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Urinary leukotriene E_4 in bronchial asthma

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ABSTRACT: Leukotriene E_4 (LTE_4) is excreted into the urine in a relatively constant proportion of 4–7% when either leukotriene C_4 (LTC_4) or LTE_4 is intravenously infused, regardless of the magnitude of the infused dose. Measurement of LTE_4 in urine is, therefore, a convenient and non-invasive method for assessing changes in the rate of total body sulphidopeptide leukotriene production. We assayed urinary LTE_4 in 17 normal subjects, 31 subjects with asthma without aspirin sensitivity, and 10 aspirin-sensitive subjects. The relationship between urinary LTE_4 and nonspecific bronchial hyperresponsiveness, as assessed by the provocative dose producing a 20% fall in forced expiratory volume in one second (PD_{20}) to inhaled histamine, was examined in 19 non-aspirin-sensitive asthmatic subjects. The urinary LTE_4 values were log-normally distributed. Urinary LTE_4 was detected in 28 of the 31 non-aspirin-sensitive asthmatic subjects, and the geometric mean (95% confidence interval (CI) of 43 (32–57) $pg \cdot mg^{-1}$ creatinine was no different to that of 34 (25–48) $pg \cdot mg^{-1}$ creatinine measured in the normal subjects. The geometric mean of 101 (55–186) $pg \cdot mg^{-1}$ creatinine measured in the aspirin-sensitive asthmatics was significantly higher than that measured in the normal subjects ($p<0.005$) and in the asthmatic subjects who were non-aspirin-sensitive ($p<0.002$), but there was considerable overlap between the three groups. There was no relationship between urinary LTE_4 and PD_{20}, or between urinary LTE_4 and baseline forced expiratory volume in one second (FEV_1) (% predicted). Thus, measurement of LTE_4 in a single sample of urine will not predict the extent of bronchial hyperresponsiveness or degree of airflow obstruction.

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The sulphidopeptide leukotrienes (LTC_4, LTD_4 and LTE_4) comprise the activity previously referred to as the slow reacting substance of anaphylaxis (SRS-A). These mediators enhance microvascular permeability [1–4], increase nonspecific bronchial hyperresponsiveness [5–8], and are potent bronchoconstrictor agents [1, 9, 10]. They have the potential to mediate events associated with airway inflammation, and to contribute to the pathophysiology of asthma.

The leukotrienes are derived from arachidonic acid by oxidative metabolism *via* the 5-lipoxygenase pathway. The unstable intermediary epoxide, LTA_4, is enzymatically metabolized to LTC_4 by the addition of glutathione at the 6 position by LTC_4 synthetase. LTC_4 in turn may be converted to LTD_4 by the removal of a glutamic acid residue through the action of γ-glutamyl transpeptidase. Finally, LTD_4 may undergo further bioconversion by cleavage of the glycine residue, by a dipeptidase, to form LTE_4.

The most stable of these three compounds, LTE_4, can be measured in nanomolar quantities in urine [11–15]. Following intravenous infusion of both synthetic LTC_4 and LTE_4 between 4–7% is excreted as LTE_4 within the two hours following infusion. Because this proportion is relatively constant and is independent of the dose infused [12, 14], the measurement of LTE_4 in urine is a convenient, non-invasive method of assessing changes in the rate of total body sulphidopeptide leukotriene production. The problem of *ex-vivo* production of leukotrienes is also minimized, since urine is essentially free of cells.

The utility of measuring LTE_4 has been demonstrated in a number of recent publications. TAYLOR *et al.* [15] and WESTCOTT *et al.* [16] reported that urinary concentrations of LTE_4 are significantly raised in patients admitted to hospital with an acute exacerbation of asthma, when compared with those measured following recovery. They also demonstrated that urinary LTE_4 concentrations rise significantly following allergen challenge [15, 17]; an observation which has now been confirmed by a number of other groups [18–20]. CHRISTIE *et al.* [21] demonstrated that aspirin challenge in aspirin-sensitive individuals resulted in an increase in urinary excretion of LTE_4. Measurement of urinary LTE_4 has, therefore, been useful in identifying an increased production of leukotrienes in response to

selected challenges, supporting the view that these eicosanoid mediators may contribute to the mechanism of the acute responses elicited.

