

TABLE 4. Reasons for Implementing NIV, Timing and Duration of NIV Use, and Initial NIV Settings (n = 47)

Reasons for NIV use [n (%)]	
Worsened atelectasis	21 (44.7)
Respiratory distress	19 (40.4)
Hypercapnia	5 (10.6)
Pulmonary edema	2 (4.3)
Time from extubation to NIV use (hours)*	2 (0-44)
Time from extubation to NIV use < 72 hours [n (%)]	43 (91.5)
Duration of NIV use (days)*	9 (2-17)
Initial NIV settings	
IPAP (cm H ₂ O) [†]	7.6 ± 1.7
EPAP (cm H ₂ O) [†]	3.9 ± 1.0
Respiratory rate (breaths/minute) [†]	25.2 ± 7.0
Spontaneous-timed mode/timed mode (n/n)	27/20

*The data are expressed as medians and first and third quartiles.

[†]The data are expressed as means and standard deviations.

TABLE 5. Reasons for Reintubation and Death

Reasons for Reintubation*	No NIV Use (n = 11)	NIV Use (n = 3)
Worsened atelectasis	3 (27.3)	2 (66.7)
Massive airway secretions	2 (18.2)	0 (0)
Hypercapnia	6 (54.5)	1 (33.3)

Reasons for Death	No NIV Use (n = 5)	NIV Use (n = 5)
Graft failure	4 (80)	4 (80)
Intra-abdominal bleeding	0	1 (20)
Pneumonia	1 (20)	0

NOTE: The data are presented as numbers and percentages.

*The time from extubation to reintubation was 7.9 ± 7.5 hours for the non-NIV group and 19.2 ± 8.6 hours for the NIV group (P = 0.06).

have systematically investigated the effects of NIV use in pediatric patients after abdominal surgery. The results of the current study showed that the need for reintubation due to RCs after surgery was significantly lower for pediatric OLT patients receiving NIV versus those not receiving NIV. The differences in the clinical backgrounds of the 2 cohorts imply that the NIV cohort had more severe conditions than the non-NIV cohort. In addition, the fact that the treatment modalities for RCs during the screening period did not change provides support for the strong association of NIV use with the reduction in the number of patients requiring reintubation. Furthermore, as we show in

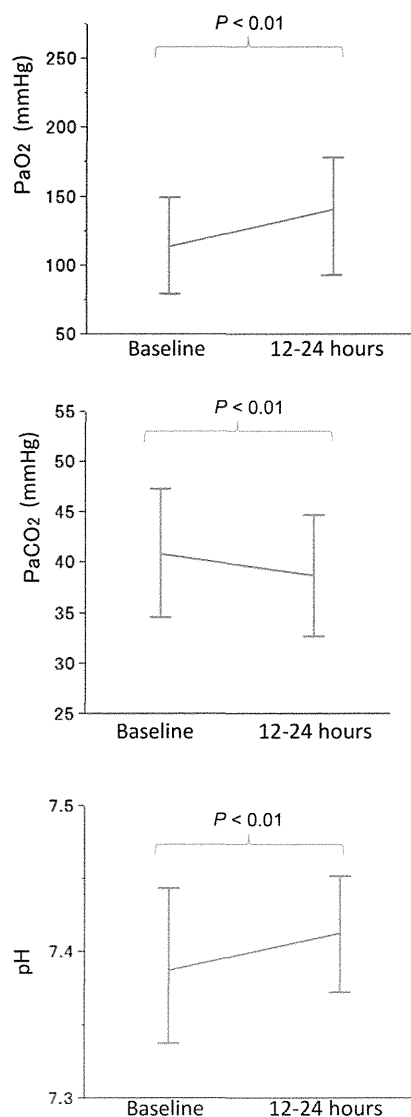


Figure 4. Changes in ABG analyses before and after NIV use.

Fig. 3, the annual changes in the rates of NIV use and reintubation also seem to imply an association of NIV use with the reduction in reintubation.

