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Hyperhomocysteinaemia predicts the decline in pulmonary function in healthy male smokers

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Running title: Hyperhomocysteinaemia predicts decline in FEV_1

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ABSTRACT: Hyperhomocysteinaemia is associated with chronic obstructive pulmonary disease (COPD). However, the relationship between plasma homocysteine (Hcy) levels and spirometric measures has not been investigated in a general population. We aimed to determine whether Hcy levels are predictive for a rapid decline in lung function among healthy current smokers.

Blood sampling and spirometry were performed on subjects (n = 3,257), participating in a community-based annual health check in Takahata, Japan, from 2004 to 2006. Spirometry was re-evaluated in 147 male current smokers in 2009.

On initial assessment, percent predicted forced vital capacity (%FVC) and forced expiratory volume in 1 s (%FEV_1) correlated inversely with Hcy levels, and were predictive for Hcy levels, independent of various clinical factors. Hcy levels were higher in subjects with restrictive, obstructive, or mixed ventilatory disorders. Additionally, Hcy levels were higher in subjects with mixed ventilatory disorders, compared with restrictive or obstructive disorders. On follow-up, subjects showing a decline in FEV_1 had higher Hcy levels than those who did not. Logistic regression analysis indicated that Hcy levels were predictive for a decline in FEV_1.

%FVC and %FEV_1 were significantly associated with Hcy levels, and hyperhomocysteinaemia predicted the annual rate of decline in FEV_1 among male smokers.

KEYWORDS: pulmonary function test, homocysteine, smokers, health check

Long-term cigarette smoking injures the respiratory system and causes disorders such as chronic obstructive pulmonary disease (COPD) [1]. Even in healthy individuals, smoking causes a decline in pulmonary function [2]. In addition, patients with COPD have various comorbidities, including atherosclerosis, cachexia, diabetes mellitus, and osteoporosis [3-8]. These comorbidities are thought to be associated with systemic inflammation due to the leakage of mediators from local inflammatory sites in the lung, and this is an important determinant of the morbidity associated with the disease [9].

Homocysteine (Hcy) plays an important role in the development of atherosclerosis [10-12], and Hcy is also suggested to be involved in the pathogenesis of COPD [13-15]. Plasma Hcy levels were found to be elevated in COPD patients and this is thought to be related to the stage of COPD [15]. However, Hcy levels are influenced by other factors such as gender [16], body mass index (BMI) [17], cigarette smoke inhalation [18, 19], blood pressure (BP) [20], diabetic nephropathy [21], hyperlipidaemia [22], liver function [23], and renal function (serum creatinine levels) [24]. Therefore, findings in relation to Hcy levels need to be carefully assessed, with consideration to variation in the

backgrounds of the subjects. To date, the relationship between Hcy levels and spirometric measures has not been investigated in a general population. Furthermore, it remains to be elucidated whether Hcy levels are predictive for a rapid decline in lung function among current smokers.

In this cross-sectional study, the relationship between spirometric parameters and Hcy levels was investigated in participants in an annual health check in Takahata, Japan. Follow-up spirometric measurements were performed in 147 male current smokers, and the relationship between a decline in lung function and Hcy levels was assessed.

METHODS

Study population

This study was part of The Takahata Study based on an annual community health check in which residents, aged 40 years or older, participated. The study was approved by the institutional ethics committee and all participants gave written informed consent [2]. Cross-sectional analyses of spirometry data for subjects who were enrolled from 2004 to 2005 (n = 3,165; 1,380 males, 1,735

females) have been reported previously [2, 25-27]. Enrolment of subjects continued through 2006, and the additional data for these subjects became available recently. A final total of 3,520 subjects (1,579 males, 1,941 females) were enrolled. Two hundred and sixty-three subjects were excluded from the analysis because their spirometry data did not meet the specified criteria. The data for a total of 3,257 subjects (1,502 males, 1,755 females) was entered into the final statistical analysis. Subjects used a self-report questionnaire to document their smoking habits. The lifetime consumption of cigarettes was expressed as the Brinkman index (number of cigarettes per day x years of smoking). Of the 523 male current smokers, 147 performed subsequent follow-up spirometry in 2009.

Measurements

Systolic and diastolic BP were measured using a mercury sphygmomanometer. Mean BP was calculated as diastolic BP plus 0.33 x [systolic BP minus diastolic BP] [25]. Blood samples were taken from the antecubital vein of subjects who had been fasting.

Total plasma Hcy concentrations were measured using an enzymatic homocysteine assay kit (MBL, Nagoya, Japan) [28].

