

Diurnal variation of urinary leukotriene E₄ and histamine excretion rates in normal subjects and patients with mild-to-moderate asthma

Koichiro Asano, MD,^a Craig M. Lilly, MD,^a Walter J. O'Donnell, MD,^a Elliot Israel, MD,^a Andrew Fischer, MD,^a Bernard J. Ransil, MD, PhD,^b and Jeffrey M. Drazen, MD^a Boston, Mass.

Background: Leukotriene E₄ (LTE₄) and histamine excreted into the urine reflect the *in vivo* synthesis and release of cysteinyl leukotrienes and histamine, respectively. We examined the diurnal variation of the excretion rate of these mediators over 4 consecutive days in normal subjects (n = 5) and patients with stable mild-to-moderate asthma (n = 8).

Methods: Sixteen consecutive 6-hour urine samples were collected over 4 days. Urinary LTE₄ concentrations were determined by reverse-phase high-pressure liquid chromatography, followed by ELISA. Urinary histamine concentrations were measured by ELISA. The excretion rates of these compounds were normalized relative to urinary creatinine content.

Results: The mean urinary LTE₄ excretion rate was 83.8 ± 38.2 pg/mg creatinine (mean $\pm$ SD) in normal subjects; in patients with asthma, the urinary LTE₄ excretion rate (110.0 ± 59.2 pg/mg creatinine) was significantly higher than that in normal subjects ($p < 0.05$). The urinary histamine excretion rate was not different between normal subjects (24.0 ± 12.5 ng/mg creatinine) and patients with asthma (31.5 ± 25.8 ng/mg creatinine). A robust and systematic within-day variation ($p < 0.01$), but no day-to-day variation, was observed in histamine excretion rate. Although the magnitude of variation in LTE₄ excretion within a day was significantly greater in patients with asthma than in normal subjects ($p < 0.05$), we could not identify any specific diurnal variation pattern in either the normal or the asthma group. No significant correlation was observed between urinary LTE₄ and histamine excretion rate within any subject.

Conclusions: Patients with asthma excrete LTE₄ in the urine at a greater rate than normal subjects. Although no systematic variation in urinary LTE₄ excretion rates over the course of a day was observed in either normal subjects or patients with stable asthma, the presence of a systematic diurnal variation of urinary histamine excretion exists in both groups. (*J ALLERGY CLIN IMMUNOL* 1995;96:643-51.)

Key words: Mediators, leukotrienes, excretion rate, urine, histamine, asthma

The recovery of inflammatory mediators from urine has the potential to provide a rapid, safe, and easily available index of mediator synthesis or

Abbreviations used

FEV ₁ :	Forced expiratory volume in 1 second
LT:	Leukotriene
RP-HPLC:	Reverse-phase high-pressure liquid chromatography

From ^aCombined Program in Pulmonary and Critical Care Medicine and ^bCharles A. Dana Research Institute and the Harvard-Thorndike Laboratory of Beth Israel Hospital, Departments of Medicine, Beth Israel Hospital, Brigham and Women's Hospital and Harvard Medical School.

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Reprint requests: Jeffrey M. Drazen, MD, Combined Program in Pulmonary and Critical Care Medicine, Brigham and Women's Hospital, 75 Francis St., Boston, MA 02115.

release. For example, it is now well established that the measurement of leukotriene (LT) E₄ excretion in the urine provides an index of cysteinyl LT

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production in patients with asthma.¹⁻⁷ In this regard, urinary LTE_4 excretion rates are known to increase during the early-phase response after allergen challenge.¹⁻⁵ In addition, the excretion rate of LTE_4 in patients with aspirin-sensitive asthma is significantly higher than that of normal subjects and patients with non-aspirin-sensitive asthma; aspirin exposure causes a striking increase in urinary LTE_4 excretion in patients with aspirin-sensitive asthma.^{5,6} We previously reported that patients who visited an emergency department for treatment of acute asthma and responded to inhaled β -agonists had high urinary LTE_4 excretion rates.⁷

