the 22 patients treated with GCV, CMV-pp65Ag decreased to undetectable levels. Seven patients treated with GCV died within 1 month before the disappearance of CMV-pp65Ag.
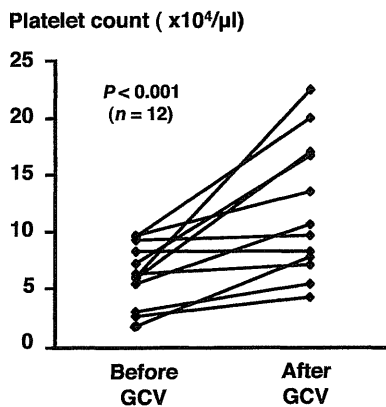
Treatment of CMV-pp65Ag antigenaemia

Thirty-four patients with CMV-pp65Ag antigenaemia received GCV for a median of 11 days (interquartile range (IQR) 7–14 days) until CMV-pp65Ag could not be detected in general. GCV therapy was tolerable except for three patients, complicated with myelosuppressive and hepatotoxic effects and received foscarnet instead. Sixteen patients received immu-

Platelet count (x10⁴/μl)

P < 0.001
(*n* = 12)

Figure 3 Thrombocytopenia in patients with clinical cytomegalovirus (CMV) disease improved significantly after ganciclovir (GCV) therapy. Platelet counts in peripheral blood before and after GCV therapy were compared using the Wilcoxon signed-rank test.

noglobulin supplementation. Granulocyte colony-stimulating factor was administered to two patients with HPS. CMV-pp65Ag decreased to undetectable levels in 19 patients after the various therapeutic approaches described previously. In five of the 19 patients, CMV-pp65Ag antigenaemia recurred,and GCV treatment more than twice was needed. In 12 patients with clinical CMV disease, thrombocytopenia improved significantly after GCV therapy (Fig. 3).

The prognosis of patients with DPLDs after diagnosis of CMV infection depended on CMV-pp65Ag levels

Multivariate analysis revealed that immunosuppressive drugs, CMV-pp65Ag and CRP levels were significantly associated with patient survival (Table 3).

DISCUSSION

CMV infection, including both clinical CMV disease and subclinical antigenaemia, occurred in 25.8% of patients with DPLDs in the present study and in about 40% of patients with collagen vascular diseases (CVDs) treated with immunosuppressants.[12,13] Thus, CMV infection is quite common in patients with DPLD and CVD who are being treated with immunosuppressants. The incidence of clinical CMV disease among patients with CMV-pp65Ag-positive PBLs was 33.3–70% in the present study and other reports on patients with CVD.[12–14] Not all subclinical antigenaemia deteriorates into clinical CMV disease; spontaneous resolution of antigenaemia may occur.[15]

Table 3 Multivariate analysis of clinical parameters at the onset of CMV infection to determine mortality after the onset of CMV infection

		Risk ratio	95% CI	*P* value
CRP, mg/L	≥10	2.703	1.387–5.504	0.0032
	<10	1	—	—
Immunosuppressants	No	2.660	1.278–5.51	0.0093
	Yes	1	—	—
CMV pp65Ag, per 5 x 10⁴ leukocytes	>7.5 (high)	2.225	1.161–4.293	0.0162
	≤7.5 (low)	1	—	—
Underlying DPLDs	IIPs	2.023	0.997–4.414	0.7444
	CVD-LDs	1	—	—
Lymphocytes, x 10⁹/L	≤0.9	1.874	0.981–3.694	0.3429
	>0.9	1	—	—
Gender	Male	1.525	0.763–3.148	0.8957
	Female	1	—	—
DM	Yes	1.168	0.587–2.429	0.664
	No	1	—	—

The risk ratio for survival after the onset of CMV infection was estimated by multivariate Cox proportional hazard analysis using seven factors for which the *P* values were <0.05, as determined by univariate Wilcoxon test on data for all patients (*n* = 69) (Table S5 in the online supporting information). Univariate analysis showed that age, smoking status, prednisolone dose at the onset of CMV infection, lactate dehydrogenase levels and rate of onset of DPLDs were not significant (Table S5).

CMV, cytomegalovirus; CI, confidence interval; CRP, C-reactive protein; DM, diabetes mellitus; CVD-LDs, collagen vascular disease-related lung diseases; DPLDs, diffuse parenchymal lung diseases; IIPs, idiopathic interstitial pneumonias.

We have encountered 23 cases of DPLD complicated by clinical CMV disease, most frequently accompanied by thrombocytopenia. Haematological abnormalities have been most frequently reported in patients with CVD receiving immunosuppressants who exhibited CMV antigenaemia.[12]

A decrease in lymphocyte numbers following immunosuppressive therapy was import.... .. ..ing the occurrence of subclinical CMV-pp65Ag antigenaemia and its deterioration into clinical CMV disease in the present study, consistent with other reports.[1,16]

Serum IgG is important in conferring protection from viruses, including CMV-related disease.[1] Serum IgG levels were lower in patients with CMV infection than in those without CMV-pp65Ag. However, serum concentrations of IgG were similar in patients with subclinical CMV antigenaemia and those with clinical CMV disease, which is in agreement with the findings of Takizawa *et al.*.[14] These results may reflect the fact that the effectiveness of immunoglobulin infusions for prevention of CMV disease is still unclear.[17-19]

In the present study, acute/subacute onset of DPLD and severe respiratory failure ($AaDO_2 \geq 60$ mm Hg) at the start of therapy were associated with a significantly increased likelihood of early onset CMV infection. This finding is in keeping with previous reports that serious CMV infection was often complicated by severe respiratory failure[20] and inflammatory disease.[21-24] There are two possible explanations for this finding; one is that severe inflammatory processes may disturb immunological reactions; the other is that tumour necrosis factor (TNF)-α, a central mediator of inflammation, which is often elevated in the serum of critically ill patients[25,26] and in acute fibrotic lung disease,[27] accompanied by high serum CRP levels, may induce replication of CMV by activating nuclear factor-κB-mediated early enhancer/promoter regions of the CMV genome.[25,26]

Based on our clinical findings, we suggest the following strategies for managing CMV infection during immunosuppressive therapy in patients with DPLDs. We recommend that DPLD accompanied by high CMV-pp65Ag levels (>7.5 cells per 5×10^4 PBLs) should be treated with GCV or other antiviral drugs. DPLD accompanied by low CMV-pp65Ag levels (≤ 7.5 cells per 5×10^4 PBLs) may not require antiviral treatment, provided CMV-pp65Ag levels are carefully monitored every 7–14 days; however, pre-emptive treatment with GCV before the onset of clinical CMV disease is preferable in patients with severe lymphocytopenia ($\leq 0.3 \times 10^9/L$), elevated CRP levels (≥ 10 mg/L), acute/subacute onset of underlying DPLD, severe respiratory failure and/or augmentation of immunosuppressive therapy directly after detection of CMV-pp65Ag. The usefulness and appropriate frequency of routine monitoring of CMV-pp65Ag should be investigated in another trial. At least monthly monitoring during the 4 to 6 months after the initiation of immunosuppressive therapy may be beneficial, considering the median interval from initiation of therapy to the onset of CMV infection.

This study had some limitations. First, CMV seropositivity of our cases before initiation of immunosuppressive therapy, which is a very important factor in determining the onset of active CMV infection,[28] is not included in our analysis. Second, this was a retrospective study, and laboratory investigations were not routinely performed for each patient. Third, the CMV-pp65Ag assay was not sufficiently well standardized, although it was reported to have high sensitivity, specificity and reproducibility.[2,11] Therefore, the cut-off value used in the present study should be verified in another trial.

In conclusion, we recommend that patients with DPLDs and CMV-pp65Ag antigenaemia of >7.5 cells per 5×10^4 PBLs should be treated with GCV. Patients with DPLDs accompanied by lower levels of CMV-pp65Ag should be carefully monitored or treated with GCV if onset of DPLD is acute/subacute or if there are other clinical indicators of a poor prognosis. Future prospective studies should focus on verifying the validity of this strategy.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Longitudinal observation of the clinical course of patients with high levels of cytomegalovirus (CMV) pp65 antigen (CMV-pp65Ag) (>7.5 cells per 5×10^4 peripheral blood leucocytes) at the initial CMV detection. Of all the CMV-pp65Ag-positive cases, 25 patients showed high levels of CMV-pp65Ag. The CMV-pp65Ag became negative in 12 out of the 22 patients treated with ganciclovir (GCV). However, three out of the 12 patients died within one month. Three out if the four patients, whose CMV-pp65Ag levels remained high in spite of GCV therapy, died within one month. Four patients died within one month after the start of GCV therapy before reevaluation of CMV-pp65Ag. Three patients died before the diagnosis of CMV infection and before GCV therapy could be initiated.

Table S1 Underlying DPLDs and CMV-pp65Ag evaluation

Table S2 Onset of underlying DPLDs

Table S3 Patient Details of CMV-pp65Ag detected and non-detected cases

Table S4 Clinical parameters at the start of immunosuppressive treatment and the interval leading to the onset of CMV infection (univariate analysis)*

Table S5 Clinical parameters at the onset of CMV infection and survival from the CMV infection (univariate analysis)*

ORIGINAL ARTICLE

Breathing irregularity during wakefulness associates with CPAP acceptance in sleep apnea

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Abstract

Purpose Individuals have different breathing patterns at rest, during wakefulness, and during sleep, and patients with sleep apnea are no different. The hypothesis for this study was that breathing irregularity during wakefulness associates with CPAP acceptance in obstructive sleep apnea (OSA).

