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Effect of acute sleep deprivation on vascular function in healthy subjects

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Sauvet F, Leftheriotis G, Gomez-Merino D, Langrume C, Drogou C, Van Beers P, Bourrilhon C, Florence G, Chennaoui M. Effect of acute sleep deprivation on vascular function in healthy subjects. *J Appl Physiol* 108: 68–75, 2010. First published November 12, 2009; doi:10.1152/japplphysiol.00851.2009.—Sleep disorders are associated with inflammation and sympathetic activation, which are suspected to induce endothelial dysfunction, a key factor in the increased risk of cardiovascular disease. Less is known about the early effects of acute sleep deprivation on vascular function. We evaluated microvascular reactivity and biological markers of endothelial activation during continuous 40 h of total sleep deprivation (TSD) in 12 healthy men (29 ± 3 yr). The days before [*day 1* (D1)] and during TSD (D3), at 1200 and 1800, endothelium-dependent and -independent cutaneous vascular conductance was assessed by iontophoresis of acetylcholine and sodium nitroprusside, respectively, coupled to laser-Doppler flowmetry. At 0900, 1200, 1500, and 1800, heart rate (HR) and instantaneous blood pressure (BP) were recorded in the supine position. At D1, D3, and the day after one night of sleep recovery (D4), markers of vascular endothelial cell activation, including soluble intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin, and interleukin-6 were measured from blood samples at 0800. Compared with D1, plasma levels of E-selectin were raised at D3, whereas intercellular adhesion molecule-1 and interleukin-6 were raised at D4 ($P < 0.05$). The endothelium-dependent and -independent CVC were significantly decreased after 29 h of TSD ($P < 0.05$). By contrast, HR, systolic BP, and the normalized low-frequency component of HR variability (0.04–0.15 Hz), a marker of the sympathetic activity, increased significantly within 32 h of TSD ($P < 0.05$). In conclusion, acute exposure to 40 h of TSD appears to cause vascular dysfunction before the increase in sympathetic activity and systolic BP.

vascular reactivity; iontophoresis; endothelial activation; blood pressure

CHRONIC SLEEP DISORDERS AND short sleep duration (≤5 h per night) are associated with an increased incidence of cardiovascular and metabolic diseases in humans (14, 19). However, the biological mechanisms that link short sleep duration and cardiovascular disease are unknown (14), and little is known about the early effects of acute sleep deprivation on vascular function.

In healthy subjects, acute total sleep deprivation (TSD) induces a significant increase *1*) in circulating levels of endothelial cell activation markers, such as soluble intercellular adhesion molecule-1 (ICAM-1) and E-selectin (13); and *2*) in pro- and anti-inflammatory markers, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), IL-1 receptor antagonist, and interleukin-6 (IL-6) (9, 13, 50). It has also been reported that a high level of IL-6, ICAM-1, or E-selectin could be linked to the development of endothelial dysfunction and cardiovascular disease (32, 44, 50). Indeed, in humans, the increase in cytokine levels (i.e., IL-6, IL-1β, TNF-α) is related to decreased arterial vascular tone via endothelial receptors (26). In particular, IL-6 inhibits endothelium-dependent, nitric oxide (NO)-mediated relaxation (37). Endothelial dysfunction is an early finding and marker in the development of atherosclerosis and associated with numerous risk factors for cardiovascular disease, including hypertension, coronary artery disease and sudden death and metabolic syndrome (51). A relationship between endothelial dysfunction assessed in the peripheral circulation and coronary artery dysfunction has also been evidenced (17, 45). Endothelial dysfunction has been reported after acute sleep restriction, although the mechanisms remain unclear (1, 46). All of these findings suggest that an acute exposure to TSD (i.e., one night) could represent a sufficient stimulus for triggering of endothelial dysfunction (35) that could, in turn, explain the relationship between sleep disorders and cardiovascular disease.

Other cardiovascular disturbances, such as an increase in heart rate (HR), blood pressure (BP), and sympathetic activity, have been suggested to affect endothelial function during both short and prolonged sleep deprivation (14, 32). In fact, contradictory findings suggest more complex mechanisms. Some studies have reported an increase in inflammatory markers after TSD in healthy subjects that was related to an increase in systolic BP (SBP) (28, 32, 36), probably mediated via an increase in sympathetic activity (52). Experimental studies have showed that an increase in both SBP and sympathetic activity decrease vascular reactivity (23) and increase plasma levels of endothelial cell activation markers (4, 18, 32, 39). Conversely, no changes in SBP after 24-h TSD were reported by others (38), suggesting that the contribution of increased SBP in the vascular inflammation during sleep deprivation remains to be established (6). Another study (28) rather reported a decrease in muscle sympathetic efferent nerve activity, but unchanged vascular resistance after TSD. These results ruled out the hypothesis that the pressor response after sleep deprivation was only mediated by a smooth muscle sympathetic vasoconstriction (28).

