

Increased urinary leukotriene E₄ excretion in obstructive sleep apnea: Effects of obesity and hypoxia

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Background: Low-grade inflammation may potentially explain the relationship between obstructive sleep apnea syndrome (OSA) and cardiovascular events. However, the respective contribution of intermittent hypoxia and confounders, such as obesity, is still debated.

Objectives: To monitor urinary leukotriene E₄ (U-LTE₄), a validated marker of proinflammatory cysteinyl leukotriene production, in OSA; to determine the influence of obesity and other confounders on U-LTE₄ concentrations; to examine the mechanisms involved through transcriptional profiling of the leukotriene pathway in peripheral blood mononuclear cells (PBMCs); and to investigate the effect of continuous positive air pressure (CPAP) on U-LTE₄ concentrations.

Methods: We measured U-LTE₄ by liquid chromatography–tandem mass spectrometry.

Results: The U-LTE₄ concentrations were increased ($P = .019$) in 40 nonobese patients with OSA carefully matched for age, sex, and body mass index (BMI) to 25 control subjects, and correlated ($r = 0.0312$; $P = .017$) to the percentage of time spent with mean oxygen saturation (SaO₂) less than 90%. In a larger cohort of patients with OSA ($n = 72$), U-LTE₄ increased as a function of BMI ($r = 0.445$; $P = .0002$). In those patients, the expression levels of 5-lipoxygenase activating protein mRNA in mononuclear cells exhibited a similar pattern. A stepwise multiple linear regression analysis performed in this cohort identified BMI ($P = .001$; regression coefficient, 3.33) and percentage of time spent with SaO₂ <90% ($P = .001$; regression coefficient, 1.01) as independent predictors of U-LTE₄ concentrations. Compared with baseline, CPAP reduced by 22% ($P = .006$) U-LTE₄ concentrations only in patients with OSA with normal BMI.

Conclusion: Obesity, and to a lesser extent hypoxia severity, are determinant of U-LTE₄ production in patients with OSA. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Leukotriene E₄, obstructive sleep apnea syndrome, nocturnal oxygen desaturation, obesity

Obstructive sleep apnea syndrome (OSA) is characterized by recurrent episodes of partial or complete upper airway obstructions during sleep. These episodes of upper airway collapse are usually associated with a desaturation-reoxygenation sequence, which is a recognized detrimental stimulus for the cardiovascular system.

Obstructive sleep apnea syndrome is associated with an increased cardiovascular morbidity and mortality.¹ Multiple causal factors have been suggested to contribute to this cardiovascular morbidity, including sympathetic activation and systemic inflammation.² However, a classic problem in clinical research addressed to characterize the cardiovascular consequences of OSA is confounding factors. In particular, OSA is often associated with overweight or obesity,^{3,4} which is an admitted confounder in the link between OSA and surrogate markers of inflammation.⁵ Indeed, obesity itself induces an inflammatory state directly because adipose tissue is an abundant source of proinflammatory cytokines (see review⁶). In this context, assessing the specific contribution of intermittent hypoxia in the underlying inflammation associated with OSA requires the difficult task of identifying cohorts of patients with OSA free of known confounders associated with inflammation (eg, hypertension, diabetes, established coronary artery disease, smoking, and obesity) that may mask specific effects of intermittent hypoxia.

Among inflammatory mediators, leukotrienes (LTs) are lipid compounds that derive from the 5-lipoxygenase (5-LOX) pathway of arachidonic acid metabolism. The LT family includes LTB₄ and cysteinyl leukotrienes (cysLTs: LTC₄, LTD₄, and LTE₄), which display specific and overlapping functions as inflammatory mediators.⁷ On the one hand, LTB₄ is a potent chemoattractant, and its role of in the pathogenesis in atherosclerosis is well established. On the other hand, cysLTs mediate vascular permeability, edema, smooth muscle cell contraction, and proliferation, and favor the expression of adhesion molecules and the activation of monocytes.^{8,9} The role of cysLTs in the pathogenesis of cardiovascular diseases has been suggested by clinical studies showing an increased urinary excretion of LTE₄ in patients with coronary artery disease,¹⁰ cardiac ischemia,¹¹ and type 1 diabetes mellitus.¹² In addition, the expression of the 5-LOX pathway (5-LOX, 5-LOX activating protein [FLAP], LTC₄ synthase, and cysLT receptors) is increased in atherosclerotic lesions at various stages of development in human aorta, coronary arteries, and

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Abbreviations used

AHI:	Apnea-hypopnea index
BMI:	Body mass index
CPAP:	Continuous positive air pressure
CRP:	C-reactive protein
cysLT:	Cysteinyl leukotriene
11-DehydroTXB ₂ :	11-Dehydro thromboxane B ₂
FLAP:	5-Lipoxygenase activating protein
hsCRP:	High sensitive CRP
5-LOX:	5-Lipoxygenase
LT:	Leukotriene
OSA:	Obstructive sleep apnea syndrome
RDI:	Respiratory disturbance index
SaO ₂ :	Oxygen saturation
U-LTE ₄ :	Urinary leukotriene E ₄

carotid arteries.¹³ More recently, Bäck et al¹⁴ identified the gene encoding FLAP as 1 of the most upregulated genes in adipose tissue derived from either transgenic ApoE^{-/-} mice with a dominant-negative TGF- β type II receptor or obese ob/ob mice, suggesting a role of LT pathway in both atherosclerosis-related and obesity-related inflammation.