Methods From a 2007–2010-database of patients with a diagnostic polysomnography (PSG) and prescribed CPAP (n=380), retrospectively, 66 patients who quit CPAP treatment at 6 months were identified. Among them, 27 OSA patients quit despite having no side effects for discontinuing CPAP (Group A) and were compared to a matched group (age, body mass index, and apnea–hypopnea index) with good 6-month CPAP adherence (Group B; n=21). Five minutes of respiratory signal during wakefulness at the initial PSG were extracted from respiratory inductance plethysmography recordings, and measured in a blinded fashion. The coefficients of variation (CV) for the breath-to-breath inspiration time (T_i), expiration time (T_e), T_i+T_e (T_{tot}), and relative tidal volume, as well as an independent information theory-based metric of signal pattern variability (mutual information) were compared between groups.

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Results The CV for tidal volume was significantly greater (p=0.001), and mutual information was significantly lower (p=0.041) in Group A as compared to Group B.

Conclusions Differences in two independent measures of breathing irregularity correlated with CPAP rejection in OSA patients without nasal symptoms or comorbidity. Prospective studies of adherence should examine traits of breathing stability.

Keywords Obstructive sleep apnea · CPAP adherence · Respiratory control · Nonlinear analysis

Introduction

Individuals breathe in different ways under strictly defined conditions during quiet wakefulness. There are also significant effects of different cognitive events such as thought, attention, and emotion on the basic breathing pattern, presumably through forebrain influences. Although forebrain activity is depressed during slow-wave sleep (non-REM), breathing individuality persists [1]. Moreover, identical twins breathe with a similar pattern [2, 3]. Thus, genetic background affects breathing pattern, and accounts for variation across individuals.

Breathing variability is measured not only through physiologic extractions of respiratory frequency and tidal volume but also using analyses of the breathing signal unrelated to the physiologic extractions. The latter includes methods such as sample entropy and mutual information that deconstruct the signal, and disclose features and patterns that reveal structural underpinnings and complexity. Such complexity occurs in expression of and differs among apnea types [4]. Previously, we reported a difference in an information theory-based metric of signal pattern variability (sample entropy) among patients with obstructive sleep apnea syndrome (OSAS) comparing those with many mixed apneas (>30 % of events being mixed apneas) to those with predominant obstructive apnea. Secondary observations in this data set suggested that breathing variability during wakefulness could be a predictor of acceptance of CPAP as a therapy [5]. While studies indicate that age, sex, severity of disease, symptoms of sleepiness, socioeconomic status, nasal symptoms, and psychological factors relate to CPAP adherence in OSAS [6–15], none have considered an individual's inherent features of respiratory control as quantified in terms of measures of variability.

Using a case–control design, we tested the hypothesis that the regularity of resting breathing during wakefulness might be a predictive feature of subsequent CPAP acceptance. Patients with nasal symptoms and comorbidity known to affect adherence were excluded, and our analysis focused on the breathing pattern during wakefulness prior to the diagnostic polysomnography (PSG). Ventilatory pattern variability was quantified using conventional (linear) statistical analysis (coefficient of variation (CV)) of breath-to-

breath tidal volume and frequency from noninvasive measures, as well as an information-based analysis of the respiratory signal using mutual information, an approach that uses the raw respiratory waveform data that does not depend on either breath depth or frequency identification.

Methods

Subjects

There was an initial exclusion of patients with an apnea–hypopnea index (AHI) <20 who had medical history of arrhythmia, cerebral infarction, and psychosomatic/psychogenic diseases or who used opioid, hypnotic medications, or antidepressant; all of which might have an influence on breathing irregularity. Figure 1 shows the ascertainment profile of the study that resulted in the final comparison
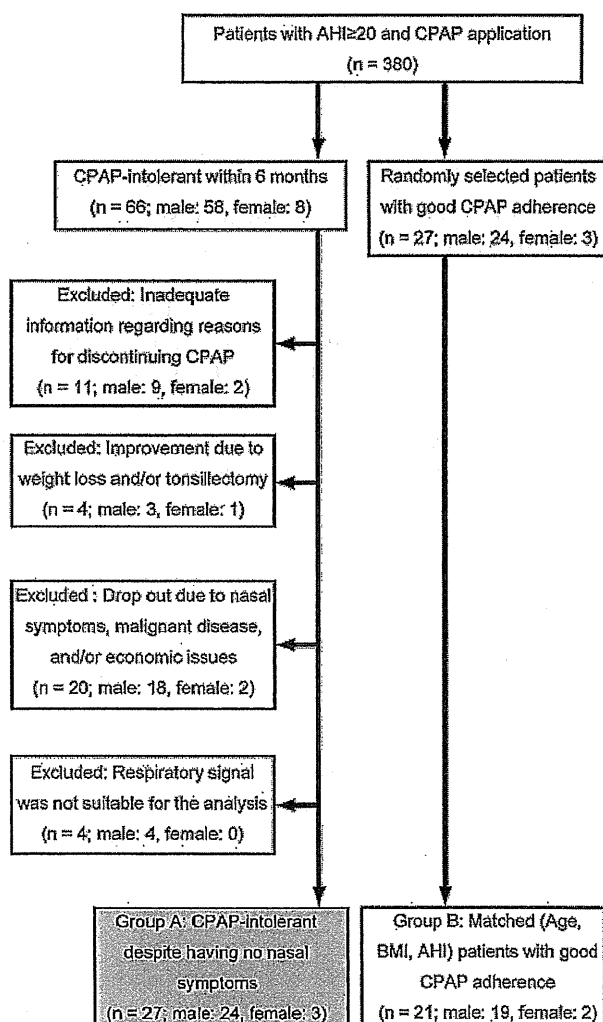

Fig. 1 Diagram for the comparison groups enrollment

groups. Among 380 patients with obstructive sleep apnea (OSA) who had a diagnostic PSG and then who were prescribed CPAP from 2007 to 2010, 66 patients quit CPAP treatment at 6 months. Among these 66 patients, 11 patients were excluded from further analysis due to loss of the information regarding reasons for CPAP dropout, 4 patients quit CPAP because sleep disordered breathing was improved by weight loss and/or tonsillectomy, 20 patients were CPAP-intolerant because of nasal symptoms, malignant diseases, and/or economic issues, and 4 patients were excluded as an adequate respiratory signal could not be obtained during wakefulness. Eventually, 27 patients were enrolled to this study analysis (Group A). We also randomly extracted 27 patients with good CPAP adherence from the same database. Fairly good adherence to CPAP was defined at a high threshold, i.e., more than 90 % of days with more than 5 h usage each night. A final group of 21 patients that were matched for age, body mass index (BMI), and AHI within the mean and standard deviation values for Group A were designated as Group B. Data were collected on the Epworth sleepiness scale (ESS) and current medications. Written informed consent was obtained from all patients, and the Human Subjects Ethics Committee of Nara Medical University approved the study.

Diagnostic PSG

Data acquisition started from 9 p.m. and continued until 6 a.m. on the following morning. Subjects were not informed that the respiratory signal before sleep onset was extracted and used for analysis. When sensors were applied and data acquisition could be initiated, subjects were instructed to close their eyes and the recording started.

The PSG was performed using a polygraph system (EEG7414; Nihon Kohden, Tokyo, Japan). electroencephalogram (EEG; C3-A2, C4-A1), bilateral EOG, submental electromyogram (EMG), ECG, and bilateral anterior tibial EMG were recorded. Airflow was monitored using an oronasal thermal sensor and/or nasal air pressure transducer. Thoracic and abdominal respiratory movements were monitored using respiratory inductance plethysmography (RIP) (Respitrace; Ambulatory Monitoring Inc., Ardsley, NY, USA). Oxyhemoglobin saturation and pulse rate were monitored using pulse oximetry with a finger probe (OLV-3100; Nihon Kohden, Tokyo, Japan). All the signals were digitized and stored on a personal computer. Apneas were defined as an episode of complete airflow cessation measured from the thermal sensor lasting more than 10 s. Hypopnea was defined by $\geq$30 % reduction in amplitude of the RIP-sum signal lasting more than 10 s with$\geq$3 % oxygen desaturation. AHI was calculated as the average number of apnea–hypopnea events per hour over the total sleep period.

CPAP

All patients were initiated on nasal CPAP (REMstar Auto; Respironics; Pittsburgh, PA, USA or GoodKnight 420E; Tyco Mallinckrodt Plaisir, France) with auto titrating mode. All patients under CPAP treatment visited our sleep laboratory every month, which is mandatory in the Japanese healthcare insurance system, and CPAP adherence was monitored every month using data extracted from the memory of the CPAP equipment. If necessary, CPAP settings including pressure range or CPAP mode (auto or fixed mode) were modified by an expert physician at the monthly visit to our laboratory. Eventually, most of the patients used CPAP with auto titrating mode during the follow up period.

Analysis of the respiratory signal

An investigator blinded to the groups chose approximately 5 min of artifact-free respiratory signal data before sleep onset, without a change in body position and scored as wakefulness, from the diagnostic polysomnography. Respiratory signals were generated by the sum of chest and abdominal signals using RIP. The sum was not calibrated to volume but adjusted to have a similar tidal displacement among subjects. The respiratory signal was identified, and the EMG (chin and limb) signal was used to detect body movements. When the amplitude of the EMG signal was high, that part of the respiratory signal was considered to be during movement and inappropriate for analysis. The part of respiratory signal in which eye movements without alpha rhythm in epochs not scored as sleep was also excluded from analysis. In the analytic phase of the study, investigators were also blinded to the group assignment, and each 5-min record of the respiratory signal during EEG-staged periods of wakefulness was analyzed for breath-to-breath inspiration time (T_i), expiration time (T_e), T_i+T_e (T_{tot}), and relative tidal volume. To avoid the fluctuation of breathing due to drowsiness, we extracted 5 min of respiratory signal if EEG were considered to be fully awake for each epoch (30 s) over a 5-min period. To assess breathing irregularity, the CV ([standard deviation / mean]$\times$100) for each parameter was calculated.

