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ORIGINAL ARTICLE

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Enhanced flow-dependent vasodilatation after bed rest, a possible mechanism for orthostatic intolerance in humans

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Abstract We investigated the alteration in flow-dependent-dilatation in the orthostatic intolerance occurring after bed-rest deconditioning. Eight men [aged mean (SEM) 32 (2) years] underwent two consecutive periods of 7 days of head-down-tilt (HDT, –6°) during bed rest. A control age and sex matched group [$n=8$, 30 (2) years], maintained its usual physical activity. Blood flow velocity (BFV) and diameter (Doppler and echotracking systems) were measured in the brachial artery, under basal conditions and during the post ischaemic hyperaemia following occlusion. The increase in BFV post-ischaemia did not change before, during and after HDT but the relative increase in the diameter was greater on the 7th day of the HDT period than before HDT [+8.8 (1.6)% compared to +3.7 (1.0)%, $P<0.001$]. After HDT, 11 of 16 standing tests (comprising eight subjects in the two HDT periods) had to be stopped because of orthostatic intolerance. The flow-dependent-dilatation measured at the end of HDT was negatively correlated with the post-bed-rest duration of orthostatic tolerance ($r=0.78$, $P<0.01$). After the sublingual administration of glyceryl trinitrate, there was no change in the increase in diameter. No significant changes were observed in the control group. Bed-rest deconditioning enhances the flow-dependent vasodilatation of large arteries and might contribute to the orthostatic intolerance observed following bed-rest.

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Introduction

Orthostatic intolerance is experienced by patients with autonomic dysfunction, but also by healthy individuals, athletes (Levine et al. 1991), astronauts returning from space flight (Bungo et al. 1985), and after bed rest (Blomqvist and Stone 1983).

In healthy individuals the underlying mechanisms of orthostatic intolerance may include blood pressure dysregulation (Watenpaugh and Hargens 1996), changes in the cardiovascular system (Levine et al. 1994, 1997), impairment of the baroreflex regulation of heart rate (Convertino et al. 1990; Fritsch et al. 1992), or alterations in cerebral autoregulation (Zhang et al. 1997). Orthostatic hypotension with syncope occurs because of a reduction in blood pressure sufficient to decrease cerebral blood flow. During orthostatic stress, cerebral vasoconstriction might also compromise cerebral blood flow (Grubb et al. 1991), aggravating the fall in cerebral blood flow and pressure related to a systemic haemodynamic collapse (Blomqvist 1990).

The endothelium is considered a key factor in the regulation of vasomotor tone (Furchgott and Zawadzki 1980; Langille and O'Donnell 1986). Orthostatic stress is characterized by acute changes in arterial blood pressure and flow, which in turn modify wall shear stress, the major physiological stimulus of the endothelium (Kamija and Togawa 1980; Langille and O'Donnell 1986; Langille et al. 1989). Endothelium-dependent and independent vasodilatation may be non-invasively investigated by increasing the velocity of arterial blood flow (Uehata et al. 1997) and by using exogenous nitric oxide (Lieberman et al. 1996; Uehata et al. 1997), respectively.

Prolonged head-down-tilt (HDT) bed rest is used to simulate the haemodynamic changes occurring when humans are exposed to microgravity and often results in orthostatic intolerance (Blomqvist and Stone 1983;

Buckey et al. 1996). We hypothesized that altered flow-dependent vasodilatation might contribute to the orthostatic intolerance. We measured flow-mediated endothelium-dependent vasodilatation in the brachial artery of healthy men before and after 7 days of HDT during bed-rest at –6° and correlated the arterial flow-dependent-dilatation with orthostatic intolerance assessed by a standing test at the end of the HDT bed-rest period.

Methods

Subjects

Flow-dependent and flow-independent dilatation were studied in the brachial artery of eight healthy men [age 32 (2) years, height 177 (3) cm and body mass 74(3) kg] [mean (SEM)] submitted to two periods of 7 days HDT (HDT group). Each subject underwent these two bed-rest periods separated by an interval of 1 month, in the first with, and in the second without, a thigh cuff on each lower limb. This was done to assess the effect of the thigh cuffs used by Russian cosmonauts to limit the thoraco-cephalic fluid shift. No effect of the thigh cuffs was found on mean blood pressure, heart rate, blood flow velocities, flow-dependent and flow-independent vasodilatation in the brachial artery. Therefore, the two bed-rest periods were considered in the present study as one experiment with eight subjects. A control age and sex matched group [$n = 8$, age 30 (2) years, height 177 (2) cm and body mass 72 (2) kg] was investigated without interrupting its usual physical activity. Endothelium-dependent and independent-vasodilatation were investigated after 1 h in the resting position on the day before and the last day of HDT. All subjects were non-smokers. No subject was under medication or had medical problems or risk factors for arterial disease. All subjects signed an informed consent document approved by the Ethics Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Toulouse, France).