The use of urinary LTE_4 excretion rates in assessing disease status is based on the tacit assumption that baseline LTE_4 excretion rate, in the absence of known activating stimuli, is relatively constant. However, the excretion rates of some hormones and inflammatory mediators, including histamine, are known to vary over the course of a day.⁸⁻¹⁰ In this study we collected 16 consecutive 6-hour urine specimens from five normal subjects and eight patients with stable mild-to-moderate asthma to measure the excretion rates of urinary LTE_4 and histamine. Our data demonstrate variable rates of both urinary LTE_4 and histamine excretion, whereas a systematic diurnal variation was observed only in histamine excretion rate.

METHODS

Subjects

Five normal male volunteers (38 to 54 years of age) and eight patients with stable mild-to-moderate asthma (4 men and 4 women; 19 to 50 years of age) were enrolled in this study. None of the normal subjects had a history of atopic or airway disease; none were taking any medication. All patients (7 atopic, 1 nonatopic [subject D]) met the American Thoracic Society criteria for the diagnosis of asthma¹¹ and were receiving treatment with β -agonists alone. They were free of asthma exacerbation for at least 2 months before the study and had not received treatment with glucocorticoids (in any form) for that period. None of the normal or asthmatic subjects had a history of aspirin sensitivity. One of the normal subjects was an ex-smoker who had stopped smoking 25 years before the study, and all of the others were nonsmokers. The protocol was approved by Brigham and Women's Hospital Institutional Review Board, and written informed consent was obtained.

Study protocol

Each subject was admitted to the General Clinical Research Center at Brigham and Women's Hospital for at least 96 consecutive hours. All subjects consumed a caffeine-free normal diet. At the beginning of the study, blood samples were collected for complete blood count and routine serum chemistry studies. Four 6-hour urine collections were taken each day from noon to 6:00 PM, 6:00 PM to midnight, midnight to 6:00 AM, and 6:00 AM to noon. Pulmonary function tests, including forced expiratory volume in 1 second (FEV_1), were performed twice daily, between 7:00 and 8:00 AM and between 4:00 and 5:00 PM with an instrument that met American Thoracic Society performance criteria. In patients with asthma, β -agonists were not used within 4 hours of spirometric testing.

Assay of urinary LTE_4

Urine containers were spiked with 0.5 ml of 1 mol/L 4-hydroxy-2,2,6,6-tetramethyl-1-piperidinyloxy free radical (4 hydroxy-TEMPO; Sigma, St. Louis, Mo.) and 0.5 ml of 10 N NaOH and kept at 4° C during collection. After collection was completed for a given interval, 1 ml of urine was aliquoted and stored at -20° C for the assay of urinary histamine and creatinine.

Tritiated LTE_4 (60,000 dpm; 154 Ci/mmol; New England Nuclear, Boston, Mass.) in 2.5 ml of ethanol was added to 10 ml of urine as an internal standard, and the sample was centrifuged at 10,000 g for 10 minutes. The supernatant was applied to a C_{18} Sep-Pak cartridge (Millipore Corp., Bedford, Mass.), which had been preconditioned by the serial addition of methanol and distilled water. The cartridge was then washed with distilled water, followed by 40% methanol; LTE_4 was eluted with 5 ml of 100% methanol. This eluate was dried under vacuum and resuspended in 0.2 ml of 65% methanol, 35% water, 0.3% glacial acetic acid, and 0.01% ethylenediaminetetraacetic acid, pH 5.6. The samples were injected onto a C_{18} reverse-phase high-pressure liquid chromatography (RP-HPLC) column (Novapak C_{18} , 0.4 × 30 mm; Millipore Corp.) and eluted under isocratic conditions at 1 ml/min with 65% methanol, 35% water, 0.3% glacial acetic acid, and 0.01% ethylenediaminetetraacetic acid, pH 5.6. One-milliliter fractions of the RP-HPLC eluate were collected; 50 μl was removed from each fraction, and the amount of radioligand was quantitated by scintillation spectrophotometry. The fractions encompassing the tritiated LTE_4 peak were dried and resuspended in ELISA buffer, and the amount of LTE_4 was quantitated with a commercially available LTE_4 ELISA kit (Cayman Chemical, Ann Arbor, Mich.). Urinary LTE_4 concentrations determined by ELISA were corrected for the recovery of tritiated LTE_4 . The recovery of tritiated LTE_4 during solid-phase extraction and HPLC was 52% $\pm$ 1% (mean $\pm$ SEM). The coefficient of variation among assays was 22%. LTE_4 excretion rates were normalized with the use

