Angiotensin II contributes to the increased baseline leg vascular resistance in spinal cord-injured individuals

Jan T. Groothuis[a,b], Dick H.J. Thijssen[a,c], Gerard A. Rongen[d], Jaap Deinum[e], A.H. Jan Danser[f], Alexander C.H. Geurts[b,g], Paul Smits[d] and Maria T.E. Hopman[a]

Objective Spinal cord-injured (SCI) individuals demonstrate an increased baseline leg vascular resistance (LVR). In addition, despite the lack of sympathetic control, an increase in LVR is observed during orthostatic challenges. On the basis of the vasoconstrictive characteristics of angiotensin II, we examined the hypothesis that angiotensin II contributes to the LVR at baseline and during head-up tilt (HUT) in SCI individuals.

Methods Supine baseline leg and forearm blood flow were measured using venous occlusion plethysmography and leg blood flow during 30° HUT using duplex ultrasound. Measurements were performed before and 4 h after an angiotensin II antagonist (irbesartan, 150 mg) administered in eight SCI individuals and eight age-matched and sex-matched able-bodied controls. Vascular resistance was calculated as the arterial–venous pressure gradient divided by blood flow.

Results Angiotensin II blockade significantly decreased baseline LVR in SCI individuals ($P = 0.02$) but not in controls, whereas no changes in forearm vascular resistance were found in both groups. Angiotensin II blockade did not alter the increase in LVR during HUT in SCI individuals nor in controls.

Conclusion Our results indicate that angiotensin II contributes to the increased baseline LVR in SCI individuals. As angiotensin II does not contribute to forearm vascular resistance, the contribution to LVR may relate to the extreme inactivity of the legs in SCI individuals. Angiotensin II does not contribute to the increase in LVR during HUT in SCI individuals nor in controls. *J Hypertens* 28:000–000 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

[a]Department of Physiology, Radboud University Nijmegen Medical Centre, [b]Department of Rehabilitation, Sint Maartenskliniek, Nijmegen, The Netherlands, [c]Research Institute for Sport and Exercise Sciences, Liverpool John Moores University, Liverpool, UK, [d]Department of Pharmacology-Toxicology, [e]Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, [f]Division of Pharmacology, Vascular and Metabolic Diseases, Department of Internal Medicine, Erasmus Medical Center, Rotterdam and [g]Department of Rehabilitation, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

Correspondence to Professor Dr Maria T.E. Hopman, Department of Physiology, RUNMC, PO Box #9101, 6500 HB Nijmegen, The Netherlands
Tel: +31 24 36 13650; fax: +31 24 35 40535;
e-mail: m.hopman@fysiol.umcn.nl

Introduction

Angiotensin II plays an important role in the regulation of vascular tone and blood pressure (BP) via binding to the angiotensin II subtype 1 (AT_1; vasoconstriction) and subtype 2 (AT_2; vasodilation) receptor [1,2]. Although angiotensin II does not contribute to vascular tone in healthy controls [3,4], it plays a pivotal role in the increased forearm vascular resistance (FVR) in individuals with an increased renin–angiotensin system (RAS) activity [3–6].

Spinal cord-injured (SCI) individuals not only demonstrate an increased RAS activity, supported by elevated renin levels [7,8], but also show an increased leg vascular resistance (LVR) [9–13]. Vascular resistance is regulated by various factors, including angiotensin II. Previous studies demonstrated that the increased LVR in SCI individuals cannot be explained through the sympathetic nervous system [10] or via endothelium-derived nitric oxide [9], whereas endothelin-1 partly contributes to the increased LVR [11]. In SCI individuals, less angiotensin II is necessary to achieve a similar increase in BP compared with controls [14], which suggests an increased angiotensin II responsiveness in SCI individuals. Angiotensin II might, therefore, play an important role in the increased LVR in SCI individuals. SCI individuals offer a unique model of nature to assess peripheral vascular adaptations to inactivity as the skeletal muscles below the lesion are paralyzed and, therefore, extremely inactive. Furthermore, supraspinal sympathetic cardiovascular control is importantly impaired, which could lead to serious complications such as hypotension, orthostatic intolerance and episodic hypertension (autonomic dysreflexia) [15]. Understanding the regulation of peripheral

vascular tone is of special interest as an increased LVR may contribute to the development of pressure sores and pour wound healing due to decreased perfusion in SCI individuals [16].