To further quantify breathing pattern variability, the mutual information of the raw respiratory signal (RIP-sum signals) sampled at 10 Hz was quantified. Mutual information is a measure of statistical dependence in a data set [16, 17]. This information theory-based metric reflects the decrease in uncertainty associated with a time-shifted data point $x(t+\tau)$ that results from knowledge of the coordinate $x(t)$. Mutual information (measured in bits) was computed as described previously [18, 19], and additional details are provided in an appendix. Due to the periodic nature of the respiratory pattern, mutual information was calculated over

multiple time delays (τ's) from unity to one cycle length. Values were averaged across time lags excluding those with high linear correlations as defined by the first minimum of the mutual information function. Average mutual information (excluding small lags) was reported for each group. In practice, higher values of mutual information suggest increased statistical dependence (decreased variability and greater predictability) in the signal, while lower mutual information is associated with more variable (less predictable) patterns [20].

Statistical analysis

Comparison of continuous variables between the groups was done by the unpaired t test, and categorical variables were compared by the chi-squared test. Differences with $p<0.05$ were considered significant. All results were expressed as means±standard deviation (SD). Statistical analysis was done with IBM SPSS Statistics 19 for Windows software (SPSS Inc., Chicago, IL).

Results

Subject characteristics

Table 1 shows subject characteristics for each group. There were no significant differences in ESS and the use of medications for hypertension, hyperlipidemia, and diabetes mellitus between groups. In Group A, the main reasons for poor CPAP acceptance were an uncomfortable feeling with CPAP or a sensation of it being hard to breathe and fall asleep. Some reported removing CPAP without awareness during sleep. Also, those who refused or could not tolerate CPAP treatment generally felt no significant improvement in presenting symptoms such as excessive daytime sleepiness,

Table 1 Subject characteristics

	Group A (n=27)	Group B (n=21)	p value
Age, year	51.6±10.1	51.3±10.0	N.S.
Sex, (male/female)	24/3	19/2	N.S.
AHI, /h	46.4±18.3	53.6±23.7	N.S.
ESS	10.4±5.7	11.4±6.0	N.S.
BMI, kg/m^2	25.2±3.2	26.8±2.4	N.S.
Hypertension	6/27 (22.2 %)	8/21 (38.1 %)	N.S.
Dyslipidemia	4/27 (14.8 %)	4/21 (19.0 %)	N.S.
Diabetes mellitus	3/27 (11.1 %)	0/21 (0.0 %)	N.S.

Data are shown as mean±SD or no. (%). Group A are the patients who dropped out of CPAP therapy; Group B are the patients with good CPAP adherence

AHI apnea–hypopnea index, *ESS* Epworth sleepiness scale, *BMI* body mass index, *N.S.* not significant

morning headache, and sleep quality. Regarding the 39 patients with poor CPAP acceptance excluded from the analysis, the AHI, age, and ESS were similar to 27 patients that were analyzed in Group A (data not shown).

Breathing irregularity during rest before sleep onset

Figure 2 shows examples of RIP-sum signals during wakefulness for two subjects with poor CPAP acceptance (Group A) and two subjects with good CPAP adherence (Group B). These tracings highlight the more irregular breathing pattern, especially in amplitude rather than respiratory frequency, prior to sleep onset in Group A as compared to Group B. Although the CV values for T_i were significantly higher in Group A (22.6±10.2 vs. 15.9±7.8 %; $p<0.05$), the CV values for T_e and T_{tot} were similar between groups (T_e, 24.0±11.3 vs. 19.5± 8.5 %; T_{tot}, 18.7±8.9 vs. 15.2±6.7 %, $p>0.05$). The CV values for tidal volume in Group A were significantly greater than in Group B (30.7±7.8 vs. 22.1±9.0 %; $p<0.01$) (Fig. 3). The independent analyses of the respiratory waveform also identified differences in breathing pattern. The mutual information was significantly lower in Group A as compared to Group B (0.94±0.16 vs. 1.16±0.52 bits, respectively, $p<0.05$) (Fig. 4). Mutual information is a measure of statistical dependence between points, so the lower value in Group A suggests a greater variability of the breathing pattern during wakefulness in subjects with poor CPAP acceptance. There were no correlations between severity of OSA (AHI) and parameters for breathing irregularity including CVs and the mutual information (data not shown).

Discussion

The present study supports the hypothesis of an association of breathing irregularity during wakefulness prior to the diagnostic sleep study, as quantified by two independent measures, to CPAP adherence. We observed that breathing irregularity is greater in the patients with OSA who could not tolerate CPAP therapy than in age-, AHI-, and BMI-matched patients with good CPAP adherence. While highly selected for the absence of nasal symptoms and confounding medical conditions, these findings suggest that during wakefulness, a pattern of individual resting breathing irregularity could be a predictive marker for CPAP acceptance.

Breathing irregularity during wakefulness is associated with genetic diseases such as RETT syndrome [21, 22], with certain environments such as high altitude [23, 24], with treatment with opioid medications [25, 26], and with medical conditions including heart failure [27–29] and cerebral infarction [30, 31]. These phenomena reflect particular features of the respiratory control system that involve respiratory rhythm generation and/or central and peripheral

Group A

Patient 1

Patient 2

Group B

Patient 3

Patient 4

30 sec
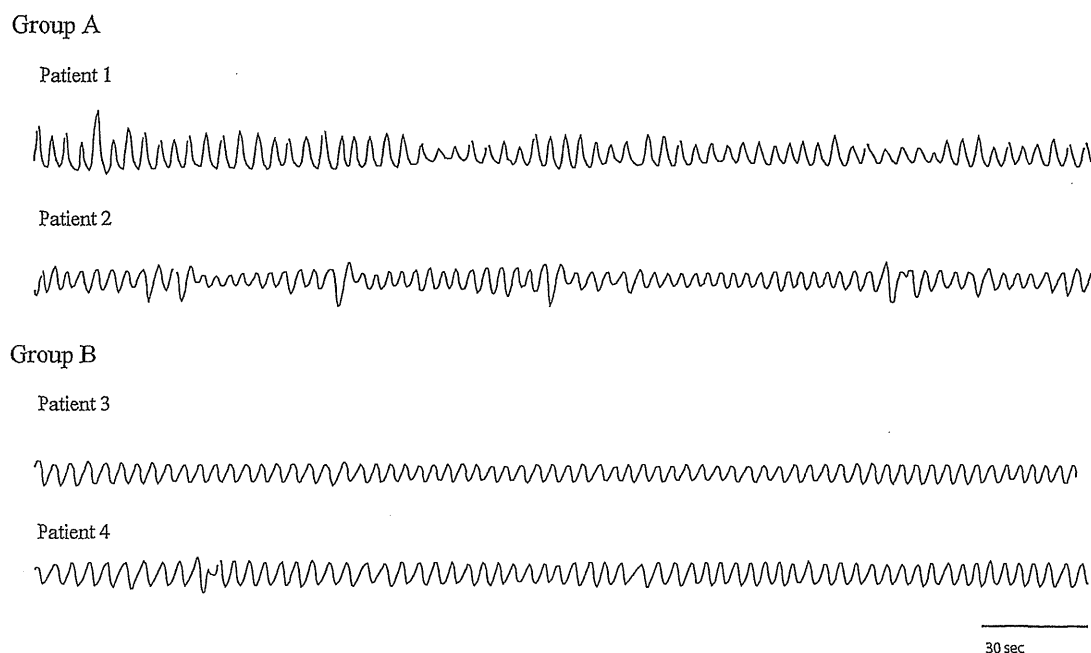
Fig. 2 Examples of RIP-sum tracings before sleep onset. Tracings of each two patients from Group A and B were presented, showing the breath-to-breath greater irregularity in tidal volume rather than respiratory frequency in Group A. *Group A* is composed of the patients who dropped out of CPAP therapy. *Group B* is composed of the patients with good CPAP adherence

chemoreception. Breathing "stability" or "instability" is operationally defined from the output of the respiratory controller. We previously reported that breathing behavior during wakefulness even in room air and in the phase of post acute hypoxic exposure as well were different between mouse strains [32–35], thus we think that the patterning of breaths over time (periodic, chaotic, etc.) around eupnea is an important feature to begin to define not only operationally but mechanistically. In a previous study, findings suggested that the central respiratory control system in mixed apnea dominant OSAS is different from obstructive apnea dominant OSAS and closer to patterning in central apnea syndrome [5]. These results taken together with the present study indicate that patients with OSA who cannot tolerate CPAP and showed irregular breathing may have somewhat of a different respiratory control system from patients with good adherence to CPAP.