Spirometric parameters (forced vital capacity [FVC] and forced expiratory volume in 1 s [FEV_1]) were measured using standard techniques, with subjects performing FVC manoeuvres on a CHESTAC-25 part II EX instrument (Chest Corp., Tokyo, Japan), according to the guidelines of the Japanese Respiratory Society (JRS) [29]. A bronchodilator was not administered prior to spirometry. The highest value from at least three FVC manoeuvres by each subject was used for the analysis. The rate of decline in spirometric measures [ΔFEV_1/year (%) and ΔFVC/year (%)] were calculated as ([value at second spirometry – value at first spirometry]/value at first spirometry) × 100/ time between observations (years).

Statistical analysis

For continuous variables, data are presented as the mean ± SD or median (interquartile range). The Student's t-test for parametric data and the Mann-Whitney U test for non-parametric data were used to analyse the differences between two groups. For multiple comparisons one way analysis of

variance was used, followed by the Student-Newman-Keuls test. Correlations between two variables were evaluated using Pearson's product moment correlation coefficient. Univariate regression analysis was used to examine the association between Hcy levels and each of the spirometric measures considered in this study. Multiple linear regression analysis was then performed to determine whether each of these spirometric measures contributed to Hcy levels after adjustment for all other variables included in the model. A stepwise variable selection method was used to retain variables that reached a 0.20 level of significance. Results of multiple logistic regression analysis were presented as odds ratio (OR) and 95% confidence interval (CI). Statistical significance was inferred for two-sided *P* values < 0.05. All statistical analyses were performed using JMP version 8 software (SAS Institute Inc., Cary, NC, USA).

RESULTS

Assessment of results from the Takahata Study, 2004-2006

The characteristics of the subjects who were enrolled from 2004-2006 are summarized in Table 1. In this study population, 64.4% of the subjects were

never-smokers, and the Brinkman index did not show a normal distribution (25% quartile, 0; median, 0; 75% quartile, 175.5). The distribution of Hcy measurements was skewed towards higher values (25% quartile, 8.7; median, 10.3; 75% quartile, 12.3). Therefore, Hcy values were logarithmically transformed before analysis. Age, Brinkman index, mean BP, and alanine aminotransferase (ALT), serum creatinine (sCr), triglyceride (TG) and log Hcy values were significantly higher in male subjects compared with female subjects. In contrast, total cholesterol (TC) levels, %FVC and $\%FEV_1$ were significantly lower in males than in females. BMI and haemoglobin A1c (HbA1c) levels did not differ significantly between men and women.

Figure 1 shows the relationship between spirometric parameters (%FVC and $\%FEV_1$) and log Hcy values. There were inverse relationships between these spirometric parameters and log Hcy levels (Fig. 1A, B, C & D).

Univariate regression analysis demonstrated that age, gender, Brinkman index, mean BP, ALT, sCr, TG, TC, %FVC, and $\%FEV_1$ were significantly associated with log Hcy levels (online supplementary Table 1). Multiple linear regression

analysis was performed to determine whether these spirometric measures contributed independently to Hcy levels (Table 2). As %FVC and $\%FEV_1$ were strongly correlated (r = 0.85, P < 0.0001), these parameters were assessed separately to avoid confounding (Table 2A & B). As shown in Table 2, %FVC and $\%FEV_1$ were predictive for Hcy levels, independent of the other factors.

The possible relationship between increased Hcy levels and impaired pulmonary function was investigated. The subjects were categorized according to their spirometry data: %FVC ≥ 80 and $FEV_1/FVC \geq 0.7$, normal spirometry; %FVC < 80 and $FEV_1/FVC \geq 0.7$, pure restriction; %FVC ≥ 80 and $FEV_1/FVC < 0.7$, pure obstruction; %FVC < 80 and $FEV_1/FVC < 0.7$, mixed ventilatory disorder (Fig. 2). Subjects with abnormal spirometry (restriction, obstruction, or mixed) had higher Hcy levels than subjects with normal spirometry (Fig. 2). Furthermore, subjects with a mixed ventilatory disorder had significantly higher Hcy levels than subjects with either a restrictive, or obstructive, disorder.

Association between longitudinal changes in pulmonary function and plasma Hcy levels in the Takahata Study

Of the 523 male current smokers who participated in the study, 147 performed follow-up spirometry in 2009 (characteristics of these are summarized in Table 3). BMI, Brinkman index, mean BP, HbA1c, ALT, sCr, TG, TC, Hcy, log Hcy, %FVC, and $\%FEV_1$ were not significantly different between the 523 male current smokers who were assessed in 2004-2006 and the 147 who performed follow-up spirometry in 2009 (data not shown). However, the mean age of these 147 subjects was significantly lower than that of the 523 subjects assessed in 2004-2006 [mean age (years): 56.40 ± 9.06 vs. 59.77 ± 10.40, $P = 0.0004$].

