

Vascular response to 1 week of sleep restriction in healthy subjects. A metabolic response? ☆,☆☆



Fabien Sauvet^{a,b,*}, Catherine Drogou^{a,b}, Clément Bougard^{a,b}, Pierrick J. Arnal^{a,b,c}, Garance Dispersyn^{a,b}, Cyprien Bourrilhon^a, Arnaud Rabat^{a,b}, Pascal Van Beers^{a,b}, Danielle Gomez-Merino^b, Brice Faraut^{b,d}, Damien Leger^{b,d}, Mounir Chennaoui^{a,b}

^a Institut de recherche biomédicale des armées (IRBA), Brétigny-sur-Orge, France

^b Université Paris Descartes, Hôtel Dieu, EA7330 VIFASOM (Vigilance, Fatigue, Sommeil), Paris, France

^c Université de Lyon, Laboratoire de Physiologie de l'Exercice, Saint Etienne, France

^d Centre du Sommeil et de la Vigilance, Hôtel Dieu, APHP, Paris, France

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ABSTRACT

Background: Sleep loss may induce endothelial dysfunction, a key factor in cardiovascular risk. We examined the endothelial function during one week of sleep restriction and a recovery period (from 3-to-13 days) in healthy subjects, and its link to autonomic, inflammatory and/or endocrine responses.

Methods: 12 men were followed at baseline (B1, 8-h sleep), after 2 (SR2) and 6 (SR6) days of SR (4-h sleep: 02:00–06:00) and after 1 (R1) and 12 (R12) recovery nights (8 h sleep). At 10:00, we assessed changes in: arm cutaneous vascular conductance (CVC) induced by local application of methacholine (MCh), cathodal current (CIV) and heat (44 °C), finger CVC and skin temperature (T_{fi}) during local cold exposure (5 °C, 20-min) and passive recovery (22 °C, 20-min). Blood samples were collected at 08:00.

Results: Compared with baseline (B1), MCh and heat-induced maximal CVC values (CVC_{peak}) were decreased at SR6 and R1. No effect of SR was observed for T_{fi} and CVC during immersion whereas these values were lower during passive recovery on SR6 and R1. From SR2 to R12, plasma concentrations of insulin, IGF-1 (total and free) and MCP-1 were significantly increased while those of testosterone and prolactin were decreased. Whole-blood mRNA concentrations of TNF-α and IL-1β were higher than B1. No changes in noradrenaline concentrations, heart rate and blood pressure were observed.

Conclusions: These results demonstrate that SR reduces endothelial-dependent vasodilatation and local tolerance to cold. This endothelial dysfunction is independent of blood pressure and sympathetic activity but associated with inflammatory and metabolic pathway responses (ClinicalTrials-NCT01989741).

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1. Introduction

Chronic decreased sleep time is notably associated with the development of cardiovascular and metabolic diseases [1,2]. However, the biological mechanisms that link short sleep duration and these diseases

Abbreviations: MCh, metacholine chloride; B, baseline (i.e., 8 h time in bed per night); CIV, cathodal induced vasodilatation; COX, cyclooxygenase; DAP, diastolic arterial pressure; EDHF, endothelium-derived hyperpolarizing factors; HR, heart rate; MAP, mean arterial pressure; NO, nitric oxide; NOS, nitric oxide synthase; PU, perfusion unit; SAP, systolic arterial pressure; SR, sleep restriction (i.e., 4 h time in bed per night); SkBF, skin blood flow; T_{arm}, arm skin temperature; T_{IB}, time in bed; TST, total sleep time; T_{co}, body temperature; T_{fi}, finger temperature.

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* Corresponding author at: NCO / Unité Fatigue & Vigilance, Institut de recherche biomédicale des armées (IRBA), BP 73, 91223 Brétigny sur Orge, France.

E-mail address: fabien.sauvet@gmail.com (F. Sauvet).

are unknown [3], and little is known about the early effects of acute sleep restriction on vascular function. In a recent study, we showed that 29 h of continuous wakefulness is a sufficient stress to decrease endothelial-dependent cutaneous vasodilatation [4] in healthy humans. Other authors have confirmed this endothelial dysfunction in healthy subjects after acute sleep deprivation [5,6] or restriction [6,7]. All these findings suggest that acute exposure to sleep loss could represent a sufficient stimulus to trigger endothelial dysfunction [1].

Endothelial dysfunction is known to be an early key factor and marker in the development of atherosclerosis and is associated with numerous risk factors for cardiovascular disease, including hypertension, coronary artery disease, vascular calcification, sudden death and the metabolic syndrome [8].

With regards the possible mechanisms linking sleep disorders to cardiovascular disease, it is thought that activation of the sympathetic nervous system induces an increase in blood pressure [9] and an endothelial dysfunction [7], probably via an increase in inflammatory

markers, as observed after acute or chronic sleep loss in healthy subjects [10–12]. However, results conflict regarding the effects of acute sleep restriction on sympathovagal balance [9,10,12]. In previous studies with healthy humans we observed that 40 h of wakefulness caused endothelial dysfunction before any increase in sympathetic activity and blood pressure [4,13], and more recently, that this endothelial dysfunction is also demonstrable in sympathectomized rats [14]. In other words, the available data still do not provide a clear picture of the role of sympathetic activation in the development of endothelial dysfunction during sleep loss and the mechanisms involved are still unclear. Moreover, little is known about the recovery kinetics of vascular dysfunction after a period of sleep loss.

As the skin is readily accessible in humans and rodents, it is ideal for assessing peripheral microvascular endothelial function and vascular reactivity using non-invasive methods [15]. The purposes of this study was to characterize the effects of sleep restriction (7 days with 4 h Time in bed, TIB), and subsequent recovery on i) vascular function, heart rate, blood pressure and sympathetic activity and ii) inflammatory and endocrinal responses.

2. Methods

2.1. Subjects

Twelve healthy men, aged 29.3 ± 5.2 yrs (mean \pm SD), with a body mass index (BMI) of 23.8 ± 2.1 kg/m², were included in the study after giving their written informed consent. The ethics committee of the Hotel Dieu – Ile de France 1 (Paris) and the Agence Nationale pour la Sécurité du Médicament (Drug safety national agency, ANSM) approved the protocol (N°ID RCB: 2012_A00399634), which was conducted according to the principles laid out in the Declaration of Helsinki of 1975, as revised in 2001.

All subjects gave a detailed medical history and underwent a full medical examination. Exclusion criteria were: shift-workers, smokers, daily consumption of alcoholic beverages and those consuming more than 400 mg of caffeine per day, subjects with a BMI greater than 30 kg/m², and those taking medication. Subjects with excessive daytime somnolence (Epworth Sleepiness Scales ≥ 9) [16] or sleep complaints (Pittsburg sleep quality index (PSQI) < 31 , or > 69) [17] were also excluded as was any volunteer not considered as an intermediate chronotype using the Horne and Orstberg questionnaire [18]. Sleep/wake patterns were checked using wrist actigraphy (Actiwatch TM, Cambridge Neurotechnology, Cambridgeshire, UK) one week before the experiment. The subjects' mean total sleep duration was 432 ± 15 min (mean \pm SD).

2.2. Sleep restriction protocol (Fig. 1)

Subjects were accommodated individually in temperature-controlled bedrooms (24 ± 1 °C) in our laboratory at the Hôtel-Dieu APHP Hospital (Paris, France) for 13 days (Fig. 1). During the first day (B0), they were familiarized with the laboratory procedures and equipped for continuous polysomnography. On B0 and the day after (B1), the subjects went to bed at 23:00 and were awoken at 07:00 (mean TST = 420 ± 18 min). The sleep restriction (SR) started on the 3rd day (SR1) and finished after 7 nights of sleep restriction (02:00 to 06:00) (mean TST = 221 ± 11 min). The subjects left the hospital after 3 recovery nights (R1 to R3; 23:00–07:00, mean TST = 447 ± 15 min) and they returned for a further 2 nights (R12, R13) after 9 days of recovery at home.

Blood samples were collected via an intravenous forearm catheter at 08:00 on B1, SR2, SR4, SR6, R1, R3 and R12. Blood samples were immediately centrifuged at 1100 G and plasma aliquots frozen and stored at -80 °C.

