Supplementary methods and data

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Healthy volunteers exposed to fourteen nights of intermittent hypoxia

We used sera from a double-blind randomized cross-over study (ClinicalTrials.gov, Identifier: NCT02058823) performed in 12 healthy volunteers, to compare exposure to intermittent hypoxia and intermittent air. Intermittent hypoxia (IH) is obtained by delivering O_2 for 15 seconds every 2 minutes during sleep in a hypoxic tent, where subjects breathed hypoxic air with a 13% FiO_2, to create 30 cycles/hour [1]. Peripheral oxygen saturation range was 85-95%. Subjects were exposed during 8_9h/night, for 14 nights. Intermittent air (IA) is obtained by exposing subjects to the same air flow but sleeping in a normoxic (FiO_2 21%) tent. Six volunteers were exposed to IH for 14 nights, followed by a wash-out period, and 14 nights of IA; while 6 volunteers were exposed to 14 nights IA first, and then to 14 nights in the 2nd period after the wash-out. Blood samples were collected from fasted volunteers in the morning, before and after 2 weeks of overnight exposure to IA or to IH, and stored at -80°C. Only seven out of the 12 volunteers completed the two periods of 14 nights and the blood collection, and were therefore included for sVE analysis. All participants provided written informed consent approved by the ethical committee Nord Ouest III (October 10th 2011, ref CPP 2011-32; EudraCT:2010-020801-34) and CNIL approval.

Chemical and inhibitors

We used 0.1% DMSO (as a vehicle control), 30µM genistein (a broad-spectrum tyrosine kinase inhibitor)[2], 10µM PP2 (a Src-kinase inhibitor)[2], 1µM saracatinib (AZD0530, a Src-kinase inhibitor) [3], 1µM 2-Methoxyestradiol (an HIF-1 inhibitor devoid of estrogen-like activity) [4, 5], 1µM acriflavine (an HIF-1 inhibitor)[6], 5µg/mL pazopanib (VEGF receptor tyrosine kinase inhibitor) [7], 100µM Tempol (a superoxide dismutase mimetic ROS scavenger) [8, 9], which were all obtained from Sigma-Aldrich (Darmstadt, Germany). Mouse anti-hVEGF blocking antibody (0.5µg/mL) [10] was obtained from R&D Systems (Minneapolis, USA). The concentrations used were chosen according to the literature, and cell viability was verified after the treatment of HAEC cells (data not shown).

Cell culture and exposure to intermittent hypoxia

Cells were exposed to fast IH cycles using a recently developed device that mimics the hypoxia-reoxygenation cycles of OSAS patients, as previously described [11]. An IH cycle includes a 5 minute normoxia phase (16% O_2) followed by a 5 minute hypoxia phase (2% O_2). CO_2 was kept constant at 5%. Special plates with gas-permeable bottom membranes (Lumox^{TM} 50mm dishes, Sarstedt, Nümbrecht, Germany; or Fluorcarbon Imaging 24-well Plates, Zell Kontakt, Gottmadingen, Germany) were coated with collagen I (0.2mg/mL, 1h at 37°C) before cell seeding. Cells exposed to IH were compared to control cells cultured at 16% constant oxygen level using the same device and same plates.

For permeability and FITC-dextran experiments, cells were cultured in permeable polystyrene membrane inserts (6.5mm diameter) with 0.4µm membrane pore size (Corning, Tewkesbury, USA) coated with collagen I (0.2mg/mL, 1h at 37°C). HAEC were seeded at $50x10^3$ cells per insert and proliferated for 3 days until they reached 100% confluence and formed a confluent monolayer with endothelial junctions before IH exposure.

For sVE detection, HAEC were seeded at 350 x 10^3 cells per dish in 60mm-dishes coated with collagen I, and cultivated for 3 days to reach 100% confluence before IH exposure.

Western Blot

Cell pellets were lysed in lysis buffer (20 mM Tris HCl, 0.27M sucrose, 0.5% Triton, 1 mM dithiothreitol, 1 mM EDTA, 1 mM EGTA, 1 mM Na3VO4, 25 µg/mL leupeptin, 1 µg/mL pepstatin A), centrifuged at 14,000g for 10 minutes at 4°C and the resulting supernatant was collected.

Sera or cell extracts were separated by 7% SDS-PAGE and transferred onto a polyvinylidene difluoride (PVDF) membrane using a Trans–Blot turbo transfer system. Membranes were incubated with a mouse monoclonal anti–VE-cadherin primary antibody (BV9 clone, 1µg/mL, Merck Millipore) or a rabbit anti-

phosphoY^{685}-VE-cadherin antibody (1µg/mL, kindly provided by Dr I. Vilgrain), or a mouse anti–β actin primary antibody. After secondary antibody incubation, membranes were revealed by chemiluminescence using a ChemiDoc XRS^{+} imager system. PVDF membranes, transfer system, enhanced chemiluminescence detection reagents and the detection system were from Bio-Rad Laboratories (Marnes-la-Coquette, France). Bands were quantified by densitometry using ImageJ software.

Supplementary table S1 : Baseline characteristics of OSAS patients and controls. N=31 for control subjects and n=43 for OSA patients.

Variables	Control subjects	OSAS patients	P-value
Male sex, number (%)	23 (71.9)	38 (88.4)	0.13
Age (years) median [Q1;Q3]	51 [46.25; 59]	54.5 [48; 63]	0.21
BMI (kg/m²) median [Q1;Q3]	24.2 [22.12; 26.12]	25.6 [23.72; 27.7]	**0.02**
sVE median [Q1;Q3]	0.52 [0.43 ; 0.92]	0.90 [0.57 ; 1.06]	**<0.01**
AHI (events/hour) median [Q1;Q3]	5.37 [3; 12.25]	33.06 [23.43; 45.45]	**<0.01**
SBP (mmHg) median [Q1;Q3]	121 [115.5; 128.5]	128.5 [117.25; 132.75]	0.1
DBP (mmHg) median [Q1;Q3]	75 [69.5; 78.5]	79 [75; 84]	**0.02**
VEGF (pg/mL) median [Q1;Q3]	237.2 [91.4; 408.3]	263.15 [137.3; 433.45]	0.41
CRP (mg/L)	0.9 [0.5; 1.4]	1.4 [0.7; 2.4]	0.07
Glycaemia (mmol) median [Q1;Q3]	4.8 [4.7; 5]	4.9 [4.7; 5.2]	0.18
Triglycerides (g/L)	0.8 [0.6; 1.2]	1.1 [0.8; 1.5]	**0.04**
Total cholesterol (g/L)	2 [1.8; 2.2]	2.2 [1.9; 2.5]	0.08

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