The present study was designed to determine, in healthy young subjects, the effect of 40 h of TSD *1*) on the endothelial-dependent and -independent reactivity of the cutaneous microvasculature, along with plasma levels of endothelial activation markers, and *2*) on BP, HR, autonomic activity, and catecholamine and cortisol levels to determine the possible mechanisms

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and physiological relevance of their changes with vascular dysfunction.

METHODS

Subjects

Twelve healthy men, aged 29.1 ± 3.3 yr (mean ± SD), with a body mass index of 23.4 ± 1.5 kg/m^2, were included in the study, after giving written, informed consent. Institutional Paris-Cochin ethics committee approved the protocol (N°ID RCB: 2008-A00170-55), which was conducted according to the principles expressed in the Declaration of Helsinki of 1975, as revised in 2001.

All subjects underwent a detailed medical history and examination. Exclusion criteria were as follows: shift-workers, smokers, daily consumption of alcoholic beverages, and those consuming >400 mg of caffeine per day, subjects with body mass index >30 kg/m^2, and those taking medication. A blood test was performed to assess biological parameters (glucose, total cholesterol, triglycerides, γ-glutamyltranspeptidase, and transaminases). Subjects with excessive daytime somnolence (Epworth Sleepiness Scales ≥ 9) (27), or sleep complaints (Pittsburgh sleep quality index <31 or >69) (3), or if they could not be considered as an intermediate chronotype on the Horne and Orstberg questionnaire (24) were also excluded. Sleep/wake patterns were checked using wrist actigraphy (Actiwatch, Cambridge Neurotechnology, Cambridgeshire, UK) 1 wk before the experiment. The mean subjects' total sleep duration was 8.1 ± 0.6 h (mean ± SD).

Protocol

Subjects were housed individually in a temperature-controlled bedroom (24 ± 1°C) for 5 days (Fig. 1) at the Percy Military Hospital (Clamart, France). During the first day (D0), they were familiarized with the laboratory procedures and equipped for continuous polysomnography. During D0 and the day after (D1), subjects went to bed at 2300 and awoke at 0700. The TSD started on D2 at 0700 and finished on D3 at 2300, corresponding to 40 h of continuous awakening. Subjects left the laboratory on D4 after one night of sleep recovery (2300-0700).

All measurements were performed supine at rest in the subject's bedroom with eyes open. Blood samples were collected via an indwelling venous forearm catheter at 0800, during D1, D3, and D4. Samples were immediately centrifuged at 1,100 *g*, and plasma aliquots were frozen and stored at −80°C. Baseline HR and BP recordings were performed before TSD at D1 after a 10-min stabilization period and recorded for 10 min at 0900, 1200, 1500, and 1800. Microvascular reactivity was also evaluated at 1200 and 1800. All measurements were repeated after TSD on D3.

During the experiment, except for an every day shower between 2200 and 2230, the subjects' waking states were continuously monitored for polysomnography (Embla, Broomfield, CO) and analyzed offline (Embla Somnologica for Windows XP Software) to ensure that they remained alert throughout the 40-h TSD. Central body temperature was measured from an ingested wireless capsule (VitalSense, Mini Mitter, Bend, OR). Laboratory illumination was maintained at 150–200 lux during the entire period of sleep deprivation. When not engaged in any specific testing or meal, subjects were allowed to read, watch videos, or play games, or converse with the staff or visitors. Subjects were prohibited from exercise, caffeine, tobacco, alcohol, and other psychoactive substances 24 h before and during the study. Meals and caloric intake were standardized for all subjects (2,600 kcal/day). Water was allowed ad libitum.

Hormone and Cytokine Assays

Plasma samples were assayed using commercially available ELISA kits [soluble ICAM-1, E-selectin, VCAM-1, high-sensitivity (hs) C-reactive protein (CRP), IL-6 (hs), IL-1β (hs), TNF-α (hs), R&D Systems, Minneapolis, MN; epinephrine, norepinephrine, LDN Labor Diagnostika Nord, Nordhorm, Germany]. Plasma cortisol concentrations were measured using a commercial radio-immunoassay (gamma-Coat cortisol RIA Kit, Diasorin, Stillwater, MN).

The lower limits of detection for ICAM-1, E-selectin, VCAM, CRP, IL-6, IL-1β, TNF-α, epinephrine, and norepinephrine ELISAs were 0.096 ng/ml, 0.009 ng/ml, 0.6 ng/ml, 0.059 ng/ml, 0.01 ng/ml, 0.039 pg/ml, 0.057 pg/ml, 8 pg/ml, and 5.4 ng/ml, respectively. Average intra-assay coefficients of variation were 5, 5.7, 3.5, 4.4, 6.9, 10.2, 8.5, 17.1, and 15.6%, respectively. Average interassay coefficients of variation for ICAM-1, E-selectin, VCAM, CRP, IL-6, IL-1β, and TNF-α were 5.3, 7.7, 7.7, 6.0, 9.6, 10.4, and 10.6%, respectively. The lower limit of detection was 5.9 nmol/l for the cortisol RIA, and the average inter- and intra-assay coefficients of variation were 9.8 and 7.7%, respectively.

