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Effects of hypobaric hypoxia exposure at high altitude on left ventricular twist in healthy subjects: data from HIGHCARE study on Mount Everest

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Aims Previous studies investigating the effect of hypoxia on left ventricle focused on its global function, an approach that may not detect a selective dysfunction of subendocardial layers that are most sensitive to an inadequate oxygen supply. In the HIGHCARE study, aimed at exploring the effects of high altitude hypoxia on multiple biological variables and their modulation by an angiotensin receptor blocker, we addressed the effects of hypobaric hypoxia on both systolic and diastolic left ventricular geometry and function, focusing on echocardiographic assessment of left ventricle twist to indirectly examine subendocardial left ventricular systolic function.

Methods and results In 39 healthy subjects, physiological and echocardiographic variables, including left ventricular twist and a simplified torsion-to-shortening ratio (sTSR), were recorded at sea level, at 3400 m, and at 5400 m altitude (Mount Everest base camp). Both left ventricular twist and sTSR were greater at 5400 m than at sea level (12.6° vs. 9.6° and 0.285 vs. 0.202, $P < 0.05$ for both), were linearly related to the reduction in arterial oxygen partial pressure ($P < 0.01$ for both), and were associated with significant changes in LV dimensions and contractility. No effects of angiotensin receptor blockade were observed on these variables throughout the study.

Conclusion Our study, for the first time, demonstrates an increase in left ventricular twist at high altitude in healthy subjects exposed to high altitude hypoxia, suggesting the occurrence of subendocardial systolic dysfunction in such condition.

Keywords high altitude • hypobaric hypoxia • left ventricular twist and torsion • subendocardial left ventricle function

Introduction

The myocardial cell response to hypoxia exposure is characterized by both contraction and relaxation abnormalities.[1] However, experiments on individuals exposed to real or simulated high altitude hypoxia (HAH) failed to demonstrate any relevant impairment of the global left ventricle (LV) systolic performance, whereas in the same experiments the early phase of diastole has been consistently shown to be impaired,[2–4] The dependence of myocardial relaxation on adequate oxygen supply[5] is a putative mechanism for the diastolic dysfunction shown under HAH. In fact, experimental data showed that reduction of high-energy phosphates metabolism induced by hypoxia is associated with an impairment of LV diastolic function.[6] Such a reduction in high-energy phosphates should also exert a negative effect on LV systolic function,[7] which, however, was never observed in men exposed to HAH. In the myocardial

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wall, subendocardial fibres are characterized by a lower oxygen supply-to-demand ratio than subepicardial ones.[8,9] However, a normal subepicardium can compensate for even a substantial impairment of contraction at subendocardial level, leaving the global LV function unaffected.[10] Our hypothesis is that HAH reduces the oxygen supply-to-demand ratio prevalently at subendocardial level, and that the consequent subendocardial fibres systolic dysfunction might be masked by increase of the global LV systolic function related to the elevated sympathetic activity which characterizes this condition.[11] In this study, we have investigated the effects of HAH exposure on LV contractile function in healthy subjects, focusing on subendocardial fibres through the analysis of LV twist (T) and on its relation with fibre shortening, an approach that allows to unveil subendocardial fibres dysfunction even in the presence of a normal global LV systolic function.[12–15] Our analysis was performed in the frame of the High Altitude (HA) Cardiovascular Research (HIGHCARE) study (www.HIGHCAREPROJECTS.eu), a scientific Himalayan expedition, the main goal of which was to explore the cardiovascular effects of HAH in healthy subjects, along with their possible modification by administration of an ARB.

Methods

The data presented in this paper are derived from the HIGHCARE trial, whose main results have been previously published.[16]

Participants

Fifty eligible healthy subjects were identified, of whom three withdrew their consent before study start. We therefore enrolled 47 volunteers (32 males, 15 females, mean age 39.9 $\pm$ 10.0, range 25–64 years, BMI 22.8 $\pm$ 2.9 kg/m^2) living permanently below 500 m above sea level (asl), who gave written informed consent to participate in the study. Exclusion criteria were known cardiovascular disease, any chronic cardiovascular therapy, repeated exposures to altitudes >2500 m asl over the 8 months preceding the expedition, history of severe mountain sickness, history of angioedema, and pregnancy. All fertile females not using an effective contraceptive method during the study and professional athletes were also excluded. Subjects underwent a general health check including stress test and echocardiography before entering this project.

