[5]. In the latter group, mortality and morbidity are high during infancy because compression of the trachea and right main stem bronchus produces severe respiratory distress within the first year of life. However, asymptomatic pulmonary artery slings are typically diagnosed incidentally in adolescence or adulthood.

Pulmonary artery sling can be repaired in infancy with low operative mortality and excellent long-term patency of the left pulmonary artery, by dividing the left pulmonary artery and implanting it into the main pulmonary artery anterior to the trachea [6]. In contrast to symptomatic patients, the prognosis for asymptomatic patients is excellent and surgical intervention is not indicated.

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Effects of fasudil in patients with high-altitude pulmonary hypertension

To the Editors:

High-altitude pulmonary arterial hypertension (HAPH) is characterised by increased pulmonary vascular resistance (PVR) secondary to hypoxia-induced pulmonary vasoconstriction and vascular remodelling of pulmonary arterioles [1]. Among the potential therapeutic targets is the RhoA/Rho kinases (ROCK) signalling pathway. The GTPase RhoA is a member of the Rho protein family, which regulates cellular functions such as contraction, motility, proliferation and apoptosis, and ROCKs are the best characterised downstream targets for RhoA [2, 3]. Activation of the small GTP-binding protein RhoA and its downstream target ROCK play a significant role in the pathogenesis of pulmonary hypertension (PH). The effectiveness of Rho/ROCK inhibition has been shown in several murine models of PH [4–7]. Fasudil, a ROCK inhibitor, is a potent vasodilatator approved in Japan for the treatment of brain vessel vasospasm induced by subarachnoid haemorrhage. It has been shown to reduce PVR in patients with severe idiopathic pulmonary arterial hypertension (PAH) [8, 9]. The aim of our study was to investigate the potential therapeutic effects of fasudil on pulmonary artery pressure (P_{pa}) in Kyrgyz highlanders with HAPH.

19 patients with HAPH, all permanent residents of the Tien-Shan Mountains (altitude 3,200–3,600 m) were studied. The study was approved by the ethics committee of the National Center of Cardiology and Internal Medicine (Bishkek, Kyrgyzstan) and all subjects gave informed consent. All subjects underwent health screening by history, physical examination, ECG, spirometry, blood pressure measurement and biochemical analysis for liver and kidney functions in order to exclude any pathology that might have potential influence on HAPH.

The effects of fasudil hydrochloride hydrate (Eril; Asahi Kasei Pharma Corp., Tokyo, Japan) and placebo (saline vehicle) on pulmonary haemodynamics were compared in a randomised, double-blind study. Each patient attended our high-altitude hospital at 3,600 m on two occasions, 1 day apart. On each occasion, echocardiographic studies were performed at baseline and after 30 min rest. Following this, fasudil or placebo were administered intravenously in a dose of 1 mg·min^{-1} for the following 30 min (total dose of fasudil 30 mg). Echocardiography was started 5 min before the fasudil or placebo infusion finished. Cardiac frequency (f_C), systemic arterial pressure and arterial oxygen saturation (S_{a,O_2}) were monitored continuously during the study (PROPAQ 102; Protocol Systems Inc., Dallas, TX, USA).

Doppler echocardiography was performed using a portable ultrasound system equipped with a 2.5-MHz probe (SpectraMax; SonoSite, Bothell, WA, USA). Systolic P_{pa} was estimated from the systolic pressure gradient between the right ventricle and the right atrium by the peak continuous-wave Doppler velocity of the tricuspid regurgitation jet velocity (TRV) using the modified Bernoulli equation plus right atrial pressure estimated from the inferior vena cava size and collapsibility with respiration. A pulsed Doppler pulmonary blood flow velocity signal was

