Relation of serum elastin peptide concentration to age, FEV_1, smoking habits, alcohol consumption, and protease inhibitor phenotype: an epidemiological study in working men

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Abstract

Background **In clinical investigations elastin peptide concentration has been proposed as one potential marker of lung elastin degradation. No epidemiological study has yet confirmed this hypothesis.**

Methods **The relation of elastin peptide concentration to some factors closely related to pulmonary emphysema (age, smoking habits, FEV_1 α protease inhibitor (PI) phenotype) and to alcohol consumption was examined in an epidemiological study of 310 working men. The elastin peptides used for obtaining antibodies and as reference in an ELISA assay were prepared from chemically hydrolysed elastin.**

Results **The elastin peptide concentration significantly decreased with age from 2·92 (1·54) μg/ml among subjects younger than 30 years to 2·18 (1·14) μg/ml among subjects older than 50. Elastin peptide concentration did not differ with smoking habits and was clearly unrelated to FEV_1. A lower elastin peptide concentration was observed in all groups of subjects with a protease inhibitor phenotype other than PI MM (PI FM, IM, MP, MS, MZ, and S phenotypes).**

Conclusions **The results cast doubts on the usefulness of the elastin peptide concentration as a marker of lung destruction in middle aged, predominantly healthy men. Blood elastin peptide concentration may reflect both elastin degradation and resynthesis. The results of this analysis suggest that several factors (age, alcohol consumption, non-PI MM phenotype) may be associated with decreased resynthesis of lung elastin. Further studies, conducted in various age groups and including estimates of the degree of lung destruction, are needed to unravel the mechanisms underlying lysis and resynthesis of lung elastin.**

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The protease-antiprotease imbalance theory, derived from the discovery of α_1 protease inhibitor deficiency linked to an increased risk of emphysema[1] and from animal models of emphysema induced by proteases,[2] has been proposed to explain lung destruction in pulmonary emphysema. Although this theory explains the pathogenesis of emphysema in patients who are homozygous for PI Z α_1 protease inhibitor deficiency, it is still not clear whether the same mechanisms underly smoker's emphysema, the most common form of the disease.[3] Elastin metabolism may be affected by risk factors, such as smoking, both through an increased elastin degradation and through a reduction in the capacity of the lung to repair itself.[3 4] The use of potential biological markers of elastin metabolism in body fluids has been proposed as an approach to the pathogenesis of emphysema. Desmosine, a cross linking amino acid of elastin and a potential marker of elastin degradation, was not found to be increased in clinically defined emphysema[5] or with smoking,[6] nor was it related to the FEV_1.[6] Besides desmosine, the plasma[7] or serum[8] elastin peptide concentration has been reported in clinical studies to be higher among patients with chronic obstructive pulmonary disease than among healthy subjects[7 8] and higher in smokers than non-smokers.[7] An increased elastin peptide concentration would therefore be related to markers and potential risk factors of emphysema. This hypothesis was tested in an epidemiological study exploring the relation of serum elastin peptide concentration to some factors closely related to pulmonary emphysema (age, smoking habits, FEV_1, protease inhibitor phenotype) and to alcohol consumption, a potential cause of poorer lung function,[9 10] which had previously been shown to be related to a decreased α_1 protease inhibitor activity in the population studied.[11]

Methods

In an ongoing epidemiological longitudinal study, 912 working men were examined in 1980–1 as part of an annual compulsory medical examination. A second survey was performed five years later on a sample of the original population with spirometric tracings satisfying European Coal and Steel Community criteria in 1980,[12] and elastin peptide concentrations were measured at that survey. Practical considerations did not allow the resurveying of all men, so we planned to resurvey three quarters of that original population. The sampling scheme was designed to include all subjects with a previous history of asthma, wheezing, any perceived symptom of bronchial

hyperresponsiveness, eczema, urticaria, and other possible markers of or factors in susceptibility to chronic airflow limitation, including PI MZ phenotype, and a similar number of controls, chosen at random among the remainder. Five hundred and ninety nine men were selected to be resurveyed. The details of the protocol of the survey are given elsewhere.[13] All subjects were interviewed with a modified British Medical Research Council-European Coal and Steel Community questionnaire. Smoking habits and alcohol consumption were recorded. The daily consumption of tobacco was calculated by considering one cigarette equal to 1 g, a cigarillo equal to 2 g, and a cigar equal to 5 g. The subjects were classified as never smokers, ex-smokers (of at least one month's duration), moderate smokers (less than 20 g of tobacco a day), and heavy smokers (20 g or more). The alcohol consumption reported by questionnaire in this study was by previously validated biological measurements.[14] Alcohol consumption was estimated for each subject in g/day according to the volume and the alcoholic strength of the various drinks consumed by the subject. Heavy consumption was defined as over 60 g alcohol/day, which corresponds to a 75 cl bottle of wine.