Cardiovascular Measurements

Vascular reactivity was determined from skin blood flow (SkBF) measurements monitored by laser-Doppler flowmetry (PF5000, Perimed AB, Stockholm, Sweden), connected to two laser-Doppler probes (481-1, Perimed AB), positioned on the ventral aspect of the forearm. The laser-Doppler flowmetry technique has been described elsewhere (43). Two laser-Doppler probes specifically designed with an embedded sponge for skin iontophoresis of agonists were fixed 5 cm apart to the skin with an adhesive patch. Before each experiment, the sponge of one probe was wetted with 0.2 ml of either acetylcholine (ACh) solution (1%, 5.5 mM, acetylcholine chloride, Sigma-Aldrich, L'Isle d'Abeau, France), and the other probe with a sodium nitroprusside (SNP) solution (2%, 67 mM, Nitriate, Serb Laboratoires, Villiers de l'Isle-Adam, France). Each probe and its Ag-AgCl reference electrode (PF 384, Perimed AB) were connected to a current intensity-regulated generator (PeriIont 382, Perimed AB) for iontophoresis.

Endothelium-dependent vasodilatation in the cutaneous microcirculation was induced by ACh iontophoresis delivered at the first probe (anodal electrode). Endothelium-independent vasodilatation was induced by iontophoresis of SNP solution delivered at the second probe (cathodal electrode). For all drugs, the vehicle was deionized water (pH = 6.5). After 5 min of a baseline period, SNP and ACh were simultaneously administered by three successive current steps of 0.1 mA each during 10 s (S1, S2, and S3) with 120-s interstimulation intervals. For each probe, the electrical charge density was 2.5 mC/cm^2, and the total electrical dose was 3 mC. This protocol was designed to avoid unwanted hyperemic effects, resulting from galvanic current (5, 12). Forearm skin temperature at recording sites was assessed by thermocouples fixed to the skin, 3 cm apart from each probe, and maintained within 33 and 34°C (5).

Arterial BP was continuously recorded from the left third finger contralateral to vascular reactivity measurements by a digital servo-photoplethysmography (Portapres, Finapres Medical Systems, Am-

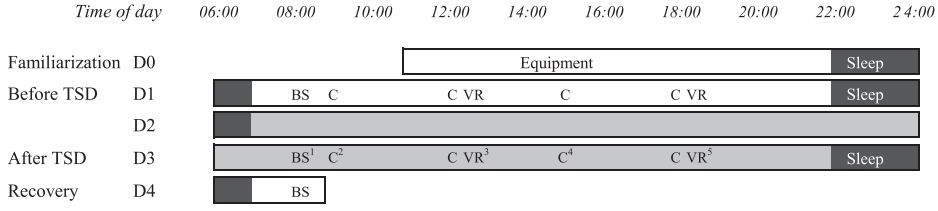

Fig. 1. Experimental design. BS, blood sample; C, cardiovascular measurements; VR, vascular reactivity assessment; D, day. [1]After 25 h of total sleep deprivation (TSD); [2]after 26 h of TSD; [3]after 29 h of TSD; [4]after 32 h of TSD; [5]after 35 h of TSD.

sterdam, The Netherlands) (25). The photoplethysmograph was automatically calibrated, and the SBP and diastolic BPs were controlled by a brachial cuff device before each test. HR was assessed by electrocardiography using a CM5 configuration (ECG 100 amplifiers, Biopac Systems, Goleta, CA). Respiratory movements of the rib cage were recorded with a piezoelectric sensor band (SS5LB, Biopac Systems).

Data Collection and Analysis

All nonfiltered signals were digitized and recorded with an analog-to-digital converter (MP150, Biopac Systems) with a sampling frequency of 1,000 Hz for offline computerized analysis.

Microvascular reactivity. Data collection began with a 5-min baseline rest period before the onset of current application and was continuously recorded for 14 min. The SkBF (expressed in arbitrary perfusion units) signal from each laser-Doppler probe was averaged every 10 s to reduce instantaneous variability owing to vasomotion. To take into account the possible changes in systemic hemodynamic conditions, cutaneous vascular conductance (CVC; expressed in perfusion units/mmHg) was calculated as the ratio of SkBF to mean BP. Baseline CVC values were calculated over the 2-min rest period before the first current stimulation (S1). Finally, all results were expressed as a percentage of the basal CVC value (5). The CVC peak was defined as the maximal value recorded after S3. The slopes of the CVC curves during the 60 s preceding and following S3 were determined by linear regression. Total SkBF was determined from the area under the curve (AUC) from the first stimulation to the end of the 5-min period after S3.