However, it has not been established whether baseline measurements of urinary LTE_4, in the absence of challenge, may also provide useful information. For example, CHRISTIE *et al.* [21] observed that the resting levels of urinary LTE_4 were higher in aspirin-sensitive asthmatic subjects than those measured in asthmatic subjects who were not aspirin-sensitive. We reasoned that it may be possible to distinguish between different groups of subjects, or perhaps to assess disease severity by measuring urinary LTE_4 during resting conditions. We have therefore assayed samples of urine from a group of 17 normal subjects, 31 subjects with asthma who had no history of aspirin sensitivity, and 10 aspirin-sensitive asthmatic subjects. In order to determine whether baseline urinary LTE_4 concentrations reflect disease severity in asthmatic patients, we also examined the relationship between urinary LTE_4 concentrations and the degree of sensitivity to the non-specific agonist, histamine, in a group of asthmatic subjects with no history of aspirin sensitivity.

Methods

Subjects

Normal subjects. Seventeen subjects (8 male, 9 female) were studied. They were aged 20–35 yrs and had no history of asthma, sensitivity to aspirin or allergic disease. None were smokers, and none were atopic.

Asthmatic subjects without aspirin-sensitivity. Asthma was defined as a clinical history of episodic wheezing and evidence of at least 15% reversibility either spontaneously or with up to 800 μg of inhaled salbutamol.

Thirty one subjects with asthma (23 male, 8 female) aged 18–34 yrs, with no history of aspirin sensitivity volunteered for the study. The mean±1 SD baseline forced expiratory volume in one second (FEV_1) (%predicted) was 86±15%. Thirty of the subjects were atopic. At the time of the study, 25 (81%) were using beta-sympathomimetics, 6 (19%) were also using inhaled corticosteroids, and 1 (3%) was using cromolyn sodium. All therapy for asthma was withheld for at least 12 h prior to each study day.

Subjects with aspirin-induced asthma. Ten subjects (8 male, 2 female) aged 21–54 yrs, with aspirin sensitivity were studied. Six were atopic. They were selected on the basis of a previous challenge with aspirin which produced a >15% fall in FEV_1. The mean±1 SD FEV_1 (%predicted) was 97±10%. At the time of the study, 8 were using beta-sympathomimetics, 5 were using inhaled steroids, 3 were using oral theophylline, 1 was using oral corticosteroids (5 $mg \cdot day^{-1}$) and one was using cromolyn sodium.

Histamine challenges

The histamine challenges were performed within 30 min after urine collection and were administered using a protocol similar to that described by YAN *et al.* [22]. Solutions of histamine dissolved in phosphate buffered saline (PBS) were delivered *via* DeVilbiss No. 45 hand-held nebulizers (DeVilbiss Co., Pennsylvania, USA). The output of four nebulizers was determined by the operator (RH), who performed all histamine challenges. Four solutions were then prepared, one for each nebulizer, such that they delivered PBS and 0.1, 0.4 and 0.8 μmol of histamine, respectively, per inhalation.

To administer the aerosol, the subjects were instructed to open their mouth widely and to exhale to slightly below their functional residual capacity (FRC). They were then instructed to inhale slowly over 1–2 s towards total lung capacity (TLC) whilst keeping their mouth widely opened. The nebulizer was placed in front of the mouth, between the teeth, and at the beginning of inspiration, the nebulizer bulb was firmly squeezed once. The subjects were instructed to hold their breath for 3 s before exhaling. Doses that required more than one inhalation were given in consecutive breaths. The FEV_1 was measured 90 s after each dose.

After taking initial measurements of FEV_1, subjects were given two inhalations of PBS. If the decrease in FEV_1 was <5%, histamine challenge was performed. The subjects were then given increasing doses of histamine until the FEV_1 had fallen by 20% of the value measured after inhalation of PBS control. The cumulative doses administered were 0.1, 0.4, 1.6, 4 and 8 μmol.

Measurement of urinary LTE_4

The method used to measure urinary LTE_4 was similar to that described by TAGARI *et al.* [13].

Reagents and standards. For high performance liquid chromatography (HPLC) separation, HPLC grade methanol and glacial acetic acid were purchased (BDH, Poole, Dorset, UK). For the HPLC buffers, water purified with the MilliQ system (Millipore, Molsheim, France) was used. All other reagents were of analytical quality and were obtained either from BDH (NaH_2PO_4, CH_3COOH) or Sigma Chemical Co., St Louis, Mo, USA (disodium edetic acid (EDTA)). [^{3}H]LTE_4, used as an internal standard, had a specific activity of 39.3 $Ci \cdot mmol^{-1}$, and was purchased from NEN-Du Pont, Boston, USA.