Spirometric measurements were made with a dry spirometer (TLc Morgan, Rainham, UK) with the subject in a sitting position and wearing a nose clip. The best of three tracings that satisfied the European Coal and Steel Community criteria[12] was used for analysis. Values were converted to BTPS. FEV_1 values were normalised (FEV_1 scores) as the observed $FEV_1 - (a\,[\text{age}] + b\,[\text{height}] + c)/\sqrt{s^2}$, where a and b are the age and height regression coefficients, c the intercept, and s^2 the residual variance. Various methods of analysis of the potential relation of elastin peptide concentration to FEV_1 have been used: we considered the continuous variable of FEV_1 adjusted for age and height (FEV_1 score), a dichotomous variable of % predicted FEV_1[12] lower than 80%, and a 10 class variable of FEV_1 adjusted for age and height (FEV_1 score deciles). FEV_1 deciles were obtained after dividing the distribution of FEV_1 into 10 groups of similar size. Using deciles allowed non-parametric analysis of the distribution of elastin peptide over the whole scatter of FEV_1.

Blood samples were taken in 1985 and frozen at −80°C until they were analysed. Of the 599 men selected from the population seen in 1980 to be resurveyed in 1985, nine were dead and 390 attended the examination. Three hundred and ten men provided serum samples for determination of elastin peptide and FEV_1 was available for 295 of them. Protease inhibitor phenotype was determined by isoelectric focusing on polyacrylamide gel.[15] Protease inhibitor typing was done for 245 subjects in 1980[16] and for the 65 subjects not typed in 1980 the protease inhibitor phenotype was determined in 1990 from the stored frozen serum. In our sample no subject was PI Z.

The elastin peptide concentration was measured in serum by an enzyme linked immunosorbent assay (ELISA). The elastin peptides used for obtaining antibodies and as reference in the assay were prepared from human aorta elastin hydrolysed with 1 M KOH in 80% aqueous ethanol (kappa (κ) elastin). The indirect ELISA procedure we used, described in detail elsewhere,[17] was found to give specific and reproducible results (coefficient of variation 8·5%). Samples were run in triplicate. The assay was sensitive from 0·3 μg to 220 μg of κ elastin equivalents per ml of serum. Results were expressed as κ elastin equivalents per ml of serum. Elastin peptide concentration scores were elastin peptide concentration normalised as the elastin peptide concentration $- (d\,[\text{age}] + e)/\sqrt{s^2}$, where d is the age regression coefficient, e the intercept, and s^2 the residual variance.

Analysis of variance, correlation, and multiple linear regression were used for the analysis.[18]

Results

No difference between the 310 men of our population sample and the 280 men lost to follow up was observed for age and smoking habits at the first survey. The subjects resurveyed had a significantly higher FEV_1 at the first survey than those who were lost to follow up (4·19 (0·60) v 4·06 (0·65) l). This difference in FEV_1 was mainly among men over 40 in 1980.

The population studied consisted of 310 men aged 28–58 (mean (SD) 43·2 (7·4) years. The FEV_1 level averaged 4·00 (0·61) (range 1·49–5·99) l. FEV_1% predicted European Coal and Steel Community 1983 values ranged from 41% to 151% (mean (SD) 106% (15%)). Ten per cent of the subjects had a FEV_1 lower than 80% of the reference predicted FEV_1. Seven per cent of the men were asthmatic, 7% reported sputum production, and 17% reported dyspnoea grade 1. Nineteen per cent of our subjects were heavy smokers ($\geq$20 g/day) and 12% of them were heavy drinkers (>60 g alcohol/day). Elastin peptide concentration, if we exclude one outlier value of 13·27 μg/ml, ranged from 0·14 to 7·24 μg/ml, with a mean (SD) of 2·55 (1·34) μg/ml. The subject with the outlier value (whose phenotype was PI MM) did not differ from others in age, smoking, drinking habits, or FEV_1. Analyses were performed before and after excluding this outlier value and our conclusions were unchanged. The results presented therefore include all values.

