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Intermittent hypoxia activated cyclooxygenase pathway: role in atherosclerosis

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Abstract

Intermittent hypoxia (IH), the main stimulus of obstructive sleep apnea (OSA), induces inflammation, leading to early atherosclerosis. Whether the cyclooxygenase (COX) pathway contributes to IH-induced atherosclerosis remains to be determined.

We studied the effects of 8 week-IH exposure on COX-pathway gene expression and atherosclerosis, and the influence of COX-1 inhibition by SC-560 on atherosclerosis progression in aortas of $ApoE^{-/-}$ mice. Urinary 11-dehydrothromboxane B_2 (11-$dTXB_2$) was assessed in 50 OSA free of cardiovascular risk factor (CVRF) matched for age and body mass index with 25 controls, and 56 OSA with CVRF.

IH significantly increased atherosclerotic lesion sizes, mRNA levels of COX-1 and thromboxane synthase (TXBS). Lesion sizes correlated to COX-1 ($r=0.654$, $p=0.0003$) and TXBS ($r=0.693$, $p<0.0001$) mRNA levels. COX-1 inhibition reduced lesion progression in IH mice only ($p=0.04$). Urinary 11-$dTXB_2$ was similar in OSA free of CVRF and controls, but was increased by 13% ($p=0.007$) in OSA with CVRF compared to OSA without.

Although OSA itself was not associated to increased urinary 11-$dTXB_2$ concentration, COX-1 pathway was activated in IH exposed mice and in OSA presenting CVRF, and may contribute to IH-induced atherogenesis. COX-1 inhibition could be of clinical interest in the prevention of cardiovascular morbidity in OSA.

Key words: thromboxane, cyclooxygenase, intermittent hypoxia, obstructive sleep apnea, atherosclerosis.

Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent episodes of partial or complete upper airway obstruction occurring during sleep, leading to chronic intermittent hypoxia (CIH). This hallmark is the main factor involved in cardiovascular remodelling in OSA[1]. In OSA patients, early signs of atherosclerosis correlated to hypoxia severity[2], even after adjustment for confounding factors. OSA is associated with increased cardiovascular morbidity and mortality, and is identified as an independent cardiovascular risk factor (CVRF)[3]. Moreover, exposure of apolipoprotein E-deficient ($ApoE^{-/-}$) mice[4, 5]and C57BL/6J mice[6] to CIH accelerated atherogenesis progression.

Atherosclerosis is a chronic inflammatory disease. Among the many inflammatory mediators involved in atherogenesis, we previously demonstrated the contribution of arachidonic acid (AA)-derived metabolites in OSA patients. Notably, OSA patients exhibited elevated levels of leukotriene B_4[7, 8] and cysteinyl-leukotrienes[9], that were associated with vascular remodelling. However, the cyclooxygenase (COX)-dependant pathway of AA metabolism was poorly studied in OSA patients, and its implication in CIH-induced atherogenesis is unknown.

Thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) are two COX-derived metabolites of AA metabolism. TXA_2 is predominantly generated by platelets through COX type 1 isoform (COX-1) and thromboxane synthase (TXBS). TXA_2 is quickly metabolized on thromboxane B_2 (TXB_2) and 11-dehydrothromboxane B_2 (11-$dTXB_2$), two metabolites respectively quantifiable in plasma and urine. TXA_2 binding on its TP receptors (TPr) induces platelet activation, vasoconstriction, vascular smooth muscle cell proliferation and increases expression of adhesion molecules[10]. The production of PGI_2 mainly depends on endothelial COX-2 and prostacyclin synthase (PGIS). PGI_2 is rapidly metabolized on 6-ketoPGF1α in plasma and 2,3-dinor-6ketoPGF1α (PGI-M) excreted in urine. In contrast to TXA_2, PGI_2 binding on its IP receptors (IPr) inhibits platelet aggregation and vasoconstriction, and

reduces chemotaxis and expression of adhesion molecules[10]. Thus, TXA_2 and PGI_2 have antagonist properties.

A recent growing body of evidence suggests a major role of the COX pathway in the pathogenesis and progression of atherosclerosis. In fact, pharmacological inhibition of COX pathway[11, 12] or genetic deletion[13, 14] delayed atherosclerosis in different mouse models of atherosclerosis. Furthermore, the urinary 11-dTXB$_2$/PGI$_2$-M ratio is enhanced in elderly subjects with past history of serious cardiovascular event (stroke and myocardial infarction)[15]. Since selective COX-2 inhibitors may have non-favourable effects on this ratio and increase cardiovascular risk[16], the role of COX-1 inhibition has received less attention. In addition, data in OSA are very limited and conflicting showing either an increased[17] or a decreased[18] urinary ratio 11-dTXB$_2$/PGI$_2$-M. Thus, the aim of the present study was to characterize the COX pathway in ApoE$^{-/-}$ mice exposed to CIH and in OSA patients, and to investigate its link with CIH-induced atherogenesis and OSA-associated early vascular remodelling.