Head-down-tilt protocol

Subjects were housed in the Space Clinic located in Rangueil Hospital during the study. There were three successive periods in the experiment:

1. A baseline period of 5 days to take pre- HDT biological samples and make haemodynamic measurements
2. An HDT period at –6°of 7 days. The HDT was maintained day and night and was monitored by video
3. A recovery period of 4 days preceded by orthostatic tests immediately at first standing.

Liquid intake was about 1.9 $l \cdot day^{-1}$, but no beverage, including tea, cafe or cola, was allowed. Energy intakes were about 2,500 $kcal \cdot day^{-1}$. Measurements were performed in the supine position 1 day before HDT (D–1), on the 1st and 7th days of the HDT period (D1 and D7, respectively) and on the 1st and 3rd days of the recovery period (D + 1 and D + 3, respectively). In the control group, subjects were ambulatory without restriction on orthostatic or work activity. Endothelium-dependent and independent-vasodilatation were investigated after 1 h of rest in the supine position on D–1 and D7.

Data acquisition

Haemodynamic monitoring

All subjects were examined in the morning in a room at an ambient temperature of 22 ± 1°C. Flow-mediated endothelium-dependent vasodilatation (Dakak et al. 1998) was measured in the brachial artery during reactive hyperaemia after 4 min of occlusion by arterial cuff. The right arm was comfortably immobilized in an extended position to allow consistent access to the brachial artery for imaging. A pneumatic cuff was placed around the wrist and ultrasound parameters (brachial diameter and blood flow velocity) were recorded before (basal conditions), during the cuff inflation (250 mmHg for 4 min) and 1 min after the sudden release of the cuff pressure which led to an acute post-ischaemic hyperaemia and an increase in blood flow rate.

The endothelium-independent vasodilatation was measured 15 min later using a sublingual glyceryl trinitrate (GTN, 400 mg) spray. Measurements of the artery diameter were made after 1, and repeated after 3 and 5 min.

Blood flow velocity and artery diameter were measured 3 to 7 cm above the elbow. Blood flow velocity measurements were obtained using a range gated 8 MHz Doppler apparatus with a pulsed repetition frequency at 33 kHz (Echovar, AL 40, Alvar, Montreuil, France), the beam axis was positioned to form a 60° angle with the long axis of the vessel. Artery diameter was measured using a wall track system (WTS, Pie Medical) composed of a two-dimensional echograph apparatus (Medical Scanner 200, Pie Medical) having a 7.5 MHz 40 mm linear array transducer and a computer system (Hoeks et al. 1997). On a conventional B-mode image, a line of observation perpendicular to the long axis of the artery was selected. Switching to M-mode forced the ultrasound system to operate with a fixed ultrasound beam position. High-frequency radio frequency signals were digitized synchronously with the emission trigger at a rate of four times the emission frequency and were temporarily stored in a high speed memory system. The data from the memory were transferred to the computer system and processed. Two sample volumes were placed along the M-mode line, one on the anterior and the other on the posterior arterial wall. Movement within the sample volumes was detected using an algorithm based on cross-correlation of the signal received from the sample volumes over subsequent observations permitting measurements of the arterial diameter and wall displacement to an accuracy of 12 μm.

Values of blood flow velocities and arterial lumen diameters were obtained by the same investigator and correspond to the mean of five measurements. Two series of paired measurements separated by a 2 h interval were compared and the relative (positive or negative) differences (Di) within each pair of measures were calculated. The agreement between these two measurements was estimated by calculating the mean and the standard deviation of Di. Repeatability of the blood flow velocities and arterial lumen diameter measurements was investigated in the eight subjects using a calculation of the repeatability coefficient (RC) (British Standards Institution 1979), i.e. according to $RC^2 = \sigma Di^2/N$, where N is the sample. This coefficient is the standard deviation of the estimated difference between two repeated measurements. The RC values for intra-observer repeatability were 1 $cm \cdot s^{-1}$ for the mean blood flow velocity and 49 μm for arterial lumen diameter measurements, which were not statistically different from 0.

