

Diurnal variation in FEV₁ after heart-lung transplantation

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ABSTRACT: We have examined the diurnal variation in forced expiratory volume in one second (FEV₁) in 25 heart-lung transplantation patients over a four week period in order to study the pathophysiological mechanisms underlying the increased mortality and morbidity which occurs at night in asthma. These patients do not have pulmonary autonomic nervous reflexes, but often have muscarinic receptor hypersensitivity. They also develop mixed cell infiltration of the lung tissue in the course of infection or rejection. Thus, they show many features in common with asthma.

Seventeen patients (68%) showed a significant diurnal variation in airway calibre (mean amplitude of FEV₁ was 4.6% (sd 3.7%)), which is similar to that reported in normal adults. One patient had a diurnal variation of 34.5% during an episode of rejection. This variation fell to 6.9% after steroid therapy, a change often seen in asthma. There was a correlation between increased amplitude of the variation and the presence in transbronchial biopsies of airway submucosal eosinophils and lymphocytes, associated with a histological diagnosis of acute rejection and with epithelial damage. No association was seen with muscarinic receptor sensitivity. The variation in FEV₁ showed no alteration from the normal day/night synchronization, and the peak values were around 1300 h.

We conclude that the diurnal variation in FEV₁ after heart-lung transplantation is not dependent on autonomic nerve reflexes or muscarinic receptor sensitivity, but is related to the consequences of inflammation described above.

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Pulmonary function displays a day/night or diurnal variation in both asthmatic and normal subjects, which is normally of the order of 24 h [1]. Explanations for this include diurnal rhythms of parasympathetic activity [2], adrenaline and cortisol concentrations [3], body temperature [4], and sleep [5]. Diurnal pulmonary function varies more in asthma than in normals, which is believed to be a result of airway inflammation and bronchial hyperresponsiveness [6, 7]. This can lead to severe nocturnal airflow limitation or even death.

To determine the role of autonomic reflex neural activity and whether airway or pulmonary inflammation are important to diurnal changes in pulmonary function, we have studied the variation of FEV₁ at home in recipients of heart-lung transplantation (HLT).

HLT offers a treatment for severe pulmonary vascular disease [8], end-stage chronic lung disease [9] and cystic fibrosis [10]. The surgery involves acute denervation of the lungs below the lower trachea [11]. Pulmonary vagal afferents do not re-innervate the lungs up to 36 months after surgery [12], and histological examination shows no irritant receptors, unmyelinated C-fibres, and vagal afferent and preganglionic efferent fibres disappear [13]. This denervation is frequently

associated with bronchial responsiveness to methacholine [14], as seen in asthma. After transplantation, the lungs experience episodes of acute rejection and infection, which can be diagnosed accurately by histology of lung tissue obtained by transbronchial biopsy [15, 16]. We, therefore, have the opportunity to study the effects of mixed cell inflammation, the absence of autonomic reflexes and muscarinic receptor hypersensitivity on diurnal changes in pulmonary function in man, which could throw light on the mechanisms underlying the increased mortality and morbidity from asthma which occur at night.

Patients and methods

Twenty five (11 male) of our HLT patients were studied. No patient had proven obliterative bronchiolitis. Their ages, original diagnoses and time since surgery are shown in table 1, together with any previous history of asthma. The forced expiratory volume in one second (FEV₁) was measured in triplicate at 8 am, 12 am, 4 pm, 8 pm and 12 pm daily, for four weeks, with the best value being recorded. All measurements were made within 15 min of the time specified, or omitted.

Table 1. — The age, original diagnosis, months after surgery, mean midday postoperative FEV₁, and history of asthma of the 25 patients studied

Patient no.	Age yrs	Original diagnosis	History of asthma	Months after surgery	Mean midday postoperative FEV ₁ % pred
1	38	CF	No	11	63.9
2	21	EIS	No	3	59.5
3	35	LEIO	No	3	44.4
4	51	PPH	No	3	64.9
5	25	CF	No	3	75.9
6	22	PPH	No	1	105.2
7	26	PPH	No	16	54.3
8	34	EIS	Yes	6	106.3
9	36	SLE	No	11	77.2
10	26	CF	No	2	82.2
11	42	EIS	No	42	85
12	22	CF	No	27	96.3
13	40	CFA	No	44	81.7
14	44	EIS	No	25	114.6
15	29	EIS	No	9	57.2
16	14	PPH	Yes	5	76.3
17	35	EIS	No	28	73.1
18	12	PPH	No	8	49.2
19	44	EMPH	No	26	31.1
20	24	CF	No	1	69.3
21	22	CF	No	2	65.3
22	11	CF	Yes	3	71.6
23	15	EIS	No	2	60.7
24	34	PPH	No	1	77.4
25	49	EMPH	No	28	88.2