Histograms showing the rates of decline in FVC [ΔFVC/year (%)] and FEV_1 [ΔFEV_1/year (%)] are presented in online supplementary Fig. 1. As the lowest quintiles of decline in FVC (online supplementary Fig. 1A) and FEV_1 (online supplementary Fig. 1B) were -1.2% and -2.5%, respectively, the cut-off values for identifying subjects showing a rapid decline in pulmonary function were defined as annual rates of decline equivalent to, or greater than, these values. Twenty-eight subjects showed a decline in FVC (Table 4A) and 29 showed a decline in FEV_1 (Table 4B). Although %FVC and $\%FEV_1$ at the first visit did not differ between those who did, or did not, show a decline in FVC, the values at the

second visit were significantly lower in those who did show a decline in FVC (Table 4A). FEV_1/FVC at the first visit was significantly lower in those who showed a decline in FVC, compared with those who did not. However, this difference was not observed at the second visit. There were no differences in age, BMI, Brinkman index, mean BP, or HbA1c, ALT, TG, TC and Hcy levels between subjects who did, or did not, show a decline in FVC, the exception being sCr values (Table 4A).

Percentage FVC and $\%FEV_1$ at the first visit did not differ between subjects who did, or did not, show a decline in FEV_1 (Table 4B). However, at the second visit, %FVC and $\%FEV_1$ were significantly lower in subjects who did show a decline (Table 4B). FEV_1/FVC at the first visit was significantly lower in subjects who showed a decline in FEV_1, and this difference was greater at the second visit. Although age, BMI, mean BP, and HbA1c, ALT, sCr, TG, and TC levels were not different, the Brinkman index and Hcy levels were significantly higher in subjects showing a decline in FEV_1 (Table 4B).

To investigate whether Hcy levels were predictive for FEV_1 decline, multivariate

logistic regression analyses were performed. Hcy levels were significantly predictive for FEV_1 decline (Table 5).

Receiver operating characteristic (ROC) curve analysis was performed to determine the optimum cut-off value of Hcy for discriminating a decline in FEV_1 among smokers. The optimum cut-off value for Hcy was 11.1 μM, with an area under the curve of 0.7, a sensitivity of 0.9, and a specificity of 0.5 (online supplementary Fig. 2). Pulmonary function values for these subjects, relative to Hcy levels (cut-off = 11 μM), are shown in online supplementary Table 2. At the first spirometric assessment, %FVC, $\%FEV_1$ and FEV_1/FVC did not differ significantly between subjects with Hcy levels ≤ 11 μM or > 11 μM. At the second spirometric assessment, %FVC and $\%FEV_1$ were significantly lower in subjects with Hcy levels > 11 μM than in those with Hcy levels ≤ 11 μM. The median annual rate of decline in FEV_1 was significantly greater in subjects with Hcy levels > 11 μM than in those with Hcy levels ≤ 11 μM.

DISCUSSION

This study demonstrated that there was a significant correlation between

spirometric parameters and Hcy levels in subjects who participated in an annual health check. Furthermore, multiple linear regression analyses revealed that %FVC and %FEV_1 were significantly predictive for Hcy levels, independent of age, gender, BMI, smoking index, mean BP, and HbA1c, ALT, sCr, TG, or TC levels. We can conclude that there are significant associations between spirometric measurements and Hcy levels in the Japanese general population. In this cohort, subjects who showed a decline in FEV_1 had higher Hcy levels than those who did not. Logistic regression analysis demonstrated that Hcy levels were a determinant of which subjects showed a decline in FEV_1.

Hcy is an intermediate metabolite in the production of cysteine from methionine [30]. The factors contributing to hyperhomocysteinaemia are vitamin B deficiency [31], ageing [16], hypertension [20], renal dysfunction [24], diabetic nephropathy [21], and cigarette smoking [18, 19]. Multiple factors may regulate Hcy levels, and as such, the main cause(s) of hyperhomocysteinaemia in individual subjects are complex. Previous studies demonstrate elevated Hcy levels in COPD subjects, although the mechanism is still unknown [14, 15, 32].

Kai et al. demonstrated elevated Hcy levels in COPD patients, and a positive, rather than an inverse correlation, between Hcy levels and %FEV_1 in a small number of COPD patients. This finding indicates that patients with mild airway obstruction may have higher Hcy levels. Also, in contrast to the inverse relationship between severity of airflow limitation and Hcy levels, the latter were positively correlated with the annual rate of decline in FEV_1 [14]. Seemungal and colleagues also demonstrated elevated Hcy levels in COPD patients. However, they demonstrated an inverse correlation between Hcy levels and %FEV_1 in a small number of COPD patients [15]. These previous studies showed contrasting results in terms of the correlation between severity of airflow obstruction and Hcy levels. The present study clarifies this issue by demonstrating in a large population that poor lung function was associated with high Hcy levels. In addition, high Hcy levels were shown to be a predictor of FEV_1 decline among male active smokers.