Few studies have investigated the activation of cysLT pathway in OSA. These studies have been performed in children and demonstrated (1) increased concentrations of cysLTs in exhaled breath condensate¹⁵ and urine,¹⁶ (2) an enhanced expression of cysLT type 1 and 2 receptors in tonsillar T lymphocytes of children with OSA,¹⁷ and (3) an increase proliferation of adeno-tonsillar cells in response to LTD₄ that is reversed by cysLT₁ receptor antagonist.¹⁸ No data are available in the completely different context of OSA in middle-age adults.

The main objective of the current study was to evaluate urinary LTE₄ (U-LTE₄) concentrations as a biomarker of inflammation in patients with OSA. Therefore, nonobese patients with OSA free of any cardiovascular history and medication were compared with carefully matched healthy subjects. Furthermore, the influence of several confounders on LTE₄ urinary excretion was examined in a larger cohort of patients with OSA, reflecting routine clinical activity. To assess the mechanisms involved, the expression levels of the mRNA of enzymes involved in LT biosynthesis were explored in circulating mononuclear cells derived from patients with OSA, and the relationships between U-LTE₄ and surrogate markers of inflammation were studied.¹⁹ Finally, the influence of continuous positive air pressure treatment (CPAP) on LTE₄ urinary concentrations was investigated.

METHODS

This study was approved by the local ethic committee in accordance with the Declaration of Helsinki. All the participants gave written informed consent.

Patients with newly diagnosed OSA were prospectively included in the study between June 2005 and April 2008. Patients were referred to the Grenoble University Hospital sleep laboratory for symptoms suggesting OSA. Controls were healthy volunteers who received no compensation for their participation in the study. All patients and controls underwent full polysomnography. They were nonsmokers and were free of symptoms or history of medical or surgical treatment for cardiovascular diseases except obesity in a subgroup of OSA. Exclusion criteria were known hypertension, disease potentially affecting blood pressure regulation (Parkinson disease, renal or cardiac transplantation, severe cardiac heart failure), atrial fibrillation or frequent premature beats ($\geq 10/\text{min}$), smoking, shift work, diabetes mellitus,

asthma, chronic obstructive pulmonary disease, history of stroke or myocardial infarction, atopy, rhinitis, arthritis, oral appliances or maxillofacial surgery, or pharmacologic treatment that could affect LT concentration, including aspirin, nonsteroidal anti-inflammatory drugs, corticosteroids, and cysLT₁ receptor antagonists.

Design of the studies

First, to assess the specific contribution of intermittent hypoxia on LTE₄ urinary concentration, 25 control subjects were carefully matched for age, body mass index (BMI), and sex with 40 non-obese patients with OSA for the comparison of LTE₄ production.

Second, the influence of obesity on LTE₄ urinary concentrations was assessed in a larger cohort of 72 patients with OSA. A total of 172 patients were newly diagnosed with OSA during the study period. One hundred were not eligible for the study because of the presence of ≥ 1 exclusion criterion; thus, 72 patients with OSA were available for analysis. Patients with OSA were stratified as normal weight ($20 \leq \text{BMI} < 25$), overweight ($25 < \text{BMI} < 30$), or obese ($\text{BMI} \geq 30$) patients.

Third, the influence of CPAP of LTE₄ urinary excretion was studied. Out of the 72 patients with OSA, 67 were treated with CPAP, and 5 refused the treatment. Among these 67 patients with OSA treated with CPAP, only 44 were adherent. Adherence to CPAP was defined as CPAP use ≥ 4 hours daily.²⁰ The median CPAP duration period was 89 (range, 49–307) days, and the median daily used of CPAP was 5.6 (range, 4.6–7.3) hours per night.

Urine sample for LTE₄ 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. In a subgroup of 24 patients with OSA, mononuclear cells were also isolated for analysis of FLAP and 5-LOX mRNA expression. Polysomnography was scored blind to biological parameters. Biochemical measurements were analyzed blind to the polysomnographic results.

Polysomnography

Nocturnal polysomnography was performed as previously described.²¹ Details on polysomnography can be found in this article's Online Repository at www.jacionline.org. Sleep apnea was defined as an apnea-hypopnea index (AHI) ≥ 5 per hour of sleep and symptoms or respiratory disturbance index (RDI) > 15 .^{21–23}

Quantification of LTE₄ and 11-dehydro thromboxane B₂ by liquid chromatography–tandem mass spectrometry

Quantification of LTE₄ and 11-dehydro thromboxane B₂ (11-dehydroTXB₂) was performed by liquid chromatography–tandem mass spectrometry. For details, see this article's Online Repository at www.jacionline.org. The lower limits of quantification were 10 pg/mL urine for LTE₄ and 100 pg/mL urine for 11-dehydroTXB₂. Interassay and intra-assay precision was $< 6\%$ for both analytes.