FEV₁: forced expiratory volume in one second; CF: cystic fibrosis; EIS: Eisenmenger's syndrome; LEIO: pulmonary artery leiomyosarcoma; PPH: primary pulmonary hypertension; SLE: systemic lupus erythematosus; CFA: cryptogenic fibrosing alveolitis; EMPH: emphysema.

A portable battery driven turbine spirometer was used (Micromedical Ltd, Rochester, Kent, UK), calibrated against a dry wedge spirometer (Vitalograph Ltd, Buckingham, UK) [17].

The mean FEV₁, amplitude % mean [18], and time of the highest value (acrophase) of the diurnal variation were determined using multivariate analysis. Several groups have used an analysis of diurnal rhythms involving fitting a cosinor curve to the data. However, recent work has cast doubt on the validity of this method [19] and, therefore, we chose to use multivariate analysis.

The data were analysed week by week and for the whole four weeks to determine the effect on the diurnal rhythm of time, and of intercurrent treatment for infection and rejection.

In ten patients, the recordings of FEV₁ were repeated between five and seven months after the first study, for a similar time period, to observe the effect of time after surgery on the diurnal rhythm and its amplitude.

Transbronchial biopsy was performed in 23 patients during the period of observation. The methods used to diagnose by histology both lung rejection and infection have been described previously [15, 16] and will not be discussed here in any detail.

A standard methacholine bronchial provocation challenge [20] was performed at the end of data collection using a Wright nebulizer. The provoking concentration of methacholine which caused a 20% fall in FEV₁ (PC₂₀) was determined.

The age of the patients varied from 11–51 yrs (mean 30 yrs), and the time from surgery from 1–44 months (mean 12.4 months) (table 1). The FEV₁ % predicted prior to the study varied from 31.1–114% (mean 72.6%).

Results

There were no differences in the results between males and females and, therefore, the combined results of both sexes are presented. When the four weeks were considered together, significant diurnal rhythm in FEV₁ was seen in 17 patients (68%) (fig. 1). The amplitude % mean of diurnal variation was 4.6% (sd 3.7%) but it ranged from 0.6–34.5% (fig. 2). The patient (no. 22) who had an amplitude of variation of 34.5%, had concurrent lung rejection and muscarinic receptor hypersensitivity (PC₂₀ of 2 mg·ml⁻¹). Treatment with steroids decreased the variation to 6.9% during the four week period. This patient had a history of asthma before transplantation.