Study design and procedures

Subjects enrolled in the study were randomized to receive either Telmisartan 80 mg or placebo once a day in the morning. After 8 weeks of double-blind treatment, the participants flew from sea level (Milan, 140 m) to Kathmandu (Nepal, 1355 m) where they stayed for 3 days. They were then brought by air transportation to Namche Bazaar (3400 m), where they stayed for another 3 days. From Namche Bazaar, they hiked over 5 days to Mt Everest Base Camp (EBC, 5400 m) where they remained for 12 days. Afterwards, they returned to sea level with a 6-day trip (*Figure 1*). Study drugs were taken throughout the expedition until the last tests were completed after return to sea level. Study tests were performed at six time points:

- at sea level before (SL1) and ~4 weeks after the beginning of treatment (SL2)
- during acute (Day 1–3) exposure to 3400 m at Namche Bazaar (Nam)
- at Mt EBC, 5400 m asl, within the third day after arrival (acute exposure, EBCa)
- at Mt EBC after prolonged (Days 9–11) exposure (EBCp)
- immediately after return to sea level (SL3)

Experiments in Nam were performed in comfortable lodge rooms, while at EBCa and EBCp, they were performed in heated tents. In all conditions, laboratory temperature, humidity, and barometric pressure were recorded. For safety reasons, mountain sickness severity was assessed daily by means of the Lake Louise Score.[17]

In each subject, at each time point, heart rate (HR), systemic systolic (S) and diastolic (D) blood pressure (BP) (by validated Microlife A100 Plus device, Microlife, Windau-Switzerland), respiratory rate (RR), body temperature (standard axillary thermometer, BT), and peripheral oxygen saturation (SpO_2, Ohmeda Tuff Sat with sensor OxyTip Finger 6051-0000-160) were recorded in the morning, after a rest period of 15 min in sitting position. Then blood samples for plasma norepinephrine (PNE) assessment (HPLC, CHROMSYSTEMS Instruments & Chemicals Gmbh, Munich,– Germany) were collected, and either immediately sent to the laboratory for analyses (in case of SL samples), or immediately centrifuged and frozen in liquid nitrogen (HA samples), to be shipped to the laboratory in Milan as soon as possible.

Standard and Doppler echocardiography

Echocardiography was performed at sea level with Vivid 7 equipment (GE Healthcare-Finland) and at HA with a portable device (Vivid I, GE Healthcare). LV endocardial and epicardial diameters along with long-axis lengths were obtained in standard way both at end-diastole(ED) and end-systole (ES). From these chamber volumes (V), stroke volume, ejection fraction, outer/inner diameter ratio (do/i), circumferential strain, and systolic stress were calculated. Doppler velocities were obtained at mitral valve tip. Details on the cardiac ultrasound examination are provided in the Supplementary data online, *Appendix S1*.

Speckle tracking echocardiography

Longitudinal systolic strain, systolic strain rate (SSR) as well as longitudinal diastolic early and late strain rates and mitral annulus diastolic motion velocities were obtained in the four-chamber apical projection through the speckle tracking echocardiography (STE) technique. LV apical and basal rotation were also obtained by the STE technique from short-axis projections at the appropriate levels. Left ventricle twist (LVT), peak untwisting rate (PUTR), and a simplified torsion-to-shortening ratio (sTSR) were then calculated as described in Supplementary data online, *Appendix S1*, where additional details of echocardiographic measurements are provided.

Calculated arterial pO_2

Since the values of peripheral arterial oxygen saturation (SpO_2) at HA lie in the flat portion of the haemoglobin dissociation curve, where small changes in peripheral saturation correspond to a greater arterial pO_2 modification, we used the Ellis' inverse solution of the Severinghaus equation[18] to calculate the arterial pO_2 (calculated arterial pO_2, $capO_2$). More details on how we calculated arterial pO_2 from SpO_2 are provided in Supplementary data online, *Appendix S2*.

Statistical analysis

Data are presented as means $\pm$ standard error (se) unless otherwise specified. Mixed multilevel linear analysis was used to assess the differences in the values of each variable between SL1 and the various study conditions (SL2, Nam,EBCa,EBCp,SL3) by setting 'observation' at first and 'subject' at second level. An alpha value of 0.05 was used to determine statistical significance (two-tail, $P < 0.05$). The dependence of LVT at HA from $capO_2$, from physiological variables (BP and HR), and from the variables describing mechanical and contractile properties of the LV was assessed by mixed multilevel multiple linear regressions between