Fimognari and colleagues demonstrated that Hcy levels were elevated in COPD patients and that low levels of folate and vitamin B12, as well as hyperglyceridaemia, were independent predictors of hyperhomocysteinaemia

[32]. The mechanism for the hyperhomocysteinaemia observed in COPD patients may simply be a decrease in folate and vitamin B12 or increased TG levels. Unfortunately, we did not measure serum levels of folate and vitamin B12 in this study. As shown in Table 2, TG levels were associated with Hcy levels, independent of other variables, although TG levels did not differ between subjects who did or did not show a decline in FEV_1 (Table 4). In a future study, assessing the relationship between TG levels and spirometric values in this population may be required.

The main cause of COPD is the long term inhalation of cigarette smoke [1]. An imbalance of oxidants and antioxidants is one of the most important mechanisms in the development of COPD [1]. Pulmonary and systemic inflammation are recognized as important features of COPD, with regard to the development of airflow obstruction, and comorbidities such as atherosclerosis, weight loss, and osteoporosis [33]. Seemungal et al. also reported an association between Hcy levels and C-reactive protein levels in COPD patients [15]. Acute administration of Hcy to rats is reported to result in elevated blood and brain concentrations of cytokines [34]. This suggests that

hyperhomocysteinaemia may partly contribute to the systemic inflammation observed in COPD patients.

Hyperhomocysteinaemia is a well-known risk factor for cardiovascular disease [10-12]. Endothelial dysfunction induced by hyperhomocysteinaemia reportedly plays important roles in the development of atherosclerotic lesions [35], and Hcy is associated with apoptosis in endothelial progenitor cells that play important roles in vascular endothelial homeostasis [36]. Therefore, it is hypothesised that lowering Hcy levels with the use of vitamin B may potentially attenuate the development of atherosclerosis. However, some randomised control trials reveal that treating hyperhomocysteinaemia with B vitamins does not decrease vascular events, suggesting that hyperhomocysteinaemia is only a marker of overall vascular risk [37-39].

Cardiovascular disease is also a frequent comorbidity in patients with COPD [40]. Accumulating evidence suggests that pulmonary endothelial dysfunction is involved in the pathogenesis of COPD [41-43]. We have previously shown that the rate of washout of ^{123}I-metaiodobenzylguanidine, a specific scintigraphic

molecule that enables measurement of endothelial function *in vivo*, was decreased in patients with COPD [41]. Many risk factors, including hypertension, hyperglycaemia, hyperglyceridaemia, hypercholesterolaemia, and smoking, contribute to the pathogenesis and development of atherosclerosis. Therefore, even if subjects receive therapy with vitamin B to lower homocysteine levels, cardiovascular events may not be reduced due to the significant effects of other risk factors such as hypertension, hyperglycaemia, hyperglyceridaemia, hypercholesterolaemia, and smoking. In contrast to atherosclerosis, the risk factors for the development of COPD are simple, and cigarette smoking is the most important risk factor. Although the results of trials of the effects of vitamin B on cardiovascular events were negative [37-39], therapy to lower Hcy levels may protect the lungs from injury induced by cigarette smoke. Clinical trials investigating the potential of Hcy-lowering therapy for COPD patients may be required. In addition, measuring Hcy levels may be useful in identifying patients with COPD, who are at higher risk of vascular events, and a more rapid decline in lung function.

As mentioned previously, Hcy levels are influenced by various other factors,

including age, gender, blood pressure, blood glucose levels, and renal function. Therefore, careful consideration of these other factors is required to assess the relationship between pulmonary function and Hcy levels. In the present study, multiple linear regression analyses were performed to control for the influence of factors other than Hcy levels on pulmonary function. Ozkan et al. previously demonstrated that serum Hcy levels were significantly higher in patients with severe sleep apnoea syndrome than in controls, suggesting that tissue hypoxia may be associated with the level of serum Hcy [44]. However, partial pressures of arterial oxygen and oxygen saturation were not measured in this population, and this was a limitation of our study.

A further limitation was sampling bias. Takahata town has a resident population of 15,222 adults, aged 40 years or older (7,109 males and 8,113 females). Data for a final total of 3,257 enrolled subjects (1,502 males, 1,755 females) was entered into the final statistical analysis. Among the 1,502 male subjects, there were 523 active smokers. However, follow-up spirometry in 2009 was only performed for 147 subjects who had continued to smoke. The remaining subjects were lost to follow-up or quit smoking before follow-up. The number of

subjects who were followed-up was not large, however, these 147 subjects appeared to be representative of the 523 male active smokers from the first assessment. The only significant difference between these groups was age (see *Results* section). In addition, workers in public institutions and many companies did not participate in this research program for reasons described previously [25]. Differences in type of employment, socio-economic status or life style, including intake of vitamin B, between participants and non-participants may have influenced the results.