The improvements in ABG data after NIV use suggest that NIV stabilized patients' respiratory conditions and gave them time to respond to other treatments. This may have allowed them to avoid reintubation and may have facilitated early discharge from the ICU. In this study, the major reasons for the implementation of NIV were worsened atelectasis and the development of respiratory distress. Atelectasis is a common postoperative complication after abdominal surgery.¹³⁻¹⁵ In particular, children are at increased risk for the development of atelectasis because of anatomical factors (eg, a lack of collateral pathways for ventilation and low conductance of the central airways).¹⁵ Because airway patency is lost abruptly after extubation, the aspiration of small amounts of mucus and oropharyngeal or gastric secretions may occlude a bronchus or bronchiole in a child, and atelectasis

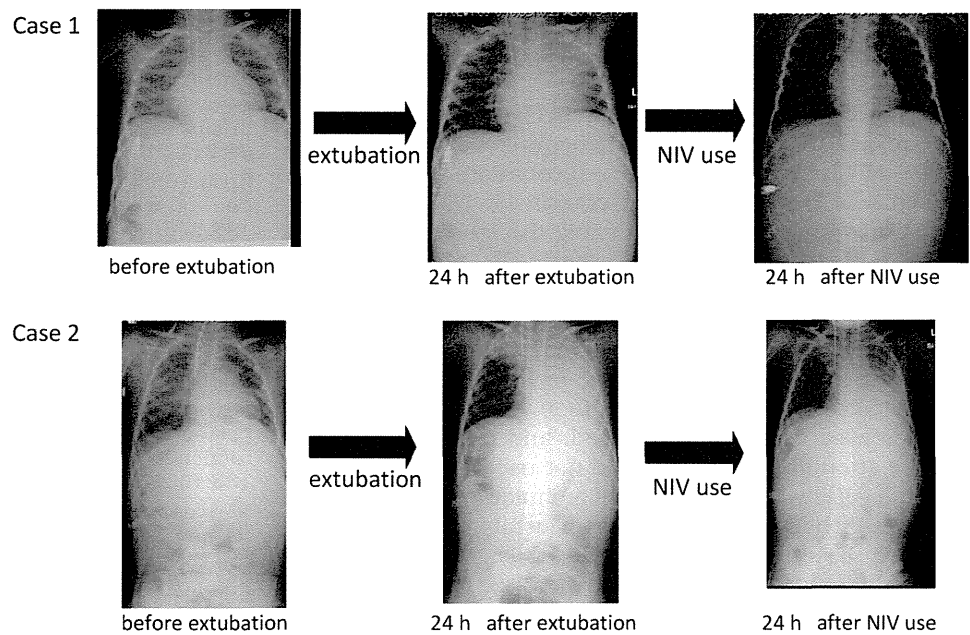


Figure 5. Typical examples of CXRs showing the deterioration of atelectasis after extubation and the amelioration of atelectasis after NIV use.

may develop or, if already present, may worsen.¹⁶ The developed atelectatic lung area decreases the functional residual capacity and increases the ventilation-perfusion mismatch. Subsequently, hypoxemia may either develop or deteriorate.¹⁷ In adult patients with postoperative hypoxemic respiratory failure, it has been shown that NIV improves ventilation, minimizes atelectasis formation, and increases the functional residual capacity.^{18,19} Squadrone et al.²⁰ showed in their prospective randomized controlled study (RCT) that NIV can decrease the need for reintubation in adults after major abdominal surgery. This benefit of NIV can be just as valid for pediatric patients.

On the other hand, the effects of NIV on postextubation respiratory distress are controversial. Keenan et al. performed an RCT in a heterogeneous adult population and found no differences in the rates of reintubation for the NIV and control groups.²¹ Esteban et al.⁴ also performed an RCT to investigate whether NIV could prevent the need for reintubation in patients with respiratory failure after extubation. Although NIV was introduced because of respiratory distress in most of their cases, they were not able to show a benefit from NIV. However, in these 2 trials, the majority of the acute respiratory failures were medical in origin, and there were a limited number of postoperative patients who had undergone abdominal surgery. In these previous trials, the efficacy of NIV appeared to differ markedly and to depend on the backgrounds of the subjects and the criteria for the initiation of NIV.