Quantification of high sensitive CRP in plasma

The quantitative determination of hsCRP was performed by nephelometry (Behring Nephelometer analyzer, Deerfield, Ill). The lower limit of quantification was 0.175 mg/L; and interassay and intra-assay precisions were $< 5.7\%$ and 4.4% .

FLAP and 5-LOX mRNA quantification in mononuclear cells from patients with OSA

Venous blood was drawn from 24 patients with OSA on citrate as anticoagulant. Mononuclear cells were isolated by Ficoll-Paque centrifugation as previously described.²⁴ mRNA levels of FLAP and 5-LOX were measured by reverse transcriptase and real-time Taq-Man PCR as previously described,¹⁴ by using primers and probes from Applied Biosystems (Foster City, Calif) (Hs00233463_m1 and Hs00167536_m1, respectively). Data

TABLE I. Baseline characteristics of the controls and nonobese patients with OSA carefully matched for age, sex, and BMI

	OSA	Controls	P value
N	40	25	
Male (%)	85	72	.2
Age (y)	48.6 (35.1-58.3)	45.0 (37.5-56.4)	.22
BMI (kg/m ²)	25.1 (21.1-28.0)	23.4 (21.6-27.7)	.29
SBP (mmHg)	126.5 (110.0-139.5)	125.0 (110.0-140.0)	.18
DBP (mmHg)	80.0 (67.5-100.0)	81.0 (70.0-93.0)	.64
AHI (events/h)	27.0 (8.8-54.2)	2.8 (0.0-7.4)	<.0001
RDI (events/h)	40.7 (22.1-63.7)	10.3 (5.0-17.5)	<.0001
Mean SaO ₂ (%)	94.0 (92.0-96.0)	95.4 (93.2-97.9)	.026
Minimal SaO ₂ (%)	85.0 (76.1-92.0)	90.0 (85.9-93.0)	.0002
SaO ₂ <90% (% TST)	1.0 (0.0-12.0)	0.0 (0.0-0.6)	.0005
RAI (events/h)	38.4 (19.1-62.2)	9.3 (2.8-11.5)	<.0001
Plasma glucose (mmol/L)	4.8 (4.5-5.8)	4.7 (4.3-5.3)	.15
Plasma insulin (μUI/mL)	6.0 (3.4-9.7)	5.0 (2.3-8.1)	.09
HOMA-R index	1.3 (0.7-2.2)	1.1 (0.5-1.7)	.55
Total cholesterol (g/L)	2.1 (1.6-2.3)	1.9 (1.6-2.4)	.22
HDL cholesterol (g/L)	0.6 (0.4-0.9)	0.6 (0.4-1.0)	.73
LDL cholesterol (g/L)	1.2 (0.7-1.5)	1.2 (0.5-1.4)	.61
Triglycerides (g/L)	1.0 (0.6-1.7)	0.8 (0.4-1.6)	.02
hsCRP (mg/L)	1.1 (0.3-3.4)	1.1 (0.6-2.8)	.57
LTE ₄ (pg/mg creatinine)	53.1 (30.6-98.4)	40.0 (23.5-66.9)	.02

DBP, Clinical diastolic blood pressure; RAI, respiratory-related arousal index; SBP, clinical systolic blood pressure; TST, total sleep time.
Values are medians (10th-90th percentiles).

were normalized to the mRNA levels of cyclophilin A (Hs99999904_m1) and expressed as $2^{-\Delta CT}$.

Statistical analysis

Statistical analyses were performed by using NCSS 97 (Kaysville, Utah). Continuous data were presented as the median, 10th, and 90th percentiles. Noncontinuous data were presented as numbers and percentages. Comparisons between patients with OSA and control subjects for continuous variables were made with the Mann-Whitney *U* test. Noncontinuous variables were compared by using a χ^2 test. Comparisons between LTE₄ production among the 3 subgroups of patients with OSA stratified on their BMI were made with the Kruskal-Wallis test. Subsequent pairwise comparisons were made with the nonparametric Bonferroni multiple comparison test. Correlations were analyzed by using the Spearman rank test. A multiple stepwise regression was performed to evaluate the impact of BMI and percentage of time spent with an oxygen saturation (SaO₂) <90% on LTE₄ concentrations. Differences between baseline and post-CPAP values were analyzed with the paired *t* test or the Wilcoxon signed-rank test. *P* <.05 was considered significant.

RESULTS

Increased LTE₄ urinary concentrations in patients with OSA

Baseline characteristics of the 25 control subjects and 40 non-obese patients with OSA carefully matched for age, BMI, and sex are described in Table I. As expected, values of sleep respiratory disturbance parameters were significantly different between patients with OSA and controls. There were no significant differences between controls and patients with OSA in terms of blood pressure, cholesterol, plasma fasting glucose and insulin, and HOMA-R index. Conversely, triglycerides were significantly higher in patients with OSA than in control subjects.