For radio-immunoassay, LTE_4 prepared by total chemical synthesis, as described previously [23], was used for the standards. The purity of the LTE_4 was confirmed using reverse phase-HPLC (RP-HPLC) on a 10 μ C18 Ultrasil ODS column (4.6 by 250 mm; Beckman Instruments Inc., Berkeley, Ca, USA) at a flow rate of 1 $ml \cdot min^{-1}$ with 62% methanol, 37.8%

water, 0.1% acetic acid and 0.1% disodium EDTA, pH 5.6. Absorbance was monitored at 280 nm with an on-line spectrophotometer linked to an integrator (Spectraphysics SP4270, San Jose, USA), and the purity of the LTE_4 was confirmed by its elution as a single peak at its unique retention time of 25 min in this solvent system. The concentration of the solution was checked by ultraviolet scanning (Philips PU8720 UV/NIS, Pye Unicam, Cambridge, UK) at 280 nm, assuming an extinction coefficient of 40,000 $cm^{-1} \cdot mol^{-1}$. Anti-leukotriene sera were kindly donated by A. Ford-Hutchinson (Merck Frosst, Canada). Reagents for the Tris-HCl radio-immunoassay buffer were obtained from BDH and were of analytical standard. From this buffer, a 1% charcoal-dextran solution was prepared for separation of the bound and free fractions. The charcoal was obtained from Sigma, and the dextran (T70) from Pharmacia (Uppsala, Sweden). The scintillation fluid (Supersolve-X) was obtained from Koch-Light (Haverhill, Suffolk, UK).

Collection and storage. Aliquots of 50 ml were taken from samples collected on arrival at the laboratory prior to measurement of baseline FEV_1 and histamine responsiveness. At the time of collection, the free radical scavenger 4-hydroxy-2,2,6,6-tetramethyl piperidinyloxy (4-OH-TEMPO) free radical (Sigma) was added to a final concentration of 1 mM, and the pH adjusted to 9.0 with NaOH to stabilize the LTE_4. The samples were then coded and stored at -70°C until analysis.

Analysis of LTE_4 and creatinine. Stored urine samples were thawed and centrifuged at 10,000×g for 10 min, to remove cellular debris. The supernatants were removed, and 100 µl of a solution containing [^{3}H]LTE_4 (39.3 $Ci \cdot mmol^{-1}$; NEN-DuPont) was added to 10 ml of urine to give 4,000 disintegrations per minute (DPM). Then 25 µl of glacial acetic acid was added to bring the pH to 3.5–3.8 and the sample was loaded directly onto a 10 µ precolumn (Ultrasil ODS, 3.4 mm × 4.5 cm, Beckman, USA), which was isolated from the main analytical column. Polar metabolites were washed from the precolumn at 2 $ml \cdot min^{-1}$ for 8 min with a phosphate buffer (0.1% NaH_2PO_4, pH 3.8) and then for 12 min with a methanol:phosphate buffer (0.1% pH 3.8) in the proportions 50:50 (v:v) at a flow rate of 2 $ml \cdot min^{-1}$. The sample was then retrogradely eluted *via* an automatic switching valve onto a reversed phase analytical column (Ultrasil ODS 4.5 mm × 25 cm, Altex) that had been equilibrated in 62% methanol:37.8% water:0.1% acetic acid:0.1% EDTA (v:v) at pH 5.6. The LTE_4 was eluted at a flow rate of 1 $ml \cdot min^{-1}$. After RP- HPLC, 250 µl of each fraction was counted in a beta liquid scintillation counter (Tricarb 1900CA, Packard Instrument Co., USA), and the peak of radioactivity containing the tritiated internal standard was identified. The remaining 750 µl of those fractions containing the [^{3}H]LTE_4, and of the two fractions eluting before and after the peak were dried under vacuum at 23°C, and resuspended in 250 µl of 10 mM Tris-HCl buffer (pH 7.4), containing 0.15 M NaCl and 0.1% gelatin. The concentrations of immunoreactive LTE_4 were assessed by radio-immunoassay as described previously [24], using synthetic LTE_4 (0.01–10 $ng \cdot ml^{-1}$) to construct the standard curve. The immunoreactivity in the fractions eluting before and after the peak of internal standard were used to calculate mean background immunoreactivity. LTE_4 concentration was expressed as $pg \cdot mg^{-1}$ creatinine and was calculated from measured immunoreactivity minus background, corrected for recovery and dilution. The recovery of LTE_4 was (74±9%, mean±SD). The intra- and inter-assay coefficients of variation are 8% and 15%, respectively, and sensitivity of the assay is 8 pg.