As shown in figure 1, elastin peptide concentration decreased with age (regression coefficient of elastin peptide concentration with age −0·03; $p \leq 0·005$). The elastin peptide concentration fell progressively across the age groups, being 2·92 (1·54), 2·69 (1·26), 2·55 (1·42), and 2·18 (1·14) μg/ml among subjects aged under 30, 30 $\leq$ age < 40, 40 $\leq$ age < 50, and age $\geq$50 years ($p \leq 0·05$).

The highest mean elastin peptide concentration was observed among ex-smokers and the lowest among heavy smokers, with intermediate values among non-smokers and moderate smokers (table 1). There was no clear

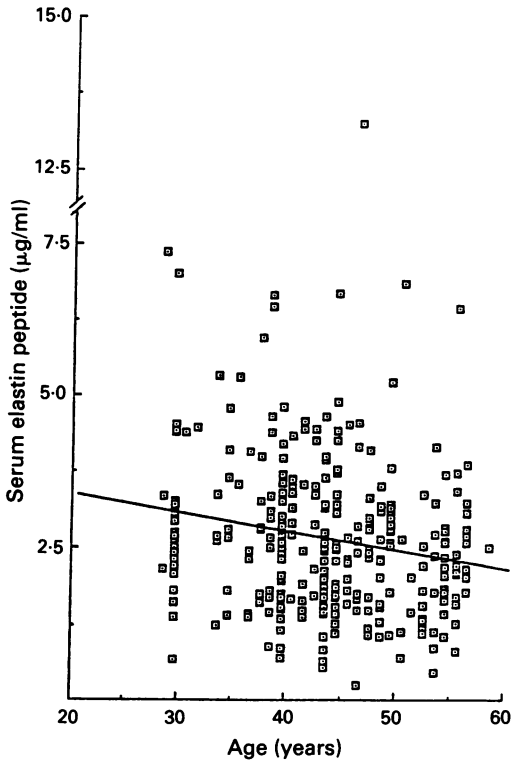

Figure 1 Elastin peptide concentration according to age (n = 310). The regression coefficient of elastin peptide concentration on age was −0·03 (p < 0·005).

trend, and the relation between smoking habits and elastin peptide concentration was not significant. Elastin peptide concentration adjusted for age also did not show any significant variation with smoking habits. These findings were unchanged in the subgroup of 200 subjects who reported the same smoking habits at the first survey, five years earlier. No association was found between smoking on the day of examination and elastin peptide concentration.

Elastin peptide concentrations were inversely related to alcohol consumption. The highest elastin peptide concentrations were observed for those without or with only a marginal consumption (≤15 g/day = 5 cl of wine/day) and the lowest values for those drinking at least the equivalent of a bottle of wine per day (>60 g/day). The difference was not, however, significant when the six class variable presented in table 1 was considered. When the heavy consumers (>60 g/day) were set against the others, the relation of elastin peptide concentration to heavy alcohol consumption became significant (elastin peptide concentration 2·13 (0·83) μg/ml among heavy consumers *v* 2·61 (1·40) μg/ml among the others; p ⩽ 0·05). As age may confound the association as it was related both to alcohol consumption and to lower elastin peptide concentration, the analysis was adjusted for age. As expected, the association of elastin peptide concentration to heavy alcohol consumption decreased but remained of borderline significance (elastin peptide concentration scores −0·28 (0·61) among heavy consumers *v* 0·04 (1·04) among the others; p > 0·05).

Elastin peptide concentration was clearly unrelated to FEV_1 even after exclusion of the asthmatic subjects. The regression of unadjusted elastin peptide concentration on FEV_1 gave a regression coefficient close to zero in the whole sample. Figure 2 shows that after adjustment of FEV_1 for age no trend appeared (regression coefficient of elastin peptide concentration on FEV_1 scores −0·05 and of elastin peptide concentration scores on FEV_1 scores −0·04). Subjects in the lower decile of FEV_1 (that is, the 10% of the men in the sample with the lowest FEV_1—D1 in the figure) had an elastin peptide concentration similar to that of the other men. Results were unchanged when % predicted FEV_1 was used. Elastin peptide concentration was similar among the 52 subjects with dyspnoea (grade 1) and among the 257 without (2·39 (0·93) *v* 2·58 (1·41) μg/ml).