In the present study, all subjects were Japanese, and those assessed for a decline in FEV_1 were 147 male active smokers. The subject number may not have been sufficient to obtain accurate cut-off levels of Hcy for discriminating those who showed a decline in FEV_1. In addition, this cut-off (Hcy > 11 μM) may not be applicable to other ethnic groups, or to females. Further investigation is required to determine the cut-off values of Hcy according to ethnicity and gender.

In conclusion, Hcy levels were significantly associated with %FVC and $\%FEV_1$

in the Japanese general population, and were identified as a significant predictive factor for decline in FEV_1 among male smokers. Measuring Hcy levels in smokers may facilitate smoking cessation by providing an indication of individual susceptibility to cigarette smoke and its effect on decline in lung function.

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STATEMENT OF INTERESTS

The authors have no conflicts of interest to disclose.

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TABLE 1 Characteristics of the study subjects

Variable	Males, *n* = 1502	Females, *n* = 1755
Age, years	62.9 ± 10.4	61.8 ± 10.3*
BMI, kg/m^2	23.5 ± 3.0	23.5 ± 3.4
Brinkman index, cigarette·years	441.0 ± 495.6	17.2 ± 89.7***
Mean BP, mmHg	99.8 ± 11.0	95.8 ± 10.9***
HbA1C, %	5.26 ± 0.75	5.26 ± 0.65
ALT, U/L	25.9 ± 15.4	21.3 ± 12.1**
sCr, mg/dL	0.78 ± 0.27	0.59 ± 0.11***
TG, mg/dL	117.7 ± 79.6	98.7 ± 47.3***
TC, mg/dL	193.6 ± 31.4	206.8 ± 31.4***
Hcy, μM	12.6 ± 6.9	10.0 ± 3.7***
Log Hcy	1.08 ± 0.13	0.98 ± 0.12***
%FVC	97.3 ± 14.9	99.8 ± 14.3***
%FEV$_1$	95.6 ± 17.5	99.7 ± 15.4***

Brinkman index was not calculated for 267 male subjects and 63 female subjects due to the lack of the information on “cigarette years” or “daily consumption of cigarettes”. Data on Hcy levels were not available for 29 male subjects and 94 female subjects. Values are mean ± SD. Differences in Brinkman index and Hcy levels between males and females were evaluated by the Mann-Whitney U test. Differences in other variables were evaluated by the Student’s t-test. *P < 0.05, ** P < 0.001, *** P < 0.0001 compared with male subjects.

BMI: body mass index; BP: blood pressure; HbA1c: haemoglobin A1c; ALT: alanine aminotransferase; sCr: serum creatinine; TG: triglyceride; TC: total

cholesterol; Hcy: homocysteine; %FVC: percent predicted forced vital capacity; %FEV_1: percent predicted forced expiratory volume in 1 s

TABLE 2 Multivariate linear regression analyses of factors predictive for plasma homocysteine levels

A. Model 1

Variable	Coefficient	SE	*P* value
Age	0.074	0.010	< 0.0001
Male gender	0.252	0.137	0.066
BMI	-0.024	0.034	0.489
Brinkman index	0.001	0.000	0.004
Mean BP	0.031	0.010	0.001
HbA_{1C}	-0.319	0.157	0.041
ALT	0.016	0.008	0.039
sCr	6.517	0.598	< 0.0001
TG	0.004	0.002	0.011
TC	-0.014	0.003	< 0.0001
%FVC	-0.017	0.007	0.012

B. Model 2

Variable	Coefficient	SE	*P* value
Age	0.073	0.010	< 0.0001
Male gender	0.250	0.137	0.069
BMI	-0.024	0.035	0.488
Brinkman index	0.001	0.000	0.007
Mean BP	0.031	0.010	0.001
HbA_{1C}	-0.298	0.156	0.056
ALT	0.016	0.008	0.033
sCr	6.548	0.598	< 0.0001
TG	0.005	0.002	0.009
TC	-0.014	0.003	<.0001
$\%FEV_1$	-0.012	0.006	0.048

BMI: body mass index; BP: blood pressure; HbA1c: haemoglobin A1c; ALT: alanine aminotransferase; sCr: serum creatinine; TG: triglyceride; TC: total cholesterol; %FVC: percent predicted forced vital capacity; $\%FEV_1$: percent predicted forced expiratory volume in 1 s

TABLE 3 Characteristics of male current smokers who performed a second pulmonary function test

Variable	Male subjects (*n* = 147)
Age, years	56.40 ± 9.06
BMI, kg/m^2	22.91 ± 2.89
Brinkman index, cigarette· years	751.7 ± 413.8
Mean BP, mmHg	98.4 ± 13.0
$HbA_{1C,}$ %	5.12 ± 0.54
ALT, U/L	26.73 ± 14.56
sCr, mg/dL	0.747 ± 0.113
TG, mg/dL	141.06 ± 88.60
TC, mg/dL	194.01 ± 29.84
Hcy, μM	11.6 (10.1, 13.6)
Log Hcy	1.07 ± 0.12
%FVC	97.87 ± 14.12
$\%FEV_1$	96.57 ± 16.58
FEV_1/FVC (%)	77.26 ± 8.43
ΔFVC/year (%)	-0.28 ± 2.42
ΔFEV_1/year (%)	-1.01 ± 2.87

Data on Hcy levels was not available for four subjects. Brinkman index data was not available for 19 subjects due to lack of information on precise smoking habits.

