Corticosteroid treatment, as in most studies with fluticasone 1000 µg twice daily and oral prednisolone 30 mg/day. A radioimmunoassay (RIA) method was used to determine serum cortisol suppression in blood with corticosteroid treatment, as in most studies published to date. However, prednisolone and its metabolites are known to be chemically similar to serum cortisol and might therefore interfere with cortisol measurements by RIA. Analytical methods involving chromatographic separation of cortisol from prednisolone and its metabolites, such as high performance liquid chromatography (HPLC), circumvent this problem of interference.

We compared serum cortisol measurements by both conventional RIA and by HPLC in the same study, which was of a double blind, double dummy, three arm parallel group design. Patients received either oral prednisolone (30 mg/day), fluticasone propionate 1000 µg twice daily (FP2000), or fluticasone propionate 250 µg twice daily (FP500), both by Diskhaler dry powder inhalation. Measurements at the start of the study and after 2 weeks of treatment were performed at the same time in the morning.

The Gilson ASTED (automated sequential trace enrichment of dialsates) system was used followed by separation with HPLC and detection by UV absorbency. The upper and lower limits of measurement were found to be 688 and 6.9 nmol/l, respectively, and the coefficient of variation ranged from 5.6% to 7.0%.

For RIA analysis samples were homogenised and diluted at +60°C. 100 µg/h (1000 Bq/100 µl) cortisol solution was added to all serum samples after which 0.2 ml of a monochlonal rabbit antiserum was added. The sensitivity of the assay was 15 nmol/l and the coefficient of variation ranged from 5% to 8%.

The number of patients with cortisol values available for both RIA and HPLC was 26 for FP2000, 23 for oral prednisolone, and 33 for FP500. There were no significant differences at baseline between the groups or between the methods of cortisol measurement. Both treatment with FP2000 and oral prednisolone significantly reduced serum cortisol levels (fig 1), but suppression of serum cortisol in the oral prednisolone group using the HPLC method (~72%) was significantly larger than with the RIA method (~34%). As expected, the difference between the cortisol levels measured by RIA and HPLC increased with higher serum prednisolone concentrations (data not shown).

The difference is fully explained by the fact that serum prednisolone levels were not separately identified from cortisol by the RIA method. This crossreactivity of prednisolone with cortisol can differ considerably between laboratories and with the RIA method (monochlonal or polyclonal) used, but is always present and ranges from 10% to 100%. There were no significant differences in the change in serum cortisol levels between the HPLC and RIA methods in the inhaled fluticasone groups (FP2000 and FP500).

We conclude that determination of serum cortisol by RIA severely underestimates serum cortisol suppression over a range of 6.9–690 nmol/l serum cortisol in the presence of prednisolone. Our study shows that cortisol suppression in the presence of prednisolone should not be assessed by conventional RIA.

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References


Smoking cessation
We welcome the study by Pelkonen et al as a further contribution to our knowledge base on smoking cessation and its effects on pulmonary function and mortality. We feel, however, that some shortcomings in the methodology may bring into question the magnitude of the results.

Our main concern relates to the difficulties in quantifying levels of tobacco exposure. Since tobacco consumption is a continuous variable, confounding factors may occur within each group when categorised too broadly. More information about duration and levels of smoking would help to avoid this problem. No information about whether intermittent quitters returned to original habits or resumed smoking at reduced levels. Beneficial effects described in this group could therefore be due to extended periods of decreased tobacco consumption rather than a period of abstinence.

There are no data provided on smoking status from 1974 to 1989. If large numbers of those classed as intermittent quitters had permanently stopped smoking by this time, the value of temporary quitting would be overestimated. Furthermore, no data exist on the duration of periods of abstention among intermittent quitters. If a significant proportion of this group exhibited prolonged periods of smoking cessation, the relevance of this study to short term quitters is debatable.