A lower average elastin peptide concentration was observed in all groups of non-PI MM subjects (FM, IM, MP, MS, MZ, and S) than in the group of PI MM subjects (table 2). The overall association between the various PI phenotypes and elastin peptide concentration was not significant. The 47 non-PI MM subjects, as a group, had a significantly lower elastin peptide concentration than the 263 PI MM subjects (elastin peptide concentration 2·15 (1·04) *v* 2·62 (1·38) μg/ml; p ≤ 0·05) but the difference was no longer significant when the 41 PI MZ, MS, and S subjects, as a group, were set against the other 269 subjects (elastin peptide concentration 2·23 (1·06) *v* 2·59 (1·38) μg/ml).

All results were unchanged after exclusion of men with asthma.

Table 1 Serum elastin peptide concentration according to smoking habits and alcohol consumption

		Elastin peptide (μg/ml), mean (SD)	
	n	*Crude value*	*Adjusted for age†*
Smoking habits			
Non-smoker	101	2·54 (1·12)	−0·01 (0·81)
Ex-smoker	87	2·62 (1·62)	0·11 (1·23)
Smoker			
<20 g /day	58	2·54 (1·35)	0·00 (1·00)
⩾20 g/day	59	2·50 (1·31)	−0·10 (0·95)
Alcohol consumption (g/day)*			
0–15	124	2·69 (1·35)	0·08 (1·01)
16–30	57	2·61 (1·71)	0·03 (1·29)
31–45	46	2·45 (1·11)	−0·03 (0·82)
46–60	45	2·51 (1·35)	0·01 (0·97)
61–75	18	2·05 (0·65)	−0·37 (0·48)
>75	19	2·20 (0·98)	−0·20 (0·72)

*Heavy drinkers (>60 g alcohol/day) had a significantly lower elastin peptide concentration than the other men (p < 0·05; after adjustment for age p > 0·05).
†Normalised residuals derived from linear regression of elastin peptide level on age.

Discussion

In this epidemiological study elastin peptide concentration decreased significantly with age. It did not show any variation with smoking habits and was unrelated to FEV_1. A lower elastin peptide concentration was observed in all groups of non-PI MM subjects than in the group of PI MM subjects. The concentration was lower, though not significantly, among heavy alcohol consumers than among other men.

Except for the study of Davies *et al* conducted among fire fighters,[6] no previous epidemiological study has included potential biological markers of emphysema. Studies have been carried out on highly selected sam-

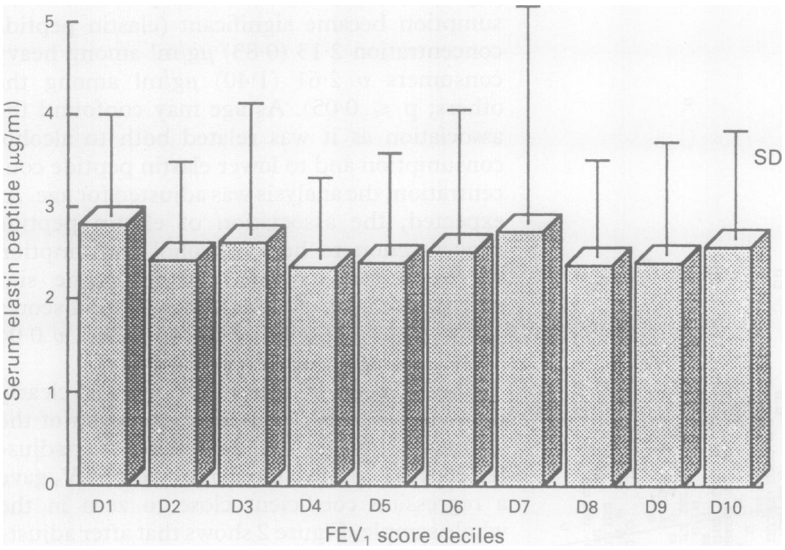

Figure 2 Elastin peptide concentration according to FEV_1 score deciles ($n = 295$). The regression coefficient of elastin peptide concentration on FEV_1 score was $-0{\cdot}05$.