Wall shear stress τ was calculated as $\tau = 4 \cdot \mu \cdot (v/r)$, where μ indicates blood viscosity estimated from the blood haematocrit (Pries and Neuhaus 1992), v is the mean blood velocity and r the radius of the lumen. Wall shear stress was expressed in dynes per centimeter squared. The relative increases in blood velocity, lumen diameter, blood flow and wall shear stress observed during reactive hyperaemia or after GTN, were expressed as percentages of their respective control values. Heart-rate, systolic, diastolic and mean arterial blood pressures were measured every minute on the left brachial artery with an automated sphygmomanometer (Dinamap monitor, Critikon Inc) for the duration of the period of ultrasound data acquisition. Blood haematocrit was measured before HDT (D–1), at the end of HDT (D7) and on the 1st day of the recovery (D + 1) from blood samples obtained from a cephalic vein.

Orthostatic stress

Orthostatic tolerance was assessed by a normalised standing test performed in the morning before (D–1) and at the end of HDT

(D + 1). After 30 min of rest in the supine position, the standing test consisted of 5 min in a sitting position, followed by 10 min in an upright standing position. At the end of HDT, the subjects stood up for the first time during the standing test. This procedure was chosen to be the same as that used by NASA after space flights. During the standing test, the subjects were told to remain without any movement. Systolic, diastolic and mean brachial arterial pressures and heart rate were automatically measured every minute at rest and then during the standing test. Criteria for stopping the standing test were either:

1. A syncope, or
2. Clinical signs of orthostatic intolerance (palor, sweating, feeling faint, dizziness), or
3. A suddenly occurring and persistent decrease in systolic blood pressure of more than 25 mmHg, or
4. A variation in heart rate of at least 15 beats·min^{-1}, including sudden bradycardia, except for the phases following changes of position, or
5. A tachycardia more than 160 beats·min^{-1}.

If one or more of these criteria appeared before the 10th min, the standing test was considered positive (orthostatic intolerance).

Nitrite determination

The NOx (nitrite/nitrate) levels were measured as the total nitrite concentration after enzymatic conversion to nitrite. Urine NOx levels were determined using a fluorimetric determination of nitrite based on the acid catalysed ring closure of 2,3-diaminonaphtalene (non fluorescent) with nitrite to form the highly fluorescent product 1-(h)-naphtotriazole. Briefly, the sample was incubated with 40 μmol·l^{-1} nicotinamide adenine dinucleotide phosphate, reduced (to initiate the reaction) and 14 mmol·l^{-1} of enzyme in a final volume of 50 μl of 20 mmol·l^{-1} TRIS, pH 7.6. The reaction was terminated after 5 min at 20°C by dilution with 50 μl of water followed by addition of the DNA reagent for determination of nitrite. Formation of the fluorescent product was measured using a fluorescent plate reader (Labsystem Laboratories) with a wavelength excitation of 365 nm and an emission of 450 nm. White opaque 96-wells plates (Dynatech Laboratories, Inc.) were used. Background values were determined and calibration curves were plotted for potassium nitrate standard dissolved in double-deionized water (linear range: 4.5–150 μmol·l^{-1}).

Statistical analysis

During the HDT the effect of the thigh cuff was analysed using a two-way analysis of variance (ANOVA). Because there was no significant effect of the thigh cuff on mean blood pressure, heart rate, blood flow velocities, flow-dependent and flow-independent vasodilatation in the brachial artery, we decided to calculate the mean value for all data of the two HDT. ANOVA for repeated measurements followed by Scheffé's tests was then used to compare:

1. The time course of changes in all parameters during the study and
2. The corresponding parameters measured under basal conditions, reactive hyperaemia and after GTN administration on the same day (i.e. D–1, D1, D7, D + 1 and D + 3).

Linear regression analysis was used to test the relationship between flow-dependent arterial dilatation and the duration of the standing test. Results are expressed as mean (SEM) and a P value of less than 0.05 was considered statistically significant. A non paired Student's t-test was applied to compare parameters measured in the HDT group and in the control group.