Urinary LTE₄ concentrations were significantly increased in non-obese patients with OSA compared with control subjects

carefully matched for age, BMI, and sex (Table I). In these patients with OSA, the urinary excretion of LTE₄ was significantly correlated to the percentage of time spent with mean SaO₂ less than 90% ($r=0.312$; $P=.0017$). No significant correlation was found between LTE₄ concentrations and metabolic variables (cholesterol, triglycerides, insulin, fasting glucose, or HOMA-R index; see this article's Table E1 in the Online Repository at www.jacionline.org).

Effects of confounders on LTE₄ urinary excretion in patients with OSA

Baseline characteristics of the cohort of the 72 subjects with OSA are described in Table II. LTE₄ was significantly correlated to BMI, RDI, mean SaO₂, percentage of time spent with SaO₂ <90%, and respiratory-related arousal index (Table III). A stepwise multiple-linear regression indicated that BMI (regression coefficient, 3.33; $P = .001$) and percentage of time spent with SaO₂ <90% (regression coefficient, 1.01; $P = .001$) were independent predictors of LTE₄ urinary concentrations.

Effects of obesity on LTE₄ urinary excretion and FLAP and 5-LOX mRNA levels in isolated mononuclear cells from patients with OSA

Leukotriene E₄ urinary concentrations were significantly higher in overweight and obese patients compared with patients with normal BMI (Fig 1, A). In addition, overweight and obesity were gradually associated with a significant increase of FLAP mRNA levels in peripheral mononuclear cells from 24 patients with OSA (Fig 1, B). Although 5-LOX mRNA levels also exhibited a trend toward an increase, this did not reach statistical significance between the 3 subgroups of patients with OSA. No significant relation was found between AHI, RDI, mean and minimal nocturnal SaO₂, and FLAP or 5-LOX mRNA levels in these samples.

Effect of CPAP on LTE₄ urinary concentrations

As shown in Table IV, CPAP treatment for at least 4 weeks significantly decreased AHI and RDI, increased the minimal nocturnal SaO₂ and mean nocturnal SaO₂, and decreased the percentage of time spent with a mean SaO₂ less than 90%. However, no significant change occurred in LTE₄ urinary concentrations after treatment with CPAP. Likewise, median BMI remained unchanged after the treatment period.

Because BMI was the major determinant of LTE₄ urinary concentration, the effect of CPAP treatment was studied in the 3 subgroups of OSA based on BMI. As shown in Fig 2, CPAP treatment significantly reduced LTE₄ concentrations in normal weight patients with OSA but not in overweight and obese patients with OSA. CPAP duration treatment and CPAP daily use were not significantly different among the 3 subgroups. Median CPAP duration treatment was 90 (range, 53-357), 96 (range, 55-354), and 73 (range, 56-139) days for normal weight, overweight, and obese patients with OSA, respectively. Median CPAP daily use was 6.8 (range, 5.1-7.5), 5.1 (range, 4.6-7.1), and 5.2 (range, 4.4-7.3) hours per day for normal weight, overweight, and obese patients with OSA, respectively.

U-LTE₄ concentration in relation to systemic inflammation in OSA

In patients with OSA, LTE₄ urinary concentrations were significantly correlated to urinary 11-dehydroTXB₂ concentrations

TABLE II. Baseline characteristics of patients with OSA stratified or not (whole population) on their BMI