TABLE I. Individual pulmonary function data at 8:00 AM and 4:00 PM

Group	Age (yr)	Gender	FEV ₁ (L)		FEV ₁ (%)
			8:00 AM	4:00 PM	
Normal					
a	51	M	4.10 ± 0.08	4.16 ± 0.16	122.4 ± 3.7
b	38	M	3.95 ± 0.10	3.88 ± 0.12	94.1 ± 2.8
c	41	M	3.22 ± 0.32	2.91 ± 0.32	87.3 ± 8.3
d	44	M	4.99 ± 0.08	5.06 ± 0.08	121.6 ± 2.0
e	43	M	3.96 ± 0.08	3.85 ± 0.04	104.9 ± 2.2
Mean	43.4				106.5
Asthmatic					
A	28	M	3.62 ± 0.18	3.50 ± 0.20	71.8 ± 3.8
B	50	F	1.82 ± 0.08	2.00 ± 0.31	72.1 ± 8.7
C	45	M	2.42 ± 0.08	2.13 ± 0.09	85.8 ± 6.6
D	33	M	2.98 ± 0.08	2.94 ± 0.08	68.7 ± 1.7
E	34	F	3.02 ± 0.02	3.00 ± 0.02	94.3 ± 0.9
F	35	M	3.05 ± 0.42	3.17 ± 0.44	78.6 ± 10.2
G	28	F	2.20 ± 0.50	2.09 ± 0.41	64.5 ± 12.9
H	19	F	1.84 ± 0.12	1.62 ± 0.19	52.0 ± 5.4
Mean	34.0				73.5

Values are expressed as means ± SD.

of urinary creatinine concentrations; creatinine concentrations were determined by the kinetic picrate method (Sigma).

Assay of urinary histamine

Urine was diluted 25- to 100-fold with phosphate-buffered saline (pH 7.4), and histamine content was determined by a commercially available ELISA kit (Immunotech International, AMAC Inc., Westbrook, Maine). The cross-reactivity of this assay was 4.2% to 4-methylhistamine and 0.8% to N α-methylhistamine, as determined by the manufacturer.

Statistical analysis

Diurnal variation of pulmonary function was examined by repeated measures analysis of variance. Urinary LTE₄ and histamine excretion rates were analyzed to determine: (1) differences between the normal and the asthma group (group factor), (2) day-to-day variation (repeated measures factor), and (3) within-day variation (repeated measures factor) with a population plot 3 factor factorial repeated measures analysis of variance. Pearson's correlation coefficient between LTE₄ and histamine excretion rates was calculated for each subject. Probability (*p*) values less than 0.05 were considered statistically significant.

RESULTS

All patients with asthma remained in stable condition during the study while using only inhaled β-agonists. FEV₁ values for each subject are given

in Table I. There were no significant differences in FEV₁ measured at 8:00 AM compared with that measured at 4:00 PM, during the course of the study in either the normal or the asthma group. All subjects had normal red cell, white blood cell, and platelet counts and normal serum chemistry values.