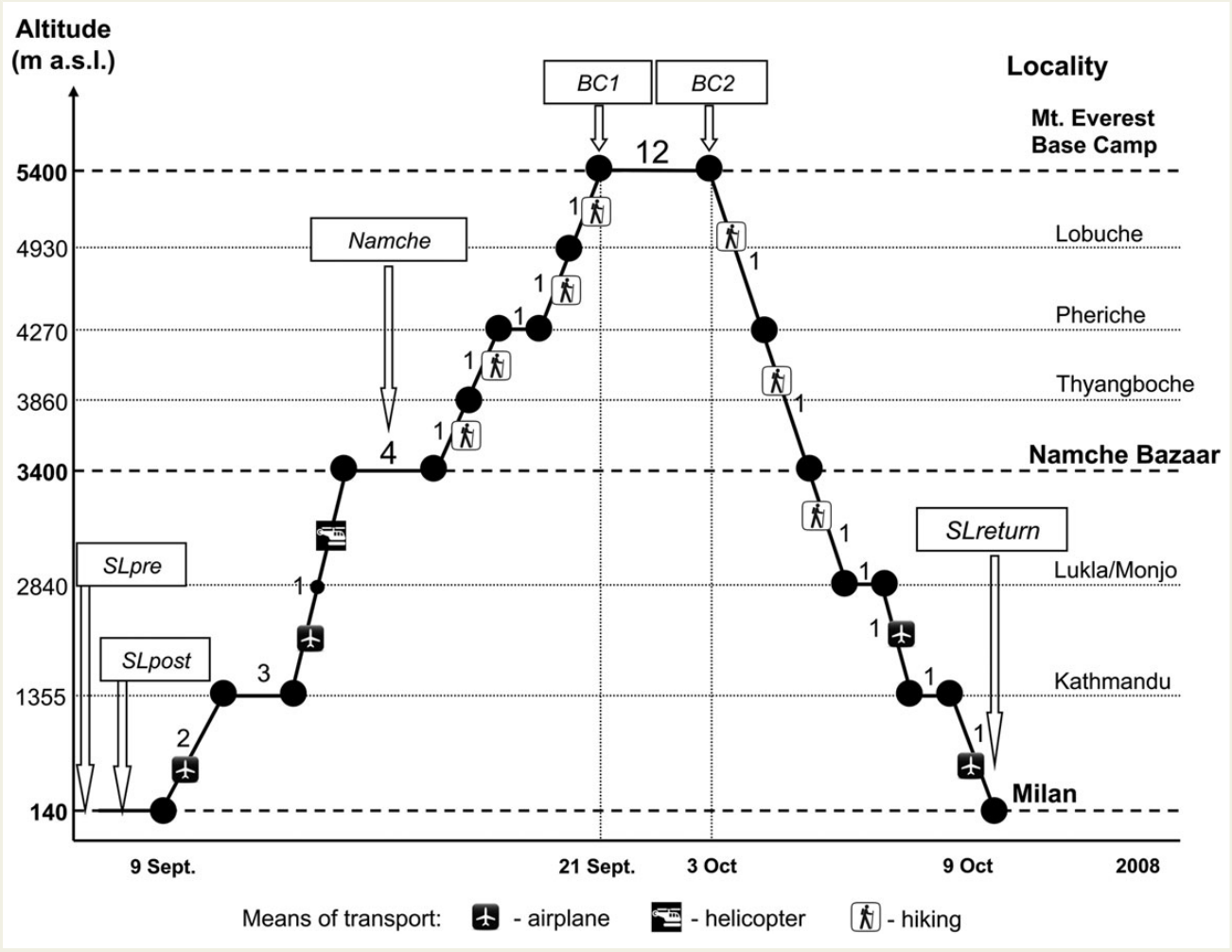

Figure 1 Timeline of the expedition. On *y*-axis altitude in meters and on *x*-axis time (in days). Numbers on the graph indicate the nights spent at each step; , airplane transport, , helicopter transport, , hiking. On the right hand of the graph names of the places. Exam refers to the time and altitude of each data collection.

LVT and the appropriate independent variables. The differences between subjects receiving active treatment and those receiving placebo were assessed by ANOVA. Commercially available statistical softwares MlWin 2.28 and Systat 12 were used.

All 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. The study protocol was approved by the Ethics Committee of Istituto Auxologico Italiano and the study was conducted in agreement with the Helsinki Declaration. The study was registered prior to its start in the Italian Medicines Agency National Monitoring Centre for Clinical Trials linked with European Clinical Trials Register (EudraCT) number 2008-000540-14.

Results

General issues and effects of angiotensin receptor blockade

None of the 47 volunteers experienced severe acute mountain sickness symptoms while ascending to and remaining at the 5400 m altitude of Mount Everest base camp. The speckle tracking echocardiographic tracings of eight subjects, whose characteristics were comparable with those of the remaining volunteers, were of poor quality and were discarded. Thirty-nine subjects (mean age 39 years, range from 25 to 65 years, 25 males, for more demographic and biochemical data, see Supplementary data online, *Table SA*) had high-quality basal echocardiographic recordings (at SL1) and at least one recording (most of them had two) suitable for twist calculation at Everest base camp. Their data were included in this analysis.