ples.[5 7 8 19] The lack of standardisation in measurement of elastin peptide concentration has led to heterogeneity of the values of these markers reported in the various studies, which have differed by a factor of 1000.[7 8 17] Comparison between the various studies is difficult because results are expressed in different units[7 8 20] and the elastin peptides identified by the various assays may differ. The ELISA procedure we used was found to give specific and reproducible results. The method avoids potential contamination of elastin peptide with elastase, which may occur when elastase is used for elastin hydrolysis, a method used in some other studies.[7 21]

AGE

The inverse relation between age, a factor highly related to emphysema,[22] and elastin peptide concentration observed in our population, aged 28–58 years, was unexpected. Nobody has reported a decrease of elastin peptide concentration with age—there has been either no relation or an increase in concentration with age.[7 17 20 23] The discrepancies observed in the various studies concerning the relation of elastin peptide to age may be accounted for by differences in the selection of subjects.

Table 2 Serum elastin peptide concentrations according to PI phenotype

		Elastin peptide (µg/ml), mean (SD)	
	n	Crude value	Adjusted for age†
PI phenotype			
MM	263	2·62 (1·38)	0·05 (1·03)
FM	1	0·72	−1·38
IM	4	2·00 (0·68)	−0·33 (0·49)
MP	1	0·93	−1·22
MS	33	2·18 (1·01)	−0·25 (0·79)
MZ	7	2·48 (1·38)	−0·07 (1·07)
S	1	2·17	−0·17

*Normalised residuals derived from linear regression of elastin peptide concentration on age.

Using the same assay as in the present study, Fülop *et al* showed a positive correlation ($r = 0{\cdot}09$) of elastin peptide concentration with age among subjects aged 18–90 years.[17] This relation was restricted, however, to a subgroup of 216 atherosclerotic patients and was not observed in 54 blood donors. Similarly, Baydanoff *et al* in a population including atherosclerotic patients found a significant increase of elastin peptide concentration with age, an association restricted to subjects aged 51–75 years.[20] In 46 patients with chronic obstructive lung disease aged 45–71 years a non-significant increase of elastin peptide concentration with age was observed.[23] Our results represent the first epidemiological study conducted in a large group of working men, and the clear decline of elastin peptide concentration with age needs to be confirmed in other unselected groups of subjects.

SMOKING HABITS

We did not detect any variation of elastin peptide concentration with different smoking habits, a finding similar to that of study[8] of 16 subjects with an age range similar to that of our study. These results are at variance with those of Kucich *et al*, who observed a significantly higher elastin peptide concentration among 51 smokers than among 83 non-smokers.[7] Changes in smoking habits cannot explain our findings as an analysis restricted to subjects with unchanged habits for at least five years gave the same results. An association between smoking habits and elastin peptide concentration could, however, be observed at an older age. On the assumption of an exclusive acute effect of smoking on lung destruction, measurement of cotinine, which precisely assesses exposure over the previous 24 hours, would be of interest in future studies, or perhaps nicotine, which assesses exposure over the last few hours.

The immunological assay used here measured elastin peptide concentration in the peripheral blood, which may be different from that in the lung. A continuous slightly increased degradation of lung elastin, due to tobacco, may be difficult to detect in peripheral blood because of a high background of extrapulmonary elastin turnover. Alternatively, these findings may suggest an episodic elastin degradation, which is acute and therefore only occasionally detectable. The hypothesis of a succession of acute events has already been put forward to explain the lack of difference in urine desmosine measurements between normal and emphysematous adults with α_1 protease inhibitor deficiency[5] and the previously observed large day to day variation.[24] Urine desmosine was not a good marker of elastin degradation, as in the only epidemiological study[6] desmosine did not increase with age, smoking, or low FEV_1 and clinical studies have produced contradictory findings.[5 19]