Urinary creatinine concentrations were measured (BM/Hitachi 737, Boehringer Mannheim, Germany) with the kinetic picrate method without deproteinization [25].

In order to validate the technique, known quantities of synthetic LTE_4 (0, 1.25, 2.5, 5 and 10 ng) were added to 10 ml aliquots of four different samples of urine from normal subjects. The purity of the synthetic LTE_4 was confirmed by its elution as a single peak at the standard retention time of 25±0.2 min (mean±SEM) for LTE_4 in this solvent system. The urine was then treated in the same way as the samples, including stabilization with 4-OH-TEMPO and NaOH, freezing and storage at -70°C, thawing, and the addition of 2,500 DPM of [^{3}H] LTE_4 as an internal standard. Endogenous production of LTE_4 was assessed in a sample which contained vehicle alone (0 ng LTE_4) and recovery of synthetic LTE_4 was calculated as total LTE_4 minus endogenously produced LTE_4. The values for synthetic LTE_4 eluted from the samples closely approximated the quantities added to the samples (fig. 1), indicating that the assay was able to reliably quantify urinary LTE_4.

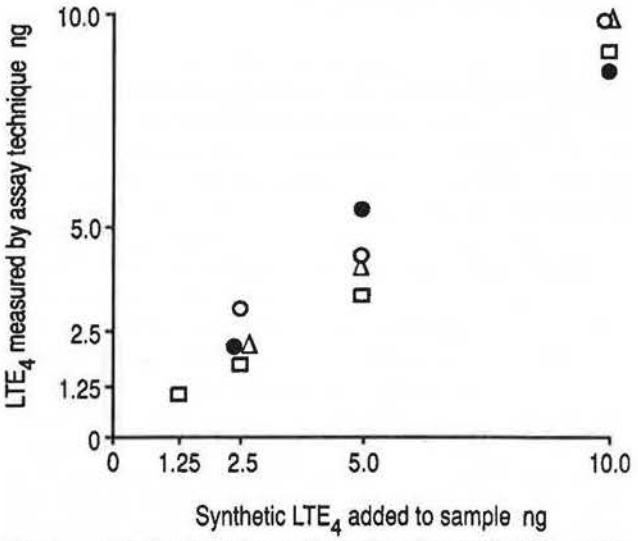

Fig. 1. – Relationship between the quality of synthetic LTE_4 added to 10 ml aliquots of urine, and the quantity measured in those samples by radio-immunoassay following separation by RP-HPLC (see Methods for details). The analysis was performed on four different samples of urine, each represented by a different symbol. LTE_4: leukotriene E_4; RP-HPLC: reverse phase high performance liquid chromatography.

Results

Distribution of values for urinary LTE_4

The distribution of values for urinary LTE_4 in the three groups is shown in figure 2. LTE_4 could be detected in the urine of all the normal subjects. Values ranged from 7–102 pg·mg^{-1} creatinine. The normality of the distribution was assessed using the "Minitab" statistics package. The distribution was highly skewed, but was normalized by log-transformation of the data. Values have, therefore, been expressed in terms of the geometric mean and 95% confidence interval (95% CI). The geometric mean value (95% CI) was 34 (25–48) pg·mg^{-1} creatinine.

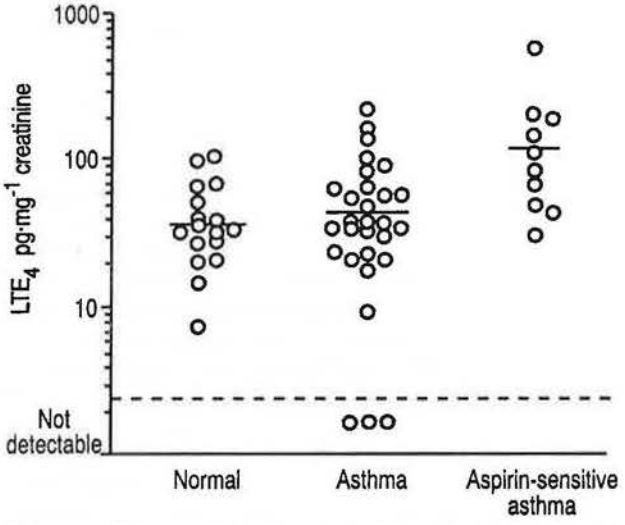

Fig. 2. – Urinary LTE_4 concentrations (pg·mg^{-1} creatinine) measured in 17 normal subjects, 31 subjects with asthma without aspirin sensitivity, and 10 aspirin-sensitive asthmatics. LTE_4: leukotriene E_4. Horizontal bars indicate geometric means.