An interaction of respiratory output with the upper airway and diaphragm may determine the expression of apnea types, such as central and obstructive [4]. The relative proportion of these components would depend on individual factors, which may be genetic or secondary to a medical condition. We speculate that patients with poor CPAP acceptance may have a relatively high central gain rather than peripheral chemoreceptor component as compared to patients with good adherence to CPAP, and this difference may be reflected by the breath-to-breath variability in tidal volume. Previously, we had compared mixed apnea

predominant to obstructive apnea dominant, and in that comparison, the differences were in the variability in respiratory frequency and, to a lesser extent, tidal volume [5]. In the present study, we focused on obstructive apnea dominant patients; and, while variability was different in tidal volume, breath-by-breath variability in respiratory frequency was similar between groups. In both studies, however, analysis of the raw signal provided insight in the direction of difference, being lower in groups will less adherence.

Although the augmented breath, sigh, is considered an important component of normal breathing [36], Baldwin et al. concluded that sighs indicate maturity and functional integrity of the neurorespiratory feedback control and proposed sighs as being important for the regulation and resetting of the neurorespiratory controller [37]. Moreover, in general, sigh could occur both during stress and negative emotions, such as panic and pain, and during positive emotions, such as relaxation and relief [38–40]. In the current study, we did not exclude sighs from the 5-min segment of respiratory data. The number of sighs might affect the CV values for tidal volume. Thus, sigh was not discarded in the analysis, because it could contribute to greater tidal volume variability and poor CPAP acceptance. This is supported by previous reports that psychological factors may relate to adherence to CPAP [15]. Therefore, differences in the respiratory control system such as a high central component or an intrinsic psychological status can explain our results; however, exploring the exact mechanism for this variability
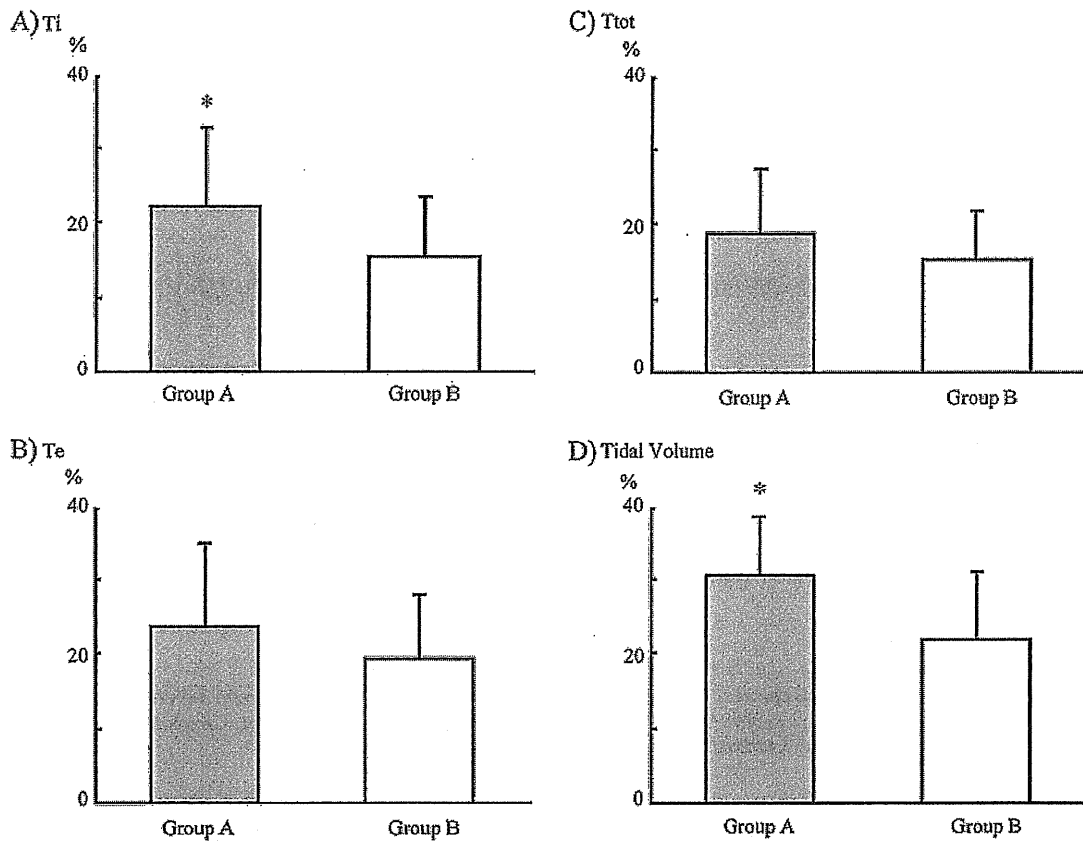
Fig. 3 Coefficients of variation for breath-to-breath respiratory variables during resting breathing before sleep onset. Values are mean±SD. a *Ti*, inspiration time; b *Te*, expiration time; c T_{tot}, T_i+T_e, d Tidal volume. *Group A* is composed of the patients who dropped out of CPAP therapy. *Group B* is composed of the patients with good CPAP adherence. The *asterisk* indicates significant difference between groups

is beyond the scope of this study. In addition, it has been recently demonstrated that arousability is one of the physiological traits that contribute to the pathogenesis of OSA [41]. Arousal due to positive airway pressure and/or CPAP discomfort may worsen CPAP adherence, thus the difference in individual arousal threshold may have contributed to our results, but further study would be needed to elucidate this issue.

A strength of the study was an ability to select a sufficient number of patients to match highly successful CPAP users to an extremely intolerant group and in both excluding known factors (stroke, opioid use, etc.) that might confound the comparisons. There are limitations in the present work. First, we cannot exclude a possible effect of hypocapnia in the poor CPAP acceptance group; however, if this were the case, then the differences between the groups would be based on a respiratory control factor such as hypercapnic responsiveness and/or apneic threshold of carbon dioxide. Second, although the statistics reveal that the CV values for tidal volume in the patients with OSAS who can not tolerate CPAP were significantly higher than in patients with good adherence, the average difference in CV values between the groups was less than 10; however, the fact that the independent measure of mutual
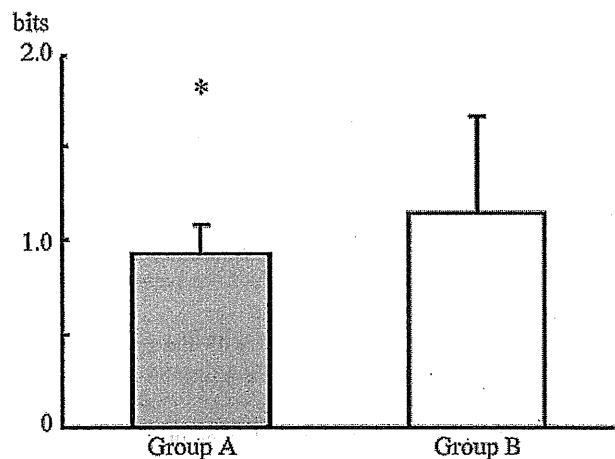
Fig. 4 The mutual information of the raw respiratory signal (RIP-sum signals). The mutual information was significantly lower in Group A as compared to Group B, suggesting a greater variability of the breathing pattern in Group A. *Group A* is composed of the patients who dropped out of CPAP therapy. *Group B* is composed of the patients with good CPAP adherence. The *asterisk* indicates significant difference between groups

information also showed such differences suggests that even small absolute differences could be important to consider. Third, although we successfully demonstrated a significant association between breathing irregularity during wakefulness and CPAP acceptance, this was a retrospective study. Thus, a prospective study will be needed to confirm that breathing irregularity predicts CPAP adherence. Lastly, the prescription of different commercial-based CPAP devices might be a confounding factor for CPAP adherence, but dropout rate was not different between these CPAP devises users.

In summary, we conclude that irregular breathing in terms of respiratory amplitude and temporal variability of the breathing signal during wakefulness may affect CPAP acceptance. This suggests that there are distinct features of respiratory control in patients who accept CPAP or cannot tolerate CPAP.

Appendix

Mutual Information: Mutual information (MI) is a measure of the statistical dependence between two time series, or two collections of points from a data set, that can arise from both linear and nonlinear sources [42]. The Mutual information between a given time series $x(t)$ and its time-shifted version $x(t+\tau)$ is computed from the joint probability distribution of $x(t)$ and $x(t+\tau)$, where τ represents a time lag. The joint probability distribution is defined as $P[x(t), x(t+\tau)]$, where $P[x(t)]$ and $P[x(t+\tau)]$ are the marginal distributions of the original and time-shifted time series, respectively. The MI can be computed as follows:

$$MI[x(t), x(t+\tau)]$$

$$= \sum_i \sum_j P[x_i(t), x_j(t+\tau)] \log \left[\frac{P[x_i(t), x_j(t+\tau)]}{P[x_i(t)] \cdot P[x_j(t+\tau)]} \right]$$

Because the breathing pattern over long time periods is strongly periodic, we computed MI for τ values from one sample (adjacent points separated by 100 ms) to one cycle length. MI tends to decrease quickly as τ is increased from a lag of one and then becomes more uniform at higher time lags, and the average MI of a given epoch was quantified excluding small lags as defined by the first minimum of the MI function.

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Distribution of Collagen Fiber Orientation in the Human Lung

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ABSTRACT

Collagen fiber, a major component of the extracellular matrix in the human lung, is crucial in maintaining the lung structure mechanically. It is necessary to study the collagen fiber orientation which the mechanical function is closely related to. In the present study the collagen fiber orientation in the lung was quantitatively measured by Osaki's microwave method. We succeeded in preparing sheet samples cut in a coronal direction from the lung for the measurements. It was found that the collagen fibers were, on average, orientated parallel to the longitudinal axis of the spine. The void spaces in the lung sample observed using an optical microscope was not circular but ellipsoidal. The direction of the long axes of ellipsoidal voids coincided with that of the collagen fiber orientation. The results suggested that collagen fiber orientation is closely related to the respiratory movement of the human lung. Anat Rec, 296:846–850, 2013. © 2013 Wiley Periodicals, Inc.