LUNG FUNCTION

No association was observed between elastin peptide concentration and FEV_1. No more

specific test of emphysema was performed in our study. The relation of elastin peptide concentration to lung function is documented in very few studies, and limited to highly selected groups of subjects. McLennan *et al*,[25] in their study of 17 subjects without interstitial lung disease (ages unspecified), did not report a relation between elastin peptide concentration and FEV_1. In that study, elastin peptide concentration was not positively associated with transfer factor, the measurement of which gives a more specific test for emphysema. By contrast, Cohen *et al* reported a significant inverse relation of elastin peptide to FEV_1 ($r = -0{\cdot}32$) among 46 patients with chronic obstructive lung disease.[23]

ALCOHOL CONSUMPTION

Epidemiological reports have shown that only heavy[9] alcohol consumption is associated with the longitudinal decline in FEV_1, independently of smoking. By contrast, a protective effect of alcohol on emphysema was reported in a study of non-selected.[26] We observed that heavy (but not moderate) alcohol consumption was related to a lower elastin peptide concentration, an association still of borderline significance after adjustment for age. The relation between α_1 protease inhibitor (synthesised by the liver) and tobacco and alcohol consumption was investigated in the 1980 survey conducted in our population.[11] Alcohol but not tobacco consumption was inversely correlated with the functionally active α_1 protease inhibitor concentration.[11 16] This suggests that alcohol has a deleterious effect on the lung by decreasing the antielastase protection. A decrease of the neutrophil elastase activity with alcohol consumption was also reported, however, suggesting that a protective effect of alcohol on emphysema in smokers may be due to the effects of alcohol on marrow precursors of neutrophils.[27] Two studies have already reported that smokers with heavy alcohol consumption had better lung function values than non drinking subjects.[9 10] Such results would suggest a protective role of alcohol among smokers. Additional studies are required to understand the complex interrelation between emphysema, elastase and antielastase activities, tobacco and alcohol consumption.

PROTEASE INHIBITOR PHENOTYPE

We did not observe any increase in elastin peptide concentration among subjects with a partial α_1 protease inhibitor deficiency (PI MS, MZ, and S subjects, whose theoretical concentration ranges from 60% to 80% of the normal mean). On the contrary, the group of PI MS, MZ, and S subjects had a lower elastin peptide concentration than other subjects. The relation between protease inhibitor phenotype and elastin peptide concentration is still uncertain and no interpretation of the decrease of elastin peptide concentration observed among subjects with the FM, IM, MP, MS, MZ, and S phenotypes can be proposed.

Interpretation of our results is limited by the fact that elastin metabolism is not fully understood. The observation that patients with chronic obstructive lung disease had higher elastin peptide concentration than healthy subjects[7] suggested that the elastin peptide concentration might be a marker of lung elastin degradation. To our knowledge no stable pool of elastin is present in blood.[28] Resynthesis of elastin could explain the increase in activity of the enzyme cross linking elastin (lysyl oxidase), which was observed after elastase injury in animal studies.[29] Such a resynthesis may be affected by smoking, among other factors, as suggested by the observation that lysyl oxidase activity was impaired in smoking animals.[4]

Animal experiments, together with the observation that the elastin content of human emphysematous lungs is normal,[30] suggest that in man also some resynthesis of elastin occurs and can be blunted by exogenous factors, particularly smoking. The elastin peptides measured by this assay may correspond to either tropoelastin, tropoelastin fragments, or mature elastin fragments. Thus elastin peptide concentration reflects not only elastin degradation but both degradation and resynthesis of elastin. Besides the protease-antiprotease imbalance hypothesis, elastin resynthesis may be important for understanding basic mechanisms underlying emphysema, primarily smoker's emphysema.[3 4 31] Low elastin peptide concentration, observed in peripheral blood, might reflect some defect in the resynthesis of elastin.

We first formed the hypothesis that an increased blood elastin peptide concentration could reflect the severity of emphysema. Our results cast doubts on the usefulness of elastin peptide concentration as a marker of lung destruction, at least in a population of middle aged, predominantly healthy men, who may have minimal lung destruction. The findings relating a lower elastin peptide concentration to several factors ight reflect decreased elastin resynthesis. Future studies will be required to unravel the mechanisms underlying lysis and resynthesis of lung elastin. More specific functional tests of emphysema, such as measurement of transfer factor, or a method of estimating the extent of lung destruction, such as computed tomography, should be included in future studies with potential biological indicators of lung elastin metabolism.

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