Despite their spinal cord lesion and concomitant sympathetic disruption, SCI individuals demonstrate an increase in LVR during orthostatic challenges to the same extent as controls [12,17]. This indicates that in SCI individuals other vasoconstrictive mechanisms contribute to the increase in LVR. In SCI individuals, renin levels rise more quickly and to higher levels during orthostatic challenges compared with controls [7,8]. Therefore, angiotensin II might play a role in the increase in LVR during orthostatic challenges in SCI individuals.

The aim of this study was to assess the contribution of angiotensin II to leg and FVR at baseline and during an orthostatic challenge in SCI individuals and controls. We hypothesized that angiotensin II contributes to the increased baseline LVR as well as to the increase in LVR during an orthostatic challenge in SCI individuals, but not in controls.

Methods
Participants
Eight male SCI individuals and eight age-matched and sex-matched able-bodied controls participated in this study (Table 1). All participants were normotensive (<140/90 mmHg; auscultatory BP measurement), free of overt cardiovascular diseases and none of the individuals smoked. Four SCI individuals used medication, none of which is known to substantially interfere with vascular reactivity or the RAS. SCI individuals had long-standing (>5 years) traumatic spinal cord injury with a motor and sensory complete spinal cord lesion (American Spinal Injury Association Impairment Scale A [18]) below the fourth thoracic spinal segment (T4). All included SCI individuals had a complete paralysis with extremely inactive and deconditioned lower limbs and normal function and activity of the upper limbs. The level of spinal cord injury was assessed by clinical examination. The study was carried out in accordance with the declaration of Helsinki and approved by the medical ethical committee of our institution (Radboud University Nijmegen Medical Centre). All participants gave written informed consent.

Experimental procedures and protocol
Three days preceding the experiment, participants followed a sodium-defined diet (2000–2400 mg sodium/day) to control the sodium intake. All participants refrained from caffeine-containing food and beverages, vitamin C supplements and alcohol for more than 12 h and from heavy physical activity for more than 24 h prior to the experiment. Participants fasted for more than 2 h and had emptied their bladder in the hour before the experiments. All experiments were performed in the morning in a quiet, temperature-controlled ($23 \pm 1°C$) room.

Participants were positioned comfortably on a manually driven tilt table and supported by a chest belt to prevent them from sliding down during the experiment. The experiment started after a supine resting period of at least 30 min. First, baseline leg and forearm blood flow were measured in supine position using venous occlusion plethysmography over a 5-min period. To examine blood flow responses during orthostatic challenges, we first measured baseline superficial femoral artery (SFA) blood flow using duplex ultrasound. Subsequently, participants were tilted manually, within 5 s, to a 10-min passive 30° head-up tilt (HUT). In the last minute of 30° HUT, SFA blood flow was measured using duplex ultrasound. After the 30° HUT, participants were returned to the supine position (Fig. 1). Participants then received an oral dose of 150 mg of irbesartan (Aprovel; Sanofi-Aventis, Paris, France), a noncompetitive selective angiotensin II subtype 1 (AT_1) receptor antagonist with a high affinity (half maximal inhibitory concentration = 1.3 nmol/l) and high mean bioavailability (60–80%) [19,20]. The measurements were repeated 4 h after ingestion of irbesartan, to match the measurements with the peak BP response to irbesartan [21–23]. Measurements were performed under the same conditions as described earlier (i.e. >2 h fasting and bladder emptying >1 h before the experiments) (Fig. 1).

Measurements
BP was measured continuously using a noninvasive BP device (Nexfin; BMEYE B.V., Amsterdam, The

Table 1 Subject characteristics of the spinal cord-injured individuals

Subject	SCI level	DOI (years)	Age (years)	Height (cm)	Weight (kg)	MAP (mmHg)	Medication
SCI 1	T7	17	39	180	84	91	Trimethoprim
SCI 2	T11	8	36	185	90	85	
SCI 3	T7	24	55	187	83	104	Methenamine, nitrofurantoin, oxazepam
SCI 4	T8	14	49	178	78	83	Solifenacin
SCI 5	T12	22	45	186	63	95	Pregabalin
SCI 6	T6	5	32	173	53	80	
SCI 7	T12	14	46	185	70	106	
SCI 8	T4	6	28	175	75	77	
SCI ($n = 8$)		14 ± 7	41 ± 9	181 ± 5	75 ± 12	90 ± 11	
Controls ($n = 8$)			42 ± 11	179 ± 7	76 ± 9	91 ± 8	

Values represent mean $\pm$ SD. DOI, duration of injury; MAP, mean arterial blood pressure; SCI, spinal cord-injured; T, thoracic spinal segment.

