Leukotriene B₄ pathway activation and atherosclerosis in obstructive sleep apnea

Francoise Stanke-Labesque, Jean-Louis Pépin, Tiphaine de Jouvencel, Claire Arnaud, Jean-Philippe Baguet, Marcelo H. Petri, Renaud Tamisier, Jean François Jourdil, Patrick Lévy, and Magnus Bäck

INSERM U1042, Grenoble, F-38042 France; Université Grenoble 1, Faculté de Médecine, IFR1, Grenoble, F-38042 France; Laboratoire de pharmacologie, Pôle rééducation et Physiologie, and Clinique de cardiologie, CHU, Hôpital A. Michallon, BP217, Grenoble, F-38043 France; INSERM U698, Paris 7 Denis Diderot University, Bichat University Hospital, 75018 Paris, France: Department of Medicine, Karolinska Institutet, 17176 Stockholm, Sweden; and Department of Cardiology, Karolinska University Hospital, CMM L8:03, 17176 Stockholm, Sweden

Abstract Leukotriene B₄ (LTB₄) production increases in obstructive sleep apnea syndrome (OSA) and is linked to early vascular remodeling, the mechanism of which is unknown. The objective of this study was to determine the molecular mechanisms of LTB₄ pathway activation in polymorphonuclear cells (PMNs) and early vascular remodeling in OSA and the specific contribution of intermittent hypoxia (IH). PMNs were isolated from 120 OSA patients and 33 healthy subjects and used for measurements of LTB₄ production, determination of mRNA and protein expression levels, or exposed for four cycles of in vitro IH. PMNs derived from OSA patients exhibited increased LTB₄ production, for which apnea-hypopnea index was an independent predictor (P=0.042). 5-Lipoxygenase-activating protein (FLAP) mRNA and protein increased significantly in PMNs from OSA patients versus controls and were associated with carotid luminal diameter and intima-media thickness. LTB₄ (10 ng/ml) increased IL-6 (P=0.006) and MCP-1 (P=0.002) production in OSA patient monocytes. In vitro exposure of PMNs from controls to IH enhanced LTB₄ mRNA levels (P=0.027) and induced a 2.7-fold increase (P=0.028) in LTB₄ secretion compared with PMNs exposed to normoxia. In conclusion, upregulation of FLAP in PMNs in response to IH may participate in early vascular remodeling in OSA patients, suggesting FLAP as a potential therapeutic target for the cardiovascular morbidity associated with OSA. —Francoise Stanke-Labesque, Jean-Louis Pépin, Tiphaine de Jouvencel, Claire Arnaud, Jean-Philippe Baguet, Marcelo H. Petri, Renaud Tamisier, Jean François Jourdil, Patrick Lévy, and Magnus Bäck

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Supplementary key words arachidonic acid • eicosanoids • granulocytes • intermittent hypoxia • lipoxygenase • vascular remodeling

Obstructive sleep apnea (OSA) patients experience recurring episodes of partial or complete upper airway obstruction during sleep. Upper airway collapse is usually associated with desaturation-reoxygenation, harming the cardiovascular system. Moderate to severe OSA patients show signs of early atherosclerosis (1–3).

Recent studies have associated the inflammatory mediators, leukotrienes (LTs), with both OSA (4–7) and atherosclerosis (8, 9). However, the mechanisms involved in the role of LTs as a link between OSA and atherosclerosis have remained largely unexplored.

LTs are synthesized on activation of 5-lipoxygenase (5-LO) (10), which interacts with nuclear membrane-bound 5-LO-activating protein (FLAP) (11), generating leukotriene A₄ (LTA₄) in inflammatory cells (12). In polymorphonuclear neutrophils (PMNs), LTA₄ is converted by LTA₄ hydrolyase (LTA₄H) into LTB₄, which modulates transcription or is secreted to mediate autocrine or paracrine effects through the BLT₁ and BLT₂ receptors. LTB₄ is a potent chemottractant, facilitating leukocyte adhesion to endothelial cell and recruitment, critical steps in atherosclerosis. Thus, LTB₄ function in the pathogenesis of atherosclerosis is well established (8, 13).

Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; CPAP, continuous positive airway pressure; CysLT, cysteinyl leukotriene; DBP, diastolic blood pressure; FLAP, 5-lipoxygenase-activating protein; hsCRP, high-sensitivity C-reactive protein; IH, intermittent hypoxia; IMT, intima-media thickness; 5-LO, 5-lipoxygenase; LT, leukotriene; LTA₄H, LTA₄ hydrolyase; OSA, obstructive sleep apnea; PMN, polymorphonuclear neutrophil; RDI, respiratory-related arousal index; RDI, respiratory disturbance index; SaO₂, oxygen saturation; SBP, systolic blood pressure; TST, total sleep time.

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To whom correspondence should be addressed.
e-mail: FStanke@chu-grenoble.fr (F.S.B.); Magnus.Back@ki.se (M.B.)

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In addition to monocytes and macrophages, PMNs mediate the onset and progression of atherosclerosis (14). As the principal source of LTB₄, the capacity of PMNs to produce LTB₄ is measured by ex vivo production of LTB₄ in response to calcium ionophore; LTB₄ production by stimulated PMNs rises in patients with a history of myocardial infarction (15) and in nonobese cardiovascular disease-free OSA patients (4).

Recent findings suggest that intermittent hypoxia (IH) induces LTB₄ activation in monocytes in vitro and that the LTB₄ pathway contributes in the development of atherosclerosis in chronic IH-exposed ApoE⁻/⁻ mice (16). However, the effects of OSA on LTB₄ pathway transcription and atherosclerosis have not been examined in humans.

The aims of the present study were: 1) to examine PMN production of LTB₄ in a large cohort of OSA patients reflecting routine clinical practice; 2) to determine the molecular mechanisms of LTB₄ pathway activation and early vascular remodeling in OSA; 3) to study the consequences of LTB₄ pathway activation in OSA in terms of paracrine effect on monocytes, another cellular type greatly involved in atherogenesis; and 4) to explore the contribution of the hypoxic component of OSA on LTB₄ pathway activation in isolated PMNs exposed to in vitro IH.

**METHODS**

**Patients**

This study was approved by the local ethics committee per the Declaration of Helsinki. All participants gave written informed consent. Patients were referred to the Grenoble University Hospital sleep laboratory for suspicion of OSA. Controls were healthy volunteers who were free of inflammatory and sleep disorders. All subjects underwent a full polysomnography, which was scored blinded to biological parameters, and biochemical measures were analyzed blinded to these results. A flow chart detailing patient inclusion in the different experiments is shown in Fig. 1.

The exclusion criteria were: cancer, infectious or autoimmune disease, diabetes mellitus, disease that potentially affected blood pressure, atrial fibrillation or frequent premature beats (>10/min), shift work, asthma, chronic obstructive pulmonary disease, atopy, rhinitis, arthritis, oral appliances, maxillofacial surgery, and pharmacological treatments that could affect LT concentration, including nonsteroidal anti-inflammatory drugs, corticosteroids.

Nocturnal polysomnography was performed in all subjects as described (5). Sleep apnea was defined as apnea-hypopnea index (AHI) >5 per h of sleep and respiratory disturbance index (RDI) including flow limitation episodes >15 and symptoms (17). OSA was considered mild (5 > AHI < 15), moderate (15 > AHI < 30), or severe (>30).

Subjects were enrolled regardless of previous cardiovascular medical history, hence reflecting routine clinical activity. They were nonoverlapping with cardiovascular disease-free subjects included in our previous study (4).

After the nocturnal polysomnography, peripheral blood was sampled. Plasma glucose and serum triglycerides levels were measured on an automat (Modular 700, Roche, Meylan, France). Serum insulin was measured by radioimmunometric sandwich assay (CIS bio international, Gif-Sur-Yvette, France). Serum high-sensitivity C-reactive protein (hs-CRP) was measured by automated immunonephelometry (Behring Nephelometer II Analyzer, Dade Behring, Germany). Carotid ultrasonography was performed in 29 OSA patients and four controls as described (1).