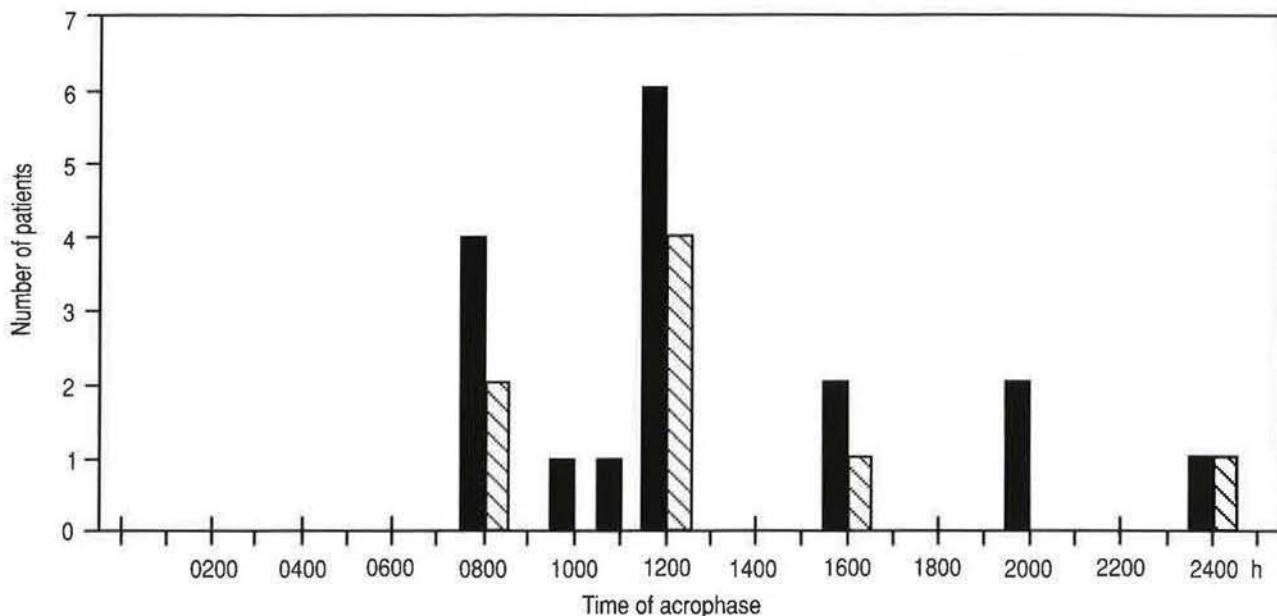


Fig. 1. — The distribution through the day of the time of the acrophase of the diurnal variation. The patients are subdivided into those who had a significant diurnal variation and those who did not. █: significant rhythm; ▨: insignificant rhythm.

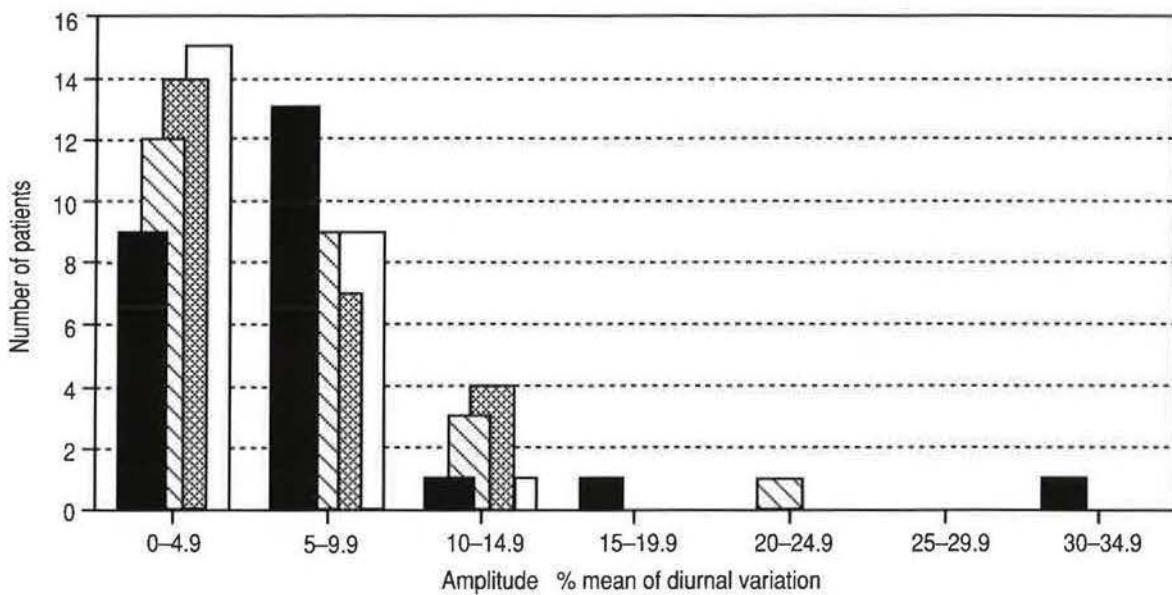


Fig. 2. — The distribution of the amplitude of the diurnal variation on a week by week basis. █: Week 1; ▨: Week 2; ▨▨: Week 3; □: Week 4.

The amplitude of the variation in FEV_1 remained constant over the four weeks in 20 patients. In five patients (nos 1, 4, 5, 6 and 22) the amplitude of variation was decreased by augmented immunosuppressive treatment for acute rejection (fig. 3). A total of nine patients were treated for rejection during the study. They received a standard course of high dose steroids [15]. The mean FEV_1 rose in all patients, but in four patients (nos 3, 20, 23 and 24) this occurred without altering the amplitude of the diurnal variation. There was no relationship between the amplitude or presence of a diurnal variation and the mean value of FEV_1 .