Materiel and methods

Experimental animal study

Male ApoE$^{-/-}$ mice (14 week-old) were purchased from the Charles River laboratories (L'Arbresle, France). All animal procedures were conducted in accordance with European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Council of Europe, European Treaties ETS 123, Strasbourg, 18 March 1986) and to the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996).

In a first series of experiments, mice were randomized to 8 weeks of CIH (cyclic 21-5% FiO_2, 60s cycle, 8h/d) or air (N, normoxic), as previously described[1] (n=15 in each group). In a second series of experiments, mice were exposed to 8 weeks of CIH or N (n=20 in each group), and randomized to receive placebo or the selective COX-1 inhibitor SC-560 (Interchim®, Montluçon, France) (15 mg/kg daily[12]) in their food, for the four last weeks of exposure. All mice were fed with a normal diet *ad libitum* during all experiment duration.

At the end of exposure, blood was collected under anaesthesia (ketamine/xylazine 100 mg/kg-10 mg/kg, intraperitoneal injection) for hematocrit and lipid measurements. Entire aortas were harvested. Abdominal aortas were placed in RNAlater (Life technologies®, Villebon-sur-Yvette, France), frozen in liquid nitrogen and stored at -80°C until analysis. Immediately after their sampling, thoracic aortas were placed in Tyrode solution (137mM NaCl, 2,7mM KCl, 0,41mM $NaHPO_4$, 2mM $CaCl_2$, 5µM $MgCl_2$, 11,9mM $NaHCO_3$, 5,5mM glucose) for prostanoid secretion measurements.

Atherosclerosis lesion size quantification

Atherosclerotic lesions of aortic roots were studied by Oil-Red-O staining, as previously described[5]. For each aorta, lipid deposition was quantified from 5 sections (8 µm thickness), separated by 80 µm from each other, using computer image analysis (NisElement®).

Aortic secretion of prostanoids

Thoracic aortas were incubated for 15 minutes, in Tyrode solution maintained at 37°C, aerated with 95% O_2 and 5% CO_2, in presence of calcium ionophore A23187 (Sigma Aldrich®, Saint Quentin Fallavier, France) at 10^{-6} M. Supernatants were immediately frozen at -80°C for prostanoid measurement, while aortas were dried for measurement of dry tissue weight.

COX-pathway gene expression

Total mRNA was isolated from aorta using the RNeasy kit (Qiagen®) as previously described[19] and reverse-transcribed using Superscript II (Invitrogen®, Carlsbad, CA) with random hexamers per the manufacturer's instructions. Quantitative TaqMan PCR was performed on a 7900 HT using primer/probe pairs designed with Assay-On-Demand™ (both Applied Biosystems) (online supplementary Table 1). Data were normalized to 18S ribosomal protein mRNA and expressed as $2^{-\Delta CT}$.

Clinical study

The local ethics committee per the Declaration of Helsinki approved this study. All participants gave written informed consent.

One hundred and thirteen newly diagnosed OSA patients were consecutively entered into the study between January 2007 and April 2011, as well as 25 controls. Patients were referred to the sleep laboratory of Grenoble University Hospital for symptoms suggesting OSA.

Exclusion criteria were: past history of stroke or myocardial infarction, known hypertension, treatment by non-steroid anti-inflammatory, aspirin, steroids, antidiabetic, antihypertensive and lipid-lowering drugs.

Design

The specific contribution of CIH on 11-dTXB$_2$ urinary concentrations was assessed in 25 controls carefully matched for age and body mass index (BMI) with 50 non-obese OSA patients (1 healthy for 2 OSA). All subjects were free of any known CVRF. A sample size of 50 OSA patients and 25 controls was calculated as adequate to show an increase of 30% in urinary 11-dTXB$_2$ excretion with at least 90% power and at the 5% significance level. This sample size calculation was based on preliminary data obtained on healthy subjects showing a mean concentration of 11-dTXB$_2$ of 647 pg/ml with a standard deviation of 287 pg/ml. Since the dispersion of 11-dTXB$_2$ was higher in patients with cardiovascular disease than in controls, we chose a mixt model including 1 healthy for 2 OSA.