Key words: collagen fiber; orientation; human lung tissue; microwave method

Collagen fiber is an important component of the extracellular matrices in the human body (Matsuda et al., 1987; Malkusch et al., 1995; Mercer and Crapo, 1990). The collagen fiber bundles in the matrix play an important role for the mechanical functions of connective tissues such as skin (Osaki, 2001) and bones (Osaki et al., 2002). The lung is also considered to be a type of connective tissue with mechanical functions because the periodic mechanical stress ascribed to respiration is applied to the lung consisting of collagen fibers, elastin, and proteoglycan. Toshima et al. (2005) reported that collagen fibers in aggregated state were observed at the alveolar orifices of the human lung using scanning electron microscope, while sac like collagen networks was observed at the alveolar septa, forming basket like networks. Collagen fibers in the collapsed lung of rat showed a wavelike configuration at the alveolar orifices and septa, while they became straight in the inflated lung (Toshima et al., 2004). Kononov et al. (2001) observed changes in morphology related to collagen fibers in the alveolar walls of rat by mechanical stretching using optical microscope and evaluated the role of collagen fiber on the mechanical property of alveolar walls. The two reports were restricted on qualitative observation in the small regions of the lung. Thus there have been few reports evaluating the collagen fiber orientation in the lung quantitatively.

It is important to evaluate the orientation of collagen fiber as related to the mechanical properties of the lung because the lung needs to maintain its structure for mechanical force accompanied with respiratory movement. However there are few reports on evaluating the
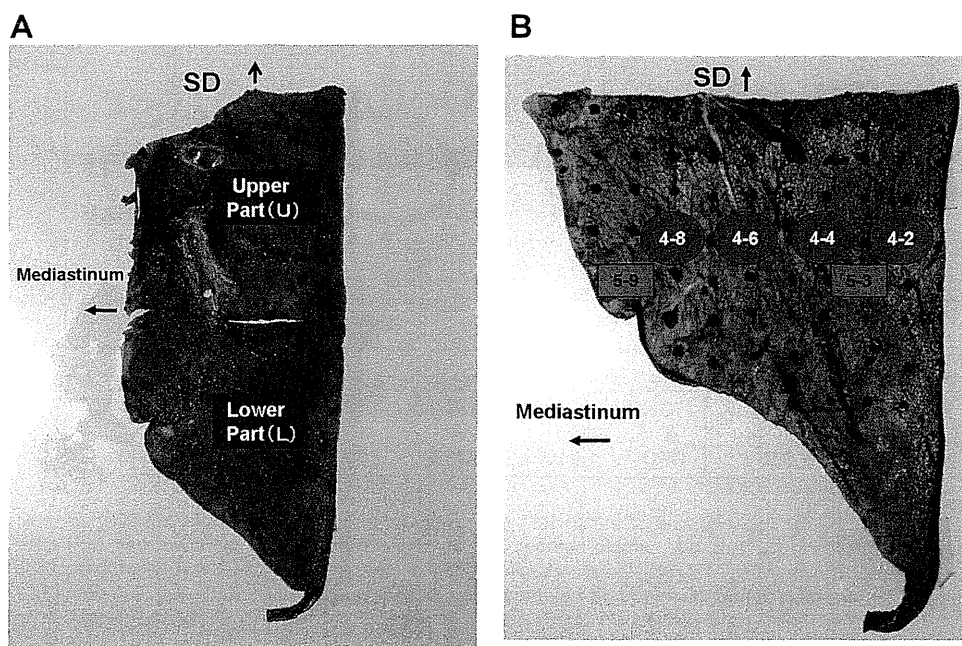
Fig. 1. **A**. The middle plate among several lobe plates 10-mm thick cut in the direction of the coronal plate from the left lower lobe of the human lung. The plate consist of upper (U) and lower (L) parts. The SD corresponds to the direction of the longitudinal axis of the spine. **B**. Human lung sheet (L part) for determining collagen fiber orientation. Collagen fiber orientation was measured at four positions (sample number 4–2, sample number 4–4, sample number 4–6, sample number 4–8). The collagen fiber orientation and optical observation were carried out at two positions (sample number 5–3, sample number 5–9).

relationship between the mechanical properties of the whole lung and the orientation of collagen fibers because staining method using specific antibody for some types of collagen is generally used not for determining the orientation of collagen fibers, but for detecting the existence of a part of collagen fibers. The numerical analysis for the orientation of collagen fibers will be required for large sections of tissues for studying the role of collagen fibers regarding the mechanical properties. Previously, one of authors (S.O.) established microwave method which is able to determine the orientation of collagen fibers in the sheet from the angular dependence of the transmitted microwave intensity (Osaki, 1987a,b, 1989, 1990a, 1997). The method was applied to human tissues such as skin (Osaki, 1990b, 1999; Osaki and Ohashi, 2004) and bones (Osaki et al., 2002; Ohuchi et al., 2003). However, this method has not been applied to the human lung, because it has been very difficult to cut human lung into several plates, which is a spheroid organ containing inspired air. And it has been also very difficult to prepare the sliced lung plate into sheet samples of about 1 mm thickness without curling ascribed to drying. Nevertheless, we finally succeeded in preparing lung sheet samples appropriate for microwave measurements.

The present study describes the preparation of sheet samples from a lower lobe of the human lung, the determination of orientation of collagen fibers, and provides the distribution of collagen fiber orientation, suggesting that the fiber orientation is closely related to the respiratory movement.

MATERIALS AND METHODS

Sample Preparation

Human lung removed on autopsy of a 60-year-old man who had died of hepatocellular carcinoma was used in the present study after his family provided written informed consent. He had no histological abnormality in the lung. Several lungs were also used in preliminary experiments. The Institutional Review Board at Nara Medical University approved our study. Samples for measurements were prepared from the lung tissue as follows: After upper and lower lobes of the left lung have been fixed in 10% formalin solution at a room temperature for a week, it was sliced in the direction of coronal plane into several lobe plates 10-mm thick by a trimming knife (FEATHER, Tokyo, Japan) used for autopsy. Middle plate from the lower lobe, which was the largest in size among the plates, was used. The plate was cut into two parts, upper (U) and lower (L) part (Fig. 1A) because it was too large to cut the whole plate into thin slices with a trimming knife (MicroGlass, WA). The lower part was used for determining collagen fiber orientation because it contained no large artery and few bronchi compared with the upper part.

After being embedded in paraffin at 40°C for fixation, the lower part was further cut into 2-mm thin slices with a trimming knife. The slices were then dipped into a 70% ethanol solution to remove the paraffin. To use sheets without curling is inevitable for the microwave measurements. Each slice was sandwiched between two pieces of wood cut such as to avoid curling. After drying
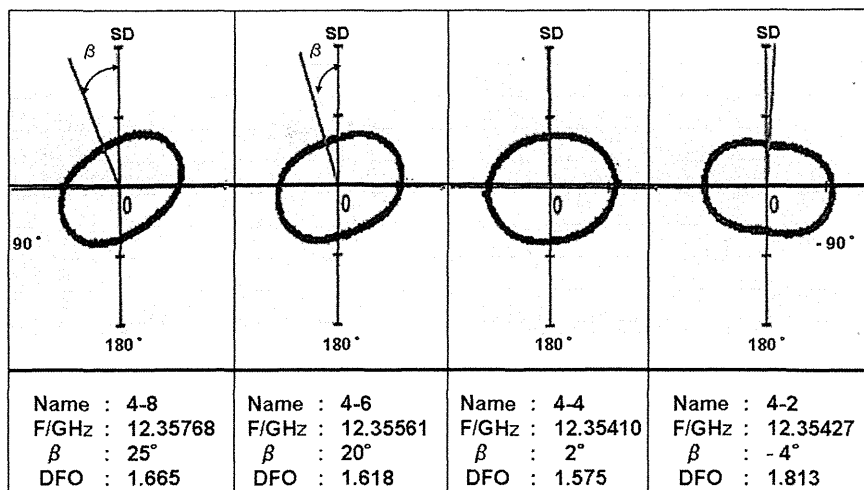
Fig. 2. The angular dependences of transmitted microwave intensity at four different positions (sample number 4-2, sample number 4-4, sample number 4-6, sample number 4-8) at the lower part of the left lower lobe as shown in Fig. 1B. SD, the standard direction corresponding to the longitudinal axis of the spine. β, the direction of fiber orientation. DFO, the degree of fiber orientation.

at a room temperature for 24 h the resulting sheet lung about 1 mm thickness was used as a sample for determining the fiber orientation using the microwave method.

Measurements of Collagen Fiber Orientation

Collagen fiber orientation was determined using the Osaki microwave method (Osaki, 1990b). The lung sheet sample was inserted into the narrow gap between a pair of waveguides constituting the cavity resonator system and was rotated around the central axis normal to the sample plane. Microwaves were irradiated to the samples and the transmitted microwave intensity of the sheet samples was measured at different rotation angles (Osaki, 1987a,b, 1989). Effective sample size to which microwaves were irradiated was 25 mm × 25 mm. The angular dependence of transmitted microwave intensity, called the orientation pattern, was measured at 12 GHz. The orientation pattern gives the orientation angle (β) and the degree of fiber orientation (DFO) (Osaki, 1987a,b, 1989). The direction at which the transmitted microwave intensity is minimal is designated as the orientation angle corresponding to the angle between the main axis of the collagen-fiber chains and the standard direction (SD). The SD corresponds to the direction of the longitudinal axis of the spine. The maximal-to- minimal ratio of transmitted microwave intensity is defined as the DFO reflecting mechanical anisotropy. The collagen fiber orientation can be explained by two factors of DFO and β.