Urinary LTE_4 could be detected in 28 of the 31 subjects with asthma who were not sensitive to aspirin. Where values could be measured, the range was 9–1,497 pg·mg^{-1} creatinine. The value of 1,497 pg·mg^{-1} creatinine was measured in a subject who arrived at the laboratory with an FEV_1 that was only 35% of the predicted value. On treatment with 100 μg of inhaled salbutamol the FEV_1 improved to 91% of the predicted normal value. Since this patient had obviously had an acute and severe attack of asthma, the data were not included in the analysis of the results. Excluding this subject, the highest value measured in this group was 212 pg·mg^{-1} creatinine, and the geometric mean value (95% CI) was 43 (32–57) pg·mg^{-1} creatinine (fig. 2). The distribution of values was, therefore, similar to that of the normal subjects, and was not significantly different (p=0.85) when compared using the Mann-Whitney U-test (fig. 2).

Urinary LTE_4 could be measured in all 10 subjects with aspirin-induced asthma and was on average three-fold greater than that measured in the other two groups (fig. 2). Values ranged from 29–550 pg·mg^{-1} creatinine. The geometric mean value was 101 pg·mg^{-1} creatinine, and the 95% confidence interval of the mean was 55–186 pg·mg^{-1} creatinine. Although there was considerable overlap, these values were significantly higher than those measured in the normal subjects (p<0.002), and the asthmatic subjects who were not sensitive to aspirin (p<0.001), when the data were compared using the Mann-Whitney U-test.

Relationship between baseline urinary LTE_4 concentrations and the degree of nonspecific bronchial hyperresponsiveness

Nineteen of the asthmatic subjects with no history of aspirin sensitivity agreed to undergo bronchial provocation with inhaled histamine.

It was not always possible to get absolute values for either responsiveness to histamine, or urinary LTE_4 concentrations. In two subjects, the FEV_1/FVC ratio was <65%, and they were not challenged. One subject did not respond with a 20% fall in FEV_1 after the final dose of histamine. Urinary LTE_4 could not be detected in three subjects. The relationship between baseline values for urinary LTE_4 and responsiveness to inhaled histamine was calculated using the Spearman's rank order correlation coefficient. The two subjects who were not challenged were not included in the analysis, since no assessment of bronchial hyperresponsiveness could be made. The subject who did not respond with a 20% fall in FEV_1 at the highest dose was ranked as least responsive. The remainder were ranked from lowest to highest according to their provocative dose producing a 20% fall in FEV_1 from baseline (PD_{20}) value. The three subjects in whom no urinary LTE_4 was detected were ranked equal first for urinary LTE_4 concentrations, and the remainder were ranked from lowest to highest according to the value for urinary LTE_4 measured.

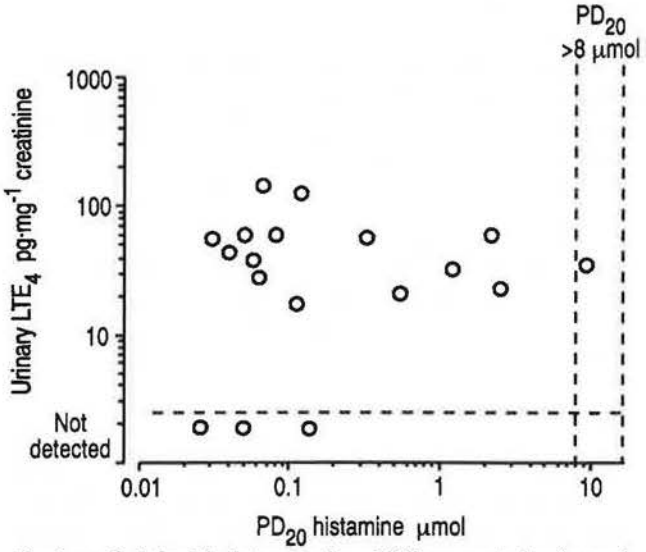

Fig. 3. – Relationship between urinary LTE_4 concentration (pg·mg^{-1} creatinine) and the cumulative dose of histamine (μmol) required to induce a 20% fall in FEV_1 (PD_{20}) in 18 asthmatic subjects without aspirin sensitivity. LTE_4: leukotriene E_4; FEV_1: forced expiratory volume in one second.