The influence of CVRF (obesity: BMI >30kg/m^2, hypertension: clinic diastolic blood pressure (BP) >90 mmHg and systolic BP >140 mmHg, dyslipidemia: LDL cholesterol >4.13 mmol/l or association of total cholesterol >5.16 mmol/l and HDL cholesterol <1.03 mmol/l, smoking, metabolic syndrome defined by criteria of International Diabetes Federation[20]) on urinary excretion of 11-dTXB$_2$ was evaluated in the whole cohort of 113 OSA subjects. Patients were stratified on the presence or not of one of these CVRF.

The influence of continuous positive airway pressure (CPAP) on urinary 11-dTXB$_2$ concentration was studied in 14 OSA free of CVRF and 21 OSA patients with CVRF who were adherent to CPAP. CPAP adherence was defined as CPAP use >4 daily hours[9].

All subjects underwent an overnight polysomnography as described[9]. Sleep apnea was defined as apnea-hypopnea index (AHI) ≥5 events per hour of sleep and symptoms or respiratory disturbance index (RDI), including flow limitation episodes >15 events per hour[21]. Urine sample for 11-dTXB$_2$ quantification and venous blood for biochemical measurements were collected at 7 AM at the end of the nocturnal polysomnographic recordings and were stored at -80 °C until later analysis.

Carotid ultrasonography was performed in 97 OSA patients and 24 controls as previously described[2].

Biological measurements

Plasma cholesterol, triglycerides, glucose and high sensitivity C reactive protein (hsCRP) concentrations were determined using enzymatic colorimetric methods on Dimension Vista® analyser (Siemens®). LDL cholesterol was calculated using the Friedewald formula [total cholesterol- HDL cholesterol- (triglycerides/5)]. Urinary 11-dTXB$_2$ of patients, TXB$_2$ and 6-ketoPGF1α in supernatants of mice were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described[9, 22]. The limits of quantitation of TXB$_2$ and 6-ketoPGF1α were respectively 14 pg/ml and 7.25 pg/ml and their inter- and intra- assay coefficients of variation were < 12% for both analytes.

Statistical analysis

Statistical analyses were performed using NCSS97 (Kaysville, Utah) or SAS (SAS 9.1, Cary, NC, USA) for the mixt model. Data were expressed as median and 10th and 90th percentiles. Normal distribution was tested using Kolmogorov-Smirnov non-parametric test. Comparisons between N and CIH mice were made with a student t test. Comparisons between OSA patients and controls matched for age and BMI (1 control for 2 OSA) were made using a mixt model.

Non continuous variables were compared using a Fisher test. Comparisons between more than 3 groups were made with Kruskal-Wallis test and subsequent pair wise comparisons were made with the Bonferroni multiple-comparison test. For patients treated by CPAP, differences between baseline and post-CPAP values were analysed with the Paired T-test or the Wilcoxon signed rank test. Relationships between two variables were studied with single regression. A multiple linear regression analysis was performed taking into account the variables that correlated with dependant variable urinary 11-dTXB$_2$ in humans. A p-value of <0.05 was considered significant.

Results

Experimental animal study

Weight, lipid levels and hematocrit

After 8 week-exposure, body weight and plasma cholesterol levels were similar in CIH and N mice (Table 1). Hematocrit was significantly higher in CIH mice compared to N mice (Table 1).

Atherosclerotic lesion sizes

Atherosclerotic lesions on aortic roots were higher (p=0.008) in CIH mice compared to N mice (CIH: 66532 (32741-163224) vs. N: 37675 (6798-75596) μm^2; p=0.03). Lesion sizes correlated with plasma total cholesterol in N mice (r=0.501, p=0.04) but not in CIH mice.

COX-pathway gene expression

COX-1 and TXBS aortic mRNA levels were significantly increased in CIH group compared to N, whereas COX-2, PGIS, TP and IP receptors aortic mRNA levels were not significantly different between both groups (Figure 1a). mRNA levels of COX-1 and TXBS significantly correlated with lesion sizes (Figure 1b).

Aortic prostanoid secretion

A23187-stimulated 6-ketoPGF1α and TXB_2 secretions and 6-ketoPGF1α/TXB_2 ratio were similar in aorta from CIH and N mice (Table 2). 6-ketoPGF1α and TXB_2 concentrations were highly correlated (r=0.842, p<0.0001).

Effects of COX-1 inhibition

In both N and CIH groups, treatment with SC-560 significantly decreased TXB_2 and 6-ketoPGF1α aortic secretion *vs*. placebo (Table 2). There was no difference between groups for total cholesterol and body weight (Table 2). Treatment with SC-560 significantly reduced lesion size by 35% in CIH mice whereas it had no effect in N mice (Figure 2).