Observation of Morphology of the Human Lung

The lung contains air spaces for gas exchange. The lung which receives the inspired air changes the void spaces during respiration, expanding on inspiration and shrinking on expiration. The morphology of the lung sample was observed by optical microscope. The direction of long axis of air spaces was determined. From the

shapes of air spaces in the sample we then compared relationship between the anisotropic shapes of air spaces and the collagen fiber orientation.

RESULTS

Collagen Fiber Orientation in Human Lung

Figure 2 shows the angular dependence of transmitted microwave intensity at four different positions (sample number 4-8, sample number 4-6, sample number 4-4, sample number 4-2) for the human lung sheet sample prepared by slicing the lower part of left lower lobe (see Fig. 1B). The angular dependences at about 12 GHz were ellipsoidal. The degree of fiber orientation (DFO) was determined to be 1.665 for sample number 4-8, 1.618 for sample number 4-6, 1.575 for sample number 4-4 and 1.813 for sample number 4-2. Here, the large value of DFO showed marked anisotropy. The orientation angle β was determined to be 25°for sample number 4-8, 20° for sample number 4-6, 2° for sample number 4-4, and −4° for sample number 4-2. The value of DFO changed slightly with changing position while the value of β changed markedly with changing position. The results show that collagen fibers are oriented anisotropically, depending on the position of lung.

Distribution of Collagen Fiber Orientation

Figure 3 shows the distribution of collagen fiber orientation at 61 different positions of the human lung sample prepared from the lower part of the coronal plates of the left lower lobe. Collagen fiber orientation is represented as a bar. Inclination of a bar gives β the deviation of fibers from SD, while the length of a bar gives DFO the degree of fiber orientation.

DFO changed from 1.120 to 2.657 while β changed from −18° to 73°. Both DFO and β change with changing position. Here the lung sample is divided into three different lesions: inner, middle, and outer lesions. The
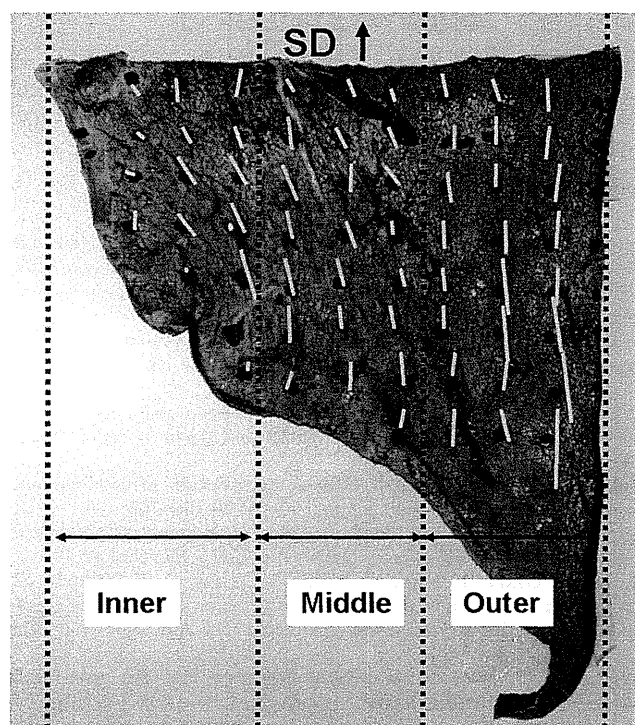
Fig. 3. Microwave measurements were carried out at 61 points in the lower part prepared from the left lower lobe, using Osaki's microwave method. The orientation of collagen fibers is represented by a bar. The inclination of a bar gives the angle of deviated from SD, while the length of a bar reflects the degree of orientation of collagen fibers.
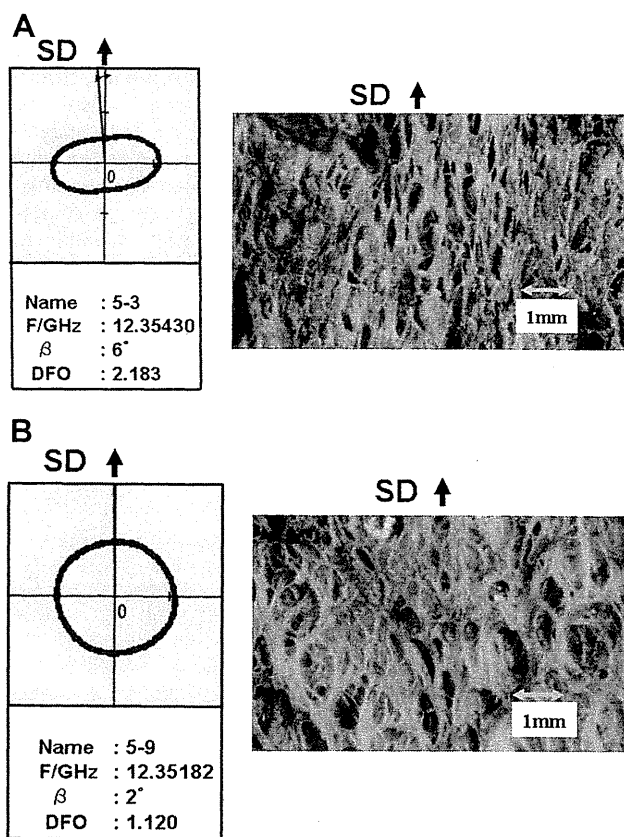

Fig. 4. The collagen fiber orientation measured using Osaki's microwave method and the fine structure using optical microscopy at two different positions (sample number 5-3 and sample number 5-9) of the lower part prepared from the left lower lobe. A, sample number 5-3, B, sample number 5-9.

DFO is relatively high in the outer part, while it is low in the middle and inner parts. β for the inner part was about −30° to SD while β for the middle and outer parts was small. That is, the collagen fibers in the middle and outer parts were almost aligned in SD. The collagen fiber orientation in the lung sheet sample changed with changing position, as shown at Fig. 3.

Fine Structure of Air Spaces in Human Lung

Fine structures at two different positions (sample numbers 5-3 and 5-9) of the lung sample were observed using an optical microscope. Shapes of airspaces were on average ellipsoidal for the sample (sample number 5-3) with large DFO (see Fig. 4A). The direction of the longitudinal axis of the ellipsoidal air spaces was parallel to that of the spine. Similarly, the bundles in the circumference of air spaces were also parallel to that of the spine (see Fig. 4A). On the other hand, air spaces were almost circular for the sample (sample number 5-9) with low DFO, while the bundles were not oriented in a fixed direction (see Fig. 4B).

The results suggest that alignment of bundles is related to the collagen fiber orientation in the human lung.

DISCUSSION

Collagen fiber, a major component of the extracellular matrix of the human lung, is crucial in keeping the mechanical function of the lung accompanying with inspiration. However, it has not been elucidated how collagen fiber should contribute to maintain the lung structure against mechanical load based on the respiratory movements. It is very important to determine the orientation of collagen fibers quantitatively for studying the mechanical function of the lung. In the present study, we succeeded in preparing sheet samples for measurements of human lung and in determining the collagen fiber orientation using Osaki's microwave method (Osaki, 1987a). It was very difficult to prepare samples as described in the Introduction because of factors such as cutting, drying, and so forth. However we measured the orientation of collagen fibers for several sheet samples from other subjects. We showed the orientational distribution for one sample since the orientational distribution of collagen fiber was roughly similar.

The results indicate that collagen fibers in the human lung are mainly orientated in the direction parallel to the spine. The angle of collagen fiber orientation (β) changed between −18° and 73°. The variation in β was very large, while the degree of orientation was relatively small. It is important to determine the distribution of collagen fiber orientation at about 60 points to make clear the orientational distribution of collagen fiber in the lung. The result demonstrated that collagen fibers were, on average, aligned in parallel to the longitudinal axis of the spine and that the degree of orientation is relatively high in the outer part of lung.

Previously one of the authors (S.O.) applied the microwave method to human bone, calf skin and cobra skin (Osaki, 1999; Osaki et al., 2002, Niitsuma et al., 2005). He demonstrated that collagen fibers were anisotropically orientated and that the anisotropy was closely related to the mechanical anisotropy. In a similar way, the present result indicates that the anisotropic orientation of collagen fiber in the human lung may be related to the mechanical anisotropy. Human lung changes its shape during respiration. On inspiration the lung dose not expand uniformly. As is well known, the shapes change remarkably in the direction longitudinal to the spine compared with the other directions while those change remarkably in the outer area compared with inner area. The anisotropy in respiratory movement may be related with those of collagen fiber orientation.

In the present study, we also observed the void structure containing air spaces in the lung sample and investigated the relationship between the void structure and the collagen fiber orientation. The void structure contains inspired air and changes during respiration. The inspiration makes the structure expand while the expiration makes it contract. Because the mechanical stress based on respiration contributes to change in the void structure in the lung, the structures should reflect the mechanical properties of the lung. Many airspace shapes were not circular but ellipsoidal in highly oriented regions. Moreover, the long axes of the air spaces were proved to be mainly parallel to that of the spine. One of the authors (S.O.) has reported that hair pores in the skin were ellipsoidal and that the longitudinal axes of the hair pores postulated the direction of collagen fibers orientation of the skin (Osaki, 2001). The direction of the longitudinal axes of the air spaces agreed with that of collagen fiber orientation of the lung sample determined by the microwave method. This result suggests that collagen fiber orientation in the human lung is markedly related to the mechanical properties.