Fig. 1
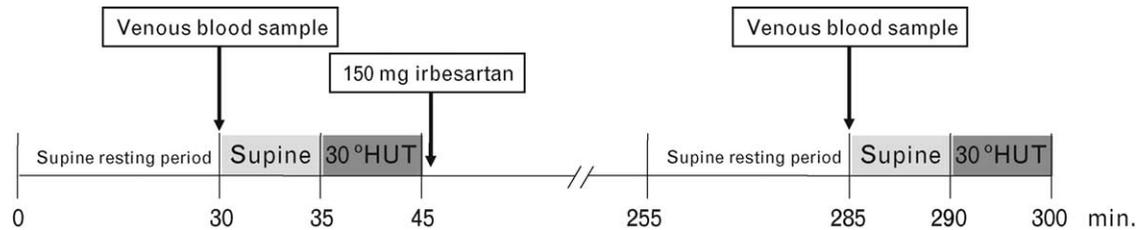
Schematic representation of the experimental protocol. 30° HUT, 30° head-up tilt.

Netherlands). A finger cuff was attached to the middle phalanx of the right third finger in order to measure finger arterial BP, which accurately reflects intraarterial BP changes [24]. A built-in heart reference system was in operation to correct for hydrostatic influences. Mean arterial BP (MAP) values were derived beat to beat and heart rate (HR) was the inverse of the interbeat interval.

Leg and forearm resistance arterial blood flow were measured by ECG-triggered venous occlusion plethysmography, using electrically calibrated [25] mercury-in-silastic strain gauges (Hokanson, Inc., Bellevue, Washington, USA), with a coefficient of variation between 6 and 13% [26]. In the supine position, the right leg and left arm were positioned approximately 5 cm above heart level to facilitate venous outflow between venous occlusions [27]. Strain gauges were placed around the upper leg, 10 cm above the patella, and around the widest circumference of the forearm. Venous occlusion cuffs, placed on the thigh approximately 5 cm above the strain gauge and around the upper arm, were simultaneously inflated with a rapid cuff inflator (Hokanson, Inc.), within 1 s, to 50 mmHg [28]. Occlusion pressures were sustained for eight heart cycles after which the cuff was deflated instantaneously (for 10 heart cycles). Blood flow was calculated as the slope of the volume change over a 4-s interval using a customized computer program (Matlab 6.1; Mathworks, Inc., Natick, Massachusetts, USA) [26].

Superficial femoral arterial blood flow during supine rest and 30° HUT was measured using duplex ultrasound, with a coefficient of variation of 14% [29]. Mean red blood cell velocity (V_{mean}) and systolic and diastolic diameter of the right SFA, approximately 2 cm distal of the bifurcation, were measured with a duplex ultrasound device (ARTLAB system; Pie Medical Imaging BV, Maastricht, The Netherlands and WAKI; Atys Medical, Soucieu en Jarrest, France). V_{mean} was calculated as the average of 20 Doppler waveforms. Automated software was used for operator-independent analyses of waveforms (Matlab 6.1; Mathworks). For diameter measurements, the average of six consecutive mean diameters was obtained. Real-time automated analyses were performed using the ARTLAB system (Pie Medical Imaging BV). Leg blood flow (LBF) was calculated with the following formula: $(\pi \times r^2 \times V_{mean}) \times 60$ ($r = 1/2 \times$ diameter of the SFA).

Venous blood samples were drawn from the right antecubital vein in supine rest before and after AT_1 receptor blockade (Fig. 1). Plasma levels of sodium, creatinine, renin and angiotensin II levels were measured before AT_1 receptor blockade and plasma levels of renin and angiotensin II were measured after AT_1 receptor blockade. Plasma renin was measured by immunoradiometric assay (CISbio International, France) and angiotensin II levels in medium were measured by radioimmunoassay (detection limit 0.5 pmol/l) as described previously [30].