A safety analysis, by two-way ANOVA, carried out separately in individuals randomized to placebo and in those assigned to active treatment with the angiotensin receptor blocker, did not show any significant difference in the effects of HAH on LVT. There were no significant interactions also between the effects of altitude and those of treatment on any variable considered. Based on this preliminary analysis, all subjects were pooled in a single database regardless of treatment allocation. Moreover, in placebo subjects, the differences in the echocardiographic assessment of LV twist under the two basal level SL1 and SL2 conditions amounted to only 6.6%.

Differences were even less for the other variables, thus demonstrating a good reproducibility of our measurements (see Supplementary data online, *Table SB*). The whole results dataset is available in Supplementary data online, *Tables SC–SE*. In the following sections, only the most relevant results are summarized. All differences, shown either as absolute values with relative standard error (se) or as percent values, are computed with reference to SL1 levels, unless otherwise specified.

Physiological measurements

At EBCa HR, SBP, and DBP increased by 20 (1.5) bpm, 12 (1.5) mmHg, and 9 (1.1) mmHg, respectively; RR increased by 4.7 (0.6) breaths per minute, BT increased by 0.5°C (0.01), and PNE increased by 617 (68) pg/dL, ($P < 0.001$ for all). Similar results were obtained at EBCp (data not shown). SpO_2 and $capO_2$ decreased by 20 (1)% and 54 (1.1) mmHg, respectively ($P < 0.001$ for both) at EBCa. However, after the acclimatization period, at EBCp, they showed a significant ($P < 0.001$) tendency to return towards baseline levels, combined with a significant decrease in RR ($P < 0.05$).

Geometrical and mechanical variables

On-going from sea level to progressively higher altitude LV diastolic and systolic short- and long-axis dimensions decreased, so that LV diastolic and systolic volumes and stroke volume decreased as well. The ratios of epicardial to endocardial diameters at end-diastole and end-systole both increased at Everest base camp, together with the sphericity index (*Figure 2*). These changes influenced LV geometry so that at EBC its cavity was smaller, its walls were thicker, and its shape more ellipsoidal. This was accompanied by a decrease in LV systolic stress (by 9% at EBCa and 16% at EBCp). The LV mass decreased by 7 and 9%, respectively, at EBCa and EBCp.

Ejection fraction, and both circumferential and longitudinal systolic strains, whose SL1 values were, respectively, 0.63 (0.01), −0.39 (0.02), and −0.20 (0.005), did not change significantly in any condition, while SSR increased at EBC.

The early diastolic mitral annulus velocity, as recorded by STE, decreased by ∼10%, at EBC while the rate of early positive diastolic longitudinal strain did not change. On the contrary, the same variables recorded in late diastole showed a significant increment at HA (P always <0.05).

Mitral inflow and right ventricular atrial gradient

Doppler transmitral peak E wave velocity decreased both at Nam and at EBC, moreover at SL3 remained lower than at SL1. Peak A wave velocity increased significantly only at EBC, while deceleration time of E wave did not change in any condition. Right ventricle to atrial pressure gradient started to increase at Nam and remained elevated even at SL3, its maximum increment being 13.8 (1.7) mmHg at EBCp.

Rotations and twist

LVT was greater already at Nam and tended to become even greater at EBCa and EBCp (by 24, 33, and 31%, respectively). These changes are due to increments in the rotation at the apex level, since the base rotation did not change. PUTR followed the twist behaviour