Clinical study

11-dTXB$_2$ and OSA

Baseline characteristics of the 25 controls and the 50 OSA patients matched for age and BMI are described in Table 3. There was no significant difference for plasma insulin, hsCRP, HDL cholesterol, HOMA'R, BP, carotid intima-media thickness (IMT) and gender ratio. As expected, polysomnographic parameters were different between OSA and control groups. Plasma glucose, total and LDL cholesterol levels were significantly increased in OSA patients versus controls. Urinary 11-dTXB$_2$ was not significantly different between OSA patients and controls (Table 3).

11-dTXB$_2$, OSA and cardiovascular risk factor

Clinical, biological and polysomnographic parameters of OSA patients with or without CVRF are shown in Table 4. OSA group with CVRF had polysomnographic parameters, BP, BMI, plasma triglycerides, total and LDL cholesterol, glucose, insulin and HOMA'R higher than OSA group free of CVRF (Table 4). Conversely, these 2 groups were similar regarding age, gender ratio, carotid IMT, HDL cholesterol and hsCRP (Table 4). Urinary 11-dTXB$_2$ was significantly increased in OSA patients with CVRF compared to OSA patients free of CVRF (Figure 3a). In OSA patients with or without CVRF, 11-TXB_2 levels were similar in patients with mild to moderate (AHI <30events/h) and severe OSA (AHI ≥30events/h) (data not shown).

To determine the CVRF involved in the increase of urinary 11-dTXB$_2$, simple regressions were performed. Hypertension (r=0.190, p=0.05) and obesity (r=0.242, p=0.01) were weakly associated with increased urinary excretion of 11-dTXB$_2$. In multiple regression model, obesity remained the sole independent predictive factor of urinary 11-dTXB$_2$ (r=0.257, p=0.04).

Effect of CPAP treatment on urinary 11-dTXB$_2$ concentration

CPAP treatment for at least 8 weeks significantly decreased AHI, RDI and respiratory arousal index, increased minimal nocturnal SaO_2 and mean nocturnal SaO_2, and decreased the percentage of time spent with a mean SaO_2 less than 90%. CPAP treatment induced no change on 11-dTXB$_2$ urinary concentration in OSA without or with CVRF (supplemental online Table 2 and Table 3).

11-dTXB$_2$, OSA and vascular remodelling

In OSA patients free of CVRF, vascular hypertrophy (defined by carotid IMT>0.8mm) was associated with increased urinary 11-dTXB$_2$ concentrations compared to subjects without vascular hypertrophy (p=0.02) (Figure 3b).

Discussion

Our study demonstrated for the first time an activation of the COX pathway in ApoE$^{-/-}$ mice exposed to CIH and in OSA patients with other CVRF; this activation being associated with higher atherosclerosis lesions in mice and with early markers of atherosclerosis in OSA patients.

We showed that CIH mice had higher atherosclerotic lesions than N mice, suggesting that, in our model, CIH might have accelerated atherosclerosis development. This result is in accordance with previous works in ApoE$^{-/-}$ mice exposed to CIH from 2 to 4 weeks[4, 5] or in C57BL/6J mice exposed to CIH for 12 weeks[6].

In the present study, plasma cholesterol levels were not significantly different between N and CIH groups and atherosclerotic lesion sizes correlated with plasma cholesterol in N mice, but not in CIH mice. These data suggested that, in our model, CIH-induced atherosclerosis might be independent of lipid disorders and contrasted with previous works showing that concomitant exposure to high-fat high-cholesterol diet and CIH aggravated both atherosclerosis and dyslipidemia in ApoE$^{-/-}$ mice[4] and in C57BL/6J mice[6, 23]. However, we recently showed that CIH also exerts pro-atherogenic effect through other contributing factors, notably the inflammatory process[5]. In agreement with this later hypothesis, our data showed an activation of the thromboxane pathway in CIH mice since mRNA levels of COX-1 and TXBS were increased in aortic tissue of CIH mice. Furthermore, the correlation between these mRNA levels and atherosclerotic lesion size was consistent with direct effects of TXA_2 on macrophages[24]. Unexpectedly, the aortic secretion of TXB_2 and 6-ketoPGF1α, as well as, the TXB_2/6-ketoPGF1α ratio were similar in CIH and N mice. These results could be explained by the fact that we measured prostanoid secretion after aorta stimulation with A23187, and not the basal aortic production. A previous study performed in LDL r-KO mice demonstrated that the acceleration of atherogenesis in response to high fat diet was associated

to increased basal levels of TXB_2 and 6-ketoPGF1α in aortic arch[25], but measurements of prostanoids were performed by ELISA, which is not a specific method.