TABLE 1 Effects of fasudil on pulmonary artery pressure in Kyrgyz highlanders

Parameters	Placebo		Fasudil	
	Baseline	30 min	Baseline	30 min
$P_{pa,sys}$ mmHg	51.9±0.7	51.9±0.8	51.8±0.7	41.5±0.6***
PAAT ms	0.087±0.001	0.086±0.001	0.087±0.001	0.102±0.001***
CO L·min^{-1}	6.2±0.2	6.2±0.3	6.1±0.3	6.6±0.2
PVR Wood Units	2.7±0.1	2.7±0.1	2.7±0.1	2.3±0.1***
P_{ra} mmHg	5.5±0.4	5.5±0.4	5.5±0.4	5.3±0.3
Blood pressure mmHg				
Systolic	125.2±4.0	125.0±4.0	125.2±4.1	122.8±4.1
Diastolic	78.7±1.5	78.7±1.6	78.61.6	76.3±1.5
fC bpm	71.3±2.6	70.8±2.7	71.4±2.6	72.0±2.8
S_{a,O_2} %	88.3±1.1	88.0±0.9	88.2±0.7	87.9±0.6

Data are presented as mean±SEM. $P_{pa,sys}$: systolic pulmonary artery pressure; PAAT: pulmonary artery acceleration time; CO: cardiac output; PVR: pulmonary vascular resistance; P_{ra}: right atrial pressure; fC: cardiac frequency; S_{a,O_2}: arterial oxygen saturation. ***: p<0.001, fasudil *versus* placebo.

sampled in the right ventricular outflow tract and a pulmonary artery acceleration time (PAAT) was defined as the time interval from the onset of forward flow in the pulmonary artery to the peak velocity of this flow. Additionally, the velocity–time integral (VTI) of the systolic flow in the right ventricular outflow tract (RVOT) was measured and PVR was calculated using the following formula: PVR=(TRV/VTIRVOT)×10+0.16 [10]. Cardiac output was calculated as a product of the area, VTIRVOT and fC. Satisfactory TRV and pulmonary flow velocity signals were obtained in all patients. All measurements were performed by an experienced echocardiographer who was blinded to the study groups. All measurements were validated by another independent observer.

Data are presented as mean±SEM. Differences between groups were assessed by ANOVA and Student Newman–Keuls *post hoc* test for multiple comparisons and a p-value <0.05 was regarded as significant.

All highlanders had a shortened PAAT, and high systolic P_{pa} and PVR at baseline, consistent with HAPH. Fasudil significantly increased PAAT by 0.015±0.001 ms (95% CI 0.012–0.017 ms, p<0.001) and decreased systolic P_{pa} by -10.37±0.97 mmHg (95% CI -12.34– -8.40 mmHg, p<0.001) compared with placebo. Although intravenous infusion of fasudil did not significantly increase cardiac output, it markedly decreased PVR by -0.39±0.05 Wood Units (95% CI -0.49– -0.29, p<0.001). No changes in systemic arterial blood pressure were noted. fC and S_{a,O_2} were not changed significantly by either fasudil or placebo infusion (table 1). Fasudil was well tolerated; one patient had facial flushing and four patients had feelings of dryness of the mouth.

Our study demonstrates a potent effect of the ROCK inhibitor fasudil on P_{pa} in HAPH. It was well tolerated, with no effect on systemic arterial blood pressure. The data are consistent with the acute effects of fasudil in animal models, which also report on the long-term effects of fasudil administration. Chronic treatment with fasudil effectively reduced PH and right ventricular hypertrophy, and improved survival in rats with monocrotaline-induced PAH [4–6]. OKA *et al.* [7] have demonstrated that intravenous fasudil injection was effective in a severe angioproliferative model of PAH. Fasudil reverses monocrotaline-induced PAH by reducing pulmonary vascular remodelling without affecting normal right ventricular function and may be more effective than bosentan and sildenafil [6]. DAHAL *et al.* [5] have reported beneficial effects from another ROCK inhibitor, azaindole-1, in two well-established models of PH. Moreover, it significantly inhibited acute hypoxic pulmonary vasoconstriction *ex vivo* and the proliferation of primary rat pulmonary artery smooth muscle cells *in vitro* [5]. There are few data in humans. Acute intravenous administration of fasudil at a dose of 1 mg·min^{-1} for 30 min effectively reduced PVR in patients with severe PAH [8, 9]. FUJITA *et al.* [11] used inhaled fasudil in patients with PAH and observed that it was as effective as nitric oxide inhalation. Our data indicate that the RhoA/ROCK signalling pathway is involved in the pulmonary vascular response to life at high altitude. A further study of chronic ROCK inhibition is now indicated.