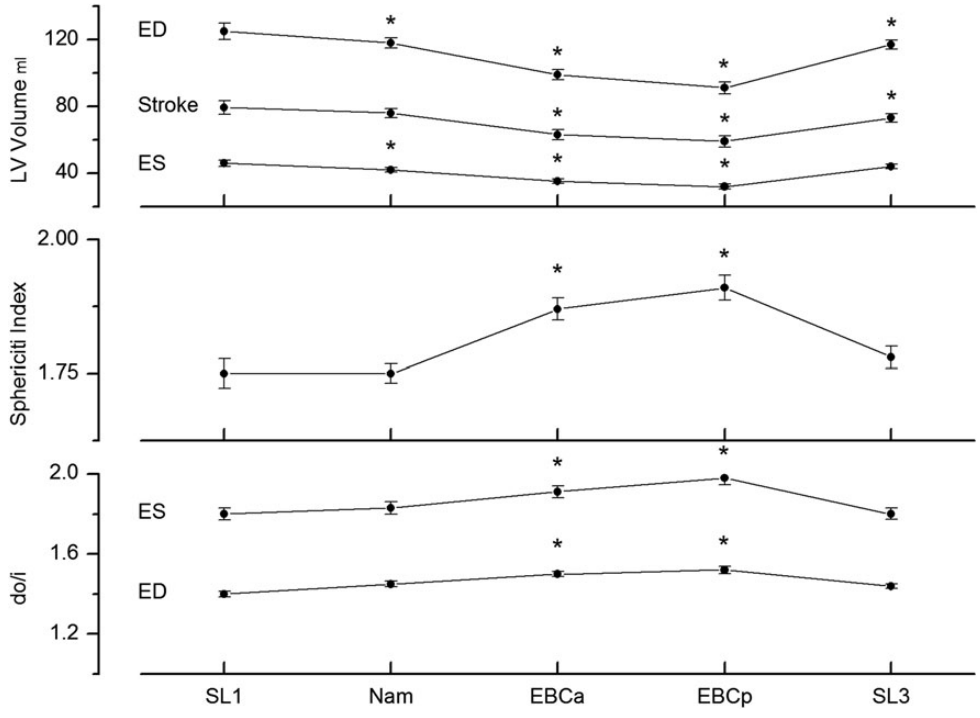

Figure 2 Left ventricular dimensions and geometry. End diastolic (ED), end systolic (ES) and stroke (Stroke) volumes, Sphericity index, end-systolic and end-diastolic outer/inner diameter ratio are separately depicted for sea level in basal conditions (SL1), Namche Bazar (Nam), Everest base camp acute (a) and prolonged (p) exposure and after the retour (SL3). Bars refer to SE; stars mean a statistical difference from SL1 with at least $P \leq 0.05$.

Table 1 Variables defining the features of LV rotation at sea level and at high altitude

	SL1		Nam			EBCa			EBCp			SL3		
	μ	SE	Δ	SE	*P*	Δ	SE	*P*	Δ	SE	*P*	Δ	SE	*P*
Base rotation°	−3.3	0.5	0	0.6	ns	0.9	0.6	ns	−0.9	0.8	ns	1.2	0.5	<0.05
Apex rotation°	7.0	0.7	1.8	0.8	<0.05	4	0.8	<0.001	3.1	0.9	<0.005	1.3	0.6	<0.05
LVT°	9.6	0.7	2.3	1.1	<0.05	3.2	1	<0.005	3	1.2	<0.05	1.4	1	ns
PUTR° s^{-1}	94	5.1	13	5.9	<0.05	43	13	<0.001	67	14	<0.001	13	7.8	ns
sTSR	0.202	0.018	0.060	0.027	<0.05	0.071	0.024	<0.005	0.083	0.038	<0.05	0.066	0.030	<0.05

Base and apex rotation, LVT, negative peak untwisting rate (-PUTR), and simplified sTSR are separately shown for the following study conditions: at sea level (SL1); at Namche Bazar (Nam); at Everest base camp (EBC; a, acute; p, prolonged exposure); at sea level immediately after return (SL3).
Data are shown as mean levels (μ) ± standard error (SE) for baseline at SL1, and as mean changes (Δ ± SE) from SL1 for the other study conditions. *P*-values refer to the statistical significance of changes from SL1.

(+13, +46, and +76% at Nam, EBCa, and EBCp, respectively) with a significant linear relation ($r = 0.56$, $P < 0.001$) between PUTR values and maximum twist values. sTSR also increased at HA, and at SL3 it remained a little higher than at SL1, at variance from LVT which returned to baseline values (*Table 1* and *Figure 3*). The timing of both peak twist and PUTR, occurring, respectively, at 98.1 (1.8) and at 119.1 (2.0)% of systole duration, did not change at any altitude.

LVT as function of physiological, geometrical, and mechanical variables and of hypoxia

The dependence of twist from physiological, geometrical, and mechanical variables potentially affecting LVT at HA was assessed by mixed multilevel linear regression equations: LVT was found to be dependent on cpO_2, SBP, DBP, HR, on EDV, ESV, and ESdo/i (of which, ESdo/i was both physiologically[19] and statistically the most relevant) and on LV contractility as expressed by SSR. Since most of them covariate at HA with cpO_2, their importance relative to hypoxia was assessed through a series of multiple linear regression analyses. From these multivariate analyses, hypoxia (cpO_2) and changes in LV dimensions (ESdo/i) resulted as the main determinants of LVT changes at altitude (*Table 2*). sTSR, which should not depend on LV dimension, was also statistically related to cpO_2 ($P < 0.01$).

Discussion

The main novel finding of our study is that at HA LVT was greater than at SL. This HAH-induced increase in LVT was caused by both LV geometrical changes and by subendocardial LV fibre dysfunction.