Results

Haemodynamics

Systemic parameters

In HDT and control groups, systolic, diastolic and mean arterial pressures measured in the supine position, did not change under basal conditions during the period of the experiment. In HDT, heart rate had increased onD + 1 [65 (3) compared to 55 (2) beats·min^{-1} at D–1, $P < 0.01$]. Heart rate had returned to baseline values on D + 3. In all subjects, the administration of sublingual GTN decreased blood pressure and increased heart rate on all days of the study compared to the corresponding basal values at an identical time (Table 1). On D7, the increase in heart rate was higher in HDT than that in the control group [81 (4) compared to 68 (3) beats·min^{-1}, $P < 0.05$].

Table 1 Time course of changes in systemic haemodynamics in basal conditions and after glyceryl trinitrate (*GTN*) administration in the head down tilt (*HDT*) group and control group [mean (SEM)]. *SBP* Systolic blood pressure, *DBP* diastolic blood pressure, *MBP* mean blood pressure, *HR* heart rate

Basal	D–1		D1		D7		D + 1		D + 3	
	GTN	Basal	GTN	Basal	GTN	Basal	GTN	Basal	GTN	Control group ($n = 8$)
SBP (mmHg)	121 (5)	113 (5)***			119 (5)	106 (4)***				
DBP (mmHg)	70 (4)	67 (5)*			69 (4)	66 (4)*				
MBP (mmHg)	87 (5)	83 (5)*			86 (4)	79 (4)***				
HR (beats·min^{-1})	61 (2)	67 (3)***			60 (2)	68 (3)***				
HDT group ($n = 8$)										
SBP (mmHg)	118 (3)	108 (4)*	114 (3)	105 (4)*	121 (3)	109 (4)***	122 (3)	110 (3)**	115 (3)	104 (3)*
DBP (mmHg)	67 (2)	58 (2)**	68 (3)	57 (3)*	69 (2)	59 (3)***	71 (2)	59 (3)**	66 (2)	57 (2)**
MBP (mmHg)	84 (2)	75 (3)*	87 (2)	74 (3)***	87 (2)	76 (3)**	88 (2)	76 (3)**	83 (2)	73 (2)**
HR (beats·min^{-1})	55 (2)	68 (3)***	57 (3)	73 (4)**	61 (3)	81 (4)**	65 (3)[a]	80 (4)**	1 (3)	75 (5)***

*$P < 0.05$
**$P < 0.01$
***$P < 0.001$ compared to basal values on the same day
[a]$P < 0.01$compared to basal values on D–1

Regional parameters

Under basal conditions, mean blood flow velocity in the brachial artery, artery lumen diameter, blood flow rate and wall shear stress measured on D–1, D1, D7, D + 1 and D + 3 were not significantly different (Table 2, Table 3).

During reactive hyperaemia, mean blood flow velocity, blood flow rate and wall shear stress were always higher than the corresponding basal values in the brachial artery on the same day but did not change during the course of the study. There was no difference between values measured in the HDT group and those in the control group on D7 (Table 2).

The relative increases in mean blood flow velocity, blood flow rate and wall shear stress during reactive hyperaemia were similar on D–1, D1, D7, D + 1 and D + 3 (Table 2). The relative increase in the diameter of the artery lumen induced by reactive hyperaemia in the control group remained unchanged: 3.7 (0.8)% on D–1 and 3.5 (0.5)% on D7 (Table 3). However, in the HDT group, the relative increase in the diameter of the artery lumen induced by reactive hyperaemia was greater on D7, compared to D–1, D1, D + 1 and D + 3 [8.8 (1.6)% compared to 3.7 (1.0)%, $P < 0.01$, 3.5 (0.7)%, $P < 0.05$, 4.7 (0.9)%, $P < 0.05$ and 3.6 (0.6)%, $P < 0.01$, respectively] and greater than the corresponding increase in the control group [8.8 (1.6)% compared to 3.5 (0.5)%, $P < 0.01$] (Fig. 1).

Each administration of GTN increased the diameter of the artery lumen as compared to the corresponding values measured under basal conditions and during reactive hyperaemia (Table 4). The diameter of the artery lumen measured after GTN administration did not change during the period of the experiment. The relative increases in lumen diameter induced by GTN were not significantly different among D–1, D1, D7, D + 1 and D + 3, and between the two groups.

Orthostatic stress

Before HDT, all orthostatic standing tests were negative except for one subject who presented a typical vaso- vagal fainting attack after 6 min in the standing position. At D + 1, 11 out of 16 standing tests (eight subjects in the two experiments) were stopped because of orthostatic intolerance (Table 5). In subjects showing a positive standing test on D + 1, there was a negative correlation between the relative increase in brachial arterial diameter during reactive hyperaemia and the duration of the standing test on D7 ($r = 0.78$, $P < 0.02$) (Fig. 2).