It is noteworthy that in our study, most reintubations and NIV introductions were performed within 72 hours of extubation. In cases of extubation failure, most reintubations are reported to be required within 48 to 72 hours of extubation, as in our study.²²⁻²⁴ Therefore, NIV use during this period appears to have a strong benefit in stabilizing a patient's respiratory condition.

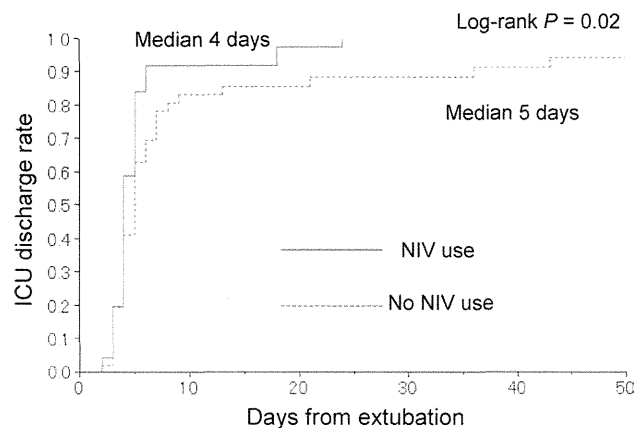


Figure 6. Kaplan-Meier analysis of rates of discharge from the ICU.

Because prospective RCTs of the respiratory management of severely ill pediatric patients are ethically difficult to perform, published reports of NIV use in children with acute respiratory failure are not abundant and are not systematic. Yañez et al.²⁵ showed in their RCT that NIV improved hypoxemia and decreased the need for intubation in pediatric patients with acute respiratory failure. In another RCT, Mayor-domo-Colunga et al.¹⁰ found that postextubation NIV was useful in preventing reintubation when it was applied immediately after extubation, whereas NIV was more likely to fail when acute respiratory failure had already developed. Although both studies reported the efficacy of NIV for pediatric patients with respiratory failure, the study patients' clinical backgrounds were not uniform. In this study, even though it was retrospective, we aimed to include patients with similar clinical backgrounds by investigating only OLT patients. Because an evaluation of the role of NIV

in supporting extubation in a clinically unified pediatric population is rare, this study can be regarded as clinically significant.²⁶

This study has some limitations. First, because of its retrospective design, the judgments about when to initiate NIV use and perform reintubation were not randomized. Even though the criteria for NIV implementation were not changed during the study period, not all patients who met the criteria for NIV implementation received NIV, and these patients composed the non-NIV cohort. It may be considered that the patients receiving NIV were somewhat sicker than the others (eg, they were more anemic or had more extensive atelectasis; Table 2). In addition, the rate of NIV use increased as the years passed, and this suggests that the attending physicians did not use NIV as readily in the early years because the technique for pediatric patients had not yet been well established. Furthermore, because ABG and CXR analyses were not performed as often in the non-NIV group, a full comparison of such data with the NIV group over a comparable time period was not possible. There is the possibility that the observed changes in ABG and CXR results in the NIV group were related to the usual clinical course rather than NIV implementation. However, the respiratory conditions (PaO₂/FiO₂ ratio, PaCO₂, and degree of atelectasis) of these patients had worsened before NIV implementation. We evaluated data before and after NIV implementation at 12- to 24-hour intervals, and it seems unlikely that the ABG data and the CXR findings would have improved significantly in such a short period without any intervention related to respiratory management. Second, during the use of NIV in our pediatric patients, we could not precisely determine oxygen concentrations with our NIV device. Therefore, when we were evaluating the ABG data, we could not calculate the PaO₂/FiO₂ ratio. Third, for the cases with atelectasis, it was impossible to judge whether they had pneumonia. Thus, we could not address the efficacy of NIV in patients with pneumonia.

In conclusion, NIV is an acceptable and promising method of respiratory management in pediatric OLT patients with respiratory failure after extubation. An NIV trial may prevent the worsening of atelectasis and contribute to the stabilization of a patient's respiratory condition. Consequently, it may decrease the need for reintubation during the postoperative period and lead to an early discharge from the ICU. It is possible that all extubated pediatric patients should be placed on NIV after abdominal surgery to prevent the development and deterioration of RCs for a short period of time. Prospective RCTs, although ethically difficult to perform, would help us to confirm these results and are warranted.