There was no correlation between baseline values for urinary LTE_4 and responsiveness to inhaled histamine (r_s=0.14, p>0.05) (fig. 3). There was also no relationship between baseline FEV_1 (% predicted) and urinary LTE_4 in this group of subjects (r_s=0.2, p>0.05) (fig. 4). Similarly, there was no relationship between baseline FEV_1 and urinary LTE_4 in the 10 aspirin-sensitive asthmatic subjects (r=0.06, p>0.05).

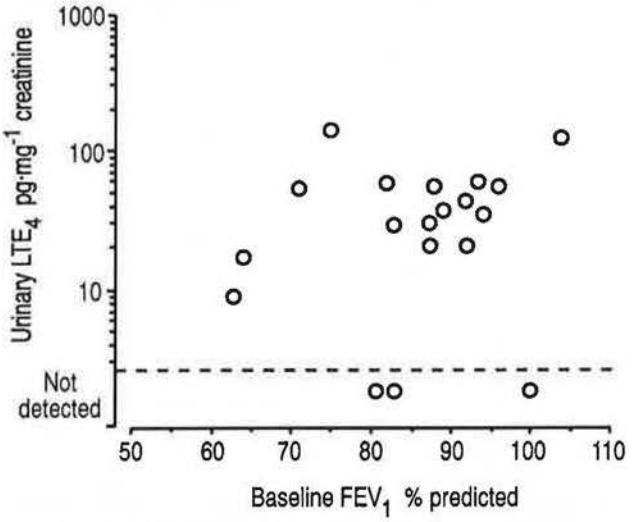

Fig. 4. – Relationship between urinary LTE_4 concentration and the baseline FEV_1 (expressed as a percentage of the predicted normal value) in 19 asthmatic subjects without aspirin sensitivity. For abbreviations see legend to figure 3.

Discussion

The values for urinary LTE_4 were log-normally distributed (fig. 2) in both the normal subjects and the asthmatic subjects without aspirin-sensitivity. This has also been reported in a group of 29 normal subjects by TAYLOR *et al.* [15]. We would, therefore, suggest that future data should be expressed in terms of the geometric mean and 95% confidence interval of the mean, rather than the mean and SEM. To date, there has been little standardization, and comparison of values reported by different groups is difficult.

The geometric mean and 95% confidence interval in the 17 normal subjects that we studied was 34 (25–48) $pg \cdot mg^{-1}$ creatinine. Using a similar technique for urinalysis of LTE_4, TAGARI *et al.* [13] reported a mean value±SEM of 35±10 $pg \cdot mg^{-1}$ creatinine in nine normal subjects, and WESTCOTT *et al.* [16] reported values which ranged from 56–81 $pg \cdot mg^{-1}$ creatinine in six normal subjects. The geometric mean values in these two studies were not reported. These values are four to sixfold lower than those reported by TAYLOR *et al.* [15] in 29 subjects (geometric mean and 95% CI 210 (176–249) $pg \cdot mg^{-1}$ creatinine).

Similar differences between studies have also been found for baseline measurements made in groups consisting of asthmatic subjects. In the 27 subjects from this study for whom urinary LTE_4 could be detected, the geometric mean value (95% CI) measured in the non-aspirin-sensitive asthmatics was 43 (32–57) $pg \cdot mg^{-1}$ creatinine. These values are similar to the geometric mean (95% CI) of 44 (34–56) $pg \cdot mg^{-1}$ creatinine that have been reported from a study of five asthmatic subjects by CHRISTIE *et al.* [21]. Considerably higher values for the geometric mean (95% CI), 208 (121–615) $pg \cdot mg^{-1}$ creatinine, have been reported by TAYLOR *et al.* [15] in eight asthmatic subjects. Although there have been values reported for asthmatic subjects in other studies, these have been reported in terms of the mean±SEM, making comparison difficult. SLADEK *et al.* [19] reported mean values of approximately 180–200 $pg \cdot mg^{-1}$ creatinine for both normal (n=6) and asthmatic (n=9) subjects, and MANNING *et al.* [18] reported a mean±SEM value of 150±50 $pg \cdot mg^{-1}$ creatinine in asthmatic subjects who demonstrated isolated responses to allergen challenge, and 67±14 $pg \cdot mg^{-1}$ creatinine in subjects who demonstrated dual responses.