Urine excretion was collected over a 24-h period preceding the experiment and urine sodium and creatinine concentrations were determined, which enabled the calculation of 24-h sodium urine output and creatinine clearance.

Data analysis
LVR and FVR were calculated as the arterial-venous pressure gradient ($P_a - P_v$) divided by blood flow. Supine venous pressure was set at 9 mmHg and during 30° HUT the arterial-venous pressure gradient was replaced by MAP, as hydrostatic pressure makes an identical contribution to leg venous as well as leg arterial pressure [31]. LVR and FVR measured using venous occlusion plethysmography are expressed in arbitrary units (AU).

Statistical analysis
Statistical analyses were performed using SPSS 16.0 (SPSS, Inc., Chicago, Illinois, USA) software. Data are presented as mean ± SD, unless otherwise stated. The level of statistical significance was set at α less than 0.05. Independent t-tests were used to assess differences at baseline. Repeated measures analysis of variance (ANOVAs) were used to examine whether the impact of AT_1 receptor blockade differed between SCI individuals and controls for angiotensin II and renin levels, and LVR and FVR. Repeated measure ANOVAs were also used to assess the effect of AT_1 receptor blockade on the

increase in LVR during 30° HUT within SCI individuals and controls. Post-hoc t-tests were performed when the ANOVA reported a significant main or interaction effect. Bonferroni's correction was used to correct for multiple comparisons.

Results

Baseline plasma angiotensin II and renin levels were significantly higher in SCI individuals compared with controls (Table 2). AT_1 receptor blockade similarly increased angiotensin II and renin levels in SCI individuals and controls (ANOVA AT_1 blockade $P = 0.03$, interaction $P = 0.35$; AT_1 blockade $P = 0.01$, interaction $P = 0.40$, respectively) (Table 2). Creatinine clearance and 24-h sodium excretion were similar between SCI individuals and controls, indicating similar sodium load (Table 2).

### Contribution of angiotensin II to baseline vascular resistance

LVR was significantly ($P = 0.01$) higher in SCI individuals (39 ± 10 AU) compared with controls (25 ± 8 AU), whereas baseline FVR was comparable between both groups (44 ± 13 and 32 ± 17 AU, respectively) (Fig. 2). AT_1 receptor blockade significantly (post-hoc $P = 0.02$) decreased LVR in SCI individuals (32 ± 10 AU), but not in controls (29 ± 11 AU) (Fig. 2). No changes in FVR were observed after AT_1 receptor blockade in both groups (46 ± 13 and 42 ± 25 AU, respectively) (Fig. 2). Baseline MAP and HR were comparable between groups and did not change after AT_1 receptor blockade (ANOVA AT_1 blockade $P = 0.07$, interaction $P = 0.29$; AT_1 blockade $P = 0.88$, interaction $P = 0.71$, respectively) (Table 3). LBF was similar between groups and significantly increased (post-hoc $P = 0.02$) in SCI individuals after AT_1 receptor blockade (ANOVA AT_1 blockade $P = 0.06$, interaction $P < 0.01$) (Table 3). Baseline SFA diameter was significantly lower in SCI individuals compared with controls and did not change after AT_1 receptor blockade (ANOVA AT_1 blockade $P = 0.27$, interaction $P = 0.71$) (Table 3).

Table 2 Plasma renin and angiotensin II levels, 24-h sodium and urine excretion and creatinine clearance in spinal cord-injured and control individuals

	SCI ($n = 8$)	Control ($n = 8$)	P
Renin (mE/l)			
Baseline	19 ± 9	11 ± 8	0.04
AT_1 blockade	$103 \pm 115^*$	$56 \pm 72^*$	0.17
Angiotensin II (pmol/l)			
Baseline	4.8 ± 1.9	2.7 ± 2.6	0.05
AT_1 blockade	$25.9 \pm 15.2^*$	$14.9 \pm 20.0^*$	0.13
24-h sodium excretion (mmol)	164 ± 43	141 ± 43	0.40
24-h urine excretion (ml)	2545 ± 984	1981 ± 702	0.30
Creatinine clearance (ml/min)	139 ± 45	140 ± 24	0.93

Values represent mean $\pm$ SD. AT_1 blockade, angiotensin II subtype 1 receptor blockade with irbesartan; SCI, spinal cord-injured. * Post hoc significantly different from baseline.