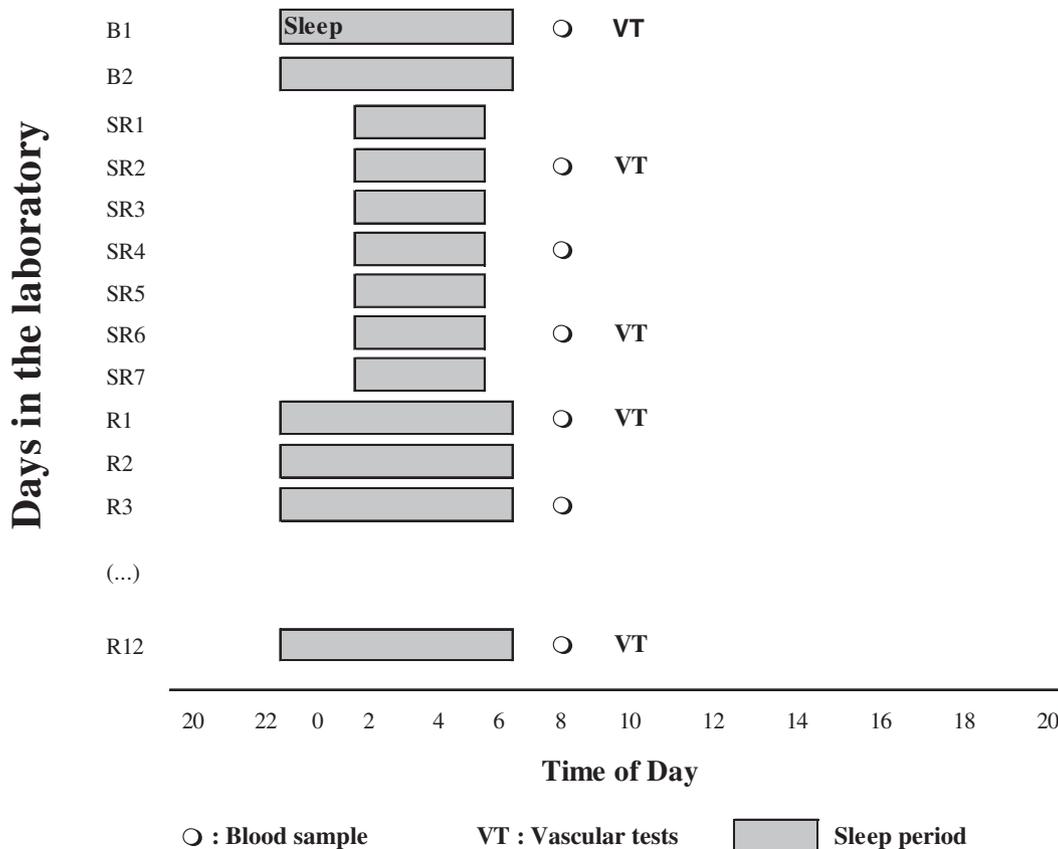


Fig. 1. Protocol for the participants. Sleep time (gray bars), blood sampling (●) and cardiovascular tests (VT) during baseline days, sleep restriction (7 days) and recovery (R).

Cardiovascular measurements were made after a 10-minute stabilization period and recorded for 10 min at 10:00 on B1, SR2, SR6, R1 and R12. Body mass (W in kg) was assessed at 07:45 on B1, SR2, SR4, SR6, R1, R3 and R12 using electronic scales (SECA se, Modèle 920, precision ± 0.2 kg). Skin folds were assessed [tricipital (Pct), underscapular (Pcs)], on B1, SR6, R3 and R12, using a Holtain skin fold caliper (Crymich, UK) (mean of five measurements of each), and the umbilical perimeter measured. Body fat and lean mass were calculated with Lohman's formula [19].

Laboratory illumination was maintained at 150–200 lx during the entire period of sleep deprivation. When not engaged in any specific testing or meals, subjects were allowed to read, watch videos, 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 (2400 kcal/day). Water was allowed ad libitum.

During the experiment the polysomnographic variables (3 electroencephalograms, electrocardiograms, 2 electrooculograms and 2 electromyogram derivations) were continuously monitored (Actiwave®, CamNtech Ltd England) and analyzed offline (Somnologica TM, Medcare, Reykjavik, Iceland).

2.3. Cardiovascular measurements

The tests were performed in a temperature-controlled room [23 ± 2 °C; 30–50% relative humidity] throughout the experiment. The subjects wore only shorts, socks and a T-shirt and rested quietly in a sitting position in a comfortable armchair.

2.3.1. Endothelial function (endothelial-dependent vasodilation) (Fig.2.)

Skin blood flow (SkBF) changes were monitored using three laser-Doppler flowmetry probes (481-1, Perimed AB) connected to a laser-Doppler unit (PF5000, Perimed AB, Stockholm, Sweden). The three probes were fixed on the ventral aspect of the right forearm with a double-sided adhesive patch.

Two laser-Doppler probes were coupled to an iontophoretic annular electrode with an embedded sponge (1.2 cm²) filled with the drugs and connected to a closed current intensity-regulated iontophoretic generator (Perilont 382, Perimed AB). Before the experiment, the first sponge was wetted with 0.2 ml of a freshly prepared 1% (10 mM) methacholine chloride solution (MCh, TCI Europe, Zwijndrecht, Belgium), dissolved in de-ionized water. The second probe was wetted with 0.2 ml of ionized water (ph = 6.5). The MCh was delivered at the anode while the ionized water was delivered at the cathode. The vascular response to MCh administration [20], a synthetic and stable ester of acetylcholine, induces cutaneous vasodilatation via endothelium-dependent releases of a variety of vasoactive substances such as nitric oxide (NO), prostacyclin (PGI₂) and endothelium-derived hyperpolarizing factors (EDHF) [21, 22]. Iontophoretic cathodal current administration, although not painful, induces current-induced vasodilation (CIV) dependent on COX-1 activation [23].

The 3rd laser Doppler probe was heated at 44 °C for 30 min. Local heat application induces cutaneous vasodilation caused initially by the axonal reflex and, for the late, part by local NO production. Low MCh and heat-induced cutaneous vasodilation are used as a surrogate marker of cardiovascular risk and endothelial dysfunction [22].

The surface temperature of the arm (Tarm) at the recording sites was monitored by thermocouples attached to the skin, 3 cm distant from the probes (MLT 422/A, 0 °C to 50 °C, accuracy ± 0.1 °C, Ad instruments, United Kingdom).

All SkBF and Tarm signals were digitized (1000 Hz sampling frequency) using a computerized acquisition system (PowerLab, ADInstruments Ltd, Oxford, UK). Experiments were performed at stable skin temperatures ranging from 33 °C to 34 °C [24]. Recordings started only if the SkBF control values were between 20 and 100 PU. Data collection began

with a 5 min control period before the heat (44 °C) was applied and continued for 40 min. Ten minutes after the recordings began, MCh and cathodal current were administered simultaneously, in 4 bursts (10-second) of iontophoretic currents of 0.1 mA with a two-minute interval between each current burst. This validated iontophoretic procedure, gives no blood flow return to baseline between sequential current applications [21]. For each probe, the electrical charge density was 2.5 mC/cm² and the total electrical dose administered was 4 mC. This protocol was designed to avoid non-specific hyperemic effects resulting from the anodal current [20].

The SkBF signal (expressed in arbitrary perfusion units — PU) 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 PU/mmHg) was calculated as the ratio of SkBF to mean blood pressure (MBP). Baseline values were obtained by averaging values for the last 2 min of the 5-min control period. Finally, all results were expressed as a percentage of the basal CVC value (% Baseline) [24]. The CVC_{peak} was defined as the maximal value recorded after the start of heat application for iontophoretic stimulation. The area under the curve (AUC) was also calculated from the end of the control period to the end of the test.

2.3.2. Local tolerance to cold (finger cold immersion test)

The skin temperature and SkBF of the pulp of the index finger on the left hand (Tfi2) were continuously recorded using, respectively, a thermocouple (MLT 422/A, 0 °C to 50 °C, accuracy ± 0.1 °C, Ad instruments, United Kingdom) and a submersible laser-Doppler probe (PF 405, Perimed, Jarfalla, Sweden) fixed with adhesive tape (Blenderm, 3 M, USA).

The subject then immersed his left index in a cold water bath maintained at 5 °C (Ministat 125, Huber, Illkirch, France) for 20 min (Finger Cold Water Immersion, CWI). Following CWI the subject removed his hand from the water bath and dried it with a towel before the 20-min passive rest rewarming period (Recovery) began.