BMI: body mass index; BP: blood pressure; HbA1c: haemoglobin A1c; ALT: alanine aminotransferase; sCr: serum creatinine; TG: triglyceride; TC: total cholesterol; Hcy: homocysteine; %FVC, percent predicted forced vital capacity; $\%FEV_1$: percent predicted forced expiratory volume in 1 s; ΔFVC/year (%): percent annual decline in FVC; ΔFEV_1/year (%): percent annual decline in FEV_1

TABLE 4 Characteristics of subjects who did, or did not, show a decline in pulmonary function

A

Variable	FVC decline *n* = 28	No FVC decline *n* = 119	*P* value
Age, years	61.79 ± 10.42	60.79 ± 8.81	0.651
BMI, kg/m^2	23.65 ± 3.53	22.73 ± 2.71	0.187
Brinkman index, cigarette·years	737.6 ± 409.8	754.4 ± 416.4	0.866
mean BP, mmHg	100.8 ± 10.4	97.8 ± 13.5	0.128
HbA_{1C}, %	5.03± 0.36	5.14 ± 0.57	0.288
ALT, U/L	26.64 ± 12.81	26.75 ± 14.99	0.782
sCr, mg/dL	0.696± 0.096	0.759 ± 0.114	0.007*
TG, mg/dL	148.57 ± 97.66	139.29 ± 86.68	0.632
TC, mg/dL	192.39 ± 29.34	194.40 ± 30.07	0.805
Hcy, μM	12.4 (11.1, 14.3)	11.9 (10.0, 14.0)	0.148
Log Hcy	1.09 ± 0.11	1.07 ± 0.12	0.148
$\%FVC_{first}$	97.79 ± 15.57	97.88 ± 13.83	0.668
$\%FVC_{second}$	90.76 ± 15.40	104.55 ± 13.58	<0.0001*
$\%FEV_{1\ first}$	92.63 ± 18.44	97.49 ± 16.04	0.236
$\%FEV_{1\ second}$	84.01 ± 19.23	98.73 ± 16.35	0.0001*
FEV_1/FVC_{first} (%)	74.27 ± 7.68	77.95 ± 8.47	0.039*
FEV_1/FVC_{second} (%)	71.99 ± 10.64	73.27 ± 9.02	0.657

B

Variable	FEV_1 decline *n* = 29	No FEV_1 decline *n* = 118	*P* value
Age, years	63.55 ± 9.50	60.20 ± 8.91	0.063
BMI, kg/m^2	23.51 ± 3.02	22.76 ± 2.68	0.326
Brinkman index, cigarette·years	863.3 ± 387.8	725.9 ± 417.1	0.028*
mean BP, mmHg	99.25 ± 11.61	98.19 ± 13.38	0.436
HbA_{1C}, %	5.09 ± 0.36	5.13 ± 0.57	0.963
ALT, U/L	28.00 ± 13.85	26.42 ± 14.77	0.377
sCr, mg/dL	0.728 ± 0.107	0.752 ± 0.115	0.3
TG, mg/dL	132.58 ± 67.66	143.14 ± 93.15	0.851
TC, mg/dL	194.86 ± 30.47	193.80 ± 29.81	0.748
Hcy, μM	13.8 (11.7, 18.4)	11.0 (9.6, 12.8)	0.001*
Log Hcy	1.13 ± 0.11	1.06 ± 0.01	0.001*
$\%FVC_{first}$	95.70 ± 16.06	98.39 ± 13.62	0.668
$\%FVC_{second}$	92.09 ± 16.17	104.34 ± 13.61	<0.0001*
$\%FEV_{1\ first}$	90.71 ± 20.94	98.01± 15.08	0.158
$\%FEV_{1\ second}$	78.44 ± 21.07	100.23 ± 14.01	<0.0001*
FEV_1/FVC_{first} (%)	73.78 ± 10.12	78.11 ± 7.77	0.019*
FEV_1/FVC_{second} (%)	65.86 ± 12.86	74.79 ± 7.28	<0.0001*

Data on Hcy levels was not available for four of the 147 subjects. Brinkman index data was not available for 19 subjects due to the lack of information on precise smoking habits.

"First" indicates spirometry data at the first visit, and "second" indicates spirometry data at the second visit.