HR and BP variability. The last 5 min of artifact-free signal recorded in the supine rest position were selected for analysis. All data acquisition and analyses were carried out in accordance with established standards (47). ECG and BP waveforms were analyzed using Matlab software (Matlab V7.3, The MathWorks, Natick, MA) to obtain temporal and frequency domains components.

R-wave peaks were automatically detected from ECG signals using a validated algorithm (31), and mean HR was calculated. Power in the low-frequency (LF; 0.04–0.15 Hz) and high-frequency (HF; 0.15–0.40 Hz) ranges was computed for HR variability (HRV) and expressed in absolute (ms^2) and normalized units (nu; in %), according to the following formula: [absolute power of the component/(LF + HF)] × 100. The normalized HF component of the HRV (HF_{RR} nu) provides an estimate of the vagal tone, whereas both sympathetic and vagal tones contribute to the normalized LF component (LF_{RR} nu) (47).

Statistical Analysis

All statistical analyses were conducted using Matlab software (Matlab V7.3, The MathWorks, Natick, MA). Biological and cardiovascular variables were analyzed using nonparametric Friedman's analysis of variance, followed, when appropriate for pairwise comparisons, by Wilcoxon's test. Unless specified, for all statistics, the significance level was set at $P < 0.05$. All data are presented as means ± SE.

RESULTS

All the subjects completed the protocol. After the experiment, EEG recordings verified that each subject remained awake during the 40 h of sleep deprivation (Fig. 1). The average sleep duration during the first, second, and fourth night were 7.34 ± 0.06, 7.51 ± 0.05, and 7.71 ± 0.03 h, respectively.

Microvascular Reactivity

Response to ACh iontophoresis (Table 1, Fig. 2). No significant differences were observed for basal CVC between D3 and D1, and between values at 1800 and 1200. Compared with D1, the CVC peak ($P = 0.03$) and AUC ($P = 0.02$) were significantly lower on D3 at 1200, but not significantly at 1800. At D1 and D3, the AUC and CVC peak were significantly higher at 1800 compared with 1200 (all $P = 0.03$). The slope of the rising part of the CVC response to S3 did not differ during D3 compared with D1 at 1200 (33.7 ± 5.8 vs. 40.9.8 ± 7.5%/s) and 1800 (34.8 ± 7.9 vs. 42.3 ± 11.3%/s). However, the slope of the falling part of the CVC response to S3 was lower at D3 compared with D1 at 1200 (−15.9 ± 7.5 vs. 32.1 ± 4.45%/s, $P = 0.03$), but no significant change was observed at 1800 (−28.4 ± 6.5 vs. 33.2 ± 9.3%/s).

Response to SNP Iontophoresis

No significant difference was observed for basal CVC observed at D3 compared with D1 and observed at 1800 compared with 1200 (Table 1, Fig. 2). Compared with D1, the AUC and CVC peaks were lower at 1200 ($P = 0.02$) and 1800 ($P = 0.02$) on D3, with no significant differences between 1200 and 1800. The slope of the rising part of the CVC response to S3 was lower on D3 at 1200 (1.6 ± 0.7 vs. 0.6 ± 0.3%/s, $P = 0.02$) and 1800 (2.1 ± 0.5 vs. 0.81 ± 0.4%/s, $P = 0.01$).

Biological Variables

Compared with D1, plasma levels of E-selectin were raised on D3 ($P = 0.04$) (Table 2). After one night of sleep recovery

Table 1. *Change in basal CVC and vascular reactivity parameters before and after total sleep deprivation at 1200 and 1800*

	Before TSD (D1)		After TSD (D3)		
	1200	1800	1200	1800	Friedman *P* Value
			CVC before and after iontophoresis of ACh		
Basal CVC, 10^2 PU/mmHg	8.2 ± 1.3	6.91 ± 0.7	8.6 ± 1.39	8.47 ± 1.6	NS
CVC peak, %basal CVC	454 ± 158	774 ± 183	283 ± 163*	608 ± 139*	<0.01
AUC, $\% \cdot s^{-1} \cdot 10^{-3}$	16.7 ± 6.5	22.2 ± 6.4	8.1 ± 2.2*	18.2 ± 4.7	0.01
			CVC before and after iontophoresis of SNP		
Basal CVC, 10^2 PU/mmHg	7.6 ± 0.7	8.7 ± 0.8	7.7 ± 1.5	8.1 ± 1.2	NS
CVC peak, %basal CVC	778 ± 158	584 ± 126	476 ± 138*	316 ± 52*	<0.01
AUC, $\% \cdot s^{-1} \cdot 10^{-3}$	17.3 ± 2.9	14.7 ± 1.9	9.1 ± 2.9*	7.5 ± 1.4*	<0.01