We chose to measure TXB_2 and 6-ketoPGF1α upon aorta stimulation with A23187 to obtain detectable prostanoid levels by our analytical technique (liquid chromatography-tandem mass spectrometry) which is highly specific but probably less sensitive. However, in our model, A23187 appears to stimulate TXB_2 and 6-ketoPGF1α release to the same extend since both levels were highly correlated, which could explain the similar TXB_2/6-ketoPGF1α ratio in aorta from CIH and N mice. We acknowledge that comparisons of basal TXB_2 and 6-ketoPGF1α production and western blot analysis of COX-1 and TXBS would have been of interest with regard to the increased mRNA levels of COX-1 and TXBS induced by CIH, and that their lack may represent limitations of our study.

Moreover, treatment with the selective COX-1 inhibitor SC-560 during the last 4 weeks of CIH exposure reduced atherosclerosis progression in CIH mice, providing further evidence for a CIH-dependent activation of the COX pathway. We already demonstrated that four weeks of CIH exposure were sufficient to induce atherosclerosis in $ApoE^{-/-}$ mice[5]; so, it was of interest to explore the effects of COX-1 inhibition on established atherosclerotic lesions. As previously described[12], treatment with SC-560 had no effect on established atherosclerotic lesions in N mice, although it was effective in inhibiting COX-1 pathway as assessed by the measurement of aortic TXB_2 and 6-ketoPGF1α secretion. Collectively, these data demonstrated that in $ApoE^{-/-}$ mice, the atherogenic process was accelerated by CIH exposure, at least in part through COX-1 pathway activation. CIH activates leukocytes[26] and OSA patients display leukocyte activation[7, 8, 27]. By regulating the interaction between leukocytes, smooth muscle cells and endothelial cells, TXA_2 promotes and PGI_2 prevents the initiation and progression of atherogenesis[14].

To extend to OSA patients the thromboxane-pathway activation observed in CIH mice, we measured the urinary excretion of 11-dTXB$_2$, a validated biomarker of systemic TXA$_2$ production[10]. Urinary 11-dTXB$_2$ concentrations of OSA patients free of CVRF were not different to those of controls carefully matched for age and BMI, two major confounding factors often present in studies focused on the underlying inflammation associated to OSA. These data suggested that OSA itself was not associated to an increased urinary 11-dTXB$_2$ excretion, an hypothesis confirmed by the observation that CPAP treatment had no influence on urinary 11-dTXB$_2$ level. However, increased urinary 11-dTXB$_2$ concentrations have previously been described in patients with cardiovascular diseases including obesity[28] and hypertension[29]. Consistent with these data, we showed that OSA patients with associated CVRF had higher urinary concentrations of 11-dTXB$_2$ than OSA patients free of known CVRF. Furthermore, among the studied CVRF, obesity was the sole independent predictor of urinary 11-dTXB$_2$ excretion, which is in agreement with a previous study demonstrating an increased urinary 11-dTXB$_2$ excretion in obese women[28]. This result provided further evidence for the major role of obesity in AA metabolism activation in OSA patients, as we previously described for the 5-lipooxygenase pathway[9]. Finally, urinary 11-dTXB$_2$ excretion was associated with carotid wall hypertrophy in OSA patients. Again, these findings were consistent with the proliferative effects of TXA$_2$ on vascular smooth muscle cells[10], although it must be kept in mind that vascular remodelling is a complex process implying many mediators and that COX pathway activation might not be the sole mechanism of vascular remodelling in OSA patients with CVRF.

In conclusion, our study showed an activation of COX-1 pathway in ApoE$^{-/-}$ mice exposed to CIH, which contributed to the acceleration of the atherogenic process induced by this stimulus. Such an activation of the COX-1 pathway was also found in OSA patients with associated CVRF. This study extends to the COX-1 pathway the activation of the AA

metabolism previously described for the leukotriene[7, 9] pathway in OSA in relation to vascular remodelling. These findings open the field to the interest of new pharmacological approaches (dual COX and 5-LOX inhibitor[30]) in the prevention of cardiovascular morbidity in OSA patients.

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Legends of the figures

Figure 1 mRNA levels of COX-pathway genes in mice exposed to chronic intermittent hypoxia a) mRNA levels in abdominal aorta. Data are expressed as fold-changes compared to normoxic mice (control). * $p<0.05$ versus normoxic group. b) Regressions between atherosclerotic lesion size and aortic mRNA levels of COX-1 and thromboxane synthase (TXBS).