The main vasoconstrictor response of the human skin exposed to cold is mediated by a rapid and selective increase in α 2-adrenoreceptor (α 2-AR) activity mediated by the Rho-associated kinase pathways [25].

All of the signals were recorded using the same method as described for Endothelial function. The baseline was obtained by averaging values for the last 2 min of the 5-min control period. For each subject CVC and temperatures (Tco, Tarm and Tfi) were calculated for the last 2 min of the control period, for the 20-min CWI period and for the 20-min rewarming period in ambient temperature (passive recovery). Local tolerance to cold, assessed by examining the following variables [13]: minimal Tfi value and maximal value observed during all the CWI period (Tfi_{min}, Tfi_{max}), the number of occurrences of cold induced vasodilation (CIVD) defined as a 0.5 °C change in skin temperature, the Tfi value at the end of the rewarming period (Tfi_{recovery}) and finally, the Tfi and CVC area under the curve (Tfi_{AUC} and CVC_{AUC}) were determined for the 20-min CWI and rewarming periods.

During the test the volunteers were asked to rate their pain using a continuous pain scale from 0 (“no pain at all”) to 10 (“unbearable pain”) every 5 min.

2.3.3. Heart rate and blood pressure measurements

During the experiment, arterial blood pressure (BP) was continuously recorded from the ring finger of the right arm using a digital servo-photoplethysmograph (Portapres, Finapres Medical Systems, Amsterdam, The Netherlands). The photoplethysmograph was automatically calibrated and the systolic and diastolic blood pressures were controlled with a brachial cuff device before each test. Electrocardiogram (ECG) was assessed by electrocardiography using a CM5 configuration (BioAmp, AdInstruments). ECG and BP waveforms were analyzed using Matlab software (Matlab V7.3, The MathWorks Inc.,

Natick, MA, USA) to obtain temporal and frequency domain components, in accordance with established standards [26]. R-wave peaks were automatically detected from ECG signals using a validated algorithm [27] 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 were computed for HR variability (HRV) and expressed in absolute (ms²) 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, with both sympathetic and vagal tone contributing to the normalized LF component (LF_{RR} nu) [26].

2.4. Biological measurements

Whole blood was collected from each subject into clot activator/plastic serum, K2EDTA (EDTA) (Vacutainer) and PAXgene Blood RNA (PreAnalytiX) tubes using a standard phlebotomy technique.

2.4.1. Hormone and cytokine assays

Plasma and serum samples were assayed using commercially available ELISA kits. IL-6 (R&D, ref HS600B), TNF-α (R&D, HSTA00D), PGE₂ (Assays Design, ADJ-900-001), IL-8 (Invitrogen, ref KHC008), IGF-1 (IDS AC-27 F1), free IGF1 (R&D DPG100), testosterone (IBL, RE52151), insulin (IBL RE53171), noradrenaline (LDN, BA E 54400) and adrenalin (LDN, BAE 5400) levels were assessed from plasma. Lower detection limits were 0.039 pg/ml, 0.106 pg/ml, 13.4 pg/ml, 0.1 pg/ml, 3.1 ng/ml, 0.015 ng/ml, 0.22 ng/ml, 2 ng/ml, 0.015 ng/ml, 0.243 nmol/l, 1.76 μU/ml and 0.243 nmol/l respectively. sE-Selectin (R&D, DSLE00), growth hormone (IBL, DB59121), MIP-1α (Invitrogen, KAC2201), MCP-1 (R&D, DCP00) and sICAM (R&D, DCD540) levels were also assessed from serum. Lower limits of detection were 0.009 ng/ml, 0.1 pg/ml, 2 pg/ml, 1.7 pg/ml and 0.096 ng/ml, respectively.

2.4.2. Whole-blood mRNA isolation and reverse transcription

RNA from blood collected in PAXgene tubes was extracted using the PAXgene Blood RNA Kit (PreAnalytiX) according to the manufacturer's instructions. RNA from EDTA blood was extracted using acidic organic phenol extraction and silica membrane clean-up. RNA was immediately stored at -80 °C. RNA quantity was measured with a Nanodrop spectrophotometer ND-1000 (Thermo Scientific). Reverse transcription was performed using the RT2 HT First Strand kit (Qiagen). The reaction was carried out using 1 μg of RNA. The cDNA was stored at -80 °C until use.

2.4.3. Real time PCR

PCR was carried out with custom RT2 Profiler Arrays in combination with RT2 SYBR Green mastermix (SA Biosciences Qiagen, Venlo, Netherlands) using 1 μL of cDNA in a final volume of 25 μL. The cDNA sequences (human) for IL-1β, TNF-α, IL-6 and IGF-1 were from GenBank (accession numbers NM_000576, NM_000594, NM_000600 and NM_000618, respectively), as were those of the two reference genes Hprt1 and RPLP0 (accession numbers NM_000194 and NM_001002). Reactions were performed on a LightCycler 480 (96-well block) and the crossing point values were calculated using LightCycler Software v3.5 (Roche Applied Science, Mannheim, Germany). Amplification specificity was checked using melting curve analysis following the

manufacturer's instructions. The genomic DNA control (GDC), the reverse-transcription control (RTC) and the positive PCR control (PPC) were tested for each sample for genomic DNA contamination, reverse transcription efficiency and the polymerase chain reaction, respectively. Assays for two housekeeping genes included in the arrays enabled normalization of data. The mean normalized mRNA (i.e., mRNA/mRNA_{housekeeping genes}) values are presented.

2.5. Statistical analysis

All statistical analyses were conducted using Matlab software (Matlab V7.3. The MathWorks Inc. Natick, MA, USA). Biological and cardiovascular variables were analyzed using non-parametric Friedman's analysis of variance (2 factors: time and day) followed, when appropriate, by pairwise comparisons using a Wilcoxon's test. For all statistics the significance level was set at p < 0.05. Variations from B1 (Δ) were calculated as [(value - B1) / B1]. Spearman rank order correlations between Δ values were then made. All data are presented as means ± SE.

3. Results

We observed a significant change in body weight (X² = 35.1, p < 0.001) with higher values after 6 and 7 days of sleep restriction compared to baseline (SR6: +0.8 ± 0.2 Kg and SR7: +0.7 ± 0.2 Kg, p < 0.05 for all). No changes in % fat mass or umbilical perimeter were observed.

3.1. Endothelial function (endothelial-dependent vasodilation)

Sleep restriction (Fig. 3) had a significant effect on MCh-induced vasodilation (Friedman X² = 33.7, p < 0.001) with lesser values of CVC_{peak} compared to baseline (B1) at 10:00 on SR6 (p < 0.05) and R1 (p < 0.05). We also observed a decrease in heat-induced vasodilation (CVC_{peak}) after 6 nights of sleep restriction (SR6) and after 1 recovery night (R1). No changes in cathodal current-induced vasodilation were observed during sleep restriction or recovery. Sleep restriction had no significant effect on baseline CVC values.

3.2. Local tolerance to cold (finger cold immersion test)

No effect of SR (Fig. 4) was found for Tfi and CVC parameters during the control period at ambient temperature or during the 20 min of immersion in the 5 °C cold water. Nevertheless, when compared to B1, lesser values of Tfir and AUC at SR6 and R1 (p < 0.05) were observed during the passive recovery period. No effect of sleep restriction and recovery was observed on Tarm or Tco values. There was a strong correlation between the maximal Tfi value in recovery (Tfi_{recov}) and the CVC_{peak} induced by local heat application (Heat 44 °C) (R² = 0.5, p < 0.001) and a significant increase in subjective pain was also observed during immersion of the finger in cold water (0.3 ± 0.3 vs. 3.3 ± 0.5, p < 0.01, Friedman Chi-square = 385.2, p < 0.001) but no effect of sleep restriction was observed.

3.3. Heart rate and blood pressure measurements

An increase of BP, HR, LFnu and LF/HF and a decrease of HFnu, SDNN and RMSSD were seen 3 min after immersion in cold water although no

Vascular tests	Parameter (unit)	5	10	15	20	25	30	35	40	45
1 Iontophoresis ^a MCh	CVC _{MCh} (PU)	Control		Ionto. ^a						
2 Iontophoresis ^a cathodal current	CVC _{Cath} (PU)	Control		Ionto. ^a						
3 Heat 44°C	CVC _{Heat} (PU)	Control	Heat 44°C							
4 Immersion 5°C	Tfi _{Cold} (°C), CVC _{Cold} (PU)	Control	Immersion 5°C			Passive recovery				

Fig. 2. Flow chart of vascular measurements. ^a4 iontophoretic electric stimulations (0.1 mA during 10 s every 2 min). Abbreviations: MCh: Methacholine, CVC: cutaneous vascular conductance, PU: perfusion unit, Tfi: finger temperature.