In conclusion, we succeeded in preparing the human lung sample for Osaki's microwave method and in determining the collagen fibers orientation in human lung tissue by Osaki's microwave method, and found that the collagen fibers in the sample from the coronal plate are generally oriented parallel to the spine. In the near future, we will present the mechanical properties of the human lung for asserting the present using microwave method.

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Cross-sectional and prospective study of the association between lung function and prediabetes

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ABSTRACT

Objectives: A growing body of evidence suggests that there is a relationship between impaired lung function and the risk of developing diabetes mellitus (DM). However, it is not known if this reflects a causal effect of lung function on glucose metabolism. To clarify the relationship between lung function and the development of DM, we examined the incidence of newly diagnosed prediabetes (a precursor of DM) among subjects with normal glucose tolerance (NGT) at baseline.

Design: Primary analysis of an occupational cohort with both cross-sectional and longitudinal data (follow-up duration mean±SD: 28.4±6.1 months).

Setting and participants: Data were analysed from 1058 men in a cross-sectional study and from 560 men with NGT in a longitudinal study.

Outcomes and methods: Impaired lung function (per cent predicted value of forced vital capacity (%FVC) or per cent value of forced expiratory volume 1 s/FVC (FEV_1/FVC ratio)) in relation to the ratio of prediabetes or DM in a cross-sectional study and development of new prediabetes in a longitudinal study. NGT, prediabetes including impaired glucose tolerance (IGT) and increased fasting glucose (IFG) and DM were diagnosed according to 75 g oral glucose tolerance tests.

Measurements and main results: %FVC at baseline, but not FEV_1/FVC ratio at baseline, was significantly associated with the incidences of DM and prediabetes. Among prediabetes, IGT but not IFG was associated with %FVC. During follow-up, 102 subjects developed prediabetes among those with NGT. A low %FVC, but not FEV_1/FVC ratio, was predictive of an increased risk for development of IGT, but not of IFG.

Conclusions: Low lung volume is associated with an increased risk for the development of prediabetes, especially IGT, in Japanese men. Although there is published evidence for an association between chronic obstructive pulmonary disease and DM, prediabetes is not associated with the early stage of COPD.

INTRODUCTION

Accumulating evidence suggests that there is a close relationship between impaired lung

ARTICLE SUMMARY

Article focus
▪ We hypothesised that lung function is associated with the development of impaired glucose metabolism. To investigate this, the data of an occupational cohort were analysed from 1058 men in a cross-sectional study and from 560 men with normal glucose tolerance (NGT) in a longitudinal study.

Key messages
▪ Low lung volume was significantly associated with the incidence of prediabetes or diabetes mellitus (DM) in both cross-sectional and longitudinal studies.
▪ Low lung volume is an independent risk factor for a particular type of prediabetes, impaired glucose tolerance rather than impaired fasting glucose. Our results suggested that prediabetes is not associated with the early stage of COPD, although there are published evidences for an association between COPD and DM.

Strengths and limitations of this study
▪ This is the first study that prospectively examined the incidence of newly diagnosed prediabetes among subjects with NGT at baseline. There are several limitations including that the subjects were limited to Japanese men and our occupational cohort may possibly be healthier than the general population.

function and diabetes mellitus (DM). Population-based studies have demonstrated associations between both obstructive and restrictive lung impairment and insulin resistance or DM.[1-9] A representative obstructive lung disease, chronic obstructive pulmonary disease (COPD), is now well known to be associated with a variety of comorbidities, including DM.[10-13] However, an accelerated decline of lung function has been observed in patients with DM.[14] The incidence rates of COPD, asthma, lung fibrosis and pneumonia

are greater in patients with DM than in those without DM.[15] The incidence of death from COPD is also increased in DM.[16]

The metabolic stage between normal glucose homeostasis and DM is called prediabetes, which the WHO divides into impaired glucose tolerance (IGT) and increased fasting glucose (IFG).[17] Both IFG and IGT are the established risk factors for DM.[18] The Diabetes Prevention Program Research Group[15] found that about 30% of subjects with prediabetes developed DM during 3–5 years of follow-up. IFG and IGT are also risk factors for cardiovascular disease (CVD), relationships that are not confounded by the development of DM.[19] [20] Subjects with prediabetes also have higher incidence rates of microvascular complications, including neuropathy, retinopathy and nephropathy, than do those with normal glucose tolerance (NGT).[21] [22]

We reported previously that smokers with airflow limitation had subclinical atherosclerosis as evidenced by carotid intima-media thickness (CIMT).[12] Although we excluded subjects with DM, the prediabetic state may influence the association, since prediabetes per se was accompanied by a modest but significant increase in the risk for developing CVD, as described above. However, there is no information regarding the association between lung function and prediabetes. Therefore, we explored the incidence of newly diagnosed prediabetes among selected subjects with NGT to further elucidate the nature of the relationship between lung function and the development of DM.

METHODS
Subjects
The subjects were recruited from 1218 men who attended the Nippon Telegraph and Telephone West Corporation Chugoku Health Administration Center for general health checkups between April 1999 and March 2006. One hundred and sixty subjects were excluded, because they did not meet the following inclusion criteria: (1) between 40 and 59 years of age at the first examination, and able to perform both a 75 g oral glucose tolerance test (OGTT) and adequate spirometric measurements (146 subjects excluded); (2) no known respiratory disease (14 excluded). Data from the remaining 1058 subjects were used for a baseline cross-sectional analysis. For the longitudinal study, subjects were restricted to those who had NGT (365 excluded), and could be followed up for more than 20 months (133 excluded). The remaining 560 subjects were included. Among these subjects, 77 were receiving medication for hypertension, 43 for dyslipidaemia and 11 for hyperuricaemia. The distributions of these subjects among the quartiles of percent predicted value of %FVC and percent value of 1 s/FVC (FEV$_1$/FVC ratio) were not significantly different.

The study was approved by the Ethical Committee of Kochi University.

75 g oral glucose tolerance test
DM and prediabetes were diagnosed according to the 2003 criteria of the WHO.[17] Subjects with prediabetes were classified into two categories: isolated IFG and IGT. Isolated IFG was defined as a fasting plasma glucose level of 6.1–6.9 mmol/l and a 2 h postload plasma glucose level of <7.8 mmol/l; and IGT was defined by a fasting plasma glucose level of <7.0 mmol/l and a 2 h postload plasma glucose level of 7.8–11.1 mmol/l. Blood samples were collected after a 10 h fast, and then 2 h after a 75 g oral glucose load.

Fasting insulin was measured by an enzyme immunoassay (Dainabot, Tokyo, Japan) with an intra-assay coefficient of variation of 3.1–4.4%. The homeostasis model assessment (HOMA) formula, (fasting insulin (mU/l)×fasting glucose (mmol/l))/22.5, was used to calculate the insulin resistance score.

Pulmonary function test
Pulmonary function was measured using a spirometer (Chest HI-801; Chest Co., Tokyo, Japan) by an experienced technician according to the recommendations of the American Thoracic Society.[23] The Japanese reference values were used.[24]

Statistical analysis
Statistical analysis was carried out using SPSS, V.18.0 (SPSS Japan Inc, Tokyo, Japan). Statistical comparisons of the baseline characteristics of each group were performed using either the χ-square test or one-way analysis of variance (ANOVA). Comparisons among the groups were performed by using post-hoc Tukey test. In the cross-sectional study, logistic regression models were used to estimate the relevant ORs. In the longitudinal study, the HR of each covariate for the risk of development of prediabetes with 95% CI was calculated using the Cox hazard model. Tests for a linear trend across increasing categories of spirometric indices were conducted by treating the categories as continuous variables in a model. In all analyses, $p<0.05$ was taken to indicate statistical significance.

RESULTS
Baseline analysis
At baseline, our study population (n=1058) consisted of 693 normal subjects, 93 with isolated IFG, 167 with IGT and 105 with DM. To examine the relationship between lung function parameters and impaired glucose metabolism, the subjects were divided into quartiles according to baseline %FVC and the FEV$_1$/FVC ratio. Some parameters, including age, body mass index (BMI), systolic blood pressure and total cholesterol, differed significantly among the quartiles (table 1). After adjustment for these parameters, impaired glucose metabolism was significantly associated with %FVC (p<0.001), but not with the FEV$_1$/FVC ratio (p=0.80). Specifically, IGT (p=0.04) and DM (p=0.008), but not isolated IFG (p=0.28), were associated with %FVC (table 2).