**PMN preparation**

Human PMNs were isolated as previously described (4) and used for one of the following protocols: 1) PMNs were resuspended in PBS, pH 7.4 containing 0.133 g/l CaCl₂ and 0.1 g/l Mg²⁺ for A23187 stimulation (68 OSA patients and 19 controls); 2) resuspended in RNAlater (Ambion) for RT-PCR (25 OSA patients and nine controls); or 3) resuspended in PBS with protease inhibitors (Sigma) for Western blot (12 OSA patients and five controls).

**LTB₄ production in A23187-treated PMNs**

LTB₄ production by PMNs was measured on stimulation with A23187 (4). Viability exceeded 98% by Trypan blue exclusion method. PMNs (2 × 10⁶ cells/ml) were incubated for 15 min at 37°C with 10 µmol/l A23187 or vehicle (PBS). In some experiments, PMNs were incubated with the 5-LO inhibitor AA861 (10 µM) or the FLAP inhibitor MK886 (10 µM) for 30 min before A23187 stimulation. Incubations were stopped by centrifugation.
from healthy subjects were analyzed by Western blot and membranes probed with rabbit GAPDH antibody (AbCam) to confirm that loading control did not differ between samples.

**In vitro exposure of PMNs from healthy subjects to IH**

Purified PMNs from eight healthy subjects underwent four cycles of IH using a modified protocol (20). In a hypoxia chamber with atmospheric pressure maintained, a 35 min hypoxic period (95% N₂ and 5% CO₂) was followed by 25 min of reoxygenation (95% O₂ and 5% CO₂), after which the cells were resuspended in RNAlater (Ambion) for LTB₄ pathway RT-PCR analysis (n = 4) or in PBS for A23187 1 µM stimulation (n = 6). Control-PMNs from the same donors were maintained in normoxic conditions for the same durations.

**Isolation of monocytes and cytokine production in response to LTB₄**

Monocytes were isolated using standard methods (21). Briefly, after separation by dextran sedimentation and centrifugation through a discontinuous ficoll, mononuclear cells were placed on plastic tissue culture dishes (Falcon 3003) precoated with pooled human serum for 15 min at 37°C. After 2 h culture at 37°C in RPMI 1640 with 10% pooled human serum, nonadherent cells were removed. Plastic adherent cells (monocytes) were collected by scraping, washed twice in RPMI, and suspended in 2 ml RPMI to be counted.

Enriched adherent cells (monocytes 2 × 10⁵ cells) were resuspended in RPMI 1640, 10% heat-inactivated FBS (Invitrogen) and 2 mM glutamine (Invitrogen), and incubated in a 96-well plate overnight at 37°C in a humidified 5% CO₂ incubator with either 10 ng/ml LTB₄ or vehicle. The supernatants were collected and stored at −80°C until use.

CCL5/RANTES, CCL2/MCP-1 (monocyte chemoattractant protein 1), IL-6, and tumor necrosis factor (TNF)α were measured by multiplex bead immunoassay (Fluorokine MAP Multiplex

**FLAP and LTA₄H proteins expression in PMNs from OSA**

PMNs were subjected to three freeze/thaw cycles in liquid nitrogen and ultracentrifuged for 30 min at 100,000 g. The supernatant (cytosolic extract) was collected, and the pellet was resuspended in 500 µL PBS. After ultracentrifugation at 100,000 g for 30 min, the pellet was resuspended in RIPA containing protease inhibitors (Sigma). Protein concentration was measured by Bradford assay.