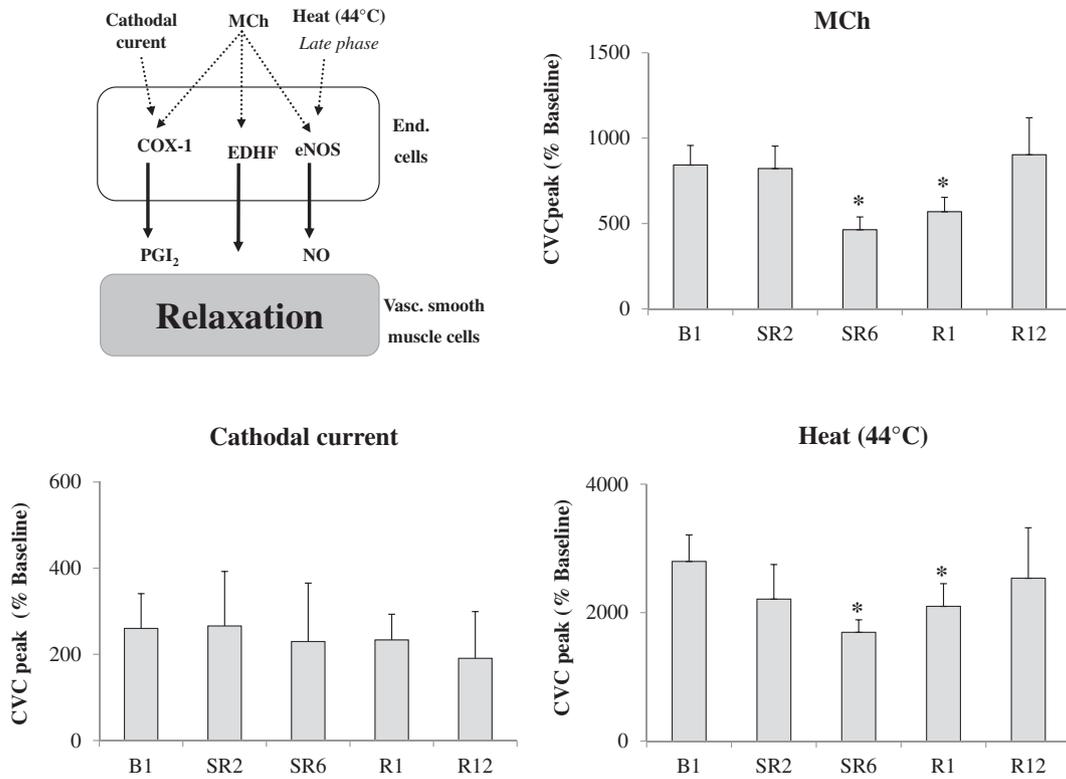


Fig. 3. Changes in cutaneous vascular conductance (CVC_{peak}, % Baseline) induced by methacholine (MCh), cathodal current and heat (44 °C) application at Baseline (B1), 2 and 6 days of sleep restriction (SR2 and SR6) and 1 and 12 days of recovery (R1 and R12). MCh stimulates both cyclooxygenase-1 (COX-1), EDHF and endothelial nitric oxide (NO) synthase (eNOS); cathodal current only COX-1 pathway and local heat application (for the late phase, up to 20–30 min) the NO production.*difference with B1 (p < 0.05).

effect of SR was observed in the control or cold water immersion values (data not shown).

3.4. Cytokine assays

MCP-1 plasma concentrations were higher on SR2, SR6 and R1 compared to B1 (p < 0.05, Friedman chi-square = 66.9, p < 0.001). We observed a significant decrease of TNF-α only on SR6 (Friedman Chi-square = 58.7, p < 0.001). No effect of SR on IL-6 and IL-8 plasma concentrations was observed (Fig. 5).

3.5. Hormone assays (Fig. 6)

Insulin plasma concentrations were higher during SR2 to R3 compared to B1 (p < 0.05, Friedman chi-square = 133.8, p < 0.001). We also showed an increase of total IGF-1 after SR2 which continued to

R12 (Friedman Chi-square = 23.7, p < 0.0) and an increase in free IGF-1 between SR2 and SR6 (Friedman Chi-square = 31.3, p < 0.05).

Compared to B1, prolactin plasma concentrations were lower from SR to SR6 and testosterone from SR4 to SR6 (Friedman Chi-square = 50.4 and 42.3, respectively, p < 0.01). No effect of SR on noradrenaline, sE-selectin, GH, VEGF, PGE₂, MIP-1 and sICAM was observed (Table 1).

Delta (Δ) values [(SR6 values – B1value) / B1value] Spearman rank order correlations coefficients are presented in Table 2. No correlations were found with prolactin or GH.

3.6. Whole-blood mRNA expression (Fig. 7)

TNF-α and IL-1β whole blood mRNA concentrations increased from SR2 to R13 compared to B1 (Friedman Chi-square = 18.1, p < 0.01 and 15.9, p = 0.01, respectively). For IL-6 mRNA concentrations, we

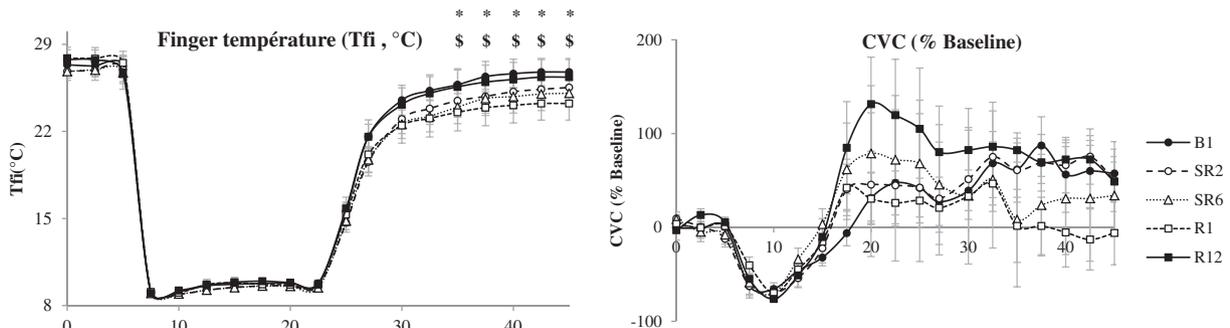


Fig. 4. Changes in finger temperature (Tfi, °C) and Cutaneous vascular conductance (CVC, % Baseline) induced by finger immersion in cold water (5 °C) and passive recovery (ambient air) at Baseline (B1), 2 and 6 days of sleep restriction (SR2 and SR6) and 1 and 12 days of recovery (R1 and R12).

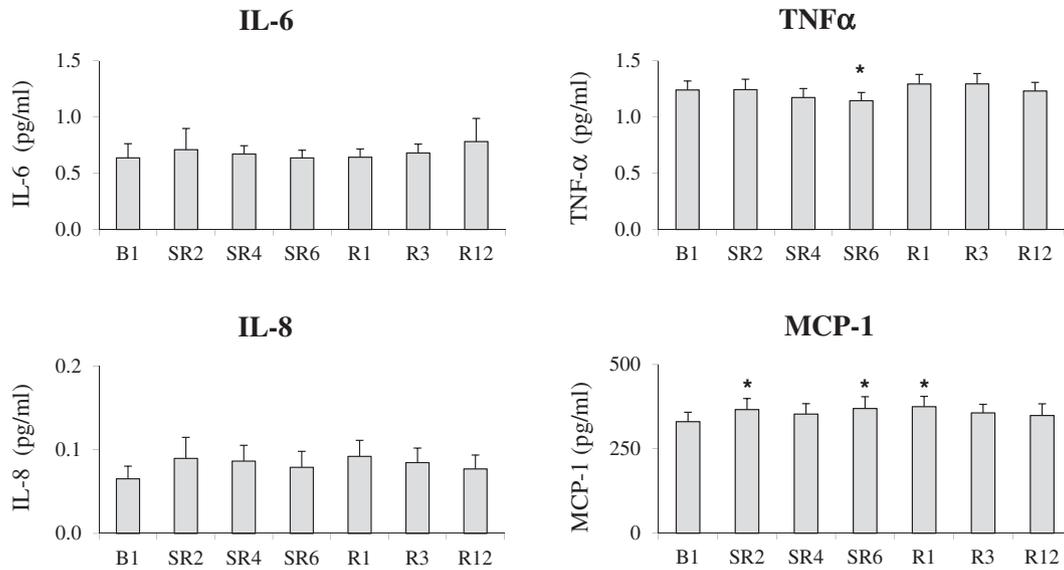


Fig. 5. Changes in circulating concentrations of IL-6, TNF- α , IL-1 β and MCP-1 at baseline (B1), during the 6 days of sleep restriction (SR2 to SR6) and the 12 days of recovery (R1 to R12). *difference with B1 value ($p < 0.05$).

observed a significant “day” effect (Friedman Chi-square = 15.9, $p = 0.001$) but no significant post-hoc result compared to B1.