Biochemistry

Blood haematocrit increased from 44.6 (0.5)% on D–1 to 47.1 (0.6)% on D7 ($P < 0.001$). Blood viscosity, estimated from blood haematocrit, increased slightly

Table 2 Time course of changes in mean velocity of arterial blood flow (*MBFV*), blood flow rate (*BFR*), and wall shear stress (*WSS*) in basal conditions (*B*) and during reactive hyperaemia (*H*), in head down tilt (*HDT*) group and control group [mean (SEM)]

	D–1		D1		D7		D + 1		D + 3	
	Basal	Hyperaemia	Basal	Hyperaemia	Basal	Hyperaemia	Basal	Hyperaemia	Basal	Hyperaemia
Control group ($n = 8$)										
MBFV ($cm \cdot s^{-1}$)	9 (1)	40 (2)***			10 (1)	40(2)***				
BFR ($ml \cdot mn^{-1}$)	79 (7)	394 (41)***			85 (9)	380(25)***				
WSS ($dyn \cdot cm^{-2}$)	5.0 (0.3)	22.2(1.3)***			5.6 (0.6)	22.8 (1.5)***				
HDT group (n = 8)										
MBFV ($cm \cdot s^{-1}$)	8 (1)	27 (1)***	12 (2)	33 (2)***	10 (1)	28 (2)***	10 (1)	32 (2)***	9 (1)	31 (2)***
BFR ($ml \cdot min^{-1}$)	88 (13)	336 (33)***	118 (19)	378 (43)***	121 (18)	386 (38)***	115 (17)	402 (46)***	107 (24)	390 (49)***
WSS ($dyn \cdot cm^{-2}$)	4.9 (0.4)	18.0 (1.4)***	8.4 (1.3)	21.4 (1.2)***	6.7 (0.9)	17.2 (1.3)***	6.6 (0.7)	20.7 (1.3)***	5.6 (0.8)	19.1 (1.2)***

*** $P < 0.001$ compared to basal on the same day

Table 3 Arterial diameter in basal conditions and during reactive hyperaemia throughout the study [mean (SEM)]. Δ Diameter: the difference between the diameter measured in basal conditions and during stimulation, *HDT* head down tilt

	D–1		D1		D7		D+1		D+3	
	Basal	Hyperaemia	Basal	Hyperaemia	Basal	Hyperaemia	Basal	Hyperaemia	Basal	Hyperaemia
Control group ($n=8$)										
Diameter (mm)	4.35 (0.15)	4.52 (0.17)***			4.30 (0.16)	4.45 (0.14)***				
Δ Diameter (mm)	–	0.17 (0.03)			–	0.16 (0.02)				
HDT group ($n=8$)										
Diameter (mm)	4.89 (0.25)	5.07 (0.27)**	4.84 (0.25)	5.01 (0.26)***	4.90 (0.19)	5.34 (0.23)***,c	4.83 (0.20)	5.06 (0.21)***	4.96 (0.21)	5.14 (0.21)***
Δ Diameter (mm)	–	0.18 (0.05)	–	0.17 (0.03)	–	0.43 (0.09)[a,b,c]	–	0.22 (0.05)	–	0.17 (0.02)

**$P < 0.01$
***$P < 0.001$ compared to basal on the same day
[a]$P < 0.05$ compared to hyperaemia on D–1, D+1 and D+3
[b]$P < 0.01$ compared to hyperaemia on D1
[c]$P < 0.01$ compared to corresponding value in control group on the same day