Differences in Brinkman index and Hcy levels between subjects who did, or did not, show a decline in pulmonary function were evaluated by the Mann-Whitney

U test. Differences in all other variables were evaluated by the Student's t-test. BMI: body mass index; BP: blood pressure; HbA1c: haemoglobin A1c; ALT: alanine aminotransferase; sCr: serum creatinine; TG: triglyceride; TC: total cholesterol; Hcy: homocysteine; %FVC: percent predicted forced vital capacity; $\%FEV_1$: percent predicted forced expiratory volume in 1 s

TABLE 5 Multivariate logistic regression analyses of factors predictive for a decline in FEV_1

	OR	95% CI		*P* value
Multivariate				
Age (per 1 year increase)	1.051	1.001	1.106	0.045
Brinkman index (per 1 cigarette·year increase)	1.121	0.960	1.318	0.150
Hcy (per 1 μM increase)	1.100	1.010	1.206	0.030

Data on Hcy levels was not available for four of the 147 subjects. Brinkman index data was not available for 19 subjects due to lack of information on precise smoking habits.

Hcy: homocysteine; FVC: forced vital capacity; FEV_1: forced expiratory volume in 1 s; OR: odds ratio; CI: confidence interval

FIGURE LEGENDS

FIGURE 1. Inverse correlations between spirometric measurements and plasma homocysteine levels

Graphs show the relationships between spirometric parameters (A and C: %FVC; B and D: $\%FEV_1$) and plasma homocysteine (Hcy) levels (A and B: males; C and D: females). Correlations between spirometric measurements and log Hcy values were evaluated using the Pearson's product moment correlation coefficient. There were inverse relationships between these spirometric parameters and plasma Hcy levels. %FVC: percent predicted forced vital capacity; $\%FEV_1$: percent predicted forced expiratory volume in 1 s