4. Discussion

Using an integrative approach, the present report describes the mechanisms by which the bioavailability of the endothelium-derived vascular reactivity is altered during acute sleep loss in healthy subjects. To our knowledge this is the first report to show that this endothelial dysfunction persists even after a recovery night. Two major findings have emerged: i) the microvascular dysfunction occurred even without changes in systolic blood pressure, heart rate and sympathetic activity as reported in the literature; ii) this was associated with important and persistent changes in metabolic and endocrine responses.

We observed a blunted vascular response to MCh and local heat application during the 6 days of SR and also during recovery after the cold exposure test, suggesting that endothelium-dependent microvascular reactivity had been altered [15,20]. This result confirms the Dettoni et al. [7] description of a decrease in ACh-induced vasodilation and the decrease of flow-mediated dilation (FMD) described by Calvin et al. [28] after one week of sleep restriction. Takase et al. [6] reported the same results in healthy students after 4 weeks of chronic sleep deprivation during an examination period. In addition, Amir et al. [5] also observed a decrease in vascular reactivity after a single 24-hour shift, particularly in subjects with a long history of working night shifts. For the first time, we have shown that this endothelial dysfunction persists after one 8-hour night of sleep recovery. The endothelial dysfunction

could in part explain the higher risk of exercise-related injuries observed after sleep restriction in healthy subjects [29]. Moreover this result also reinforces the idea that endothelial dysfunction could be an independent predictor of cardiovascular events [30].

Since both heat and MCh-induced vasodilation were decreased, but not the COX-1, our results suggest that the NO and EDHF pathways are altered by sleep restriction. The NO pathway is the main pathway for heat and MCh-vasodilation in the micro-vascular cutaneous vessels [24] although in vivo studies have indicated that EDHF may serve as a back-up and compensatory mechanism in conditions associated with altered bioavailability of NO particularly in the elderly [31].

It has been suggested that the endothelial dysfunction observed after SR could be triggered by factors such as inflammation or increased sympathetic activity [32]. In our study, we found both increased MCP-1 plasma concentrations and whole-blood mRNA production of IL-1 β and TNF- α pro-inflammatory cytokines after 2 and 6 nights of sleep restriction and after one night of sleep recovery, but no effect on the circulating IL-1 β and TNF- α concentrations that signal a low-grade inflammatory response. Recently Irwin et al. [33] observed that a single night of sleep restriction (4 h of sleep) activates cellular inflammation (IL-6 and TNF- α monocyclic spontaneous expression) and the monocyte intra-nuclear signal transducer and activator of the transduction family protein (STAT 1 and 5) expression. These changes persist after a recovery night (8 h of sleep).

The results presented here differ from previous studies on total sleep deprivation in which authors observed a higher inflammatory response with increased concentrations of circulating markers of endothelial cell

Table 1

Changes in circulating concentrations of sE-selectin, VEGF, PGE₂, MIP-1 α and sICAM.

	B1	SR2	SR4	SR6	R1	R3	R12
sE-selectin, ng/ml	17.0 \pm 1.9	17.6 \pm 2.2	16.1 \pm 2.3	16.1 \pm 2.1	19.1 \pm 2.6	16.6 \pm 1.7	17.6 \pm 2.1
VEGF, pg/ml	18.8 \pm 4.0	17.9 \pm 3.4	15.5 \pm 3.3	16.2 \pm 3.6	15.7 \pm 3.1	17.9 \pm 3.8	18.2 \pm 3.5
PGE ₂ , pg/ml	389 \pm 57	369 \pm 68	374 \pm 49	392 \pm 69	421 \pm 91	337 \pm 67	397 \pm 66
MIP-1 α , pg/ml	1.3 \pm 0.2	1.4 \pm 0.3	1.3 \pm 0.3	1.3 \pm 0.3	1.7 \pm 0.4	1.5 \pm 0.4	1.5 \pm 0.3
sICAM, ng/ml	196 \pm 25	203 \pm 25	199 \pm 21	205 \pm 24	206 \pm 24	206 \pm 23	199 \pm 22

Values are mean \pm SE. Abbreviations: VEGF = Vascular endothelial growth factor, PGE₂ = Prostaglandin E2, Macrophage inflammatory protein 1 alpha = MIP-1 α , sICAM = intercellular adhesion molecule 1.

Table 2
Delta (Δ) values Spearman rank order correlation coefficients.

	Δ Heat _{peak}	Δ T _{recov}	Δ Weight	BMI	Fat mass	Δ Total IGF-1	Δ Free IGF-1	Δ MCP-1	Δ Testost.	Δ Insulin
Δ MCh _{peak}	0.22	0.42*	-0.46*	-0.36	-0.61*	0.55*	0.40*	0.37	-0.63*	0.25
Δ Heat _{peak}		0.52*	-0.39*	0.21	-0.23	0.18	-0.44*	0.17	0.25	0.18
Δ T _{recov}			0.21	-0.24	-0.16	0.41*	-0.10	-0.20	-0.12	0.61*
Δ Weight				0.35	0.57*	0.23	-0.37*	-0.11	0.40*	0.11
BMI, kg.m ⁻²					0.50*	-0.43*	-0.62*	-0.61*	0.66*	-0.15
Fat mass, %						-0.26	-0.36	-0.17	0.57*	-0.22
Δ Total IGF-1							0.87*	0.35	-0.59*	0.34
Δ Free IGF-1								0.29	-0.52*	0.15
Δ MCP-1									-0.65*	-0.14
Δ Testosterone										0.14

N = 48. *p<0.05. Δ = (SR6value - B1value) / B1value. NO correlation was found with prolactin.