Fig. 2
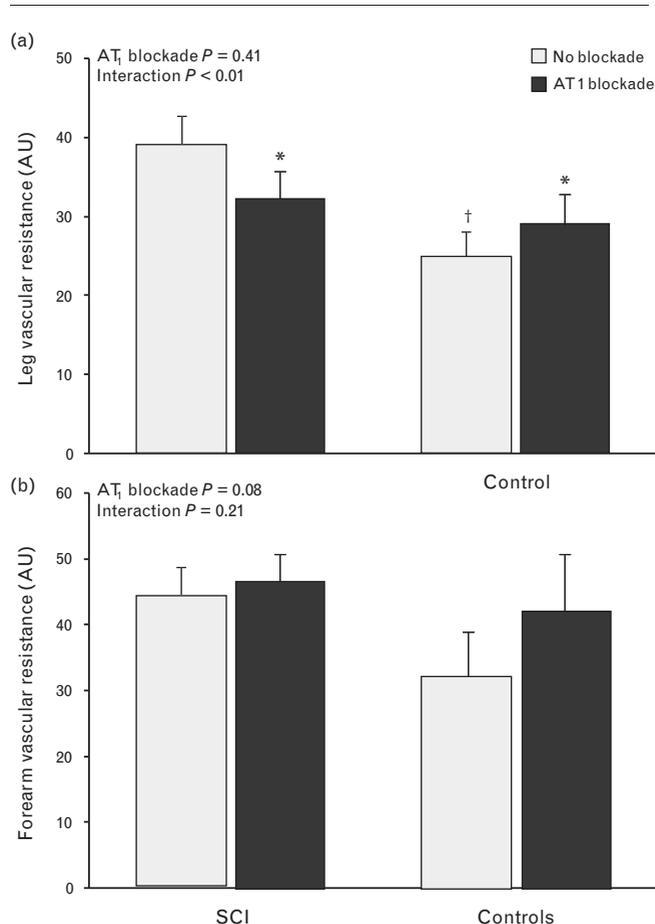
Leg (a) and forearm (b) vascular resistance in spinal cord-injured individuals and controls before and after angiotensin II subtype 1 receptor blockade. P values for two-way repeated measures ANOVA are indicated. ANOVA, analysis of variance; AT_1, angiotensin II subtype 1; AU, arbitrary units; SCI, spinal cord-injured. *, Post hoc significantly different from no blockade. $\dagger$, post hoc significantly different from SCI individuals during no blockade.

Contribution of angiotensin II to changes in vascular resistance during 30° head-up-tilt

30° HUT significantly increased LVR in SCI individuals and controls (Fig. 3). AT_1 receptor blockade did not alter the increase in LVR during 30° HUT in both groups (Fig. 3). MAP did not change during 30° HUT in SCI individuals (ANOVA 30° HUT $P = 0.10$, interaction $P = 0.17$), but significantly increased in controls after AT_1 receptor blockade (ANOVA 30° HUT $P = 0.01$, interaction $P = 0.26$) (Table 3). HR significantly increased during 30° HUT in SCI individuals before AT_1 receptor blockade (ANOVA 30° HUT $P < 0.01$, interaction $P = 0.91$), but not in controls (ANOVA 30° HUT $P = 0.08$, interaction $P = 0.96$) (Table 3). LBF decreased during 30° HUT in both groups and the decrease was comparable before and after AT_1 receptor blockade within SCI individuals and controls (ANOVA

Table 3 Effect of angiotensin II blockade on leg vascular parameters measured using duplex ultrasound in spinal cord-injured and control individuals in supine and during 30° head-up tilt