Ten micrograms of proteins (cytosolic and membrane extract) was resolved by 12% SDS-PAGE; transferred onto nitrocellulose membranes; blocked with 5% milk powder in TBS, pH 7.4, containing 0.1% Tween 20; probed with polyclonal anti-FLAP for membrane extract or goat anti-LTA₄H for cytosolic extract (200 ng/ml, Santa Cruz Biotechnology) and peroxidase-conjugated secondary anti-rabbit or -goat (1:25,000 Jackson ImmunoResearch Laboratories); and detected by ECL as previously described (19). In separate experiments, cytosolic fractions from PMNs derived from healthy subjects were analyzed by Western blot and membranes probed with rabbit GAPDH antibody (AbCam) to confirm that loading control did not differ between samples.

**Fig. 2.** Representative typical LC-MS/MS chromatogram of LTB₄, 6-trans-LTB₄, and 6-trans-12-epi LTB₄ obtained from PMNs stimulated with A23187 10 µM.
LTB₄ production in OSA patients and controls

Table 1 shows the baseline characteristics of subjects in whom A23187-induced stimulation of LTB₄ was measured, stratified by AHI. A23187-induced LTB₄ production increased with OSA severity, rising significantly in severe OSA patients versus controls (Table 1). Pretreatment with AA861 or MK886 inhibited A23187-mediated LTB₄ synthesis (data not shown).

Confounders of LTB₄ production in OSA patients

LTB₄ production correlated significantly with AHI and percentage of time spent with SaO₂ < 90% (Table 2). LTB₄ concentrations were unrelated to age, body mass index (BMI), or metabolic variables. Gender, smoking status, and different drug treatments (lipid-lowering, antihypertensive, or antiplatelet treatments) did not influence LTB₄ concentration. A multiple-linear regression including pharmacological treatments, AHI, glycemia, and LDL-cholesterol (variables with P-value < 0.2 in the univariate analysis) indicated that AHI (P = 0.042) was an independent predictor of log LTB₄ concentrations but this model merely explained 14% of the variance.

Increased FLAP mRNA expression in OSA patient PMNs

Supplementary Table II shows the baseline characteristics of the nine controls and 25 OSA patients included in the RT-PCR experiments. Among the patients included in the mRNA study, 13 were severe, eight were moderate, and four were mild OSA patients. FLAP mRNA levels were significantly higher in PMNs derived from OSA patients versus controls (Fig. 3A). Conversely, 5-LO, LTA₄H (Fig. 3A),

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**Specific effect of IH on the \( \text{LTB}_4 \) pathway**

In vitro exposure of PMNs from healthy subjects to IH consisting of four cycles of 35 min hypoxia followed by 25 min reoxygenation increased FLAP and \( \text{LTA}_4 \text{H} \) mRNA levels (Fig. 4A) versus PMNs under normoxic conditions. In contrast, IH did not alter 5-LO (Fig. 4A) or BLT\(_1\) or BLT\(_2\) receptor mRNA (data not shown). As shown in Fig. 4B, \( \text{LTB}_4 \) secretion in response to A23181 challenge was significantly 2.7-fold enhanced in PMNs exposed to IH (ng/ml/2.10^6 cells): 1.6 ± 0.4 (normoxia conditions) versus 3.3 ± 0.4 (hypoxic conditions).

**Associations of the \( \text{LTB}_4 \) pathway with atherosclerosis**

The correlations between PMN mRNA and protein levels for FLAP and \( \text{LTA}_4 \text{H} \) with measures collected at carotid artery sonography are shown in **Table 3**. FLAP mRNA and protein in circulating PMNs correlated significantly with mean luminal diameter and mean intima-media thickness (IMT) of common carotid arteries (Table 3). Similarly,