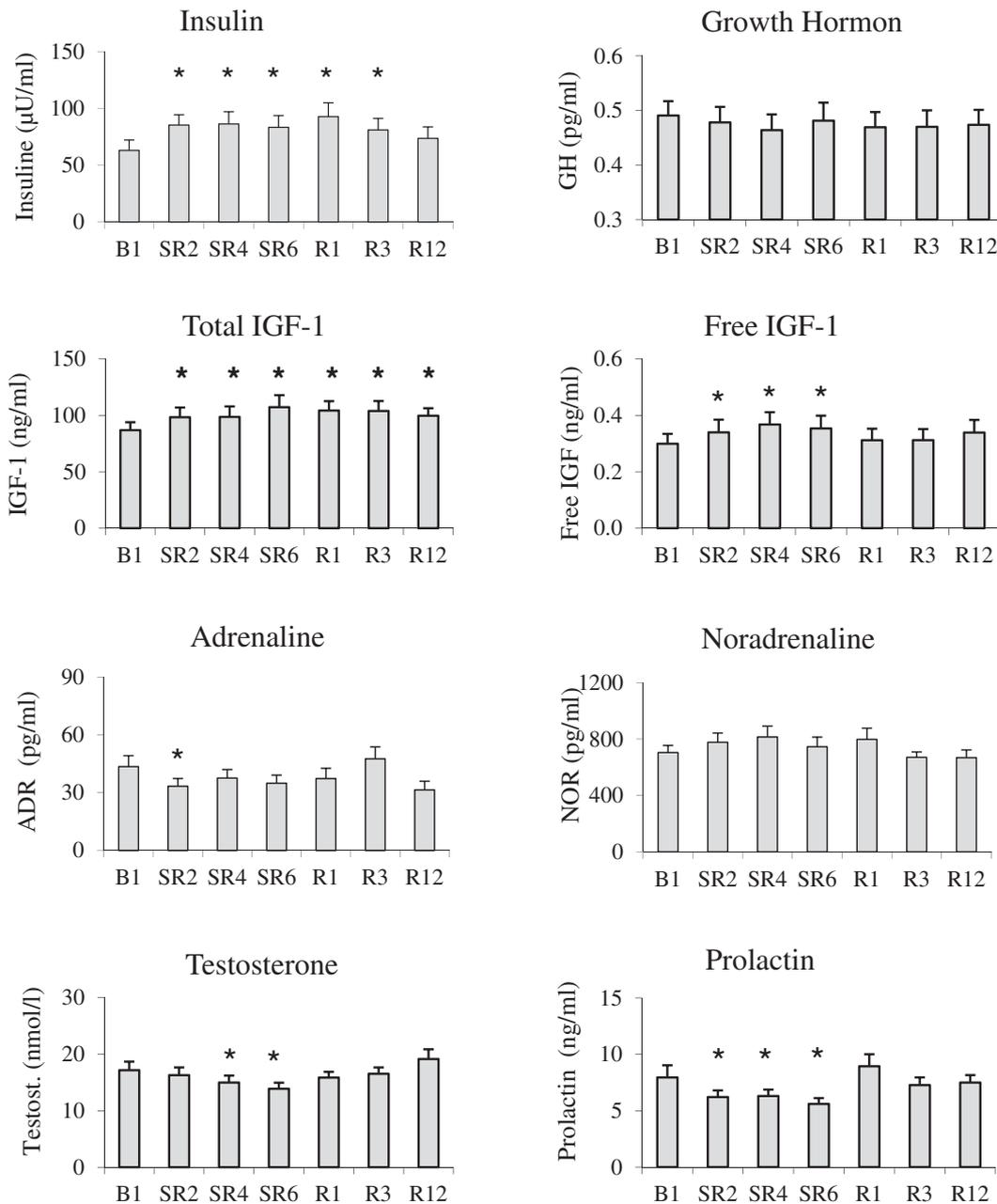


Fig. 6. Changes in circulating concentrations of insulin, GH, total IGF-1, free IGF-1, adrenaline, noradrenaline, testosterone and prolactin at baseline (B1), during the 6 days of sleep restriction (SR2 to SR6) and the 12 days of recovery (R1 to R12). *difference with B1 ($p < 0.05$).

activation such as ICAM-1 and E-selectin [4,34] and in pro- and anti-inflammatory cytokines such as TNF- α , IL-1 β , interleukin-1 receptor antagonist (IL-1Ra) and interleukin-6 (IL-6) [29,34,35]. We can thus suggest that 4 h of sleep per night seems to limit inflammatory response. As several cytokines (i.e., IL-6, TNF- α , IL-1- β ...) modulate arterial vascular tone via endothelial receptors, it has been suggested that inflammation is an important trigger of endothelial dysfunction [36,37] and it was suggested that the inflammatory response observed after acute total sleep deprivation or restriction [35,38] was implicated in endothelial dysfunction [4,14]. In the present study we have shown that 6 days of sleep restriction in healthy subject induce endothelial dysfunction, independent of any marked inflammatory responses.

We also observed increased circulating concentrations of insulin and IGF-1 (total and free) during the sleep restriction period, and for the first time we have demonstrated the persistence of increased insulin concentrations even after 3 recovery nights. Many studies have demonstrated that acute sleep restriction induces an insulin resistance associated with increased basal and post-prandial concentrations of circulating glucose [39,40]. Taken together, these findings suggested the existence of a robust link between decreased slow wave sleep (SWS) and glucose tolerance. Decreased glucose tolerance appears to result from decreased glucose utilization both by the brain and by the peripheral tissues associated with an increased food intake [41]. Indeed sleep loss increases leptin and ghrelin plasma concentrations and hunger [40,41]. As proposed by Copinschi et al. [41], increased insulin concentrations are probably an adaptive response to increased insulin resistance and hyperglycemia.

Recent studies have demonstrated that IGF-1 receptors are a critical negative regulator of insulin sensitivity and NO bioavailability in the endothelium [42]. The effects of sleep restriction on IGF-1 are not well known. Contrary to our results, a decrease in IGF-1 plasma concentration after total sleep deprivation or chronic sleep restriction [38,43,44] has been observed. We could therefore hypothesize that, as with insulin, the increased concentrations of IGF-1 (both total and free) observed after one week of sleep restriction are linked to an adaptive response to IGF-1 receptor resistance.

Our results also show that metabolic responses are associated with changes in testosterone and prolactin concentrations that could favor endothelial dysfunction. As observed previously, we confirm that five nights of sleep restriction decrease morning testosterone and prolactin concentrations [45,46] although Reynolds et al. [47] observed no change ($p = 0.09$) in total testosterone after 5 nights of sleep restriction (4 h of sleep: 04:00–08:00).

Low testosterone concentration is an independent determinant of endothelial dysfunction in men via its direct effect on the endothelium through sex hormone receptors in the cytosol and nuclear compartment [48,49]. This explains the significant correlation observed in our study between the variation in testosterone plasma concentrations and MCh_{peak} ($R^2 = 0.62$, $p < 0.05$). Testosterone is also an important modulator of insulin sensitivity and mitochondrial function in men and low testosterone concentrations are known to be associated with high insulin resistance though deregulation of fatty acid metabolism [50,51].

The circadian rhythm of prolactin is altered by total sleep deprivation and sleep restriction [46,52]. We observed decreased prolactin concentrations after SR, whereas other authors observed increased concentrations [53]. Diurnal fluctuations of prolactin concentrations are associated with decreased endothelial function occurring early in the morning in men with hypertension [54].

Few studies have assessed the effect of acute sleep restriction on blood pressure and autonomic activity in healthy subjects. Contrary to total sleep deprivation that induces increased HR, blood pressure [4] and altered cardiovascular reactivity to acute stressors in humans [55], we observed no effect of sleep restriction on the vascular parameters during rest measurements or the response to local cold exposure. An increased HR has been observed after a longer sleep restriction protocol (10 nights) [11]. However, after one [56] or 5 [7] nights of sleep restriction in real life conditions in the workplace, these authors observed an increase in catecholamine concentrations and the HRV sympathetic index whereas we showed no changes in adrenaline and decreased concentrations of noradrenaline in our laboratory-controlled conditions.

Increased sympathetic activity is a well-known trigger for endothelial dysfunction [57] and increased plasma concentration markers of

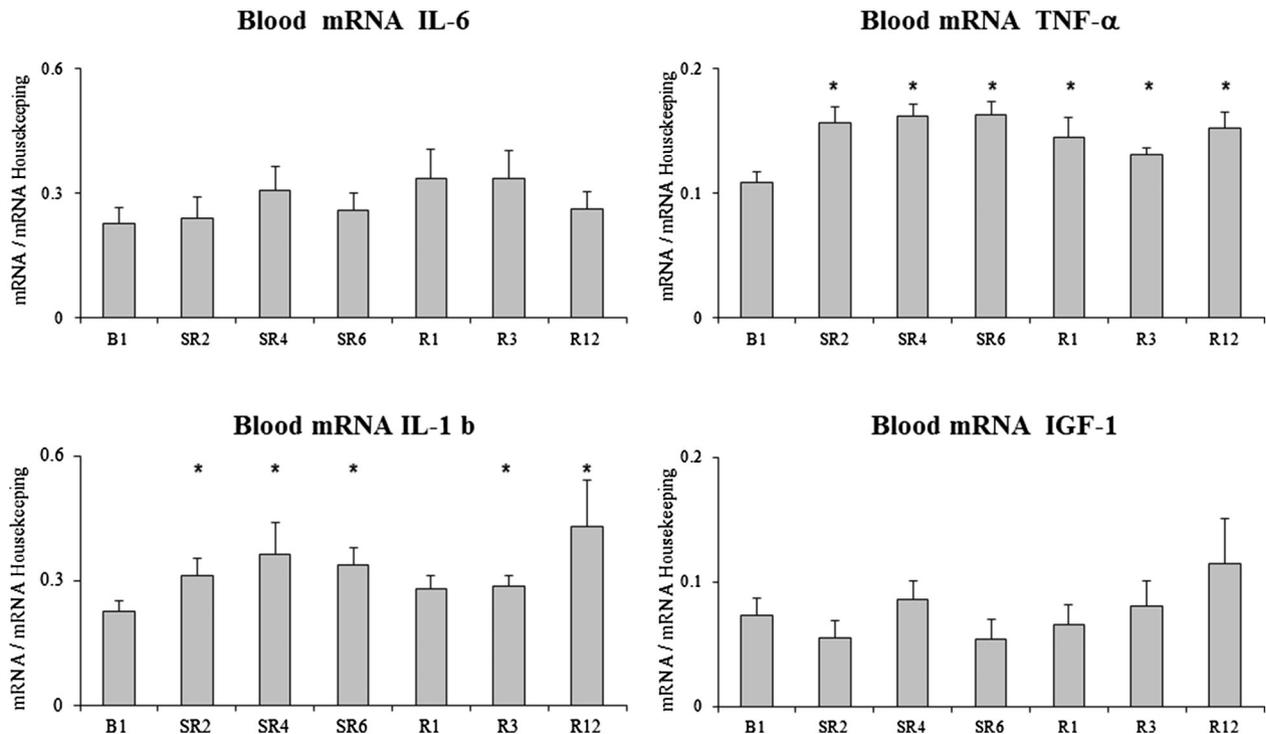


Fig. 7. Changes in IL-6, TNF- α , IL-1 β , and IGF-1 whole-blood mRNA concentrations observed at baseline (B1), during the 6 days of sleep restriction (SR2 to SR6) and the 12 days of recovery (R1 to R12). *difference with B1 value at ($p < 0.05$).