Table 1 Baseline characteristics of subjects with NGT, isolated IFG, IGT and DM in the cross-sectional study

	NGT	Isolated IFG	IGT	DM	p Value
Number of subjects	693	93	167	105	
Current smokers (%)	48	42	45	50	0.54
Age (years)	49.5±5.5	50.9±5.3*	51.1±5.3**	52.2±4.7***	<0.001
Height (cm)	169.9±5.7	168.8±5.8	169.1±6.0	168.4±5.0*	0.03
BMI (kg/m^2)	23.1±2.5	23.9±3.1**	24.6±2.8***	24.8±3.2***	<0.001
Systolic BP (mm Hg)	126.4±16.3	135.1±16.4***	135.9±18.2***	140.2±16.3***	<0.001
Pack-year smoking	30.5±15.6	38.0±22.6*	31.1±17.3	38.0±18.5**	0.002
FEV$_1$/FVC (%)	80.1±7.0	79.6±7.8	80.9±7.4	79.4±8.5	0.36
%FVC	97.9±14.2	96.5±12.9	92.0±13.3***	89.2±15.7***	<0.001
Fasting glucose (mmol/l)	5.3±0.4	6.3±0.2***	5.9±0.5***	8.1±1.6***	<0.001
120 min glucose (mmol/l)	5.7±1.0	6.5±0.8***	8.8±0.8***	12.4±4.0***	<0.001
HbA1c (%)	5.10±0.33	5.34±0.36***	5.37±0.41***	6.57±1.20***	<0.001
HOMA-R	1.08±0.56	1.91±2.23**	1.56±0.88***	2.33±1.41***	<0.001
C reactive protein (mg/l)	0.11±0.29	0.09±0.14	0.14±0.28	0.18±0.46	0.13
T-chol (mg/dl)	202.1±32.6	210.0±28.7*	209.5±36.3*	214.8±32.2***	<0.001

Values are numbers, percentages (%) or means ±SD.
*p<0.05.
**p<0.01.
***p<0.001 vs NGT.
BMI, body mass index; BP, blood pressure; CRP, C reactive protein; DM, diabetes mellitus; HbA1c, glycated haemoglobin; HOMA-R, homeostasis model assessment of insulin resistance; IFG, increased fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T-chol, total cholesterol.

Frequencies of newly diagnosed prediabetes in subjects with NGT

After the observation period (mean±SD: 28.4 ±6.1 months), there were 44 subjects with isolated IFG and 58 with IGT among those previously categorised as NGT (n=560), but no subject developed DM. As shown in table 3, there were significant differences in several parameters at baseline, including height, BMI, systolic blood pressure and %FVC, but not in FEV$_1$/ FVC ratio.

Table 2 ORs*(95% CI) of prediabetes and DM according to the quartiles of %FVC† or FEV$_1$%‡ in the cross-sectional study

	I	II	III	IV	p for trend
IFG					
%FVC	1.0	4.60 (1.29 to 16.39)	2.03 (0.53 to 7.79)	2.57 (0.69 to 9.60)	0.06
FEV$_1$/FVC	1.0	1.00 (0.32 to 3.12)	1.39 (0.49 to 3.93)	1.81 (0.67 to 4.90)	0.53
IGT					
%FVC	1.0	1.35 (0.57 to 3.19)	2.18 (1.02 to 4.05)	2.59 (1.17 to 5.69)	0.04
FEV$_1$/FVC	1.0	0.60 (0.35 to 1.15)	0.62 (0.37 to 1.16)	0.50 (0.30 to 1.02)	0.12
IFG or IGT					
%FVC	1.0	2.18 (1.08 to 4.42)	2.09 (1.04 to 4.18)	2.55 (1.28 to 5.09)	<0.001
FEV$_1$/FVC	1.0	0.56 (0.31 to 1.07)	0.63 (0.35 to 1.14)	0.65 (0.36 to 1.17)	0.29
DM					
%FVC	1.0	3.77 (1.29 to 11.03)	1.28 (0.41 to 3.99)	2.50 (0.87 to 7.16)	0.02
FEV$_1$/FVC	1.0	2.08 (0.72 to 5.99)	3.05 (1.12 to 8.31)	2.13 (0.76 to 6.00)	0.18
IFG or IGT, or DM					
%FVC	1.0	3.32 (1.71 to 6.42)	2.04 (1.06 to 3.94)	3.33 (1.74 to 6.38)	<0.001
FEV$_1$/FVC	1.0	0.74 (0.40 to 1.35)	0.98 (0.56 to 1.75)	0.84 (0.48 to 1.49)	0.70

*OR was adjusted for age, BMI, pack-year smoking, systolic BP and T-chol.
†%FVC quartile; I (highest group) (≥104.2%), II (96.0%≤%FVC<104.2%), III (86.4%≤%FVC<96.0%), IV (lowest group) (%FVC<86.4%).
‡FEV$_1$/FVC quartile; I (highest group) (≥85.0), II (81.1%≤FEV$_1$/FVC<85.0%), III (76.5%≤FEV$_1$/FVC<81.1%), IV (lowest group) (FEV$_1$/FVC<76.5%).
BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; IFG, impaired fasting glucose; IFG, increased fasting glucose; IGT, impaired glucose tolerance; T-chol, total cholesterol.

Low lung volume is a risk factor for prediabetes

Figure 1 Incidences of newly diagnosed prediabetes, isolated IFG and impaired glucose tolerance (IGT) according to quartiles of % FVC and the FEV_1/FVC ratio. The incidence of prediabetes was significantly associated with %FVC, but not with the FEV_1/FVC ratio (p=0.01). Among subjects with prediabetes, lower %FVC was significantly associated with a higher incidence of IGT (p=0.04), but not of IFG (p=0.47).
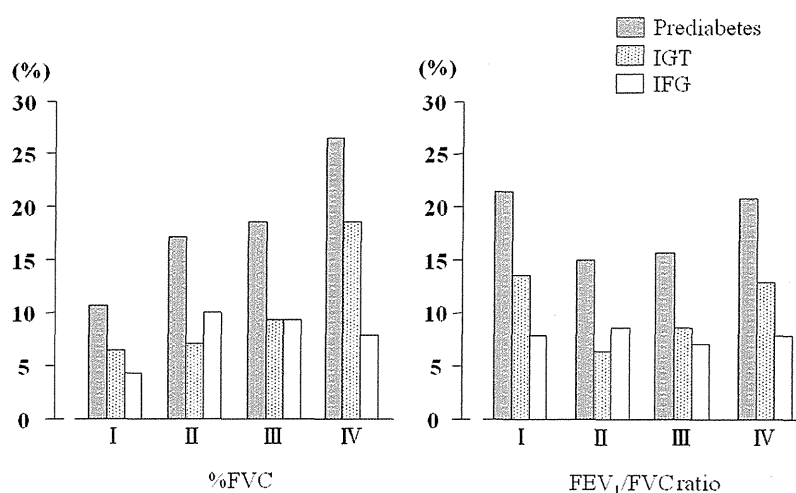
Lung function parameters were divided into quartiles according to baseline %FVC and the FEV_1/FVC ratios. Among the quartiles the parameters, including age, BMI and systolic blood pressure, were significantly different (data not shown). Both in the crude model and following adjustment by age, BMI, pack-year smoking and systolic blood pressure, the development of prediabetes occurred significantly more frequently in the lowest quartile of % FVC, but not in that of the FEV_1/FVC ratio (table 4). Among prediabetes, IGT, but not isolated IFG, was significantly associated with %FVC, as in the baseline cross-sectional analysis (table 4; figure 1).

DISCUSSION

In the baseline cross-sectional study, we found that a low %FVC, but not a low FEV_1/FVC ratio, was significantly associated with increased prevalences of prediabetes and DM. As lung function might be impaired by DM, a causal effect of lung function on DM could not be established by these data. Therefore, we also explored prospectively the effect of lung function on the development of newly diagnosed prediabetes in the population with normal glucose metabolism, as evidenced by the results of an OGTT. We found that reduced lung volume (%FVC), but not airflow limitation (FEV_1/FVC ratio), was significantly associated with the future development of prediabetes.

This study demonstrated that IGT, but not IFG, was closely associated with lower lung volume in both cross-sectional and longitudinal settings. Our finding was supported by previous studies conducted in an Asian population with relatively low BMI but high smoking

Table 3 Baseline characteristics of subjects who remained NGT, developed isolated IFG and IGT in the longitudinal study.

	NGT	Isolated IFG	IGT	p Value
Number of subjects	458	44	58	
Current smokers (%)	48	30*	50	0.05
Age (years)	49.3±5.7	50.2±4.4	50.5±4.9	0.14
Height (cm)	169.9±5.6	170.2±4.9	167.1±6.7**	0.01
BMI (kg/m²)	23.0±2.5	23.8±2.3*	23.7±3.0*	0.04
Systolic BP (mm Hg)	125.4±16.7	130.5±16.9*	129.3±14.5	0.048
Pack-year smoking	29.9±15.6	31.1±12.1	30.1±18.5	0.97
FEV_1/FVC (%)	80.1±7.1	79.7±6.3	79.9±7.9	0.95
%FVC (%)	97.5±14.2	93.0±14.7*	90.0±16.0***	<0.001
Fasting glucose (mmol/l)	5.3±0.4	5.6±0.2***	5.5±0.3**	<0.001
120 min glucose (mmol/l)	5.6±0.9	6.0±1.2	6.4±0.9***	<0.001
HbA1c (%)	5.07±0.33	5.31±0.37***	5.19±0.30*	<0.001
HOMA-R	1.04±0.53	1.19±0.61	1.31±0.64**	0.001
C reactive protein (mg/l)	0.10±0.23	0.18±0.42	0.16±0.30	0.26
T-chol (mg/dl)	201.4±34.5	205.3±27.1	212.5±28.6*	0.05
Duration (month)	28.6±6.2	28.5±5.1	27.6±5.6	0.13

Values are number, percentage (%) or mean±SD.
*p < 0.05.
**p<0.01.
***p<0.001 vs NGT.
BMI, body mass index; BP, blood pressure; CRP, C reactive protein; HOMA-R, homeostasis model assessment of insulin resistance; IFG, increased fasting glucose; IGT, impaired glucose tolerance; NGT, normal glucose tolerance; T-chol, total cholesterol.

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