LTE₄ excretion

The LTE₄ excretion rate for normal subjects was 83.8 ± 38.2 pg/mg creatinine or 148 ± 58 ng/day (mean ± SD). In patients with asthma, the excretion rate was 110.0 ± 59.2 pg/mg creatinine or 189 ± 76 ng/day; these rates were significantly higher than those of normal subjects (*p* < 0.05). Individual values are given in Fig. 1, A. No significant day-to-day or within-day variation was observed in either group. Interestingly, the magnitude of within-day coefficient of variation was significantly greater in the asthma group than in the normal group (43.7% ± 17.8% vs 29.9% ± 10.2%, *p* < 0.05). However, as shown in Fig. 2, A, no systematic periodicity (i.e. diurnal variation) was identified in either the normal or the asthma group.

Histamine excretion

The mean histamine excretion rate was 24.0 ± 12.5 ng/mg creatinine or 44.2 ± 26.4 μg/day in

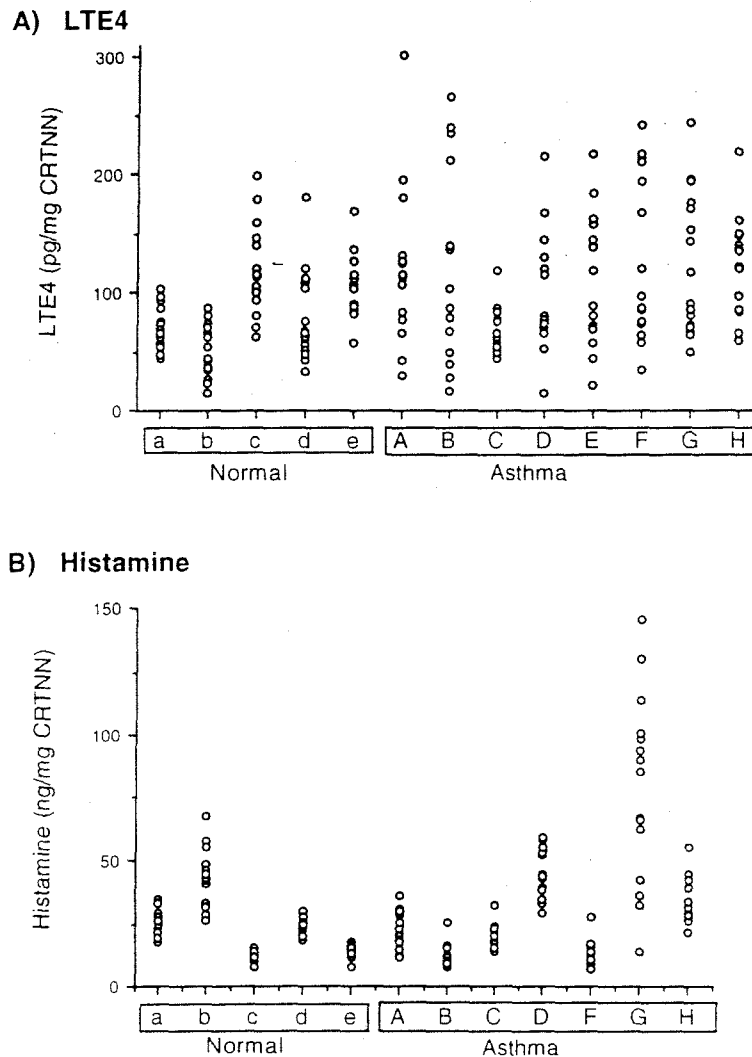


FIG. 1. Distribution of urinary LTE_4 (A) and histamine (B) excretion rates for individual subjects. Normal subjects are represented by lower case letters (a to e) and patients with asthma are represented by capital letters (A to H). CRTNN, Creatinine.