| TABLE 2. Correlation coefficients between \( \text{LTB}_4 \) production and age, BMI, and polysomnographic and metabolic variables (n = 87) |
|-------|-------|
| \( R \) | \( P \) |
| Age | 0.145 | 0.189 |
| BMI | 0.072 | 0.505 |
| AHI | **0.280** | **0.0095** |
| RDI | 0.182 | 0.092 |
| Mean nocturnal \( \text{SaO}_2 \) | -0.186 | 0.084 |
| Minimal nocturnal \( \text{SaO}_2 \) | -0.160 | 0.111 |
| \( \text{SaO}_2 \text{e90\%}, \% \text{TST} \) | **0.347** | **0.0013** |
| RAI | 0.208 | 0.066 |
| Fasting plasma glucose | 0.160 | 0.139 |
| Fasting plasma insulin | -0.038 | 0.728 |
| HOMA-R index | 0.031 | 0.773 |
| Total cholesterol | -0.035 | 0.749 |
| HDL-cholesterol | 0.089 | 0.412 |
| LDL-cholesterol | -0.158 | 0.148 |
| Triglycerides | 0.086 | 0.427 |

BMI, body mass index; AHI, apnea-hypopnea index; RDI, respiratory disturbance index; \( \text{SaO}_2 \), \( \text{O}_2 \) saturation; TST, total sleep time; RAI, respiratory-related arousal index. Bold values indicate significant associations.

and BLT\(_1\) and BLT\(_2\) receptor mRNA (data not shown) did not differ significantly between the groups. Although FLAP mRNA and OSA severity did not correlate significantly, there was a trend correlation of FLAP mRNA with percentage of time spent with \( \text{SaO}_2 < 90\% \) \((r = 0.358, P = 0.0613)\). FLAP mRNA was unrelated to BMI \((P = 0.276)\).

**Increased FLAP protein expression in OSA patient PMNs**

Because FLAP and \( \text{LTA}_4 \text{H} \) mRNA levels rose in OSA patients, protein expression of these \( \text{LTB}_4 \) pathway components was examined by Western blot. Supplementary Table III shows the baseline characteristics of the five controls and 12 OSA patients included in the Western blot experiments. In line with the PCR results, FLAP increased in the membrane fraction of PMNs derived from OSA patients versus controls, whereas cytosolic \( \text{LTA}_4 \text{H} \) did not significantly differ (Fig. 3B). FLAP expression correlated with AHI \((r = 0.536, P = 0.03)\) but not with RDI, min \( \text{SaO}_2 \), and mean \( \text{SO}_2 \). FLAP \((r = 0.664, P = 0.01)\) and \( \text{LTA}_4 \text{H} \) \((r = 0.677, P = 0.0098)\) levels were associated with BMI.

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DISCUSSION

Our results point to an important role of the LTB₄ pathway in PMNs for atherosclerosis associated with OSA. Transcriptional alterations of the LTB₄ pathway in PMNs, with a major contributing role of IH, may represent a potential molecular mechanism of leukotriene-induced atherogenesis in OSA. In particular, this study is the first to correlate subclinical atherosclerosis with expression levels of the LTB₄ pathway in PMNs and to show that IH increased LTB₄ production in PMNs associated with greater FLAP mRNA and protein expression in OSA patients. Collectively, these data suggest an important role of the LTB₄ pathway in sleep apnea-related atherosclerosis.

Increased LTB₄ production in A23187-stimulated PMNs has previously been demonstrated in patients with a history of myocardial infarction (15) and in cardiovascular disease-free OSA patients (4). The present study showed, for the first time, increased LTB₄ production in A23187-stimulated PMN 5-LO mRNA was associated with carotid luminal diameter and IMT (Table 3).

5-LO mRNA levels were greater \((P = 0.049)\) in subjects with atherosclerotic plaques \((n = 17)\) versus those without plaque \((n = 18)\) and higher \((P = 0.024)\) in patients with carotid wall hypertrophy, defined as IMT > 0.8 mm \((n = 13)\) than in subjects without hypertrophy \((n = 19)\).

Although LTA₄H mRNA levels in PMNs did not correlate with these markers of early vascular remodeling, LTA₄H protein in PMNs was associated with right carotid luminal diameter and left IMT (Table 3). BLT₁ and BLT₂ mRNA in PMNs and monocytes was unrelated to these markers (see supplementary Table IV).