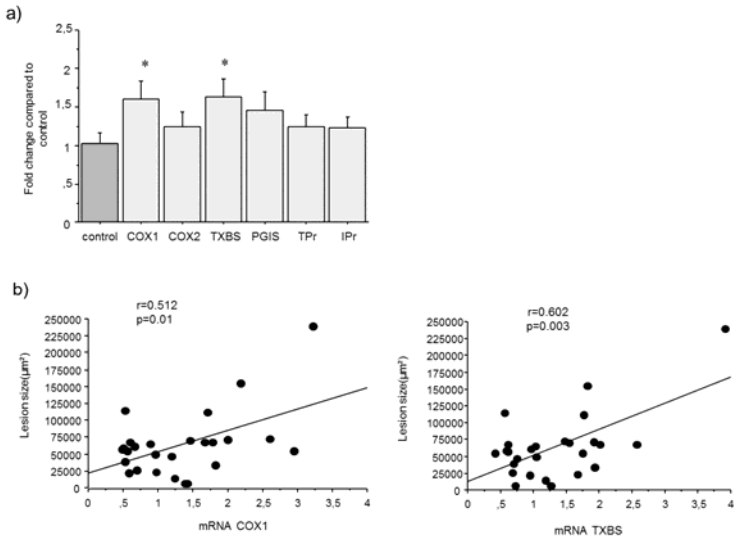

Figure 1

Figure 2 Effects of COX-1 inhibition on atherosclerosis in mice exposed to chronic intermittent hypoxia (CIH) or normoxia (N). a) Lesion sizes: boxes represent values within the interquartile range; whiskers, the data range; and the line across the boxes, median values. NS, non-significant. b) Representative photographs of Oil-Red-O staining.

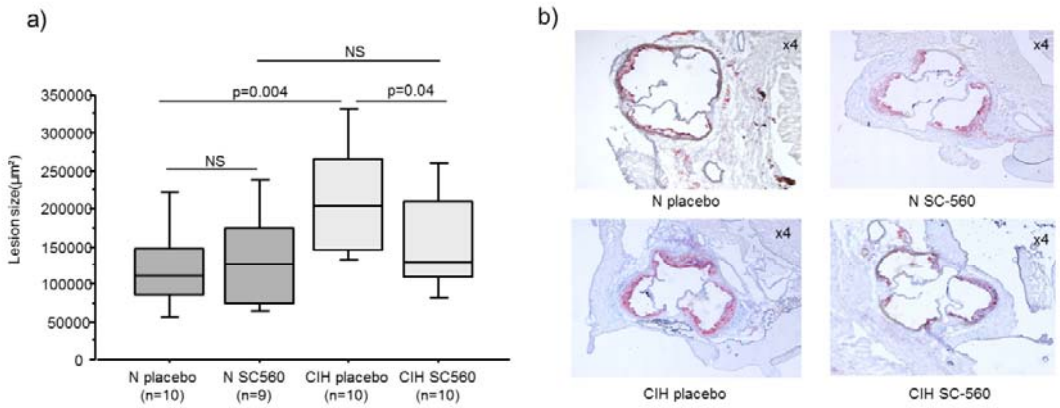

Figure 2

Figure 3 a) Urinary 11-dTXB$_2$ concentrations in OSA patients with (CVRF+) and without known cardiovascular risk factor (CVRF-). b) Urinary 11-dTXB$_2$ concentrations in OSA patients with or without carotid wall hypertrophy. Boxes represent values within the interquartile range; whiskers, the data range; and the line across the boxes, median values.

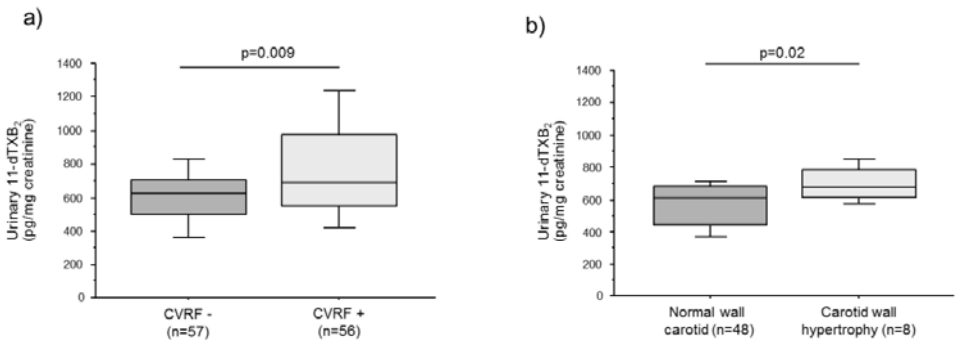

Figure 3

Table 1: Body weight, plasma cholesterol level and hematocrit of ApoE$^{-/-}$ mice exposed for 8 weeks to chronic intermittent hypoxia (CIH) or normoxia (N). Data are presented as median (10^{th} and 90^{th} percentiles).