normal subjects and 31.5 ± 25.8 ng/mg creatinine or 54.7 ± 30.6 $\mu\text{g/day}$ in patients with asthma* (not significant compared with normal subjects). The distribution of histamine excretion rates from each subject is shown in Fig. 1, B. Urinary histamine excretion rates demonstrated a significant within-

day variation ($p < 0.01$) without day-to-day variation. When analyzed separately for the normal subjects and patients with asthma, the statistical significance of within-day variation was borderline ($p = 0.06$ for normal subjects, $p = 0.04$ for patients with asthma) because of the small number of subjects, however, diurnal variation was observed in both groups (Fig. 2, B). The lowest urinary histamine excretion rates were observed in the morning (either midnight to 6:00 AM or 6:00 AM to noon), and peaks were observed in the afternoon and evening (either noon to 6:00 PM or 6:00 PM to midnight). In spite of the presence of systematic diurnal variation, the mean coefficient of variation

* One of the female patients with asthma (subject E) showed extremely high histamine excretion rates (>200 ng/mg creatinine). In female subjects, asymptomatic urinary tract infection can cause artificially high urinary histamine levels by bacterial decarboxylation of histidine.¹² Therefore the urinary histamine excretion rate data from this subject were excluded from the analysis.

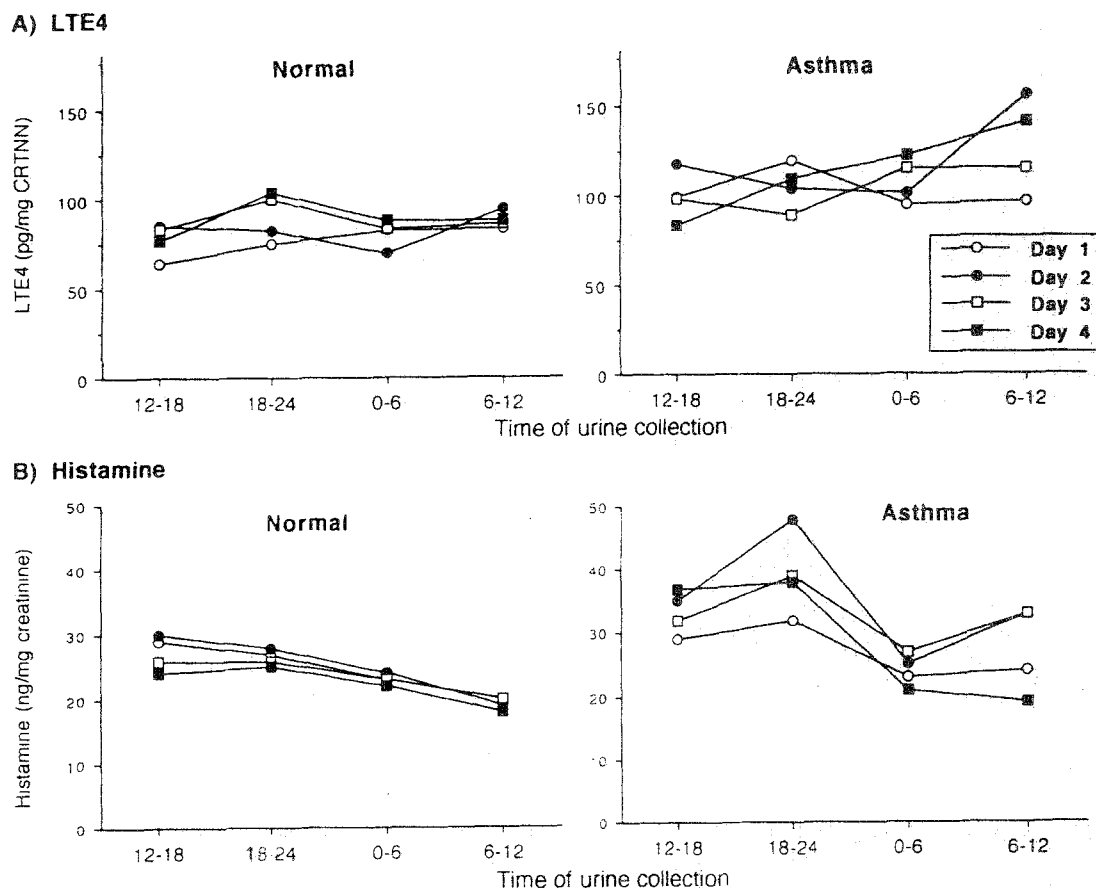


FIG. 2. Diurnal variation of urinary LTE₄ (A) and histamine (B) excretion rates in normal subjects ($n = 5$) and patients with asthma ($n = 8$ [LTE₄], $n = 7$ [histamine]). Each point represents the mean of 4 samples collected during the same time interval on the same day. Urinary LTE₄ excretion rate did not differ significantly among the samples collected at different times of day in either the normal or the asthma group. Urinary histamine excretion showed a significant diurnal variation with higher levels in the afternoon and evening (noon to 6:00 PM, 6:00 PM to midnight) than in the morning (midnight to 6:00 AM, 6:00 AM to noon) in both normal and asthmatic subjects. CRTNN, Creatinine.