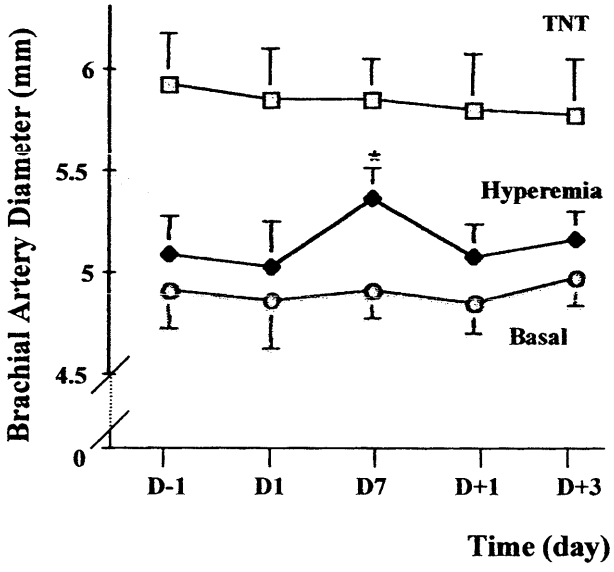

Fig. 1 Time course of changes in the relative increase in diameter of the lumen of the brachial artery during reactive hyperaemia (endothelium-dependent vasodilatation) and after glyceryl trinitrate (*GTN*) administration (endothelium-independent vasodilatation). Measurements were performed in the brachial arteries of eight men before, during and after a 7 day period of head-down-tilt (HDT). Results are expressed as mean (SEM). *$P < 0.05$ in comparison to the values on D–1, D1, D+1 and D+3.D–1 indicates 1 day before HDT, D1 and D7 the 1st and 7th days of HDT, D+1 and D+3 the 1st and 3rd days of the recovery period

from 0.0312 (0.0006) poise on D–1 to 0.0344 (0.0008) poise on D7 ($P < 0.001$). The urinary nitrite excretion did not significantly change during the whole period of the experiments [D–1: 72 (7), D1: 62 (7), D7: 68 (5), D+3: 53 (5) mmol·h^{-1}).

Discussion

The main finding of the present study was that 7 days of HDT bed rest enhances flow-induced-endothelium-dependent vasodilatation in brachial artery (more than twofold) but does not affect the endothelium independent maximal dilatation in healthy subjects.

Endothelial function has been evaluated using the rat submitted to hindlimb unloading, an experimental model of microgravity (Delp et al. 1995). That study reported a decreased endothelium-dependent relaxation to acetylcholine in aortic rings. In contrast the present study, performed in humans, showed an increased dilatation of large arteries which had been submitted to a high shear stress.

The increased flow-dependent dilatation at the end of the bed-rest period (D7) was not related to changes in systemic haemodynamics as arterial pressure and heart rate remained unchanged during the bed-rest period, despite the hypovolaemia suggested by the increase in haematocrit between the 1st and the last days of the HDT period. The wall shear stress values calculated in the brachial artery under basal conditions remained stable throughout the study. In addition, the maximal increase in shear stress induced by reactive hyperaemia was similar from D–1 to D+3. Therefore, the increase in flow-dependent dilatation recorded on the 7th day of the HDT period cannot be attributed to either differences in basal or in maximal levels of endothelial shear stress. In addition, this increase in flow-dependent dilatation cannot be attributed to time-dependent variability of the vasodilatation response, as values of endothelial shear stress in the HDT group on D7 were comparable with values found in the control group. Similarly, the increased arterial dilatation in response to reactive hyperaemia recorded on D7 cannot be attributed to an increased sensitivity of arterial smooth muscle cells to nitric oxide (NO). Indeed, the maximal dilatation of the brachial artery induced by GTN, an exogenous NO donor, was similar throughout the experiment in all subjects (about 20%).

The endothelium-dependent-dilatation showed a correlation with orthostatic intolerance: higher increases in brachial artery diameter during reactive hyperaemia

Table 4 Arterial diameter in basal conditions and after glyceryl trinitrate (*GTN*) administration during the study [mean (SEM)]. Δ Diameter: the difference between the diameter measured in basal condition and during stimulation, HDT head down tilt

Basal	D–1		D1		D7		D+1		D+3	
	GTN	Basal	GTN	Basal	GTN	Basal	GTN	Basal	GTN	Control group ($n=8$)
Diameter (mm)	4.35 (0.15)	4.97 (0.17)***			4.30 (0.16)	5.03 (0.22)***				
Δ Diameter (mm)	–	0.62 (0.10)				0.75 (0.10)				
HDT group ($n=8$)										
Diameter (mm)	4.89 (0.18)	5.92 (0.25)**	4.84 (0.23)	5.81 (0.25)***	4.90 (0.14)	5.84 (0.21)***	4.83 (0.15)	5.79 (0.28)***	4.96 (0.14)	5.78 (0.28)***
Δ Diameter (mm)	–	1.01 (0.18)	–	0.98 (0.08)	–	1.00 (0.08)	–	1.03 (0.13)	–	0.77 (0.13)