	N (n=15)	CIH (n=15)	p
Body weight after exposure (g)	31.7 (28.5-33.9)	30.3 (27.8-32.6)	0.08
Total cholesterol (mmol/l)	12.2 (5.24-18.7)	14.6 (7.10-19.1)	0.46
Hematocrit (%)	45.0 (41.4-47.0)	47.0 (44.2-55.0)	**0.006**

Table 2: Body weight, plasma cholesterol level, hematocrit and aortic prostanoid secretion of $ApoE^{-/-}$ mice exposed for 8 weeks to chronic intermittent hypoxia (CIH) or normoxia (N), with treatment by SC-560 or placebo during the 4 last weeks of exposure. Data are presented as median (10^{th} and 90^{th} percentiles).

	N		CIH	
	Placebo (n=10)	SC-560 (n=9)	Placebo (n=10)	SC-560 (n=10)
Body weight after exposure (g)	28.8 (25.8-31.0)	28.9 (27.1-32.6)	28.9 (25.9-31.5)	31.2 (28.4-32.0)
Total cholesterol (mmol/l)	14.2 (9.13-20.2)	11.1 (8.44-17.6)	15.3 (12.8-21.0)	12.3 (8.46-16.1)
Hematocrit (%)	44.5 (42.5-45.0)	45.0 (41.8-47.0)	46.0 (45.0-47.6)‡	45.5 (43.5-48.5)
Aortic secretion of TXB_2 (ng/l/mg)	55.2 (35.3-94.7)	3.13 (1.80-22.6) *	56.7 (44.0-80.8)	7.86 (2.65-11.86) †
Aortic secretion of 6ketoPGF1α (ng/l/mg)	778.3 (559.7-1059.1)	91.8 (45.9-285.7) *	802.1 (638.3-1152.8)	107.9 (68.1-198.6) †
Aortic 6ketoPGF1α/TXB_2 ratio	13.4 (9.9-21.6)	24.1 (9.3-40.8)	14.0 (11.6-18.1)	17.0 (10.9-26.7)

*$p<0.05$ normoxic mice treated by placebo versus normoxic mice treated by SC-560; † $p<0.05$ hypoxic mice treated by placebo versus hypoxic mice treated by SC-560; ‡ $p<0.05$ normoxic mice treated by placebo versus hypoxic mice treated by placebo.

Table 3: Baseline characteristics of controls and OSA patients free of any known cardiovascular risk factor, matched for age and BMI. Data are presented as median (10^{th} and 90^{th} percentiles) or as percentage.

	controls (n=25)	OSA patients (n=50)	p
Males (%)	76.0	88.0	0.20
BMI (kg/m^2)	25.0 (21.6-27.3)	24.9 (21.8-27.9)	0.83
Age (yrs)	51.2 (42.7-62.6)	51.1 (43.8-60.9)	0.99
Clinical systolic BP (mmHg)	127 (112-148)	125 (110-146)	0.72
Clinical diastolic BP (mmHg)	85 (76-97)	80 (69-93)	0.29
AHI (events/h)	5.0 (0.0-9.2)	35.4 (14.9-59.4)	**<0.0001**
RDI (events/h)	6.6 (1.0-15.7)	35.9 (24.1-71.6)	**<0.0001**
Minimal SaO_2 (%)	90.0 (85.4-92.5)	84.5 (75.3-90.0)	**<0.0001**
SaO_2<90% (%TST)	0.00 (0.00-1.00)	1.00 (0.00-13.2)	**<0.0001**
RAI (events/h)	4.9 (1.9-11.0)	28.0 (12.0-49.1)	**<0.0001**
Plasma glucose (mmol/l)	4.7 (4.3-5.3)	5.1 (4.5-6.1)	**0.03**
Plasma insulin (µUI/ml)	4.2 (2.2-7.11)	5.48 (2.85-8.57)	0.11
HOMA-R index	0.86 (0.50-1.68)	1.21 (0.63-2.22)	0.13
Total cholesterol (mmol/l)	4.77 (3.41-6.17)	5.68 (4.41-6.62)	**0.004**
LDL cholesterol (mmol/l)	2.76 (1.57-3.92)	3.55 (2.13-4.36)	**0.003**
HDL cholesterol (mmol/l)	1.52 (0.98-2.40)	1.52 (1.10-2.13)	0.94
Triglycerides (mmol/l)	0.89 (0.52-1.90)	1.11 (0.68-1.84)	0.07
hsCRP (mg/l)	1.00 (0.38-1.74)	0.95 (0.30-3.30)	0.61
Right carotid IMT (mm)	593 (515-653)	604 (508-805)	0.44
Left carotid IMT (mm)	622 (527-780)	647 (515-872)	0.32
Mean carotid IMT (mm)	606 (523-710)	620 (539-838)	0.52
Overweight/normal BMI (%)	48 / 52	46 / 56	0.87
Smoking (%)	0	0	1
Hypertension (%)	4	6	1
Metabolic syndrome (%)	0	0	1
Dyslipidemia (%)	0	20	**0.03**
Urinary 11-$dTXB_2$ (pg/mg creatinine)	609.0 (265.8-945.3)	629.3 (266.4-933.1)	0.56