endothelial cell activation [11]. However, in this study (in humans) and a previous study with rodents we have shown that endothelial dysfunction and decreased local tolerance to cold occur after sleep deprivation without any increased sympathetic activity and/or increase in blood pressure [4,13,14]. So our results confirm that in healthy subjects 6 days of sleep loss are a sufficient stress to induce a sympathetic-independent endothelial dysfunction. Nevertheless, increased sympathetic activity, as observed in real-life sleep restriction in a stressful condition could be an aggravating factor for endothelial dysfunction.

5. Conclusions

Our data suggest that acute SR 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 metabolic and moderate inflammatory responses to sleep deprivation. Even if vascular dysfunction induced by acute SR is mild and subclinical, our results point to possible interference with thermoregulatory defense mechanisms. Furthermore, we hypothesize that chronic repetition of transient sleep loss could elevate basal inflammatory mediators and insulin resistance, thereby priming the development of cardiovascular and metabolic diseases [11,58]. Further studies are needed to elucidate the precise mechanisms of the vascular dysfunction and metabolic response induced by sleep restriction and their recovery profile. This is important for improved understanding of the deleterious cumulative effects of sleep loss and also the link between cardiovascular diseases and sleep disorders.

Contributors

FS made a contribution in the conception and design, acquisition of data, analysis and drafting the article. CD, CB, PA, GD, CB, AR, PVB, and BF made a contribution in data acquisition and analysis. DG-M made a contribution in data analysis and drafting the article, DL made a contribution to the design of the study. MC made a contribution in the conception and design of the study, drafting and final approval of the article.

Conflict of interest

This was not an industry supported study. The authors report no relationships that could be construed as a conflict of interest.

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References

- [1] J.M. Mullington, M. Haack, M. Toth, J.M. Serrador, H.K. Meier-Ewert, Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation, *Prog. Cardiovasc. Dis.* 51 (2009) 294–302.
- [2] A. Dominguez-Rodriguez, P. Abreu-Gonzalez, The link between sleep duration and inflammation: effects on cardiovascular disease, *Int. J. Cardiol.* 173 (2014) 600–601.
- [3] J.E. Gangwisch, S.B. Heymsfield, B. Boden-Albala, R.M. Buijs, F. Kreier, T.G. Pickering, et al., Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey, *Hypertension* 47 (2006) 833–839.
- [4] F. Sauvet, G. Leftheriotis, D. Gomez-Merino, C. Langrume, C. Drogou, P. Van Beers, et al., Effect of acute sleep deprivation on vascular function in healthy subjects, *J. Appl. Physiol.* 108 (2010) 68–75.
- [5] O. Amir, S. Alroy, J.E. Schliamser, I. Asmir, A. Shiran, M.Y. Flugelman, et al., Brachial artery endothelial function in residents and fellows working night shifts, *Am. J. Cardiol.* 93 (2004) 947–949.
- [6] B. Takase, T. Akima, A. Uehata, F. Ohsuzu, A. Kurita, Effect of chronic stress and sleep deprivation on both flow-mediated dilation in the brachial artery and the intracellular magnesium level in humans, *Clin. Cardiol.* 27 (2004) 223–227.
- [7] J.L. Dettoni, F.M. Consolim-Colombo, L.F. Drager, M.C. Rubira, C. Cavasin de Souza SB, M.C. Irigoyen, et al., Cardiovascular effects of partial sleep deprivation in healthy volunteers, *J. Appl. Physiol.* 113 (2012) 232–236.
- [8] J.A. Vita, J.F. Keane Jr., Endothelial function: a barometer for cardiovascular risk? *Circulation* 106 (2002) 640–642.
- [9] P. Lusardi, A. Zoppi, P. Preti, R.M. Pesce, E. Piazza, R. Fogari, Effects of insufficient sleep on blood pressure in hypertensive patients: a 24-h study, *Am. J. Hypertens.* 12 (1999) 63–68.
- [10] M. Kato, B.G. Phillips, G. Sigurdsson, K. Narkiewicz, C.A. Pesek, V.K. Somers, Effects of sleep deprivation on neural circulatory control, *Hypertension* 35 (2000) 1173–1175.
- [11] H.K. Meier-Ewert, P.M. Ridker, N. Rifai, M.M. Regan, N.J. Price, D.F. Dinges, et al., Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk, *J. Am. Coll. Cardiol.* 43 (2004) 678–683.
- [12] Y. Ogawa, T. Kanbayashi, Y. Saito, Y. Takahashi, T. Kitajima, K. Takahashi, et al., Total sleep deprivation elevates blood pressure through arterial baroreflex resetting: a study with microneurographic technique, *Sleep* 26 (2003) 986–989.
- [13] F. Sauvet, C. Bourrilhon, Y. Besnard, A. Alonso, J.M. Cottet-Emard, G. Savourey, et al., Effects of 29-h total sleep deprivation on local cold tolerance in humans, *Eur. J. Appl. Physiol.* 112 (2012) 3239–3250.
- [14] F. Sauvet, G. Florence, P. Van Beers, C. Drogou, C. Lagrume, C. Chaumes, et al., Total sleep deprivation alters endothelial function in rats: a nonsympathetic mechanism, *Sleep* 37 (2014) 465–473.
- [15] E. Tesselar, F. Sjöberg, Transdermal iontophoresis as an in-vivo technique for studying microvascular physiology, *Microvasc. Res.* 81 (2011) 88–96.
- [16] M.W. Johns, A new method for measuring daytime sleepiness: the Epworth sleepiness scale, *Sleep* 14 (1991) 540–545.
- [17] D.J. Buysse, C.F. Reynolds III, T.H. Monk, S.R. Berman, D.J. Kupfer, The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research, *Psychiatry Res.* 28 (1989) 193–213.
- [18] J.A. Horne, O. Ostberg, A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms, *Int. J. Chronobiol.* 4 (1976) 97–110.
- [19] T.G. Lohman, R.A. Boileau, B.H. Massey, Prediction of lean body mass in young boys from skinfold thickness and body weight, *Hum. Biol.* 47 (1975) 245–262.
- [20] F. Sauvet, G. Mahe, M. Chennaoui, C. Langrume, M. Vasseur, P. Abraham, et al., Acetylcholine chloride as a potential source of variability in the study of cutaneous vascular function in man, *Microvasc. Res.* 82 (2011) 190–197.
- [21] H. Debbabi, P. Bonnin, P.H. Ducluzeau, G. Leftheriotis, B.I. Levy, Noninvasive assessment of endothelial function in the skin microcirculation, *Am. J. Hypertens.* 23 (2009) 541–546.
- [22] C. Puissant, P. Abraham, S. Durand, A. Humeau-Heurtier, S. Faure, G. Leftheriotis, et al., Reproducibility of non-invasive assessment of skin endothelial function using laser Doppler flowmetry and laser speckle contrast imaging, *PLoS ONE* 8 (2013) e61320.
- [23] S. Gohin, D. Sigaudo-Roussel, A. Conjard-Duplany, L. Dubourg, J.L. Saumet, B. Fromy, What can current stimulation tell us about the vascular function of endogenous prostacyclin in healthy rat skin in vivo? *J. Invest. Dermatol.* 131 (2011) 237–244.
- [24] J.L. Cracowski, C.T. Minson, M. Salvat-Melis, J.R. Halliwill, Methodological issues in the assessment of skin microvascular endothelial function in humans, *Trends Pharmacol. Sci.* 27 (2006) 503–508.
- [25] S.R. Bailey, A.H. Eid, S. Mitra, S. Flavahan, N.A. Flavahan, Rho kinase mediates cold-induced constriction of cutaneous arteries: role of alpha2C-adrenoceptor translocation, *Circ. Res.* 94 (2004) 1367–1374.
- [26] Task Force. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, *Circulation* 93 (1996) 1043–1065.
- [27] A.I. Manriquez, Q. Zhang, An algorithm for QRS onset and offset detection in single lead electrocardiogram records, *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2007 (2007) 541–544.
- [28] A.D. Calvin, N. Covassin, W.K. Kremers, T. Adachi, P. Macedo, F.N. Albuquerque, et al., Experimental sleep restriction causes endothelial dysfunction in healthy humans, *J. Am. Heart Assoc.* 3 (2014).
- [29] M. Chennaoui, P.J. Arnal, F. Sauvet, D. Léger, Sleep and exercise: a reciprocal issue? *Sleep Med. Rev.* 20 (2015) 59–72.
- [30] P.O. Bonetti, L.O. Lerman, A. Lerman, Endothelial dysfunction: a marker of atherosclerotic risk, *Arterioscler. Thromb. Vasc. Biol.* 23 (2003) 168–175.
- [31] M.L. Gaubert, D. Sigaudo-Roussel, M. Tartas, G. Berrut, J.L. Saumet, B. Fromy, Endothelium-derived hyperpolarizing factor as an in vivo back-up mechanism in the cutaneous microcirculation in old mice, *J. Physiol.* 585 (2007) 617–626.
- [32] L. Palagini, R.M. Bruno, A. Gemignani, C. Baglioni, L. Ghiadoni, D. Riemann, Sleep loss and hypertension: a systematic review, *Curr. Pharm. Des.* 19 (2013) 2409–2419.
- [33] M.R. Irwin, T. Witaranta, M. Caudill, R. Olmstead, E.C. B., Sleep loss activates cellular inflammation and signal transducer and activator of transcription (STAT) family proteins in humans, *Brain Behav. Immun.* (2014) <http://dx.doi.org/10.1016/j.bbi.2014.09.017> (in press).
- [34] D.J. Frey, M. Fleshner, K.P. Wright Jr., The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults, *Brain Behav. Immun.* 21 (2007) 1050–1057.
- [35] A.N. Vgontzas, E. Zoumakis, E.O. Bixler, H.M. Lin, H. Follett, A. Kales, et al., Adverse effects of modest sleep restriction on sleepiness, performance, and inflammatory cytokines, *J. Clin. Endocrinol. Metab.* 89 (2004) 2119–2126.
- [36] P.O. Iversen, A. Nicolaysen, K. Kvernebo, H.B. Benestad, G. Nicolaysen, Human cytokines modulate arterial vascular tone via endothelial receptors, *Pflugers Arch.* 439 (1999) 93–100.
- [37] J.M. Orshal, R.A. Khalil, Interleukin-6 impairs endothelium-dependent NO-cGMP-mediated relaxation and enhances contraction in systemic vessels of pregnant rats, *Am. J. Physiol.* 286 (2004) R1013–R1023.
- [38] M. Chennaoui, C. Drogou, F. Sauvet, D. Gomez-Merino, D.E. Scofield, B.C. Nindl, Effect of acute sleep deprivation and recovery on Insulin-like Growth Factor-I responses