$^{**}P<0.01$
$^{***}P<0.001$ compared to basal on the same day

Table 5 Duration of standing test before and after head down tilt (*HDT*). Time course of changes in systemic haemodynamics in basal conditions and after the standing test in *HDT* group [mean (SEM)]. For other definitions see Table 1

	Before HDT period (D–1)		After HDT period (D+1)	
	Basal	End	Basal	End
Duration (min)	10 (0)			7 (1)[a]
SBP (mmHg)	129 (3)	122 (6)	135 (5)	115 (9)
DBP (mmHg)	71 (2)	71 (4)	71 (2)	67 (5)*
MBP (mmHg)	91 (2)	91 (5)	90 (2)	90 (7)
HR (beats·min^{-1})	66 (4)	97 (5)***	73 (4)[a]	116 (5)***

$^{*}P<0.05$
$^{***}P<0.001$ Compared to basal on the same day
[a] $P<0.01$ Compared to D–1

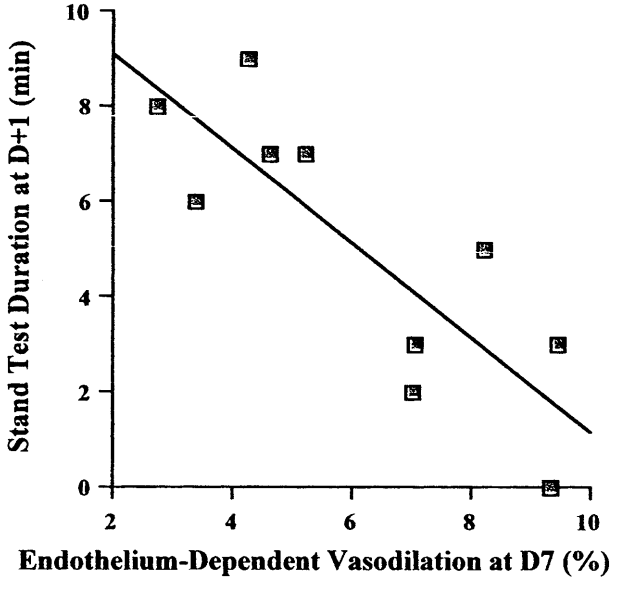

Fig. 2 Correlation between the duration of the standing test and the relative increase in diameter of the lumen of the brachial artery during reactive hyperaemia. The duration of the standing test measured at the first standing test on D+1 was significantly and negatively correlated with the relative increase in diameter of the brachial artery during reactive hyperaemia on D7 in 11 of 16 standing tests (eight subjects in two HDT periods) in the head down tilt group ($r=0.78$, $P<0.01$)

were negatively correlated with the duration of the standing test in symptomatic subjects. No correlation between flow-dependent-dilatation and tolerance to the standing test was found in subjects before the HDT period. When subjects stood up for the first time after 7 days of HDT, the acute decrease in systemic blood pressure resulted in tachycardia in order to maintain the arterial pressure and cerebral perfusion. Thus increases in arterial blood flow velocity would have led to an increase in endothelium stimulation. If the enhanced flow-dependent-dilatation seen in large arteries could be extended to resistance arteries, we could speculate that in patients with orthostatic intolerance, decreased systemic vascular resistances during standing-test could not be compensated by tachycardia or by increase in cardiac output, leading to a systemic collapse with syncope. This alteration in flow-dependent-dilatation is likely to occur in addition to an impairment of blood pressure regulation (Watenpaugh and Hargens 1996), and of the cardiovascular system (Levine et al. 1994, 1997), or an alteration of the baroreflex regulation of heart rate (Convertino et al. 1990; Fritsch et al. 1992), or cerebral autoregulation (Zhang et al. 1997) after bed-rest deconditioning.

In conclusion, bed rest simulating microgravity enhances flow induced-endothelium-dependent vasodilatation. This effect seems to be independent of

haemodynamic status and might explain, at least in part, the orthostatic intolerance seen following exposure to bed rest of long duration and after periods spent in situations of microgravity.