	Whole population of OSA	OSA		
		Normal weight	Overweight	Obese
N	72	27	33	12
Male (%)	80.5	81	91	50*†
Age (y)	51.4 (36.1-63.2)	51.4 (44.4-60.2)	51.1 (37.6-61.5)	52.6 (33.0-65.3)
BMI (kg/m ²)	26.2 (21.7-31.2)	23.2 (20.8-24.9)	27.6 (25.6-29.4)*	31.2 (30.4-36.4)*†
SBP (mmHg)	128 (113-136)	124 (106-135)	128.5 (114.9-138.0)	130 (116-140)
DBP (mmHg)	80.5 (69.7-92.3)	80.0 (67.2-97.0)	81.0 (72.9-91.1)	87.0 (69.6-92.4)
AIH (events/h)	30.6 (8.4-60.7)	24.4 (7.25-54.0)	34.1 (8.0-58.5)	34.1 (10.7-66.7)
RDI (events/h)	42.1 (24.0-70.7)	35.4 (23.9-56.6)	44.0 (24.0-69.3)	54.2 (32.5-87.2)*
Mean SaO ₂ (%)	94.0 (91.0-96.0)	94.1 (93.0-96.0)	93.1 (91.0-96.0)	92.0 (89.1-94.0)*
Minimal SaO ₂ (%)	84.0 (76.0-90.0)	85.8 (81.0-91.8)	82.9 (65.5-90.1)	85.0 (76.0-87.0)
SaO ₂ <90% (% TST)	1.0 (0-17.9)	0.3 (0.0-5.8)	1.3 (0.0-21.2)	3.8 (0.56-41.6)
RAI (events/h)	41.9 (27.7-59.9)	40.0 (25.3-59.4)	42.0 (29.1-52.9)	50.0 (21.6-72.6)
Fasting plasma glucose, (mmol/L)	5.0 (4.4-6.2)	4.7 (4.3-5.9)	5.2 (4.5-6.3)	5.1 (3.9-6.4)
Fasting plasma insulin (μU/mL)	6.2 (3.6-10.8)	5.3 (3.2-8.9)	6.2 (3.9-9.4)	8.0 (4.1-14.2)*†
HOMA-R index	1.3 (0.7-2.3)	1.3 (0.7-2.1)	1.4 (0.8-2.2)	2.4 (0.4-5.1)*
Total cholesterol (g/L)	2.0 (1.7-2.5)	2.2 (1.6-2.8)	2.0 (1.8-2.3)	2.0 (1.7-2.6)
HDL cholesterol (g/L)	0.6 (0.4-0.9)	0.6 (0.5-1.0)	0.6 (0.4-0.8)	0.6 (0.4-0.7)
LDL cholesterol (g/L)	1.2 (0.7-1.5)	1.2 (0.7-1.5)	1.1 (0.7-1.5)	1.2 (0.9-1.8)
Triglycerides (g/L)	1.1 (0.7-1.9)	0.9 (0.6-1.6)	1.1 (0.7-3.2)	1.3 (0.7-1.8)
hsCRP (mg/L)	1.1 (0.3-3.9)	0.9 (0.2-2.9)	1.1 (0.3-2.9)	3.0 (1.1-12.3)*†

DBP, Clinical diastolic blood pressure; RAI, respiratory-related arousal index; SBP, clinical systolic blood pressure; TST, total sleep time.

Values are medians (10th-90th percentiles).

Normal weight indicates 20 < BMI < 25; overweight, 25 ≤ BMI < 30, obese, BMI ≥ 30. Comparisons of variables among the 3 subgroups of OSA stratified on their BMI were made with Kruskal-Wallis, followed by the Bonferroni adjusted *t* test.

**P* < .05 vs respective normal weight;

†*P* < .05 vs overweight.

TABLE III. Correlation coefficients between LTE₄ production and age, BMI, polysomnographic or metabolic variables in the cohort of 72 patients with OSA

	<i>r</i>	<i>P</i>
Age	-0.045	.70
BMI	0.445	.0002
AHI	0.178	.133
RDI	0.253	.034
Mean SaO ₂	-0.283	.017
Minimal SaO ₂	-0.076	.523
SaO ₂ <90% (% TST)	0.285	.0164
RAI	0.318	.013
Fasting plasma glucose	0.022	.083
Fasting plasma insulin	0.209	.083
HOMA-R index	0.136	.318
Total cholesterol	-0.027	.821
HDL cholesterol	-0.128	.286
LDL cholesterol	0.041	.734
Triglycerides	0.204	.683

RAI, Respiratory-related arousal index; TST, total sleep time.

Statistically significant differences appear in boldface.

(*r* = 0.33; *P* = .018; *n* = 52) and plasma hsCRP (*r* = 0.33; *P* = .014; *n* = 55).

DISCUSSION

This is the first demonstration of enhanced urinary concentrations of LTE₄ in middle-age patients with OSA compared with matched controls. Furthermore, the results point to synergistic role of obesity and hypoxia as determinants of LT production in OSA. Finally, transcriptional profiling of the LT pathway in

circulating monocytes suggests a systemic inflammatory response, which may influence the comorbidities of OSA, such as cardiovascular disease.

Leukotriene E₄ is the major urinary metabolite of cysLTs and provides a validated marker of *in vivo* whole-body synthesis of cysLT production.²⁵ By using this biomarker, previous studies demonstrated an activation of the 5-LOX pathway in patients with cardiovascular diseases, including instable angina,¹⁰ myocardial infarction,¹¹ or poor metabolic controlled diabetes mellitus.¹² In the current study, we demonstrated that, compared with healthy subjects carefully matched for age, sex, and BMI, the urinary concentrations of LTE₄ were significantly enhanced in non-obese patients with OSA free of classic cardiovascular events, including known hypertension, myocardial infarction, and stroke. In this carefully selected population, we were able to demonstrate an increased production of LTE₄ selectively associated with OSA, particularly in relation to percentage of time spent with SaO₂ <90%.

To identify potential confounders, a larger cohort of patients with OSA was characterized for several parameters. In this population, multiple regression analysis demonstrated an increased production of LTE₄ selectively associated with obesity and with percentage of time spent with SaO₂ <90%. Moreover, a stepwise multiple linear regression analysis indicated that BMI and percentage of time spent with SaO₂ <90% were independent predictors of LTE₄ urinary concentrations, with BMI influencing LTE₄ production 3.3-fold more than hypoxia severity. These data suggested cumulative effects of obesity and oxygen desaturation on cysLT pathway activation.