Values are means ± SE. Acetylcholine (ACh) and sodium nitroprusside (SNP) were delivered in three (S1, S2, S3) 10-s galvanic current period (0.1 mA), with an interstimulation interval of 120-s (see Fig. 2). Basal cutaneous vascular conductance (CVC) was calculated as the average CVC observed during the 120 s before S1. Vascular reactivity parameters are CVC peak and area under the curve (AUC). CVC peak is the maximal observed within 10-s CVC following galvanic current stimulations. AUC was computed by integrating the curve from S1 to the end of the recovery period (5 min after S3). TSD, total sleep deprivation; D1 and D3, *day 1* and *day 3*, respectively; PU, perfusion units; NS, nonsignificant. *$P < 0.05$, significant vs. before TSD value observed at the same time of day.

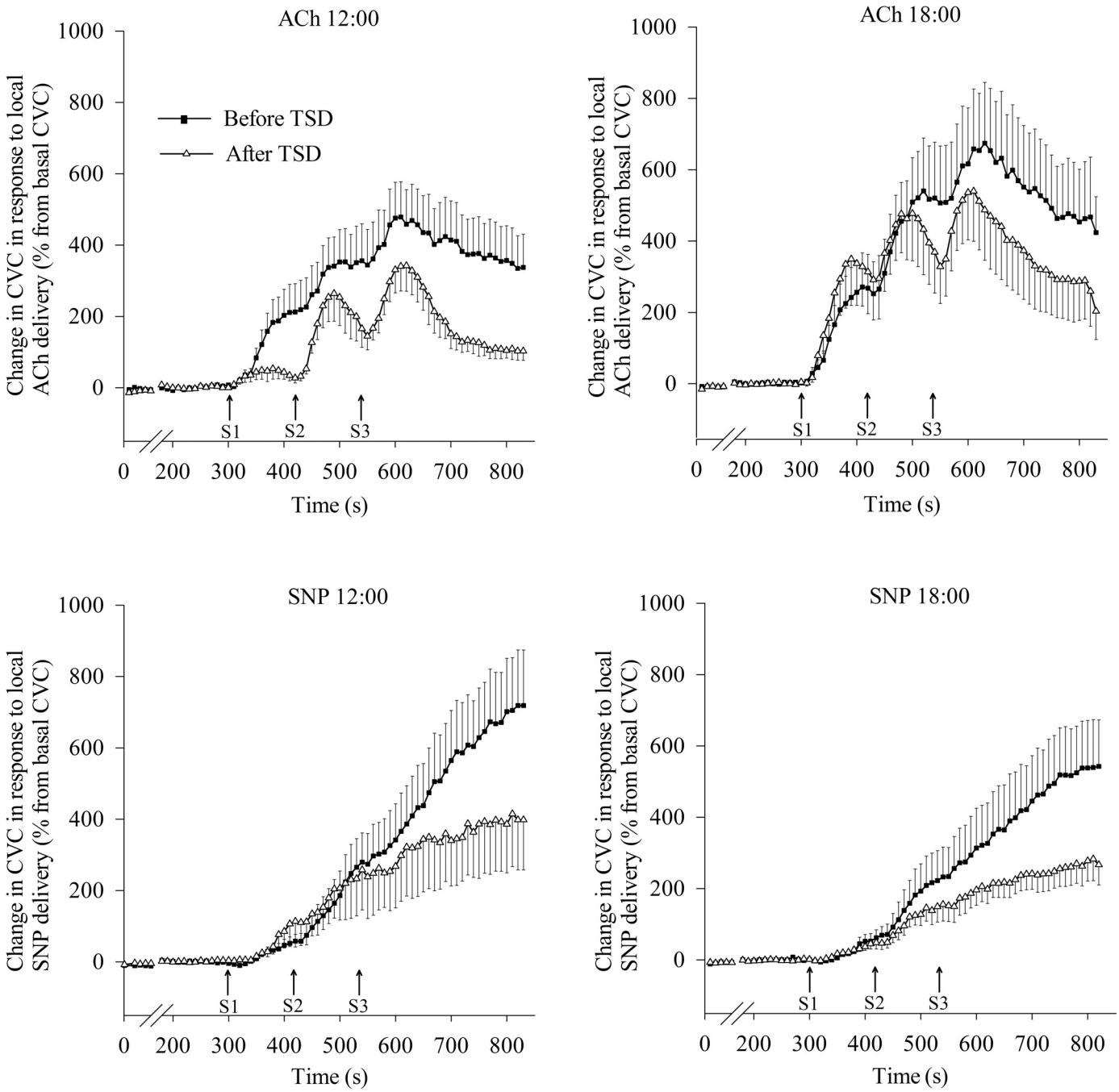

Fig. 2. Average relative increase in cutaneous vascular conductance (CVC) before and after acute sleep deprivation (TSD), after iontophoresis application of acetylcholine (ACh) and sodium nitroprusside (SNP) at 1200 and 1800. ACh and SNP were delivered in three (S1, S2, S3) 10-s current period (0.1 mA, 0.1 mC/min) with an interstimulation interval of 120 s. Basal CVC was calculated as the average CVC observed during the 120-s before S1. Values are presented as means ± SE.