Geometrical and functional factors that influence LVT at HA

LVT is a consequence of the helical arrangement of myocardial wall fibres, (counterclockwise at subepicardial and clockwise at subendocardial level) so that opposite forces roughly tangent to LV short-axis plane are generated at epicardial and endocardial levels. Their effect, beyond reducing LV dimensions, is to create torque moments whose magnitude is the cross product of the force acting on the myocardium and its lever arm (i.e. the local radium, the fulcrum being at the centre of LV cavity).[19] The overall counterclockwise LVT direction is dictated by the torque moment at epicardium, since LV radius at this level is, obviously, greater than at endocardial level. The opposite torque moment at endocardial level, however, modulates the final degree of LVT. So the magnitude of LVT depends on LV geometry, particularly on the outer to inner radii ratio, the relative strength of subendocardial and subepicardial forces, i.e. the local contractility at these two levels, and the final extent of the whole LV wall fibres systolic shortening which in turn depends on LV preload, afterload, and global contractility.[20]

In our subjects due to the well-known reduction of plasma volume at HA,[21] diastolic and systolic LV dimensions decreased. Since LV EF, circumferential and longitudinal strain did not change, the value of absolute LV fibre shortening decreased as well. LV systolic

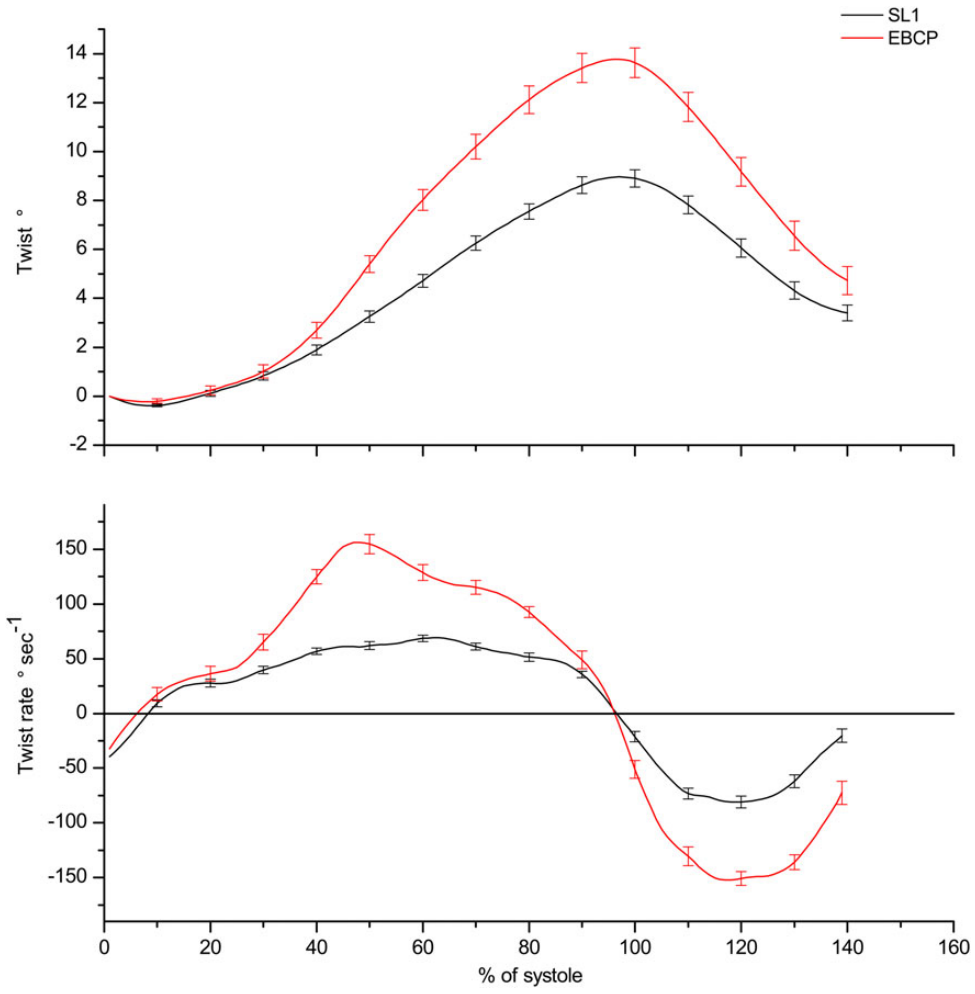

Figure 3 Twist and twist rate at sea level and Everest base camp. Mean values (± se) of LVT and twist rate at SL1 (Sea Level, baseline, black lines) and at EBCp (Everest Base Camp, after prolonged exposure, red lines) are shown. Values on the horizontal axis indicate per cent of systole duration.