Paracrine effects of LTB₄ pathway activation in OSA patients

LTB₄ (10 ng/ml) increased IL-6 and MCP-1 production in OSA patient monocytes (Fig. 5). Conversely, LTB₄ did not alter TNF-α or RANTES secretion from monocytes.

Table 3. Correlation between FLAP, 5-LO, and LTA₄H mRNA and protein expression with early vascular remodelling.

|                      | FLAP | 5-LO | LTA₄H |
|----------------------|------|------|-------|
| mRNA                |      |      |       |
| Right carotid luminal diameter | \(r = 0.550, P = 0.001\) | \(r = 0.663, P = 0.011\) | \(r = 0.176, P = 0.18\) |
| Left carotid luminal diameter | \(r = 0.457, P = 0.004\) | \(r = 0.426, P = 0.048\) | \(r = 0.079, P = 0.644\) |
| Right common carotid IMT | \(r = 0.576, P = 0.01\) | \(r = 0.645, P = 0.002\) | \(r = -0.072, P = 0.682\) |
| Left common carotid IMT | \(r = 0.584, P = 0.002\) | \(r = 0.512, P = 0.017\) | \(r = 0.182, P = 0.302\) |
| Mean common carotid IMT | \(r = 0.617, P = 0.007\) | \(r = 0.637, P = 0.002\) | \(r = 0.276, P = 0.11\) |

| Protein | FLAP | LTA₄H |
|---------|------|-------|
| Right carotid luminal diameter | \(r = 0.555, P = 0.007\) | \(r = 0.609, P = 0.05\) |
| Left carotid luminal diameter | \(r = 0.685, P = 0.04\) | \(r = 0.527, P = 0.11\) |
| Right common carotid IMT | \(r = 0.15, P = 0.671\) | \(r = 0.150, P = 0.671\) |
| Left common carotid IMT | \(r = 0.933, P = 0.008\) | \(r = 0.700, P = 0.047\) |
| Mean common carotid IMT | \(r = 0.783, P = 0.026\) | \(r = 0.617, P = 0.081\) |

Fig. 4. A/mRNA levels of 5-LO, FLAP, and LTA₄H in PMNs from healthy subjects exposed in vitro to intermittent hypoxia (IH) or normoxia (NX). (results were calculated as \(2^{ΔCt}\), and expressed as fold change compared with NX) B/A23187-mediated LTB₄ production by PMNs exposed to in vitro IH or NX.
PMNs from severe OSA patients presenting cardiovascular comorbidities as seen in clinical practice and its correlation with AHI and percentage of total sleep time with SaO\textsubscript{2} < 90%.

Although the present and previous (4, 15) studies demonstrate LT\textsubscript{B\textsubscript{4}} pathway activation in PMNs and suggested an association with cardiovascular disease and OSA, the molecular mechanisms have remained largely unexplored. In the present study, the increase in LT\textsubscript{B\textsubscript{4}} production in OSA was associated with an increased mRNA expression of FLAP, suggesting transcriptional activation of LT\textsubscript{B\textsubscript{4}} pathway components in OSA patients, which was also translated into higher protein levels. Our in vitro exposure of PMNs to IH, which increased LT\textsubscript{B\textsubscript{4}} production and induced FLAP upregulation, suggests that IH directly participates in LT\textsubscript{B\textsubscript{4}} pathway activation in OSA. The latter results are consistent with recent findings in another cell type, showing that in vitro exposure of monocyte THP-1 cells to IH increases expression levels of LT\textsubscript{B\textsubscript{4}} synthesizing enzymes (23). Although IH in vitro might not reflect the repetitive desaturation-reoxygenation sequences in OSA, hypoxic conditions in vitro are linked to NF-κB and HIF1α activation, delaying PMN apoptosis (20) and upregulating FLAP (24).