(D4), ICAM-1 ($P = 0.03$), IL-6 ($P = 0.02$), and norepinephrine ($P = 0.02$) were significantly increased compared with D1. All other changes in biological variables were nonsignificant. However, >50% of IL-1β samples were below the lower limit of detection.

Cardiovascular Variables and Variability

Compared with D1, HR was increased after TSD at 1500 and 1800 (all $P = 0.02$) (Table 3). No significant changes were observed for LF+HF_{RR}, but LF_{RR} nu was higher ($P < 0.01$), whereas HF_{RR} nu was lower ($P < 0.01$) after TSD, compared with D1.

BP and Variability

At D3, we observed an increase in SBP only at 1500 ($P = 0.03$) (Table 3). During the day before TSD (D1), SBP was decreased at 1500 compared with 1200 and 1800 ($P = 0.02$). This decrease was not observed after TSD. Concerning DBP

Table 2. *Change in plasma levels of biological parameters at 0800*

	Before TSD (D1)	After TSD (D3)	Recovery (D4)	Friedman *P* Value
ICAM-1, ng/ml	215 ± 13	227 ± 15	253 ± 16*	0.02
VCAM, ng/ml	491 ± 27	489 ± 24	513 ± 34	NS
E-selectin, ng/ml	35 ± 6	54 ± 8*	42 ± 6	0.02
CRP, μg/ml	1.22 ± 0.46	0.55 ± 0.13	0.61 ± 0.14	NS
IL-6, pg/ml	0.60 ± 0.13	0.62 ± 0.10	1.20 ± 0.23*	0.03
TNF-α, pg/ml	0.88 ± 0.32	1.05 ± 0.30	0.88 ± 0.24	NS
Cortisol, mmol/l	524 ± 42	491 ± 27	503 ± 16	NS
Epinephrine, pg/ml	81 ± 16	68 ± 10	57 ± 13	NS
Norepinephrine, pg/ml	757 ± 110	885 ± 121	1,113 ± 125*	0.04

Values are means ± SE. Plasma sample were collected at 0800 before TSD, after TSD, and after one night of recovery. ICAM-1, intercellular adhesion molecule-1; CRP, C-reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α. *$P < 0.05$, significant vs. before TSD value observed at the same time of day.

and mean BP, we observed no significant changes between the values observed before and after TSD at the same hour.

DISCUSSION

To our knowledge, this is the first report on microvascular reactivity after an acute exposure to TSD. Two major findings emerged: *1*) 40 h of acute TSD decreased endothelial-dependent and -independent cutaneous vascular reactivity with an increase in plasma levels of endothelial cell activation markers; and *2*) this microvascular dysfunction occurred well before the changes in SBP, HR, and sympathetic activity reported in the literature (28, 52).

Vascular Dysfunction

Acute TSD blunted the SkBF response to ACh and SNP after TSD, suggesting that both endothelium-independent and -dependent microvascular reactivities were altered (2, 17, 33). Amir et al. (1) observed a similar decrease in vascular reactivity after a 24-h shift, including night duty, which was more marked in subjects with a longer history of night-shift duty and in those reporting fewer sleeping hours during the shift. Takase et al. (46) reported the same results in healthy students after 4 wk of chronic sleep deprivation during an examination period. In these two studies, vascular reactivity was assessed by measurement of the brachial artery flow-mediated dilatation (FMD), a surrogate marker of large-conductance artery reactivity, while our study focused on microvascular cutaneous reactivity, reflecting the reactivity in resistance vascular beds.

There are major physiological differences between the mechanisms implicated in vasodilatation induced by shear stress (i.e., FMD) and ACh iontophoresis (22, 29). Endothelium-dependent dilatation of arterioles triggered by ACh might involve NO, prostacyclin, and endothelium-derived hyperpolarizing factor, both inducing smooth muscle relaxation and vasodilatation (29). Although ACh-induced dilatation is mediated mainly by NO in large vessels via smooth muscle cyclic GMP (cGMP), the NO/cGMP pathway is not essential for ACh-induced dilatation of arterioles and for basal BP regulation (29). In vivo studies have indicated that endothelium-derived hyperpolarizing factor may serve as a backup and compensatory mechanism in conditions associated with an altered bioavailability of NO (15). This compensatory mechanism could explain why, in our study, as early as 35 h of TSD, the impairment of endothelium-independent reactivity was not associated with a significant alteration of endothelium-dependent reactivity. Moreover, acute TSD influenced the kinetics of the response to ACh, mainly the falling part of the slope of the CVC curve recorded during the third stimulation, suggesting a change in the time course of vasodilator mechanisms. For example, it has been shown that impaired prostaglandin bioavailability is characterized by a decrease in the time efficiency of ACh-induced vasodilatation (10).

