagonist, BMS-182874, reduces acute hypoxic pulmonary hypertension in pigs in vivo. *Cardiovasc Res*. 1998;37:765–771.
22. Sartori C, Vollenweider L, Loffler BM, Delabays A, Nicod P, Bartsch P, Scherrer U. Exaggerated endothelin release in high-altitude pulmonary edema. *Circulation*. 1999;99:2665–2668.
23. Ghofrani HA, Wiedemann R, Rose F, Schermuly RT, Olschewski H, Weissmann N, Gunther A, Walmrath D, Seeger W, Grimminger F. Sildenafil for treatment of lung fibrosis and pulmonary hypertension: a randomised controlled trial. *Lancet*. 2002;360:895–900.
24. Scherrer U, Vollenweider L, Delabays A, Savcic M, Eichenberger U, Kleger GR, Fikrle A, Ballmer PE, Nicod P, Bartsch P. Inhaled nitric oxide for high-altitude pulmonary edema. *N Engl J Med*. 1996;334:624–629.
25. Ghofrani HA, Reichenberger F, Kohstall MG, Mrosek EH, Seeger T, Olschewski H, Seeger W, Grimminger F. 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.
26. Toga H, Ibe BO, Raj JU. In vitro responses of ovine intrapulmonary arteries and veins to endothelin-1. *Am J Physiol*. 1992;263:L15–L21.
27. Oelz O, Maggiorini M, Ritter M, Waber U, Jenni R, Vock P, Bartsch P. Nifedipine for high altitude pulmonary oedema. *Lancet*. 1989;2: 1241–1244.
28. Hackett PH, Roach RC, Hartig GS, Greene ER, Levine BD. The effect of vasodilators on pulmonary hemodynamics in high altitude pulmonary edema: a comparison. *Int J Sports Med.* 1992;13(suppl 1):S68–S71.
29. Zeidel ML, Brady HR, Kone BC, Gullans SR, Brenner BM. Endothelin, a peptide inhibitor of Na+/K+-ATPase in intact renal tubular epithelial cells. *Am J Physiol*. 1989;257:C1101–C1107.
30. Oishi R, Nonoguchi H, Tomita K, Marmo F. Endothelin-1 inhibits AVP-stimulated osmotic water permeability in rat inner medullary collecting duct. *Am J Physiol*. 1991;261:F951–F956.
31. Tomita K, Nonoguchi H, Terada Y, Marmo F. Effects of ET-1 on water and chloride transport in cortical collecting ducts of the rat. *Am J Physiol*. 1993;264:F690–F696.
32. Kohan DE. Endothelins: renal tubule synthesis and actions. *Clin Exp Pharmacol Physiol*. 1996;23:337–344.
33. Simonson MS. Endothelins: multifunctional renal peptides. *Physiol Rev*. 1993;73:375–411.
34. Channick RN, Simonneau G, Sitbon O, Robbins IM, Frost A, Tapson VF, Badesch DB, Roux S, Rainisio M, Bodin F, Rubin LJ. Effects of the dual endothelin-receptor antagonist bosentan in patients with pulmonary hypertension: a randomised placebo-controlled study. *Lancet*. 2001;358: 1119–1123.
35. Chen YF. Atrial natriuretic peptide in hypoxia. *Peptides*. 2005;26: 1068–1077.


## CLINICAL PERSPECTIVE

The increase in pulmonary artery and capillary pressure that follows rapid ascent to high altitude without adequate acclimatization may force liquid across the endothelial barrier into the interstitial space, with consequent respiratory problems. Tens of thousands of tourists travel to a variety of high-altitude destinations around the world on a yearly basis, so that notwithstanding the fact that different vasodilators have been found useful in the treatment of high-altitude pulmonary edema, the search for new strategies to prevent high-altitude–induced pulmonary hypertension can be considered a priority. In the present study, 1-day treatment with a mixed endothelin (ET)-1 receptor A and B antagonist (bosentan) induced a significant reduction of systolic pulmonary artery pressure and a mild increase of arterial oxygen saturation versus placebo in healthy subjects who rapidly ascended to the Regina Margherita hut (Monte Rosa, Italian Alps, 4559 m). The second main result of the study, however, is the clear demonstration that both urinary volume and free water clearance were reduced significantly versus placebo after 2 days of ET-1 antagonism. Therefore, ET-1 participates in renal fluid handling, and the early beneficial effect of bosentan on pulmonary hypertension is followed by potentially detrimental effects on diuresis that may preclude its use as a pulmonary artery pressure–lowering agent at high altitude.