		SCI individuals ($n = 8$)		Controls ($n = 8$)	
	Position	Baseline	AT$_1$ blockade	Baseline	AT$_1$ blockade
SFA diameter (mm)	Supine	5.3 ± 0.9	5.3 ± 0.9	$7.7 \pm 0.8^{\dagger}$	$7.6 \pm 0.8^{\dagger}$
	30° HUT	5.3 ± 0.7	5.2 ± 0.8	$7.5 \pm 0.8^{\dagger}$	$7.5 \pm 0.8^{\dagger}$
LBF (ml/min)	Supine	112 ± 64	$160 \pm 75^{\ddagger}$	117 ± 19	106 ± 35
	30° HUT	$84 \pm 57^{*}$	$111 \pm 58^{*}$	$80 \pm 30^{*}$	$86 \pm 35^{*}$
LVR (mmHg/ml per min)	Supine	1.17 ± 0.83	$0.65 \pm 0.30^{\ddagger}$	0.79 ± 0.14	0.95 ± 0.57
	30° HUT	$1.80 \pm 1.17^{*}$	$1.16 \pm 0.79^{*}$	$1.49 \pm 0.89^{*}$	$1.34 \pm 0.66^{*}$
MAP (mmHg)	Supine	98 ± 15	96 ± 16	98 ± 8	93 ± 9
	30° HUT	103 ± 22	97 ± 17	100 ± 9	$97 \pm 9^{*}$
HR (bpm)	Supine	69 ± 10	71 ± 13	59 ± 8	59 ± 10
	30° HUT	$73 \pm 11^{*}$	75 ± 13	62 ± 10	61 ± 9

Values represent mean $\pm$ SD. 30° HUT, 30° head-up tilt; AT$_1$ blockade, angiotensin II subtype 1 receptor blockade with irbesartan; HR, heart rate; LBF, leg blood flow; LVR, leg vascular resistance; MAP, mean arterial blood pressure; SCI, spinal cord-injured; SFA, superficial femoral artery. * *Post hoc* significantly different from supine. † Significantly different from SCI individuals. ‡ *Post hoc* significantly different from baseline.

30° HUT $P < 0.01$, interaction $P = 0.29$ and 30° HUT $P < 0.01$, interaction $P = 0.14$, respectively) (Table 3).

Discussion

This study examined the contribution of angiotensin II, a strong vasoconstrictor, to the LVR and FVR in SCI individuals and controls. The major findings are that angiotensin II contributes to the increased vascular resistance of the paralyzed legs, but not to the vascular resistance of the normally innervated forearms in SCI individuals; angiotensin II does not contribute to LVR or FVR in controls and AT$_1$ receptor blockade does not significantly contribute to the increase in LVR during 30° HUT in SCI individuals or controls.

This study is the first to demonstrate a decrease in baseline LVR in SCI individuals after selective AT$_1$ receptor blockade. This indicates that angiotensin II contributes, at least partly, to the increased vascular resistance in the extremely inactive legs of SCI individuals. In contrast, AT$_1$ receptor blockade did not alter FVR in SCI individuals. These observations demonstrate a localized effect of angiotensin II, which suggests that the extreme physical inactivity in the deconditioned legs of SCI individuals relates to the increased contribution of angiotensin II to vascular resistance. This notion is supported by increased angiotensin II levels after bed rest [32,33], whereas exercise training reduces angiotensin II levels in heart failure [34] and angiotensin II-mediated vasoconstriction in coronary artery disease [35]. To further support the idea that physical inactivity relates to the increased vascular resistance, we have consistently demonstrated that electrical stimulation-assisted exercise training of the paralyzed lower limbs in SCI individuals can normalize the vascular adaptations caused by the SCI [11,13,36]. Electrical stimulation-assisted exercise could even completely reverse the contribution of endothelin-1 to the increased LVR in SCI individuals [11].

Our observations raise questions about the mechanism behind the increased contribution of angiotensin II to the

LVR in SCI individuals. A potential explanation relates to the elevated angiotensin II and renin levels in SCI individuals compared with controls in our study, a finding similar to previous studies [7,8] in SCI individuals with high cervical lesions. However, the circulating angiotensin II levels in SCI individuals were certainly not excessive [37], and angiotensin II blockade did not affect FVR. This argues against a major role for plasma angiotensin II *per se*. A local phenomenon is, therefore, more likely to underlie our observations. Angiotensins are known to be produced locally in the vascular wall [38]. Such production depends entirely on the uptake of renal renin [37,39]. Potentially, the leg vasculature of SCI individuals may display a larger renin uptake compared with the arms. Alternatively, an increase in angiotensin-converting enzyme (ACE) availability could increase the local formation of angiotensin II [39]. However, individuals carrying the D-allele of the *ACE* gene did not have higher angiotensin II levels, despite having 60% higher ACE levels [40]. The simplest explanation might be an alteration at the level of the angiotensin II receptors.