afterload decreased, too, since the increase in systolic blood pressure intervening at HA was offset by the concomitant LV geometrical changes (reduced radii and increased wall thickness). These changes occurred together with increased concentration of plasma catecholamines, whose inotropic effects on the heart resulted in a faster longitudinal SSR. Overall, at HA, LV fibre shortening was reduced, while LV had smaller dimensions, more ellipsoid shape, increased wall thickness, increased contractility, and reduced afterload. Changes in all the above variables could have theoretically influenced the extent of LVT.[22–26] However, in our study, only those variables related to LV systolic dimensions and to LV contractility were associated with LVT increase at HA, Their relative importance was assessed by a multiple linear regression, which showed that the effect of geometrical factors as estimated by ESdo/i was preponderant over those of contractility as estimated by SSR (*Table 2*).

Therefore, our data, in line with the results of animal and human experimental studies,[22,26] suggest that the reduction in LV dimensions was, among the geometrical and functional factors in play, the main determinant of LVT at HA.

Effects of hypoxia

To the best of our knowledge, the possibility that acute exposure to hypobaric hypoxia at altitude might affect the degree of LVT has never been explored so far. This issue is for the first time addressed in our study, and our results show a significant inverse relation of both LVT and sTSR with SpO_2 and, even more so, with $capO_2$. In normal conditions, the helical arrangement of myocardial fibres and the consequent LV torsion are responsible for the even distribution of strain across the myocardial walls.[27] LV torsion and myocardial fibres strain are so closely related to each other that a nearly fixed value of the ratio between LV torsion and LV fibre shortening is constantly observed in normal conditions.[28] A mild impairment of subendocardial function, not reflected by measurable changes in global LV function, increases the extent of twist in relation to the extent of shortening, thus increasing this ratio. This is why the sTSR, with LVT normalized for LV dimensions and for their modification during systole, as proposed by Art *et al.*,[14,15,28] is a better indicator of subendocardial fibre dysfunction than LVT *per se*. Our calculation of the LV sTSR, although approximated, is based on the same

Table 2 Effects of physiological factors, geometrical LV variables and LV contractility on LV twist in two multivariate models

Model	PAR	VAR	Estimate	SE	*P*
twist = $\beta_{0j} + \beta_{1j}VAR_{ij} + e_{ij}$	β_{1j}	cpO_2	0.071	0.020	<0.001
	β_{1j}	SBP	0.073	0.030	<0.05
	β_{1j}	DBP	0.102	0.038	<0.01
	β_{1j}	HR	0.052	0.026	<0.05
	β_{1j}	EDV	−0.51	0.024	<0.05
	β_{1j}	ESdo/*i*	8.9	2.9	<0.005
	β_{1j}	SSR	5.1	2.5	<0.05
twist = $\beta_{0j} + \beta_{1j}\,VAR1_{ij} + \beta_{2j}\,VAR2_{ij} + e_{ij}$	β_{1j}	SBP	0.009	0.045	ns
	β_{2j}	cpO_2	0.068	0.022	0.005
	β_{1j}	DBP	0.007	0.064	ns
	β_{2j}	cpO_2	0.072	0.023	<0.005
	β_{1j}	HR	0.04	0.041	ns
	β_{2j}	cpO_2	0.086	0.026	<0.001
	β_{1j}	ESdo/*i*	8.5	3	0.005
	β_{2j}	EDV	0.026	0.038	ns
	β_{1j}	ESdo/*i*	7.5	2.9	0.01
	β_{2j}	SSR	−4.5	2.5	ns
	β_{1j}	cpO_2	−0.05	0.02	0.02
	β_{2j}	ESdo/*i*	6.8	2.9	0.02

Mixed-level linear regressions analysing the dependence of twist from physiological factors (cpO_2, SBP, DBP, HR), geometrical left ventricle (LV) variables (end diastolic volume, EDV; end systolic outer/inner diameter ratio ESdo/i) and contractility (systolic strain rate, SSR). Model: regression equation (single or multiple) used; PAR: the values estimated by the equation; VAR: variable(s) in the equation; Estimate: the actual value obtained for each parameter; se: standard error of estimates; p: the probability for a parameter of being different from 0; e:residuals.

principles of the TSR calculation proposed by Art *et al.* Thus, the increase of sTSR in our subjects at HA indicates the occurrence of negative changes in subendocardial fibres contractility. The relation between LVT increase and hypoxia at HA, however, is less straightforward, since the substantial changes in LV dimensions occurring in this condition can also affect LVT. The results of our multiple linear regression analysis, in which hypoxia (quantified by $capO_2$) and systolic dimensions (quantified by ESDdo/i) both contribute to LVT increase, confirm an independent role of hypoxia in determining LVT at HA.