of histamine excretion rate within an individual ($18.4 \pm 2.6\%$ for normal subjects, $31.2 \pm 16.6\%$ for patients with asthma) was smaller than that of LTE₄ excretion rate ($35.4\% \pm 5.1\%$ for normal subjects, $49.1\% \pm 13.1\%$ for patients with asthma).

Urinary histamine/LTE₄ ratio

No significant correlation was observed between urinary LTE₄ and histamine excretion rates within any individual. The molar ratios of urinary histamine concentration to urinary LTE₄ concentration were distributed in a log-normal fashion as confirmed by the Wilk-Shapiro test. The geometric mean of this ratio was 1057 (95% confidence interval, 933-1197; Fig. 3) and was not different

between normal and asthmatic subjects (1109 for normal subjects vs 1020 for patients with asthma).

DISCUSSION

The recovery of inflammatory mediators from the urine has the potential to provide a rapid, safe, and easily available method of estimating mediator synthesis and release. However, because it is well established that many endogenous biomolecules are not excreted constantly over the course of a 24-hour period,⁸ it is important to determine whether a given mediator is excreted at a constant rate. In this study we examined urinary excretion of two mediators of bronchoconstriction, LTE₄ and histamine, thought to be of importance in anaphy-

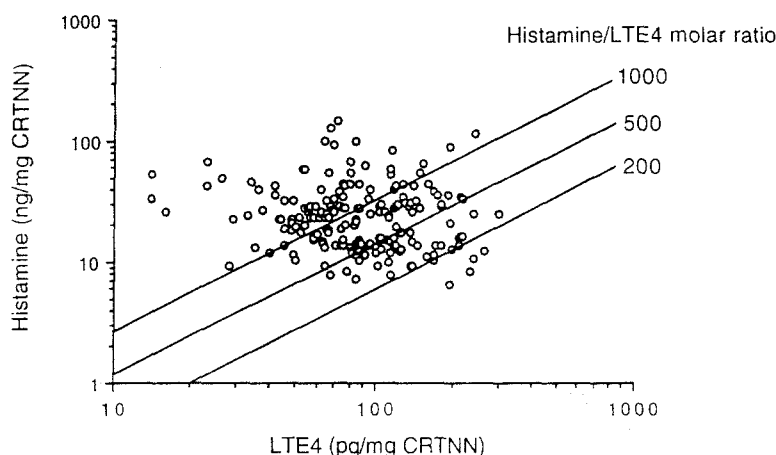


FIG. 3. Correlation between urinary LTE_4 and histamine excretion rate. There was no significant correlation between the two parameters. More than 98% of the total samples (204 samples/208 samples) demonstrated a histamine to LTE_4 molar ratio of greater than 200. CRTNN, Creatinine.

laxis, allergic rhinitis, bronchial asthma, and other diseases. Our data demonstrate that in both normal subjects and patients with stable mild-to-moderate asthma, the LTE_4 excretion rate does not vary systematically over the course of a day, but there is a diurnal variation in histamine excretion rate.