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References

Blomqvist CG (1990) Orthostatic hypotension. In: Parmley WW, Chaterjee K (eds) Cardiology. Lippincott, Philadephia, Pa., pp 1–20

Blomqvist CG, Stone HL (1983) Cardiovascular adjustments to gravitational stress. In: Shepherd JT, Abboud FM (eds) Handbook of physiology, section 2. The cardiovascular system. American Physiological Society, Bethesda, Md., pp 1025–1063

British Standards Institution (1979) Precision of test method (BS5497, part 1). British Standards Institution, London

Buckey JC, Lane LD, Levine BD, Watenpaugh DE, Wright SJ, Moore WE, Gaffney FA, Blomqvist CG (1996) Orthostatic intolerance following spaceflight. J Appl Physiol 8:7–18

Bungo MW, Charles JB, Johnson PC (1985) Cardiovascular deconditioning during space flight and the use of saline as a countermeasure to orthostatic intolerance. Aviat Space Environ Med 56:985–990

Convertino VA, Doerr DF, Eckberg DL, Fritsch JM, Vernikos-Danellis J (1990) Head-down bed rest impairs vagal baroreflex responses and provokes orthostatic hypotension. J Appl Physiol 68:1458–1464

Dakak N, Husain S, Mulcahy D, Andrews NP, Panza JA, Waclawiw M, Schenke W, Quyyumi AA (1998) Contribution of nitric oxide to reactive hyperemia: impact of endothelial dysfunction. Hypertension 32:9–15

Delp MD, Brown M, Laughlin MH, Hasser EM (1995) Rat aortic vasoreactivity is altered by old age and hindlimb unloading. J Appl Physiol 78:2079–2086

Fritsch JM, Charles JB, Bennett BS, Jones MM, Meehan RT (1992) Short duration spaceflight impairs human carotid baroreceptor-cardiac reflex responses. J Appl Physiol 73:664–671

Furchgott RF, Zawadzki JV (1980) The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373–376

Grubb BP, Gerard G, Roush K, Temesy-Armos P, Montford P, Elliot L, Hahn H, Brewster P (1991) Cerebral vasoconstriction during head-upright tilt-induced vasovagal syncope: a paradoxic and unexpected response. Circulation 84:1157–1163

Hoeks AP, Willekes C, Boutouyrie P, Brands PJ, Willigers JM, Reneman RS (1997) Automated detection of local artery wall thickness based on M-line signal processing. Ultrasound Med Biol 23:1017–1023

Kamiya A, Togawa T (1980). Adaptative regulation of wall shear stress to flow change in the canine carotid artery. Am J Physiol 239:H14-H21

Langille BL, O'Donnell F (1986) Reduction in arterial diameter produced by chronic decreases in blood flow are endothelium dependent. Science (Washington) 231:405–407

Langille BL, Bendeck MP, Keely FW (1989). Adaptations of carotid arteries of young and mature rabbits to reduced carotid blood flow. Am J Physiol 256:H931–H939

Levine BD, Buckey JC, Fritsch JM, Yancy CW, Watenpaugh DE, Eckberg DL, Blomqvist CG (1991) Physical fitness and cardiovascular regulation: mechanisms of orthostatic intolerance. J Appl Physiol 70:112–122

Levine BD, Giller CA, Lane LD, Buckey JC, Blomqvist CG (1994) Cerebral versus systemic hemodynamics during graded orthostatic stress in humans. Circulation 90:298–305

Levine BD, Zuckerman JH, Pawelczyk JA (1997) Cardiac atrophy after bed rest deconditioning: a non-neural mechanism for orthostatic intolerance. Circulation 96:517–525

Lieberman EH, Gerhard MD, Uehata A, Selwyn AP, Ganz P, Yeung AC, Creager MA (1996) Flow-induced vasodilation of the human brachial artery is impaired in patients <40 years of age with coronary artery disease. Am J Cardiol 78:1210–1214

Pries AR, Neuhaus D (1992) Blood viscosity in tube flow: dependence on diameter and hematocrit. Am J Physiol 263:H1770–1778

Uehata A, Lieberman EH, Gerhard MD, Anderson TJ, Ganz P, Polak JF, Creager MA, Yeung AC (1997) Noninvasive assessment of endothelium-dependent flow-mediated dilation of the brachial artery. Vasc Med 2:87–92

Watenpaugh DE, Hargens AR (1996) The cardiovascular system in microgravity. In: Handbook of physiology. Environmental physiology, section 4, vol I, chapter 29. American Physiological Society, Bethesda, Md., pp 631–674

Zhang R, Zuckerman JH, Pawelczyk JA, Levine BD (1997) Effects of head-down-tilt bed rest on cerebral hemodynamics during orthostatic stress. J Appl Physiol 83:2139–2145