There was no relationship between the amplitude or

presence of a diurnal rhythm and the time since surgery, or a previous history of asthma.

The acrophase of the diurnal variation in FEV_1 ranged between 0800 h and 2400 h with a mean value of 1300 h ($\text{SD} 4.7$ h) (fig. 1). The ten patients studied twice did not show any significant alteration of the mean acrophase between the studies (from 1306 h ($\text{SD} 5$ h) to 1445 h ($\text{SD} 5$ h)), nor did the mean amplitude change (from 3.97% ($\text{SD} 2.5\%$) to 4.03% ($\text{SD} 1.8\%$)).

There was an association between the amplitude of variation in FEV_1 and the presence or absence of cellular infiltration with lymphocytes, eosinophils, plasma cells and neutrophils or with the degree of epithelial damage.

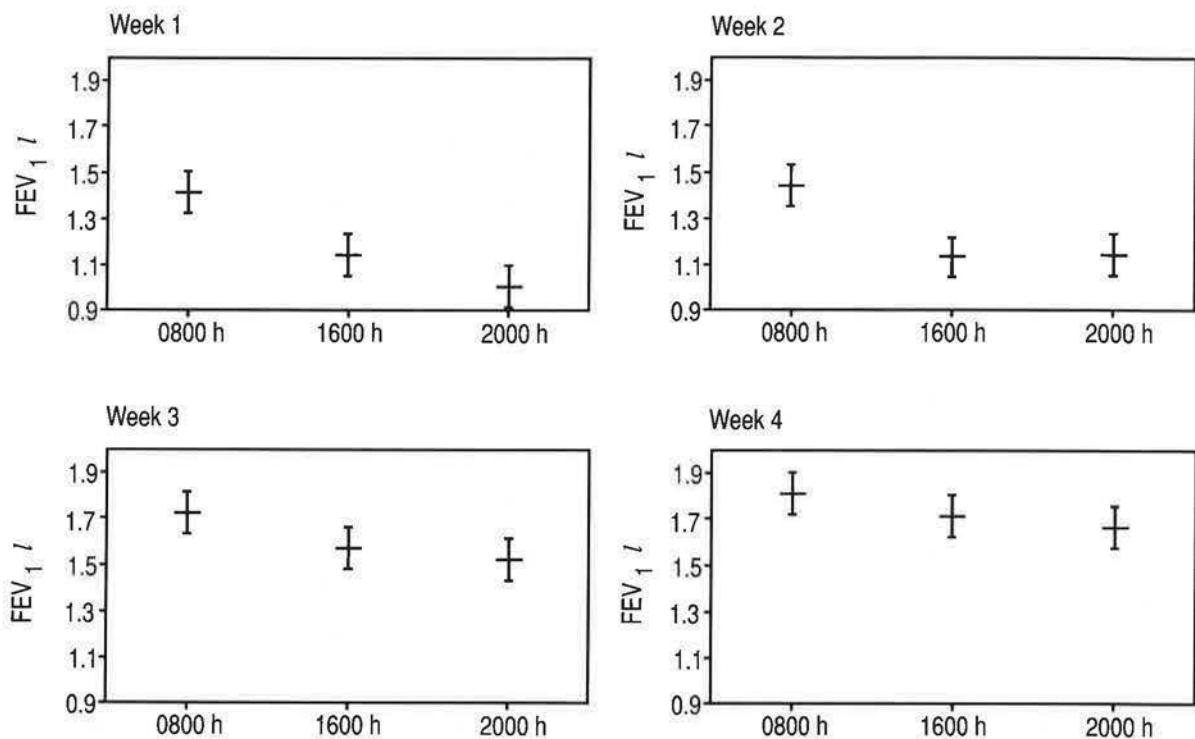


Fig. 3. – An example of the effect of steroid treatment on the mean FEV₁, together with the amplitude of the diurnal variation in patient no. 22. The mean oral dose of prednisolone for week one was 27 mg, for week two 20 mg, week three 18 mg and week four 11 mg. FEV₁: forced expiratory volume in one second; ━: 95% confidence limit; —: mean.