A number of investigators have reported values for urinary LTE_4 excretion rate in normal subjects¹³⁻²⁴; these are summarized in Table II. Our geometric mean for urinary LTE_4 excretion rate of 75 pg/mg creatinine in normal subjects corresponds to values reported by most investigators. Although an increased LTE_4 excretion rate during either provoked or spontaneous asthma is well appreciated,¹⁻⁷ it is controversial whether there is an enhanced baseline LTE_4 excretion in patients with non-aspirin-sensitive asthma. Smith et al.²² compared LTE_4 values in urine samples from 17 normal subjects and 31 patients with asthma without aspirin sensitivity and found no significant differences. Our data from eight patients with asthma showed a mean LTE_4 excretion rate of 110.0 ± 59.2 pg/mg creatinine; this mean was greater than the mean of the values from the normal subjects. Although the mean value for LTE_4 excretion was greater in the patients with asthma, the wide variability in excretion rate among both normal and asthmatic subjects makes it difficult to discern these two subgroups on the basis of a single measure of urinary LTE_4 excretion. However, it is interesting to note that no single sample in the normal subjects showed an

LTE_4 excretion rate of more than 200 pg/mg creatinine, whereas seven of eight patients with asthma had at least one LTE_4 sample with a value greater than 200 pg/mg creatinine during the 4-day study period. No patient in our study was known to be aspirin-sensitive, and no symptomatic episodes of bronchoconstriction were observed during the study. These data demonstrate both a higher average LTE_4 excretion rate in patients with asthma compared with normal subjects and greater variability of LTE_4 excretion in the asthma group.

Plasma histamine levels have been used to assess histamine release in vivo.²⁵ However, this approach is complicated by the short biologic half-life of histamine in plasma and by the release of histamine from basophils during blood sampling. For these reasons, the assay of histamine or its metabolites in urine has potential advantages over the assay of plasma histamine. The immunoassay for histamine we used has been extensively validated. McBride et al.²⁶ reported a mean urinary histamine level of 20.9 ± 11.2 ng/mg creatinine in 10 normal subjects, which is similar to the mean urinary histamine level of 24.0 ± 12.5 ng/mg creatinine for normal subjects in our study.

The presence of a diurnal variation in urinary histamine excretion rate in normal subjects is still controversial. In children, no significant diurnal variation in the excretion rate of histamine or histamine metabolites has been observed.^{12, 27} In contrast to the data obtained in children, Dunér and Pernow⁹ reported higher urinary histamine levels in the daytime than at night in adults; this

TABLE II. Urinary LTE₄ excretion rate in normal subjects

Study	Year	n	Urinary LTE ₄ excretion rate (pg/mg creatinine)		
			Arithmetic mean ± SEM	Geometric mean (95% CI)	Range
Taylor et al. ¹³	1989	29		179 (152-215)	
Tagari et al. ¹⁴	1989	9	35 ± 10		
Huber et al. ¹⁵	1989	7	67		34-101
Westcott et al. ¹⁶	1990	6	68 ± 4		56-68
Bernard et al. ¹⁷	1991	12	35 ± 10		16-57
Fauler et al. ¹⁸	1991	16	34 ± 11		
Carry et al. ¹⁹	1992	10	103 ± 42		0-458
Fauler et al. ²⁰	1992	11		37 (30-47)	
Kikkawa et al. ^{21*}	1992	10	113 ± 12	107 (80-141)	45-156
Smith et al. ²²	1992	17		34 (25-48)	
Manso et al. ²³	1992	6	108 ± 32		
Denzlinger et al. ²⁴	1993	21	33†		4-94†
This study		5	84 ± 4	75 (67-84)	14-198

CI, Confidence interval.