Even accepting the beneficial effects of intermittent quitting, we question the importance of this finding in a public health setting. Surely the main health advantage must remain the same: permanent smoking cessation should remain the goal and is superior to intermittent quitting. However, we recognise that this finding could provide encouragement to those who have relapsed following...
an attempt to quit smoking and reassure them that their efforts have not been in vain. This could provide the motivation needed for a second and possibly successful attempt to quit.

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References
2 Leon DA. Failed or misleading adjustment for confounding. Lancet 1993;342:479–81.

Authors’ reply
Lorna Dunn and Alleen Ogilvie make an important point that the confounding effect of tobacco consumption on the decline in pulmonary function may occur when the levels of tobacco exposure are categorised too broadly. They think that the benefit of intermittent quitting on the decline in FEV0.75 in our study might be explained by decreased tobacco consumption after periods of abstinence rather than by the periods of abstinence per se. They also point out that, if a considerable proportion of intermittent quitters stopped smoking permanently between 1974 and 1989, it would have led to overestimation of the value of temporary quitting. The third question concerns the duration of periods of abstinence.

In our study the data on smoking habits were recorded at baseline and in subsequent re-examinations by a standard questionnaire. The interval between examinations was usually 5 years. Intermittent quitters were either baseline past smokers who smoked in at least one of the subsequent re-examinations or baseline smokers who were quitters in one or more re-examinations but relapsed back to smoking later. To be recorded as a quitter in an examination a subject had to have given up smoking more than a year previously. During the first 15 years, 27 of 75 intermittent quitters were recorded as quitters in one examination (corresponding to at least 1 year of abstinence), 32 were recorded as quitters in two examinations (corresponding to at least 2 years of abstinence), and 16 were recorded as quitters in three examinations (corresponding to at least 3 years of abstinence).

During the first 15 years intermittent quitters reduced the number of cigarettes smoked daily compared with continuous smokers, although not significantly. To measure tobacco consumption more precisely, a new variable was constructed by computing the mean reported daily cigarette consumption at each examination point. For intermittent quitters only, the data from the examinations when they reported smoking were used in making up this variable. When we then additionally adjusted our analyses for this new variable, the decline in FEV0.75 during the first 15 years was significantly less among intermittent quitters than in continuous smokers (data available from the authors on request). The benefit of intermittent quitting on the decline in pulmonary function therefore also seems to be mediated through periods of abstinence.

Among both intermittent quitters and continuous smokers there were study subjects who stopped smoking permanently between 1974 and 1989. The proportion of such study subjects was greater among intermittent quitters than among continuous smokers. However, when we made additional adjustments for both the mean daily tobacco consumption during the first half of the follow up period and for quitting smoking during the latter half of the follow up period, intermittent quitters still lost less FEV0.75 during the whole 30 years than continuous smokers (data available from the authors on request).

In conclusion, it seems that some protection may be gained from periods of abstinence, although we agree that the main goal should be permanent smoking cessation.

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Postscript

We agree with the authors that further studies are necessary to provide evidence of a trend towards an association between ILD and smoking which could be adequately powered to show no statistically significant association between RA associated ILD and smoking. In the study by Cortet et al16 68 patients with RA were prospectively studied with HRCT scanning and smoking was less prevalent than in the North of England and the ratio of smokers to non-smokers was 1:3. No statistical association was seen between linking smoking to ILD. No statistically significant association between RA associated ILD and smoking. In the study by Cortet et al13 68 patients with RA were prospectively studied with HRCT scanning and smoking was less prevalent than in the North of England and the ratio of smokers to non-smokers was 1:3. No statistical association was seen between linking smoking to ILD. No statistically significant association between RA associated ILD and smoking.