Overweight or obesity is the most important risk factor for OSA.³ In children with OSA, LTE₄ was associated with an

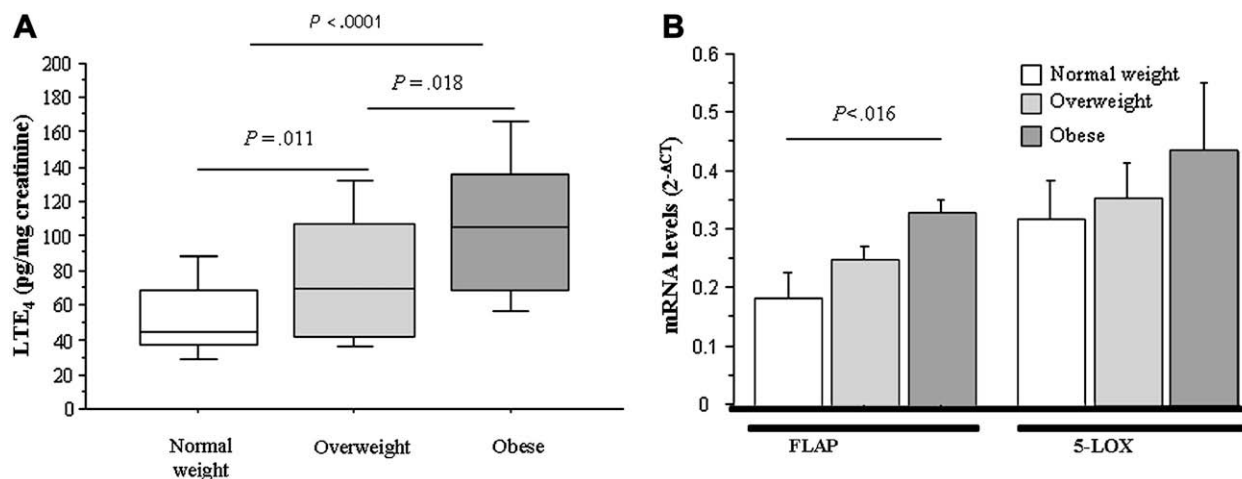


FIG 1. **A**, Compared with patients with normal weight, LTE₄ urinary concentrations are significantly higher in overweight and obese patients with OSA. Boxes represent values within the interquartile range; whiskers, the data range; and the line across the boxes, median values. **B**, mRNA levels of FLAP and 5-LOX from isolated peripheral mononuclear cells from patients with OSA stratified on their BMI (normal weight, n = 7; overweight, n = 9; obese, n = 8). Obesity was associated with an increase of FLAP mRNA levels in peripheral mononuclear cells.

TABLE IV. Characteristics before and after treatment with CPAP in 44 patients with OSA

	Before CPAP	After CPAP	P
BMI (kg/m ²)	27.7 (21.6-35.1)	27.5 (22.7-33.8)	.72
AHI (events/h)	31.4 (9.9-58.4)	0.3 (0-8.7)	.0003
RDI (events/h)	42.5 (27.0-67.1)	1.4 (0.0-39.0)	.0007
Mean nocturnal SaO ₂ (%)	93.9 (91.0-95.7)	94.9 (93.4-96.3)	.038
Minimal nocturnal SaO ₂ (%)	82.9 (75.9-90.1)	91.0 (83.3-93.9)	.0018
SaO ₂ <90% (% TST)	1.9 (0-21.4)	0.0 (0-6.4)	.04
RAI (events/h)	34.0 (20.0-50.2)	2.5 (0.2-12.8)	.002
LTE ₄ (pg/mg creatinine)	66.0 (35.8-111.2)	54.9 (26.7-122.4)	.67

RAI, Respiratory-related arousal index; TST, total sleep time.

Values are medians (10th-90th percentiles).

The median age was 59 (48-67) years.

increased BMI z score in the subgroup of children with moderate-to-severe OSA.¹⁶ The current study demonstrated that LTE₄ urinary concentrations increased as a function of BMI in patients with OSA. Previous studies have identified obesity as an inflammatory state,⁶ and the LT pathway is expressed in macrophages infiltrating adipose tissue in mouse models of atherosclerosis and obesity.¹⁴ To examine the mechanisms involved in the increased LTE₄ production associated with obesity, mRNA was isolated from circulating monocytes. Interestingly, overweight and obesity were gradually associated with an increase of FLAP mRNA levels in mononuclear cells from patients with OSA. FLAP is one of the key proteins involved in LTE₄ synthesis, suggesting that circulating mononuclear cells could represent a major cellular origin of LTE₄.

Because percentage of time spent with SaO₂ <90% was a weak independent predictor of U-LTE₄, we evaluated whether reversion of hypoxia by CPAP treatment could alter U-LTE₄ excretion. Although CPAP significantly ameliorated the hypoxia parameters, no significant change occurred in U-LTE₄ concentrations

after CPAP treatment compared with baseline. However, because BMI influenced LTE₄ production 3.3-fold more than hypoxia severity, the patients were evaluated in separate subgroups defined through their BMI. Interestingly, CPAP therapy reduced U-LTE₄ concentration only in patients with OSA with normal weight who regularly used their CPAP. This last observation provides further evidence for the contribution of both intermittent hypoxia and obesity in LTE₄ production in patients with OSA and underlines that a systematic weight loss management strategy associated CPAP treatment is desirable.