As binding of angiotensin II to the AT$_1$ receptor mediates vasoconstriction [1,2], an increased sensitivity or density of the AT$_1$ receptors in the legs would be sufficient to explain our observation in SCI individuals. The exaggerated blood pressor response to angiotensin II in SCI individuals [14] supports this concept. However, a down-regulation or a decreased sensitivity of the AT$_2$ receptors, which mediate vasodilation [2], may also contribute to our findings. Although the effect of inactivity on AT$_2$ receptors is unknown, exercise training increases AT$_2$ receptor expression [35]. As vascular changes due to inactivity seem to mirror the effects of exercise, inactivity might decrease AT$_2$ receptor expression. Future studies should further examine the exact mechanism explaining the contribution of angiotensin II to the increased LVR in SCI individuals.

We recently reported that endothelin-1 contributes to the increased LVR in SCI individuals, probably through the

Fig. 3

(a)

(b)
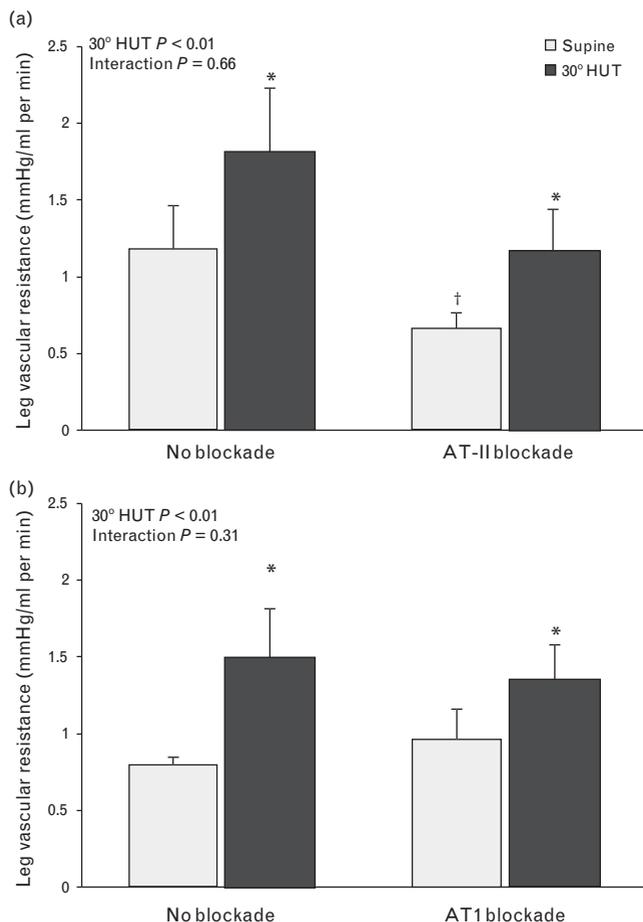
Supine and 30° head-up tilt leg vascular resistance in spinal cord-injured individuals (a) and controls (b) before and after angiotensin II subtype 1 receptor blockade. P values for two-way repeated measures ANOVA are indicated. 30° HUT, 30° head-up tilt; ANOVA, analysis of variance; AT_1, angiotensin II subtype 1. *, *Post hoc* significantly different from supine. †, *post hoc* significantly different from no blockade.

endothelin A (ET_A) receptor [11]. Interestingly, endothelin-1 and angiotensin II are closely connected. Angiotensin II stimulates the endothelin-1 release in cultured endothelial cells [41] and might mediate hypertensive effects in humans by stimulation of renal endothelium ET_A receptors [42]. Furthermore, angiotensin II-induced hypertension in rats can be attenuated by ET_A receptor blockade [43]. Although angiotensin II and endothelin-1 play an important role in the increased LVR in SCI individuals, the link between both vasoconstrictor mechanisms in SCI individuals remains unknown.

Angiotensin II levels did not contribute to baseline vascular resistance in healthy controls. Although this finding reinforces previous observations in the forearm of healthy controls [3,4], our data add to the current knowledge that angiotensin II does not contribute to the LVR in healthy controls either. This observation is of special interest, as the magnitude of the effect of

endothelium-dependent and endothelium-independent dilators are limb dependent [44], suggesting a limb-dependent balance between vasoconstrictors and vasodilators in controls, which determines the vascular tone in upper and lower limbs.