BMI, body mass index; BP, blood pressure; AHI, apnea/hypopnea index; RDI, respiratory disturbance index; SaO_2, arterial oxygen saturation; TST, total sleep time; RAI, respiratory arousal index; HOMA'R, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C reactive protein; IMT, intima-media thickness; 11-$dTXB_2$, 11-dehydrothromboxane.

Table 4: Baseline characteristics of OSA patients without (CVRF-) or with associated cardiovascular risk factors (CVRF+). Data are presented as median (10^{th} and 90^{th} percentiles) or as percentage.

	OSA		
	CVRF- (n=57)	**CVRF+** (n=56)	p
Males (%)	84.5	84.0	1
BMI (kg/m^2)	25.6 (21.5-28.4)	28.2 (23.0-34.6)	**<0.0001**
Age (yrs)	51.3 (44.5-65.3)	53.1 (39.9-66.6)	0.72
Clinical systolic BP (mmHg)	124 (110-138)	132 (118-160)	**0.0002**
Clinical diastolic BP (mmHg)	80 (65-90)	88 (72-108)	**<0.0001**
AHI (events/h)	30.0 (17.0-50.0)	40.2 (19.4-67.0)	**0.0004**
RDI (events/h)	35.8 (24.3-64.3)	51.0 (27.7-80.3)	**0.0002**
Minimal SaO_2 (%)	85.0 (76.0-90.0)	82.0 (74.9-88.2)	**0.04**
Mean SaO_2 (%)	94.0 (92.0-96.0)	93.0 (90.0-95.0)	**0.0009**
SaO_2<90% (%TST)	1.0 (0.0-11.5)	4.0 (0.0-35.0)	**0.002**
RAI (events/h)	27.9 (12.4-44.2)	35.0 (12.6-59.2)	**0.003**
Plasma glucose (mmol/l)	4.9 (4.5-5.9)	5.2 (4.7-6.4)	**0.02**
Plasma insulin (µUI/ml)	4.8 (2.7-9.0)	6.4 (3.7-12.2)	**0.0002**
HOMA-R index	1.0 (0.6-2.1)	1.5 (0.9-3.1)	**0.0001**
Total cholesterol (mmol/l)	5.47 (4.02-5.91)	5.81 (4.45-6.73)	**0.006**
LDL cholesterol (mmol/l)	3.35 (2.01-3.95)	3.66 (2.59-4.55)	**0.008**
HDL cholesterol (mmol/l)	1.55 (1.14-2.19)	1.42 (0.98-1.91)	0.13
Triglycerides (mmol/l)	1.01 (0.62-1.59)	1.27 (0.79-2.11)	**0.002**
hsCRP (mg/l)	1.30 (0.20-3.42)	1.30 (0.60-3.74)	0.50
Right carotid IMT (mm)	596 (498-821)	602 (457-815)	0.66
Left carotid IMT (mm)	613 (512-876)	663 (527-897)	0.20
Mean carotid IMT (mm)	608 (520-848)	628 (531-848)	0.21
Obesity/overweight/normal BMI (%)	0 / 46 / 54	39 / 34 / 27	**<0.0001**
Smoking (%)	0	2	**0.001**
Hypertension (%)	0	25	**<0.0001**
Metabolic syndrome (%)	0	30	**<0.0001**
Dyslipidemia (%)	0	50	**<0.0001**
Urinary 11-$dTXB_2$ (pg/mg creatinine)	612.6 (289.0-866.3)	677.9 (389.5-1247.6)	**0.008**

BMI, body mass index; BP, blood pressure; AHI, apnea/hypopnea index; RDI, respiratory disturbance index; SaO_2, arterial oxygen saturation; TST, total sleep time; RAI, respiratory arousal index; HOMA'R, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; hsCRP, high-sensitivity C reactive protein; IMT, intima-media thickness; 11-$dTXB_2$, 11-dehydrothromboxane.