- and inflammatory gene expression in healthy men, *Eur. Cytokine Netw.* 25 (2014) 52–57.
- [39] L. Klingenberg, J.P. Chaput, U. Holmback, T. Visby, P. Jennum, M. Nikolic, et al., Acute sleep restriction reduces insulin sensitivity in adolescent boys, *Sleep* 36 (2013) 1085–1090.
- [40] K. Spiegel, K. Knutson, R. Leproult, E. Tasali, E. Van Cauter, Sleep loss: a novel risk factor for insulin resistance and Type 2 diabetes, *J. Appl. Physiol.* 99 (2005) 2008–2019.
- [41] G. Copinschi, R. Leproult, K. Spiegel, The important role of sleep in metabolism, *Front. Horm. Res.* 42 (2014) 59–72.
- [42] A. Abbas, H. Imrie, H. Viswambharan, P. Sukumar, A. Rajwani, R.M. Cubbon, et al., The insulin-like growth factor-1 receptor is a negative regulator of nitric oxide bio-availability and insulin sensitivity in the endothelium, *Diabetes* 60 (2011) 2169–2178.
- [43] C.A. Everson, W.R. Crowley, Reductions in circulating anabolic hormones induced by sustained sleep deprivation in rats, *Am. J. Physiol. Endocrinol. Metab.* 286 (2004) E1060–E1070.
- [44] M. Monico-Neto, H.K. Antunes, M. Dattilo, A. Medeiros, H.S. Souza, K.S. Lee, et al., Resistance exercise: a non-pharmacological strategy to minimize or reverse sleep deprivation-induced muscle atrophy, *Med. Hypotheses* 80 (2013) 701–705.
- [45] R. Leproult, E. Van Cauter, Effect of 1 week of sleep restriction on testosterone levels in young healthy men, *JAMA* 305 (2011) 2173–2174.
- [46] S.M. Schmid, M. Hallschmid, K. Jauch-Chara, H. Lehnert, B. Schultes, Sleep timing may modulate the effect of sleep loss on testosterone, *Clin. Endocrinol.* 77 (2012) 749–754.
- [47] A.C. Reynolds, J. Dorrian, P.Y. Liu, H.P. Van Dongen, G.A. Wittert, L.J. Harmer, et al., Impact of five nights of sleep restriction on glucose metabolism, leptin and testosterone in young adult men, *PLoS ONE* 7 (2012) e41218.
- [48] M. Akishita, M. Hashimoto, Y. Ohike, S. Ogawa, K. Iijima, M. Eto, et al., Low testosterone level is an independent determinant of endothelial dysfunction in men, *Hypertens. Res.* 30 (2007) 1029–1034.
- [49] J.M. Orshal, R.A. Khalil, Gender, sex hormones, and vascular tone, *Am. J. Physiol.* 286 (2004) R233–R249.
- [50] N. Pitteloud, V.K. Mootha, A.A. Dwyer, M. Hardin, H. Lee, K.F. Eriksson, et al., Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men, *Diabetes Care* 28 (2005) 1636–1642.
- [51] S.A. Kaplan, E.D. Crawford, Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men, *Diabetes Care* 29 (2006) 749 (author reply -50).
- [52] P. Schussler, M. Uhr, M. Ising, J.C. Weikel, D.A. Schmid, K. Held, et al., Nocturnal ghrelin, ACTH, GH and cortisol secretion after sleep deprivation in humans, *Psychoneuroendocrinology* 31 (2006) 915–923.
- [53] M.L. Andersen, P.J. Martins, V. D'Almeida, M. Bignotto, S. Tufik, Endocrinological and catecholaminergic alterations during sleep deprivation and recovery in male rats, *J. Sleep Res.* 14 (2005) 83–90.
- [54] K.S. Stamatelopoulos, G.A. Georgiopoulos, P.P. Sfikakis, G. Kollias, E. Manios, E. Mantzou, et al., Pilot study of circulating prolactin levels and endothelial function in men with hypertension, *Am. J. Hypertens.* 24 (2011) 569–573.
- [55] H. Yang, J.J. Durocher, R.A. Larson, J.P. Dellavalla, J.R. Carter, Total sleep deprivation alters cardiovascular reactivity to acute stressors in humans, *J. Appl. Physiol.* 113 (2012) 903–908.
- [56] E. Tobaldini, C. Cogliati, E.M. Fiorelli, V. Nunziata, M.A. Wu, M. Prado, et al., One night on-call: sleep deprivation affects cardiac autonomic control and inflammation in physicians, *Eur. J. Intern. Med.* 24 (2013) 664–670.
- [57] M.L. Hijmering, E.S. Stroes, J. Olijhoek, B.A. Hutten, P.J. Blankestijn, T.J. Rabelink, Sympathetic activation markedly reduces endothelium-dependent, flow-mediated vasodilation, *J. Am. Coll. Cardiol.* 39 (2002) 683–688.
- [58] P.M. Ridker, High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease, *Circulation* 103 (2001) 1813–1818.