During orthostatic challenges a baroreflex-mediated increase in sympathetic activity will increase HR, cardiac contractility and peripheral resistance in order to maintain BP [45]. Despite their spinal cord lesion and concomitant sympathetic disruption, our SCI individuals demonstrate a similar increase in LVR during 30° HUT compared with controls, which is in agreement with previous studies [12,17]. This indicates that other vasoconstrictor mechanisms compensate for the lack of sympathetic control. Although renin levels increase more rapidly in SCI individuals compared with controls [7,8], we found no impact of AT_1 receptor blockade on the vascular responses during 30° HUT in both groups. We studied SCI individuals with (low) thoracic spinal lesions compared with high cervical lesions in previous studies [7,8]. All SCI individuals in our study demonstrated increased baseline renin and angiotensin II levels, indicating an increased RAS activity. Our observations indicate that angiotensin II does not contribute to the increase in LVR during 30° HUT. One explanation relates to an insufficient orthostatic stress. However, a 30° HUT causes significant cardiovascular effects [12,17], which are comparable to higher HUT angles [46]. Another explanation relates to the duration of our orthostatic challenge (10 min), as angiotensin II only contributes to vascular responses during prolonged orthostatic challenges (>25 min) in controls [47]. During 10-min orthostatic challenges, angiotensin II does not contribute to the increase in LVR in controls [47] and in our SCI individuals. The observed increase in LVR, therefore, is mediated by other vasoconstrictor mechanisms. The vasoconstrictors endothelin-1, vasopressin and aldosterone unlikely contribute to our observations, as they do not increase during orthostatic challenges [47,48]. In a recent study [17], we demonstrated that the myogenic response, at least partly, contributes to the increase in LVR during orthostatic challenges in SCI individuals.

Limitations

As AT_1 receptor blockers for intraarterial human use are not available, we used an oral selective AT_1 receptor blocker. SCI individuals and controls demonstrated similar increases in angiotensin II and renin levels after ingestion, indicating successful AT_1 receptor blockade. A single dose of irbesartan (150 mg) has a receptor occupancy of approximately 90% [21,22], which means that AT_1 receptors were not fully blocked. Nonetheless, we found a localized impact on the leg vasculature in SCI individuals.

Our experiments took place in the morning with a 4-h difference between before and after AT_1 receptor

blockade measurements (Fig. 1). The diurnal rhythm of RAS activity, with a peak in the early morning (0200–0800 h) and a relatively stable plateau during the awake hours [49], could have influenced our measurements. We started the experiments between 0900 and 1000 h in both controls and SCI individuals. The diurnal rhythm of RAS activity, therefore, unlikely explains the localized impact of AT_1 receptor blockade on the LVR in SCI individuals.

As venous occlusion plethysmography is suggested to require an empty venous system to guarantee full venous compliance, we measured LVR during 30° HUT using duplex ultrasound. In the supine position, we used venous occlusion plethysmography, as simultaneous measurements of different limbs are possible with a single device. Both methods have a good reproducibility [26,29] and there is a good agreement between the two methods with a correlation coefficient of 0.86 [46]. Possible differences at baseline and in the magnitude of angiotensin II contribution to LVR might be explained by the fact that both methods measure a different part of the cardiovascular system. Although venous occlusion plethysmography measures blood flow in resistance arteries, duplex ultrasound measures conduit artery blood flow. Distinct adaptations at both levels have been documented in response to exercise as well as physical inactivity [36].

Perspectives
SCI individuals are prone to develop pressure sores and suffer from poor wound healing below the level of the spinal cord lesion, which are associated with the increased LVR [16]. On the basis of the vasodilator effect in the paralyzed legs of SCI individuals, AT_1 receptor antagonists may improve or even prevent these secondary complications. Moreover, prolonged treatment may even improve cardiovascular function and health [1]. Finally, our results suggest a detrimental role for physical inactivity to increase the angiotensin II-mediated vascular tone. Accordingly, physical inactivity may contribute, at least partly, to the increased angiotensin II-mediated tone in other disease states such as heart failure and cirrhosis.

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