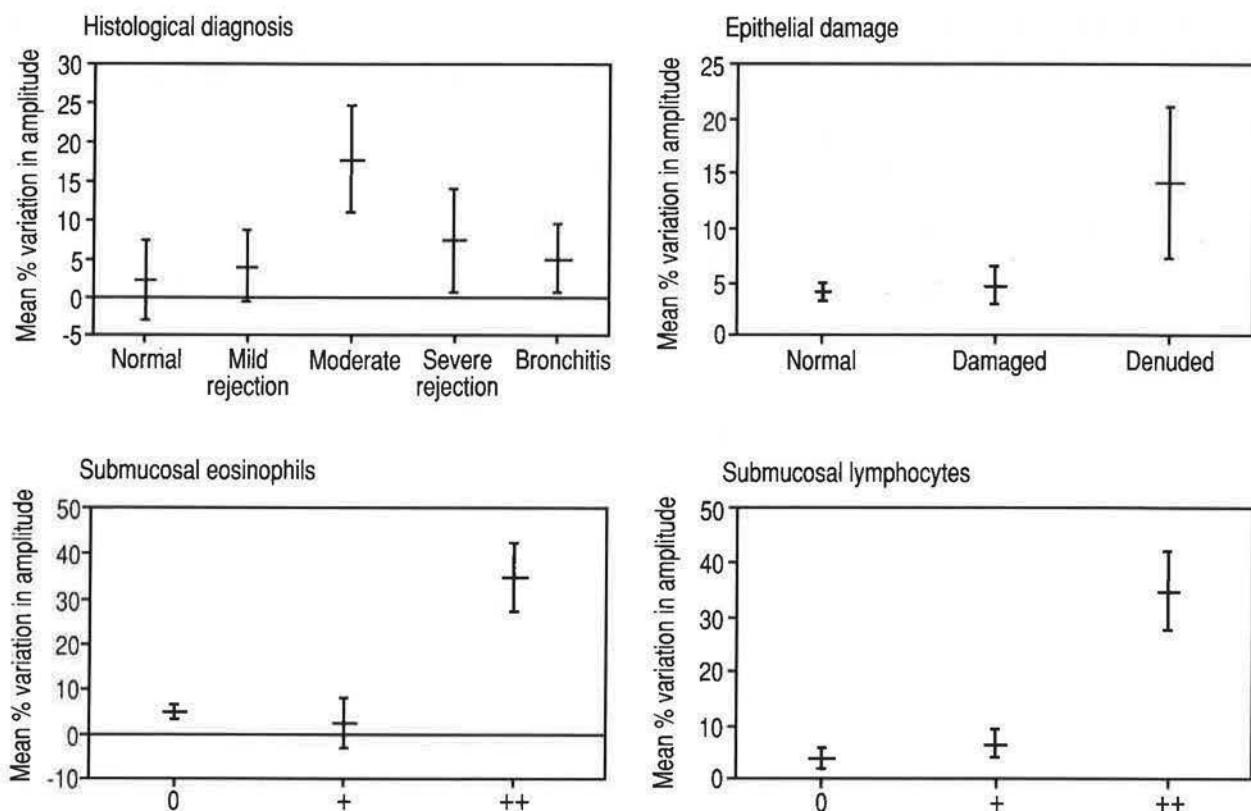


Fig. 4. – The relationship between the histological findings on transbronchial and mucosal biopsy and the mean % variation in amplitude of the diurnal variation. 0: normal; +: moderate infiltration; ++: heavy infiltration; ━: 95% confidence limit; —: mean.