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Nuclear magnetic resonance-based metabolomics of exhaled breath condensate: methodological aspects

To the Editors:

Due to the lack of standardised procedures for exhaled breath condensate (EBC), a noninvasive technique for investigating lung inflammatory mediators [1], the between-laboratory comparison of results is difficult. Moreover, different collecting devices have been reported to influence the EBC content [2, 3].

The analysis of metabolic profiles (''metabolomics'') of EBC using nuclear magnetic resonance (NMR) spectroscopy discriminates between chronic obstructive pulmonary disease (COPD) patients and healthy subjects (HS) [4]; asthmatic children and HS [5]; and patients with stable cystic fibrosis and unstable cystic fibrosis and HS [6].

This approach has recently been questioned as NMR-based metabolomics of EBC collected using a condenser with reusable parts was reported to be affected by cleaning procedures, generating artificial signals that were not related to the endogenous metabolites of the lungs [7].

In this study we assessed the effects of a different cleaning procedure of a reusable-part condenser on EBC metabolomics; the possible time and carry-over effects when the same device is repeatedly used; technique sensitivity; the ability of NMR spectroscopy of EBC to discriminate between COPD patients and HS; and the potential of NMR spectroscopy in identifying selective EBC metabolites.

If the cleaning procedure produces artificial signals in the NMR spectra of EBC, the separation between COPD patients and HS reported previously [4] is certainly surprising, as the residual signals derived from the disinfectant Descogen (Antiseptica chem.-pharm. Produkte GmbH, Pulheim/Brauweiler, Germnay) should have randomly affected both groups. Moreover, using a different reusable-part condenser, CARRARO *et al*. [5] reported that NMR-based metabolomics of EBC differentiates asthmatic children from HS with a success rate of 86% [5].

To verify the influence of the disinfectant on EBC metabolomics, we modified the proposed cleaning procedure [4] by replacing Descogen with Milton (Milton Pharmaceutical UK Ltd, Gloucester, UK), a pure sodium hypochlorite solution. The reusable parts of the EcoScreen condenser (Jaeger, Hoechberg, Germany) were soaked for 15 min with a 3.55 mM Milton solution (according to manufacturer guidelines), and then flushed for 15 min with deionised distilled water. ^{1}H-NMR spectra were recorded at 600 MHz (14.1 T) on a Bruker Avance spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) equipped with a 5-mm CPTCI CryoProbe^{TM} (Bruker BioSpin GmbH).

Figure 1a reports the NMR spectrum of the Milton solution. Except for the reference 0.1 mM sodium 3-trimethylsilyl [2,2,3,3-$^{2}H_4$]propionate (TSP) signal at 0.0 ppm, no other signals were present in the spectrum. The absence of signals (implying the absence of contaminant) was confirmed in the vertical expansion of the spectrum (fig. 1b). To exclude the presence of contaminants in the deionised water used for rinsing the collection set-up, we acquired the spectrum of the soaking water after rinsing for 5 min (fig. 1c). No peak was observed, indicating that the disinfection and the rinsing steps of the EBC collection set-up do not affect the EBC spectra. These data exclude the fact that cleaning procedure based on Milton solution affects NMR spectroscopy of EBC, indicating that the observed profiles of EBC metabolites are not artificial signals but represent endogenous metabolites.

When a reusable-part condenser (EcoScreen) was repeatedly used [8], the measurement of several EBC biomarkers detected no carry-over effects from previous sampling periods. If carry-over effects were present, the metabolites would display an ''average