We acknowledge that this point is a limitation of our study because examining responses to specific weight loss strategies on LTE₄ urinary excretion in OSA would provide definitive evidence linking obesity and LTE₄. Previous studies have shown that weight loss strategies significantly reduced 11-dehydroTXB₂ urinary excretion in obese women, indicating a potential link between arachidonic acid metabolism activation and obesity.²⁶ Further studies are required to determine the influence of the combination of CPAP treatment and weight loss on LTE₄ urinary concentrations. However, the previous demonstration in obese patients (BMI > 34) with asthma, that the cysLT₁ receptor antagonist montelukast provided a better control of asthma than beclomethasone or placebo, reinforces the link between obesity and the cysLT pathway.²⁷

LTE₄ urinary concentrations are significantly correlated with hsCRP, which is an independent predictor of cardiovascular risk.¹⁹ As for LTE₄, obesity and nocturnal oxygen desaturation appeared to contribute to its increased level in OSA.^{5,28} In addition, LTE₄ correlated with urinary 11-dehydroTXB₂ concentrations, confirming activation of the proinflammatory pathways of arachidonic acid metabolism in OSA. In line with these findings, a recent study demonstrated in culture tonsillar cells from children with OSA that LTD₄ induced the release of TNF-α, IL-6, and IL-12 via cysLT receptor stimulation.¹⁸ In addition, an enhanced expression of COX-2 has been reported in freshly harvested endothelial cells from obese patients with OSA without clinical evidence of cardiovascular disease.²⁹ All these data

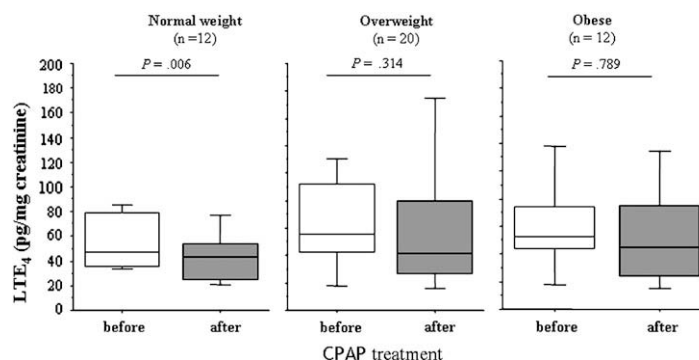


FIG 2. CPAP reduced LTE₄ urinary concentrations in normal-weight subjects but not in overweight or obese patients with OSA who were adherent to CPAP. Boxes represent values within the interquartile range; whiskers, the data range; and the line across the boxes, median values.

suggest that cysLT pathway activation contributes to the underlying background systemic inflammation associated with OSA. The findings that in obese patients with OSA, effective CPAP therapy failed to reduce circulating cardiovascular risk factors,^{30,31} are consistent with our data and provide further evidence that obesity is a main determinant of the underlying background systemic inflammation associated with OSA.

In conclusion, the current study demonstrated an increased U-LTE₄ in OSA, suggesting an activation of the cysLT pathway mainly in relation to obesity, and to a lesser extent in relation to nocturnal oxygen desaturation. Because of their vasomotor and proinflammatory properties and their link with established predictors of cardiovascular diseases, cysLTs could contribute to the cardiovascular morbidity associated with OSA.

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Clinical implications: U-LTE₄ excretion is increased in obstructive sleep apnea syndrome, in relation to obesity, and to a lesser extent, hypoxia severity. This could contribute to the cardiovascular morbidity associated with OSA.

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METHODS

Polysomnography

The diagnosis of OSA was established by full polysomnography and included recording of oronasal flow and thoracoabdominal movements, submental and pretibial electromyography, electro-oculography, electroencephalography (EEG), and transcutaneous measurement of arterial oxygen saturation. An apnea was defined as a complete cessation of airflow for ≥ 10 seconds and a hypopnea as a reduction of $\geq 50\%$ in the nasal pressure signal or a decrease between 30% and 50% associated with either oxygen desaturation of $\geq 3\%$ or an EEG arousal (defined according to the Chicago report),¹ both lasting for ≥ 10 seconds. The AHI was calculated as the total number of apneas and hypopneas (obstructive or mixed apneas plus obstructive hypopneas) per hour of sleep. The RDI was calculated and defined as the number of total respiratory events (obstructive or mixed apneas plus obstructive hypopneas plus inspiratory flow limitation episodes) per hour of sleep (full polysomnography) or per hour of recording (polysomnography without EEG recording). Sleep apnea was defined as a AHI ≥ 5 per hour of sleep and symptoms or RDI > 15 .^{E1,E2}

Quantification of LTE₄ and 11-dehydroTXB₂ by liquid chromatography–tandem mass spectrometry

Quantification of LTE₄ was performed by LC-MS/MS according to a method previously described^{E3,E4} with minor modifications.