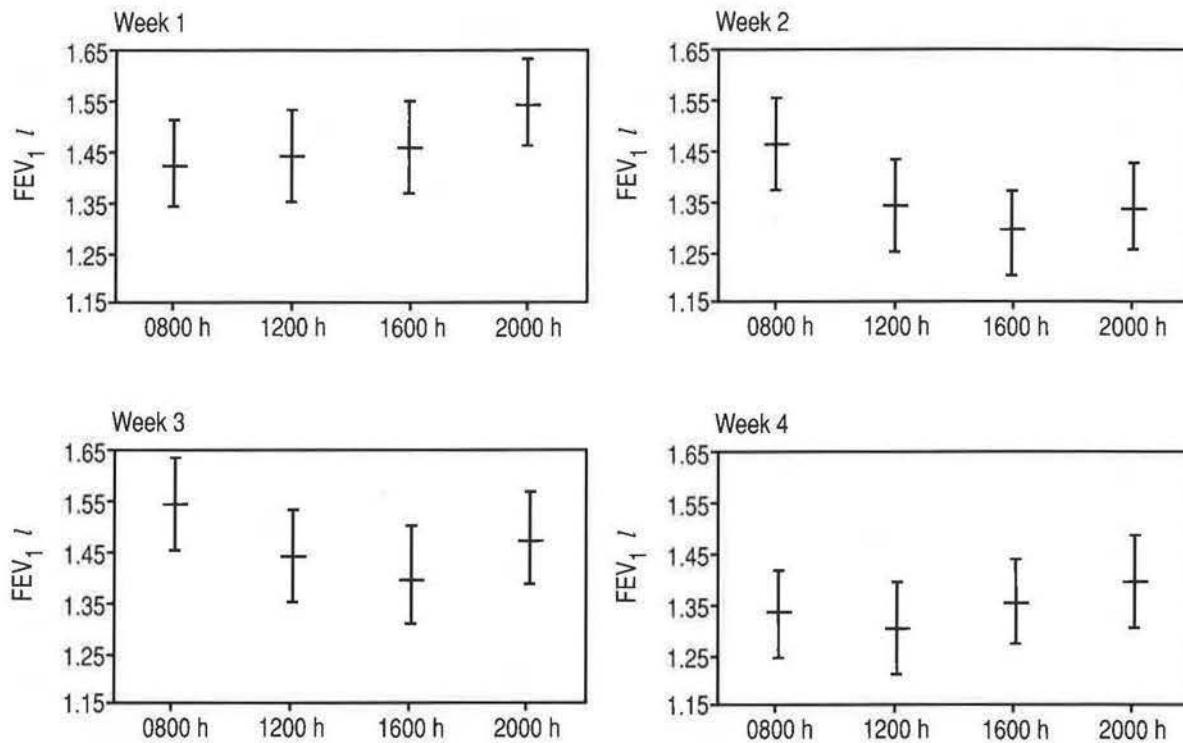


Fig. 5. — An example of variation of the time of the acrophase from week to week in patient no. 3. FEV₁: forced expiratory volume in one second; I: 95% confidence limit; —: mean.

Twelve patients had evidence of acute rejection on transbronchial biopsy specimens, with both perivascular lymphocytic infiltrates and mixed cell inflammation of the airways. Rejection was assessed histologically as being mild, moderate or severe. A diagnosis of moderate rejection was associated with an increased amplitude of diurnal variation ($p=0.015$). There was also an association between a higher amplitude of diurnal variation in FEV₁ and the presence in the mucosa of lymphocytes ($p<0.01$) and eosinophils ($p<0.01$). The degree of epithelial damage was similarly associated ($p=0.02$) (fig. 4).

There was no relationship between the amplitude of variation in FEV₁ and the PC₂₀ methacholine. Nor did the PC₂₀ of methacholine correlate with the presence or absence of mixed cellular infiltration or epithelial damage. There was no correlation between the FEV₁ % predicted and the PC₂₀ methacholine or the amplitude of the diurnal variation.

The length of the day of the diurnal rhythm was 23.9 h, suggesting no loss of control of the factors determining the biological day length after transplantation. However, four patients showed a significant variation in the time of acrophase from week to week (fig. 5), whilst keeping the length of the day around 24 h.

Discussion

A significant diurnal variation in FEV₁ was seen in 68% of our HLT patients with an amplitude of change of 4.6%, which is comparable in number and

magnitude of variation to studies of normal and asthmatic individuals [7]. This variation in airway calibre occurs in patients in whom there is no evidence of pulmonary vagal afferent nerves. This evidence is both physiological: the absence of coughing in response to ultrasonically nebulized distilled water (USNDW) for up to 36 months after surgery [12]; and histological: the absence of afferent and preganglionic efferent nerves. The absence of a cough response following HLT suggests a failure of vagal re-innervation below the tracheal anastomosis, which has also been found in re-implantation studies of canine lungs [21]. It may, therefore, be concluded that intact pulmonary vagal reflexes are not essential for the diurnal variation in airway calibre in HLT patients.