Effects of angiotensin receptor blockade

ANOVA analysis did not show any effect of treatment with ARB on LV twist either at sea level or at HA; however, a type II error affecting our conclusion cannot be totally excluded due to the relatively small sample size of our study. As a matter of fact, ARB-treated subjects at HA had a lower diastolic BP and showed a trend to a lower HR, compared with individuals randomized to placebo. The differences in the values of diastolic BP and HR at HA between individuals randomized to placebo and those randomized to ARB treatment (−4 mmHg and −4 bpm, respectively) were, however, small and of questionable relevance. Moreover, even though univariate linear regression analysis showed significant effects of heart rate and diastolic pressure on LVT, their statistical significance disappeared when cpO_2 was added in a multivariate model, probably because of their covariance with cpO_2 (*Table 2*).

In conclusion, our data do not appear to support any relevant effect of ARB treatment on LVT.

Effects of HA exposure on LV diastolic function

We also observed an increase of LV untwisting rate, proposed as an index of diastolic function, at HA. However, its strong relation with LV systolic twist limits its reliability as a marker of diastolic function in the conditions of our study. On the other hand, early diastolic mitral inflow velocity and mitral annulus motion velocity showed a reduction at HA, whereas atrial contraction-dependent velocities increased. Thus, at HA, the early phase of diastole was impaired, in spite of the reduction of LV end-systolic dimensions and the increase in LV contractility which should have been enhanced it.[29,30] These findings, which are in line with a number of previous reports,[2–4] confirm the harmful effects of hypoxia exposure on LV diastolic function also in our subjects.

Was the level of hypoxia enough to uphold a cardiomyocytes systolic dysfunction?

In our subjects, the $capO_2$ at HA, as calculated from SpO_2, decreased to levels as low as 42 mmHg. It can be argued that changes of blood pH and pCO_2, simultaneously intervening at HA, could have influenced the haemoglobin dissociation curve invalidating the results of the Ellis' equation. At any rate, our HA $capO_2$ values are consistent with those directly measured intra-arterially at similar, real or simulated, altitudes.[31,32] An alteration in the high-energy phosphate metabolism [measured as phosphocreatine (PCr)/ATP ratio by magnetic resonance spectroscopy] is already evident after a 17-h exposure to normobaric hypoxia with SpO_2 around 80%,[6] a level slightly higher than the one reached by our subjects at EBCa. More relevant for our results, a 18% reduction of PCr/ATP was found in healthy volunteers within 4 days since their return from a 17-day trek to the Everest Base Camp.[33] In both these groups of subjects, the reduction in high phosphate energy was associated with altered diastolic function. Such a reduction of energy supply can interfere with myocytes contractile activity as well. Indeed, comparably low levels of PCr, ATP, and PCr/ATP have been shown to be

related to reduced systolic ventricular function in patients with coronary stenosis or dilated cardiomyopathy.[12] Based on our sTSR and LVT analysis results, we suggest that oxygen tension at HA was low enough to impair the myocytes high-energy phosphate balance prevalently at subendocardial level, where the oxygen consumption is higher and coronary vasodilation capacity is lower than at subepicardial level.[34]

Study limitations

We have to acknowledge a few limitations of our study. First, subendocardial fibres shortening was not directly measured, but only inferred from LVT behaviour. However, we are not aware of any non-invasive and easily applicable alternative method to assess subendocardial fibre dysfunction suitable to the challenging environmental conditions of our study. Second, the arterial oxygen partial pressure was not directly measured, but mathematically calculated from SpO_2 through the reverse Ellis' equation. Theoretically, this could have influenced the results of our regression equations. However, even in such a case, the HA respiratory alkalosis shifting the haemoglobin dissociation curve to the left should have, if anything, led to an underestimation of the real pO_2 values decrease at HA, thus making our results even more significant.

Conclusions

Our study, to the best of our knowledge, for the first time provides evidence of an alteration of LV systolic function under exposure to HA hypoxia, which however appears not to be uniformly distributed throughout the ventricular wall, being prominent at subendocardial level. This is apparently in contrast with previous studies addressing myocardial systolic function at HA in humans[2,35] which focused on global LV systolic function and reported it to be normal or even increased in this condition. A normal global systolic function, however, might well conceal the occurrence of a substantial degree of a selective subendocardial fibres dysfunction.[10] Administration of an angiotensin receptor blocker did not affect LV function changes under HA exposure in the conditions of our study.

Conflict of interest: None declared.

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Supplementary data

Supplementary data are available at *European Heart Journal – Cardiovascular Imaging* online.

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