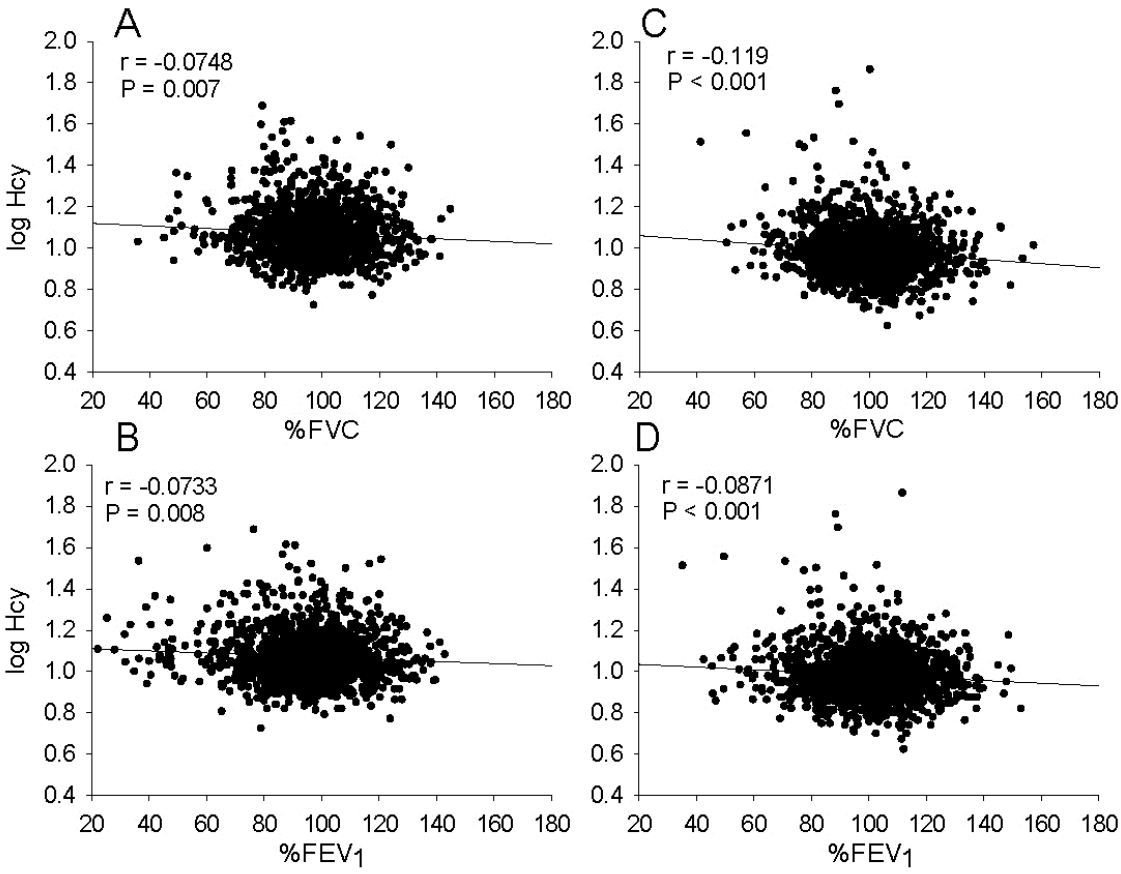


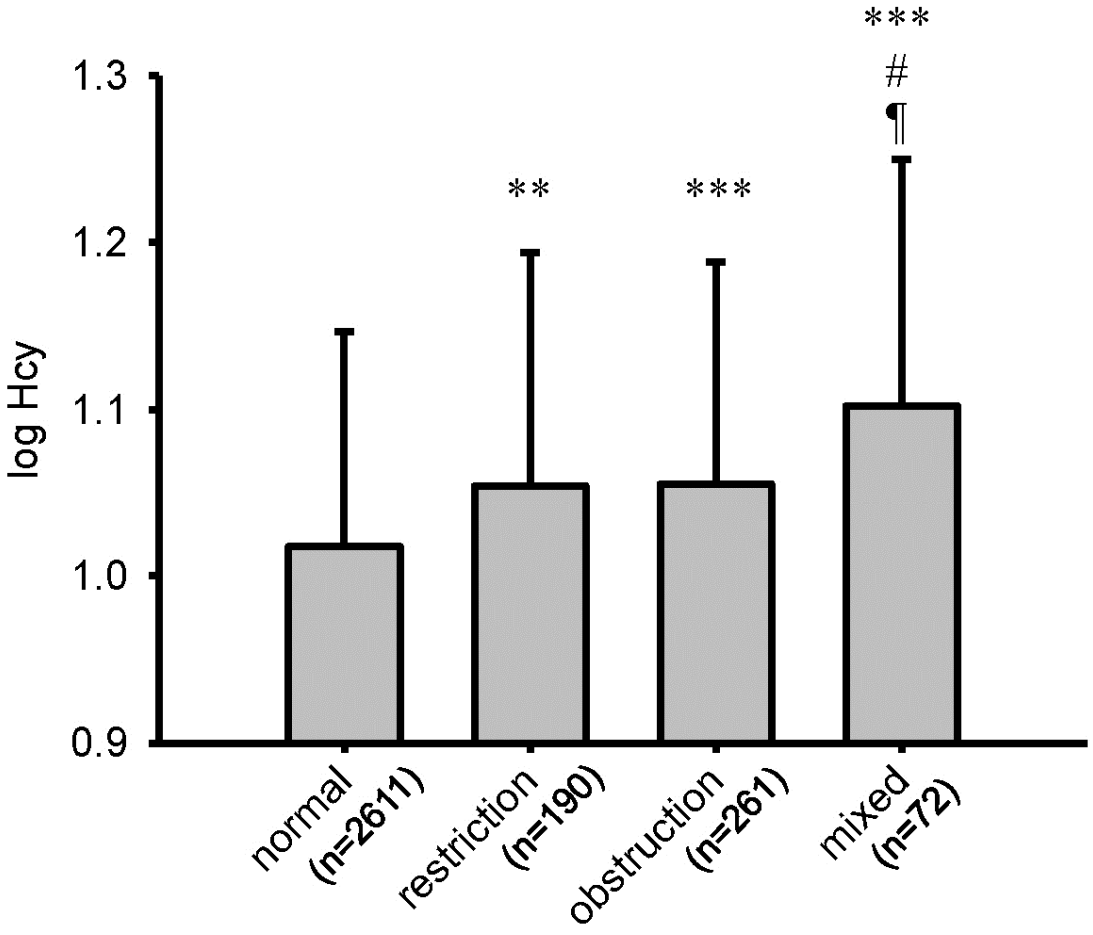
Figure 1

Figure 2. Plasma homocysteine (Hcy) levels according to the pattern of pulmonary function impairment

Subjects were classified into four groups according to their pulmonary function: %FVC ≥ 80 and $FEV_1/FVC \geq 0.7$, normal; %FVC < 80 and $FEV_1/FVC \geq 0.7$, restriction; %FVC ≥ 80 and $FEV_1/FVC < 0.7$, obstruction; %FVC < 80 and $FEV_1/FVC < 0.7$, mixed. Subjects with restrictive, obstructive or mixed impairment had higher Hcy levels than subjects with normal spirometry. In

addition, subjects with mixed impairment had higher Hcy levels than subjects with either restrictive or obstructive impairment.

Data on Hcy levels was not available for 123 subjects. Comparisons were performed by one-way analysis of variance followed by the Student-Newman-Keuls test. ** $P < 0.01$ vs. “normal”, *** $P < 0.001$ vs. “normal”, # $P < 0.01$ vs. “restriction”, ¶ $P < 0.05$ vs. “obstruction”.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#
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**
***
1.3
1.2
1.1
1.0
0.9
log Hcy
normal (n=2611)
restriction (n=190)
obstruction (n=261)
mixed (n=72)

Figure 2