In addition to this skin microvascular dysfunction, we also found changes in systemic biological markers of vascular endothelial cell activation and endothelial dysfunction (7). As reported by others after 32 h of TSD, we observed an increase in E-selectin levels after TSD (13), followed by an increase in ICAM-1 and IL-6 levels after one night of sleep recovery (i.e., D4). IL-6 is a sensitive marker of acute inflammatory response and vascular activation and high IL-6 plasma levels being associated with an increased mortality in patients at high risk of myocardial infarction (16). We found no significant changes in

Table 3. *HR and BP temporal and spectral components before and after TSD at 0900, 1200, 1500, and 1800*

	Before TSD (D1)				After TSD (D3)				
	0900	1200	1500	1800	0900	1200	1500	1800	Friedman *P* Value
HR, beats/min	65 ± 4	58 ± 2	59 ± 2	58 ± 2	66 ± 3	60 ± 2	67 ± 3*	62 ± 2*	0.001
LF+HF_{RR}, $10^2/ms^2$	22 ± 5	19 ± 5	16 ± 4	20 ± 5	20 ± 7	18 ± 3	12 ± 2	23 ± 4	NS
LF_{RR} nu,%	52 ± 6	60 ± 5	58 ± 5	52 ± 6	67 ± 5	70 ± 5	74 ± 3*	76 ± 4*	0.005
HF_{RR} nu,%	48 ± 6	40 ± 5	42 ± 5	48 ± 6	33 ± 5	30 ± 5	26 ± 3*	25 ± 4*	0.003
SBP, mmHg	109 ± 6	114 ± 4	105 ± 5	117 ± 3	114 ± 5	125 ± 7	120 ± 5*	119 ± 5	0.008
DBP, mmHg	64 ± 4	71 ± 2	61 ± 4	72 ± 4	65 ± 3	80 ± 5	72 ± 4	75 ± 4	NS
MBP, mmHg	79 ± 4	85 ± 2	75 ± 4	87 ± 4	82 ± 4	95 ± 5	88 ± 4	90 ± 4	0.012

Values are means ± SE. Changes are shown in heart rate (HR), spectral components of HR variability, systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean blood pressure (MBP). LF_{RR}, power in low-frequency range of HRV; HF_{RR}, power in high-frequency range of HRV; nu, normalized unit. *$P <$ 0.05 vs. before TSD value observed at the same time of day.

IL-6 levels after 24 h of TSD, a result consistent with previous studies (44, 50), although one study reported a decrease in IL-6 levels within 24–33 h of TSD (13). After a 2-day TSD, an increase in IL-6 was consistently reported (44, 50), whereas, in our study, an increase in IL-6 levels was observed only after the night of sleep recovery (i.e., D4). Our results suggest that at least 40 h of TSD are sufficient to increase IL-6 levels with a remnant effect after one night of sleep recovery. In our study, the delayed increases of IL-6 and ICAM-1 levels could be explained by the pattern of release of circulating adhesion molecules that has been characterized in patients with acute ischemic stroke (11). Indeed, circulating adhesion molecules might be detectable in peripheral venous blood samples between 12 and 72 h after onset of acute ischemic stroke, although with large interindividual variability (11).

BP Changes

One explanation for the vascular dysfunction after TSD could be linked to the changes in BP. In cardiovascular diseases, an increase in BP raises endothelial shear stress, inducing endothelial production of inflammatory mediators (4). However, our results rather indicated that vascular dysfunction occurred before the increase in BP. Indeed, we observed an increase in E-selectin levels after 25 h of TSD and a decrease in vascular reactivity after 29 h of TSD, without significant changes in BP and HR. Our results related to BP and HR are consistent with the findings of a recent study in controlled experimental conditions (38), where, in the absence of psychological or physical stresses, 24 h of TSD were not sufficient to induce autonomic disturbances and an increase in BP or HR. Consistent with other studies (28, 52), we found a significant increase in BP, HR, HRV, and plasma level of norepinephrine, suggesting a significant activation of sympathetic activity within 32 h of TSD.

These findings underline the fact that the vascular dysfunction induced by TSD occurred before the increase in BP or sympathetic activation. It is important to consider the circadian changes in BP in the vascular dysfunction induced by TSD (6). Indeed, sleep disturbances strongly affect both nighttime BP levels and the blunted nocturnal decline in BP (i.e., the "nondipping profile") (30). The nondipping profile has been reported to be associated with increased circulating levels of soluble endothelial adhesion molecules (6), indicating an early inflammatory vascular endothelium activation (51). Therefore, the lack of nocturnal decrease in SBP in our study could explain the acute increase in E-selectin observed as early as 24 h of TSD. In addition, a blunted nocturnal BP decline is associated with increased generation of reactive oxygen species, which are powerful mediators of atherogenesis and alter NO synthesis (41).