*Data from children aged 7 to 12 years.

†Includes both LTE₄ and N-acetyl LTE₄.

finding was confirmed by Imamura et al.¹⁰ Our data support a diurnal variation in urinary histamine excretion rate in normal adults; the highest excretion rate occurs in the afternoon.

In this study there was no correlation between the excretion rates of LTE₄ and histamine. Although this is the first study to report the urinary excretion rates of both cysteinyl LTs and histamine in normal subjects, a relationship between cysteinyl LT and histamine levels in bronchoalveolar lavage fluid has been established in patients with asthma.^{28,29} Wenzel et al.²⁸ reported a positive correlation between LTC₄ and histamine levels in bronchoalveolar lavage fluid obtained 5 minutes after endobronchial allergen challenge. In their report the mean histamine concentration was 8830 ± 1306 pg/ml, and LTC₄ plus LTD₄ concentrations ranged from 212 to 476 pg/ml after antigen challenge.²⁸ Miadonna et al.²⁹ also examined the concentrations of histamine and cysteinyl LTs during the early-phase response after endobronchial allergen exposure; in their study the histamine concentration was 20,200 ± 5,800 pg/ml, and the cysteinyl LT concentration was 820 ± 210 pg/ml.²⁹ In both studies the molar ratio of histamine to cysteinyl LTs recovered was approximately 100 to 250. In our study the molar ratio of urinary histamine to LTE₄ is 5 to 10 times greater, namely 1056, the geometric mean, (95% confidence interval, 933-1197). The recovery of histamine in urine is known to be 1% to 3%,³⁰ whereas recovery of LTE₄ in

urine is known to be 4% to 6%,³¹ which suggests that a greater amount of histamine per cysteinyl LT is available for renal clearance than is available in the lung during the early-phase asthmatic response. On the basis of these data, it seems reasonable to postulate that the cellular sources, which remain unknown, of histamine and cysteinyl LTs recovered from the urine are different from those in the lungs of allergen-exposed patients with asthma. Mast cells and basophils are most probably the major cellular sources of histamine and cysteinyl LTs during the early-phase response in patients with allergic disease. Data obtained from in vitro experiments can be used to estimate the relative amounts of histamine and LTC₄ produced during cellular activation. For example, isolated skin mast cells, stimulated by anti-IgE, release both histamine and LTC₄; the availability of the two mediators is clearly linked, with a molar ratio of histamine to LTC₄ of 350.³² Nonimmunologic stimuli, such as neuropeptides or somatostatin, induce predominantly histamine release from skin mast cells; as a result, the ratio of histamine to LTC₄ recovered increases, but the recovery of the two mediators remains closely linked.³² The lack of correlation of urinary histamine and LTE₄ excretion in our study implies the presence of a source of histamine or cysteinyl LTs other than mast cells.

Gastric mucosa is a possible nonallergic source of histamine release.³³ Histamine exists in both mast cells and enterochromaffin cells in the gastric

mucosa, and the latter are important for the histamine release induced by gastrin.³³ Although the relationship between food intake and the increase in urinary histamine excretion rate is still controversial,^{10, 12, 34} Imamura et al.¹⁰ demonstrated a significant increase of histamine recovery after meals and a significant decrease after gastrectomy. We believe that histamine release from the gastric mucosa after alimentation could explain the higher histamine excretion rate in the afternoon observed in this study and in others.^{9, 10}

Our data demonstrate an apparent diurnal variation in urinary histamine excretion in normal subjects and patients with mild-to-moderate asthma, confirming the importance of collecting samples at the same time of a day, preferably before breakfast. On the contrary, high sample-to-sample variability within an individual, in spite of the lack of an apparent systematic diurnal variation, complicates the significance of urinary LTE₄ levels, underlining the importance of evaluating multiple samples or longer urine collection rather than a single sample collected during a short period.

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