The liquid chromatography system consisted of a LC20-AD pump (Shimadzu, Kyoto, Japan), a CTO-20AC column temperature controller, a prominence SIL-20AC autosampler (Shimadzu Japan) and a Kromasil C8, 3.5 μm (70 $\times$ 2 mm) column (Macherey-Nagel, Düren, Germany) maintained at 30°C. The mobile phase consisted of 10 mmol/L ammonium formate (phase A) and methanol (phase B) and was delivered using the following stepwise gradient elution program: 60% of B at 0 minutes, 95% of B at 4 minutes with a flow rate of 330 $\mu\text{L}/\text{min}$. The second part of the run included a re-equilibration step to the initial solvent conditions up to 7 minutes.

Quantification of 11-dehydroTXB₂ was performed on 2 mL urine. After centrifugation at 4000g for 10 minutes, the sample was adjusted to pH 3.5 with 1 N hydrochloric acid and allowed to stand at room temperature for 1 hour to lead 11-dehydroTXB₂ to the lactone form.^{E5}

11-dehydroTXB₂-d₄ (6 ng) was added on each urine sample as an internal standard. Solid-phase extraction was performed by using an C18 (EC) cartridge (100 mg/10 mL), purchased from International Sorbent Technology (UK). The cartridge was washed with distilled water adjusted to pH 2 (4 mL) and petroleum ether (4 mL). 11-dehydroTXB₂ was eluted with methanol. Extracts were dried under nitrogen flow at room temperature and reconstituted in 40 μL mobile phase: methanol-10 mmol/L ammonium formate (80:20, vol/vol). A total of 10 μL was injected in the liquid chromatography–tandem mass spectrometry system. The mobile phase consisted of 10 mmol/L ammonium

formate (phase A) and methanol (phase B) that were delivered using the following stepwise gradient elution program: 40% of B at 0 minutes, 75% of B at 6 minutes with a flow rate of 330 $\mu\text{L}/\text{min}$. The second part of the run included a re-equilibration step to the initial solvent conditions up to 11 minutes.

Mass spectrometry analyses were performed on an API-3200Qtrap tandem mass-spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Canada) equipped with a TurboIonSpray source. Acquisitions were made in the negative-ion mode using multiple reaction monitoring of the m/z transitions 438.2 $\rightarrow$ 333.0 for LTE₄ and m/z 441.1 $\rightarrow$ 336.1 for LTE₄-d₃ (11, 22), 367.1 $\rightarrow$ 161.0 for 11-dehydroTXB₂, and m/z 371.2 $\rightarrow$ 165.0 for 11-dehydroTXB₂-d₄. Calibration curves were constructed using weighted (1/x) linear least-square regression.

FLAP and 5-LOX mRNA quantification in mononuclear cells from patients with OSA

Mononuclear cells were first washed in 10 mL PBS pH 7.4 containing 0.133 g/L CaCl₂ and 0.1 g/L Mg²⁺ (Sigma, Saint Quentin Fallavier, France) and then resuspended in 750 μL of this buffer in the presence of 250 μL RNA later (Ambion). Total RNA was extracted using the RNeasy Mini kit (Qiagen, Hilden, Germany) with an on-column DNase digestion step previously described.^{E6} RNA quantity and quality were assessed by a Bioanalyzer capillary electrophoresis system (Agilent Technologies, Palo Alto, Calif). cDNA was synthesized by using Superscript II (Invitrogen, Carlsbad, Calif). Quantitative Taq-Man PCR on 2.5 μL cDNA was performed with the ABI Prism 7900HT sequence detector with the following primer/probe pairs obtained as Assay-on-demand from Applied Biosystems (Foster City, Calif): Hs00233463_m1 and Hs00167536_m1, for FLAP and 5-LOX, respectively.

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TABLE E1. Correlation coefficients between LTE₄ urinary excretion and age, BMI, polysomnographic or metabolic variables in the 40 patients with OSA matched on age, sex, and BMI with controls

	<i>r</i>	<i>P</i>
Age (y)	0.02	.91
BMI (kg/m ²)	0.31	.054
AHI (events/h)	−0.09	.59
RDI (events/h)	0.03	.87
Mean SaO ₂ (%)	−0.18	.27
Minimal SaO ₂ (%)	−0.231	.15
SaO ₂ < 90% (% TST)	0.312	.017
RAI (events/h)	−0.13	.46
Fasting plasma glucose (mmol/L)	0.15	.33
Fasting plasma insulin (μU/mL)	−0.18	.91
HOMA-R index	0.02	.91
Total cholesterol (g/L)	0.05	.74
HDL cholesterol (g/L)	−0.12	.46
LDL cholesterol (g/L)	0.09	.54
Triglycerides (g/L)	0.06	.68

RAI, Respiratory-related arousal index; TST, total sleep time.