Inflammatory Responses

While in our study IL-1β did not reach detectable levels, some studies have shown that sleep deprivation induces immune cell production of proinflammatory markers, such as IL-1β, TNF-α, and IL-6 (13, 32). This inflammatory response, in particular for IL-1β, could also explain the decrease in endothelial-dependent and -independent vascular reactivity. Several cytokines (i.e., IL-6, TNF-α, IL1-β, IL-10) modulate arterial vascular tone via endothelial receptors (26), and cultured cells of aortic smooth muscles exposed to IL-1β displayed a reduction in SNP-stimulated cGMP levels (40). This decrease in cGMP accumulation could limit the biological effect of SNP, a NO donor (40). In IL-6-infused pregnant rats, Orshal and Khalil (37) demonstrated a reduction of the NO-cGMP pathway activity in systemic vessels that was the consequence of a decreased activity of NO synthase and guanylate cyclase of smooth muscles (37). In our study, after one night of sleep recovery, the increase in norepinephrine levels, a reliable marker of the increase in sympathetic nervous system activity induced by TSD, could also contribute to elevated inflammation via multiple pathway (35). Indeed, an increase in catecholamines is associated with increased inflammatory mediators (i.e., IL-6) in plasma (39) and in vivo models, with norepinephrine being able to upregulate, in human microvascular endothelial cells, the production of IL-6 and TNF-α (18). However, we observed no change in CRP and cortisol levels after TSD. CRP is a stable marker of the development of cardiovascular disease (42, 48) that increases with chronic sleep loss (32). There are contradictory findings on the effects of an acute TSD in healthy subjects, with some studies reporting an increase (32), a decrease (13), or no change (8). We surmised that our sleep deprivation conditions may not have lasted long enough to have an effect on CRP. Also, the effects of TSD on cortisol levels are unclear, with some studies reporting an increase (34), a decrease (49), or no change (21). Most of the discrepancies probably stem from differences in experimental conditions, such as timing of the blood samplings (i.e., circadian rhythm of cortisol) or nature of sampling (i.e., saliva vs. blood) (21).

The main limitation of our study is the lack of a control group. However, our findings did not differ from the crossover study of Pagani et al. (38), who evaluated the impact of 24 h of TSD on various hemodynamic and autonomic variables. Another limitation was the lack of continuous BP monitoring, mostly during the night, to check for possible alterations in circadian BP profile. Compared with the pre-TSD night and because of the prolonged activity during TSD, the observed increase in SBP likely reflected the lack of a decrease in SBP normally expected during sleep (30). Body posture, emotional stress, and cognitive and physical workload also influence regulatory factors, such as catecholamines that control BP and can exert significant effects on study outcomes (52). Although brachial artery FMD and the magnitude of the ACh-induced skin perfusion increase correlate (20), the present results are limited to the skin resistance vasculature. Indeed, there are major physiological differences in the mechanisms implicated in vascular reactivity between the conductance and resistance vessels and, in particular, the relative importance of the NO/cGMP pathway (29). Moreover, dysfunction of the peripheral vascular endothelium was shown to constitute an independent predictor of cardiovascular events (2). Finally, the time course of our findings are limited to the day after TSD (i.e., D4) when the changes reflecting an alteration in sympathovagal balance became significant.

In conclusion, in our controlled environmental conditions, without cognitive and physical workloads, our data suggest that acute TSD is a stimulus of sufficient strength to trigger dysfunction in the peripheral resistance vasculature of healthy subjects. This short-term response could initially result from alteration in circadian BP modulations and acute inflammatory

activation rather than an increase in SBP (i.e., not a hypertensive response). Although this is an open study, our results are relevant to human health, health risk management, and disease. Even if vascular dysfunction induced by acute TSD was mild and subclinical, we hypothesize that chronic repetition of transient sleep loss could elevate basal inflammatory mediators, thereby priming the development of cardiovascular disease (32, 42). Furthermore, the present data obtain in the cutaneous vasculature point to possible interference with thermoregulatory defense mechanisms. It should be borne in mind that our observations are limited to healthy young subjects and cannot be extrapolated to patients with already altered vascular function (e.g., with atherosclerosis, hypertension, smokers, etc.) (14). Further studies will be needed to elucidate the precise mechanisms for the vascular dysfunction and inflammatory activation induced by sleep deprivation and their recovery profile. This is important for the further understanding of the deleterious cumulative effects of sleep deprivation and also the link between cardiovascular diseases and sleep disorders.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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