LT\textsubscript{B\textsubscript{4}} pathway activation in OSA may help to explain the association between OSA and atherosclerosis. In the present study, the expression levels of LT\textsubscript{B\textsubscript{4}} synthesizing enzymes correlated with findings on carotid ultrasound, linking, for the first time, transcription of the LT\textsubscript{B\textsubscript{4}} pathway in peripheral leukocytes with atherosclerosis and early vascular remodeling. For example, 5-LO transcript levels were higher in PMNs derived from subjects with carotid atherosclerotic plaques compared with those derived from subjects without atherosclerosis. The latter finding is in line with the proinflammatory and proatherogenic effects of LT\textsubscript{B\textsubscript{4}}, which have been previously established (8, 9). In addition, the correlation between FLAP and 5-LO expression in PMNs with carotid wall hypertrophy, measured as IMT, is consistent with the direct chemotactic and proliferative effects of LT\textsubscript{B\textsubscript{4}} on vascular smooth muscle cells (25).

The association of transcriptional levels of the LT\textsubscript{B\textsubscript{4}} pathway in PMNs with atherosclerosis and vascular remodeling is in line with PMNs being a major source of LT\textsubscript{B\textsubscript{4}} production. However, because monocytes/macrophages are major effectors in atherosclerosis, it is also important that our results indicate that LT\textsubscript{B\textsubscript{4}} production in PMNs may act in a paracrine way to induce proinflammatory IL-6 and MCP-1 in monocytes. These findings are supported by the upregulation of leukotriene B\textsubscript{4} receptors by IH in THP-1 cells (16). Similarly, proatherosclerotic MCP-1 levels rise in monocytes after sleep in severe OSA patients (26) and IH-increased MCP-1 expression is markedly attenuated by BLT\textsubscript{1} receptor antagonist (16). Thus, LT\textsubscript{B\textsubscript{4}}-induced proinflammatory monocyte signaling might be a link between PMN-derived LT\textsubscript{B\textsubscript{4}} and atherosclerosis.

Although our results link IH in OSA patients and LT\textsubscript{B\textsubscript{4}} pathway activation in PMNs, leading to LT\textsubscript{B\textsubscript{4}}-induced proinflammatory effects in monocytes and atherosclerosis, it must be acknowledged that atherosclerosis is a multifactorial disease. Due to confounders and cardiovascular risk factors in our patients, we cannot exclude that LT\textsubscript{B\textsubscript{4}} pathway activation by IH may not be the sole mechanism of vascular remodeling. Notably, LTA\textsubscript{4}H expression correlated with BMI but not oxygen desaturation, whereas FLAP expression was influenced by oxygen desaturation and obesity as reported (5, 18). Similarly, FLAP mRNA and protein levels were significantly higher in PMNs derived from OSA patients versus controls, which supports the suggested mechanism, but our data demonstrated only trend correlation of FLAP mRNA with percentage of time spent with SaO\textsubscript{2} < 90%. In addition, because the protein analysis did not include an internal control for each sample, it cannot be fully excluded that subtle differences in protein loading may have influenced the evaluation of protein levels. Finally, the presence of cardiovascular risk factors in our...
population may contribute to blunt the correlation between hypoxia severity and FLAP mRNA levels. In particular, our observations support that obesity is a major confounding factor of the inflammatory state in OSA patients. However, considering BMI and other potential cardiovascular comorbidities, AHI remained an independent predictor of LTB4 production in OSA patients by multivariate analysis.

In summary, we have demonstrated activation of the LTB4 pathway in PMNs from OSA patients through transcriptional upregulation, which correlated with carotid atherosclerosis and IMT. LTB4-induced proinflammatory monocyte signaling might be a link between PMN-derived LTB4 and atherosclerosis. Together with the upregulation of FLAP and LTA4H in PMNs exposed in vitro to IH, our data provide evidence that IH is a major feature of OSA involved in LTB4 pathway activation underlying vascular remodeling and atherosclerosis. Our results implicate LTB4 pathway, notably FLAP, as a therapeutic target in OSA-associated metabolic and cardiovascular morbidity.

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