The mean acrophase of peak expiratory flow rate reported in normal subjects ranges between 1000 h and 1600 h depending on the duration of the study and the method of analysis used [7, 22–24]. The mean acrophase of FEV₁ in our HLT patients falls within these ranges, therefore vagal efferent nerves are not important in synchronizing the circadian rhythm in pulmonary function in man.

We have no data on circadian rhythms of catecholamines or endogenous cortisol in these patients; however, the acrophases for these hormones in normal and asthmatic subjects are, respectively, 1600 h and 0900 h [3], which show no relationship to the acrophases seen in our HLT patients. There is no evidence that the circadian rhythm of the hypothalamic-adrenal axis or resting catecholamines should alter after HLT. Heart transplant patients who have undergone

similar surgery and who are on comparable immunosuppressive treatment show a normal resting and catecholamine response to exercise [25]. There was some change in immunosuppressive therapy including steroids between the two studies, however, there was no consistency between this change of therapy and the timing of the acrophase, which may imply that endogenous cortisol is unlikely to have an effect. However, these factors require further investigation.

There are insufficient data on the diurnal variation in FEV₁ in normal subjects to know whether the variation of the time of the acrophase seen in four of the patients is a normal phenomenon or one unique to HLT patients. However, in general, a rhythm in an individual subject shows remarkable stability [26]. This may imply that in some subjects neural influences may synchronize the diurnal rhythm. Two central "clocks" in the hypothalamus are thought to drive circadian rhythms. One clock governs body temperature control and the other the sleep/wake cycle. In normal conditions the two are synchronized, but, in total isolation experiments, the two desynchronize after about 15 days, with the sleep/wake cycle lengthening to 33 h, whilst the body temperature cycle remains at about 24 h [26]. The circadian rhythm of airway calibre seems to be synchronized to the 24 h day by humoral or other factors, and not neural factors in the HLT patient. Perhaps it may be related to the circadian change in core temperature, which closely follows the temperature of the bronchi. Alteration of airway temperature has been demonstrated to diminish nocturnal asthma [4]. Alternatively, there may be a natural endogenous rhythm of the bronchial calibre with a cycle of about 24 h, which shows some synchronization to the sleep/wake cycle.

We were able to relate the amplitude of the diurnal rhythm in FEV₁ to the presence of submucosal infiltrates with lymphocytes and eosinophils, and also to epithelial damage and a histological diagnosis of acute rejection in these patients. Epithelial damage due to the release of major basic protein from activated eosinophils is considered important as a causative factor of the bronchial hyperresponsiveness underlying asthma [27, 28]. Our observations tend to confirm this, but also indicate the importance of activated T-lymphocytes [29]. Treatment of acute lung rejection is associated with a reduction in numbers of activated lymphocytes, neutrophils and eosinophils [30]. In five of the nine patients treated with augmented immunosuppression there was not only a rise in mean FEV₁ as previously reported [31], but the amplitude of the variation in FEV₁ decreased (fig. 3), which suggests a strong link of inflammation with an increased diurnal variation of airway calibre.

We were unable to relate the bronchial responsiveness to methacholine to the pulmonary inflammation or degree of epithelial damage seen on biopsy. This is consistent with our earlier observations on bronchial hyperresponsiveness in HLT patients, in which the methacholine responsiveness was unassociated with pulmonary inflammation or epithelial damage [32].

From this we infer that the amplitude of the diurnal variation in airway calibre is not dependent on muscarinic receptor hypersensitivity, with the latter phenomenon probably related to denervation.

One of our patients had an amplitude of variation in FEV₁ of 34.5% and 10 had PC₂₀ values below 8 mg·ml⁻¹, both of which are frequent features of asthma. There was no relationship between these findings and an earlier history of asthma in the recipient. Donors with an obvious history of asthma are excluded in our selection procedure [33], but as asthma is under-diagnosed in the community [34], it is likely that some donors had asthma. Many of the mucosal abnormalities seen on transbronchial biopsy specimens are often described in asthma [35], for example eosinophil, lymphocyte and neutrophil infiltration and epithelial damage.

We have, therefore, excluded pulmonary autonomic reflexes and airway smooth muscle muscarinic receptor hypersensitivity as causes for diurnal variation in airway calibre in HLT patients. We have demonstrated that epithelial damage and submucosal inflammation with eosinophils and lymphocytes are associated with increased diurnal variation in FEV₁, which confirms an intimate relationship between inflammation and increased variation in airway calibre in man.

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