Although there are differences in absolute values reported from different centres for urinary concentrations of LTE_4, there are no differences in the data from these centres when the values are considered in terms of the proportionate change from baseline values and there has been no discrepancy in the conclusions that have been drawn from these studies. The reasons for these differences in absolute values measured by various groups are not clear, and cannot be entirely explained by the difference between expressing the data in terms of the arithmetic and geometric mean. The technique that we have used in the present study, and that used by TAGARI *et al.* [13] have both been validated by showing that known quantities of synthetic LTE_4 added to urine can be reliably quantified following separation by RP-HPLC, and measurement by radioimmunoassay (fig. 1). The values reported from both of these centres, using similar techniques [13, 18, 19] are comparable, and are low compared with other values reported in the literature.

This study confirms the previous findings of CHRISTIE *et al.* [21], that urinary LTE_4 levels are significantly higher in aspirin-sensitive asthmatics than those measured in asthmatic subjects who are non-aspirin-sensitive. Although the values were on average threefold greater than those observed in the non-aspirin-sensitive asthmatic subjects and the normal control subjects, there was considerable overlap between the three groups. Thus, baseline measurements of urinary LTE_4 alone will not indicate aspirin sensitivity in many aspirin-sensitive patients.

The observation that there is on average a higher urinary LTE_4 concentration in aspirin-sensitive subjects is, however, interesting and suggests that there may be increased eicosanoid production in these patients, even in the absence of provocation.

There was no relationship between baseline concentrations of urinary LTE_4 and the degree of nonspecific bronchial hyperresponsiveness in the non-aspirin-sensitive asthmatic subjects studied. Similarly there was no relationship between concentrations of urinary LTE_4 and baseline FEV_1 in either non-aspirin-sensitive or aspirin-sensitive subjects. These findings are consistent

with those recently reported by WESTCOTT *et al.* [17]. Together with the observation that baseline values are not different from those measured in normal subjects, these findings indicate that leukotriene excretion is not abnormally high in the absence of provocation or an acute attack of asthma. However, we cannot exclude the possibility that the metabolism and/or clearance of leukotrienes may be different in asthmatic and normal individuals, and that leukotriene production is increased, but is not reflected in urinary concentrations of LTE_4.

It is possible that the patient's asthma therapy, particularly corticosteroid use, may have influenced the production of sulphidopeptide leukotrienes. However, there was no relationship between corticosteroid use and urinary LTE_4 concentrations. The patients with undetected urinary LTE_4 were not on any treatment other than inhaled beta-sympathomimetics.

The measurements of urinary LTE_4 were made from a single sample taken at the time of the visit to the laboratory. It is possible that a cumulative assessment of LTE_4 excretion over time may have been more informative.

In summary, measurement of sulphidopeptide leukotrienes in urine is a convenient and non-invasive way to measure total body leukotriene production. Following acute challenge with either allergen [15, 17–20], or aspirin [21], urinary LTE_4 increases, suggesting a role for the sulphidopeptide leukotrienes in the immediate response to these challenges. Recent studies with specific and potent leukotriene antagonists support this conclusion, since both allergen-induced acute bronchoconstrictor responses [26, 27] and aspirin-induced asthma [28] can be inhibited by these drugs. However, the contribution of the leukotrienes to the chronic signs and symptoms of asthma remains to be established. Preliminary evidence suggests that long-term administration (6 weeks) of potent and specific leukotriene antagonists improves baseline FEV_1 [29] and symptom scores [30] in asthmatic subjects. These treatment effects have been modest, however, and clearly further studies are required to establish the role of leukotrienes in the aetiology of asthma. In the absence of bronchial provocation, it is not possible to predict either the degree of nonspecific hyperresponsiveness or baseline FEV_1 from the concentration of LTE_4 in the urine of asthmatic subjects.

We have confirmed the findings of CHRISTIE *et al.* [21], that urinary LTE_4 concentrations are raised in patients with aspirin-induced asthma. There was considerable overlap with values measured in the normal and asthmatic subjects, and although this finding is of interest in terms of the mechanism of aspirin sensitivity, it is unlikely to be useful in the clinical setting for predicting aspirin sensitivity.

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