References

Authors’ reply
We are pleased to receive the letter from Saravanan and Kelly in response to our recent publication in Thorax.1 This is an interesting one. There is no consistent finding in the literature of smoking and RA associated FA and, as far as we are aware, no prospective RA based study has shown a statistically significant association between RA associated FA and smoking. In the study by Cortet et al126 68 patients with RA were prospectively studied with HRCT scanning and smoking was less prevalent than in the North of England and the ratio of smokers to non-smokers was 1:3. No statistical association was seen between linking smoking to ILD. A prevalence of 20% of ILD (17% ground glass pattern and 2.9% reticular pattern) was still found. It is true that in our study the absolute risk of ever smoking cannot be excluded since the factor for RA as the number of lifelong non-smokers is small; however, the pack year data are adequately powered to show no statistically significant difference.

With regard to the paper by Rajasekaran et al13 6, we feel it necessary to point out that the patients in their study with FA and RA had the diagnosis confirmed by HRCT scanning and, in addition, were suspected of having interstitial lung disease. We are sure this will provide very valuable information about the progression of FA in patients with RA but it will not add to our knowledge on the outcome of HRCT changes detected at a subclinical stage. Rajasekaran et al found honeycombing on the HRCT scan in three of 18 patients with RA associated ILD and in four of 18 patients with CFA; this difference is not statistically significant. None of these patients was rheumatoid factor positive. This finding is particularly interesting given that there is evidence in the literature of smoking being associated with seropositivity for rheumatoid factor in patients with and without RA.12 14 We

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It is noted that the blood granulocytes are isolated by use of a density gradient. Density gradients may interfere with some neutrophil functions and this must be borne in mind when interpreting these results. Additionally, BAL granulocytes from horses were isolated by use of a density gradient, whereas this was not used for the diseased horses. This difference of methods introduces a potential bias into the study. We have previously attempted to isolate neutrophils from human BAL fluid with no success (unpublished observations) and would be interested to know if the authors achieved this separation easily. We are also surprised at the viability of >90%. Cell viability is likely to diminish with increasing rates of apoptosis, and it is notable that the BAL granulocytes from healthy horses have apoptotic rates of around 40%. This study is interesting, but the methodological issues raised must be considered in interpreting the results.

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References

Authors’ reply
We thank Dr Kelly and colleagues for their interest in our paper. In the past equine heaves was called COPD but, because equine heaves is completely different from human COPD, specialists in the field have recommended avoiding the erroneous term “COPD” for designating this disease. Indeed, it is now clear that equine heaves is very close to atopic asthma and these diseases share important characteristic features including hypersensitivity to aeroallergens, Th2 type immune response, chronic airway inflammation, reversible airway obstruction, non-specific airway hyperresponsiveness, and production of specific IgE against those allergens. Indeed, neutrophils are the predominant inflammatory cells in equine heaves, but this does not exclude the use of this model in asthma studies. Indeed, neutrophils are known to play an important role in asthma; whereas recent studies have questioned the importance of eosinophils in this disease.

In our study only small amounts of granulocytes were recovered from the lung of the horses so we were only able to use one method to assay these cells for apoptosis. We chose the method that has been found to be the most sensitive marker of granulocyte apoptosis—the annexin V (AV)/propidium iodide (PI) method. The results obtained with this method were interpreted as follows: AV+/PI− cells were considered alive, AV−/PI+ cells were considered apoptotic, and AV+/PI+ cells were considered necrotic. This is the first time we have heard of controversy surrounding the interpretation of the results obtained with this method, probably because they have not been published in scientific journals. According to the archives we have read using the websites addresses provided by Dr Kelly and colleagues, it appears that this controversy exclusively concerns the status of AV+/PI+ cells. Such cells are uncommon and were not observed in our study.

We agree that density centrifugation may interfere with neutrophil function. To the best of our knowledge there is no other way of separating granulocytes from other cell types. As mentioned in the Methods section of our paper, cell viability of freshly isolated granulocytes was evaluated by trypan blue (TB) exclusion. The cells were then cultured for different times and assayed for apoptosis using AV/PI. Cells in an early state of apoptosis are AV+ and PI+. It is not surprising to find 40% apoptotic (AV+) cells in a population where nearly all the cells (>90%) are TB−.

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References