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Transient Increase in Epileptiform Discharges after the Introduction of Nasal Continuous Positive Airway Pressure in a Patient with Obstructive Sleep Apnea and Epilepsy

Takefumi Hitomi^{1,2}, Toru Oga¹, Tomomasa Tsuboi¹, Chikara Yoshimura¹, Takeo Kato³, Akio Ikeda², Ryosuke Takahashi² and Kazuo Chin¹

Abstract

In patients with obstructive sleep apnea (OSA) and epilepsy, the frequency of generalized spike and wave complexes (GSWCs) usually decreases after the initiation of nasal continuous positive airway pressure (nCPAP) therapy. However, we herein report a patient who had a transient increase in GSWCs following nCPAP treatment. A woman with epilepsy underwent polysomnography, who showed severe OSA and 30 GSWCs during the sleep study. Polysomnography at the introduction nCPAP showed that the GSWCs increased to 94 times during the monitoring period, despite improvement of her OSA. Polysomnography was again performed four months later, and the GSWCs had decreased to 23 times. Physicians should therefore be cautious regarding a possible increase in epileptiform discharges and seizures immediately after the introduction of nCPAP.

Key words: transient increase in epileptiform discharges, epilepsy and obstructive sleep apnea, nasal continuous positive airway pressure (nCPAP) introduction

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Introduction

Both sleep disordered breathing (SDB) and epilepsy are common disorders. SDB was observed in 24% of adult men and 9% of adult women (1) and the prevalence of epilepsy in the general population is approximately 1% (2). Therefore, epilepsy is often accompanied by SDB, mainly obstructive sleep apnea (OSA). In one study, one-third of medically intractable epilepsy patients had a respiratory disturbance index greater than 5 (3), and another study showed that about 10% of the adult epilepsy patients had OSA (4). In patients with SDB and epilepsy, the frequencies of seizures (5-7) and epileptiform discharges (8) are usually decreased after the treatment of SDB, such as by nasal continuous positive airway pressure (nCPAP) and other means.

This secondary beneficial effect of SDB treatment on epilepsy may be associated with a decrease in sleep fragmentation, sleep stage transition, and desaturation, since focal and generalized seizures are more likely to occur during light sleep or soon after awakening (9) and epileptiform discharges tend to be activated by sleep in some epilepsy patients.

As mentioned above, treatment for SDB usually ameliorates not only SDB, but also epilepsy, in patients with both conditions. However, we herein report a patient who experienced a transient increase in epileptiform discharges immediately after beginning nCPAP treatment.

Case Report

An 18-year-old woman began to have monthly complex

¹Department of Respiratory Care and Sleep Control Medicine, Graduate School of Medicine, Kyoto University, Japan, ²Department of Neurology, Graduate School of Medicine, Kyoto University, Japan and ³Department of Pediatrics, Graduate School of Medicine, Kyoto University, Japan

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Correspondence to Dr. Kazuo Chin, chin@kuhp.kyoto-u.ac.jp

Table. Polysomnogram Analysis of the Effect of Introducing nCPAP on OSA and GSWCs

	Before nCPAP	Just after nCPAP	4mo after nCPAP
Sleep duration (min)	399	336	414
Sleep onset (min)	11	14	36
Sleep efficacy (%)	84.1	75.6	88.2
Stage I (%)	13.4	3.6	6.2
Stage II (%)	36.7	36.3	49.1
SWS (%)	39.0	55.2	35.4
REM (%)	10.9	4.9	9.3
Arousal index/h	19.7	22.0	15.8
Awakenings/h	5.6	8.4	2.5
Micro-arousals/h	14.1	13.6	13.3
AHI/h	52.2	5.7	3.0
Obstructive apnea/h	13.7	0.5	2.2
Mixed apnea/h	0.3	0.4	0.0
Central apnea/h	0.6	0.7	0.1
Hypopnea/h	38.5	4.1	0.7
REM-AHI	53.8	3.6	0.0
NREM-AHI	52.0	5.8	3.4
Minimum SpO ₂ (%)	80	89	91

nCPAP: nasal continuous positive airway pressure, OSA: obstructive sleep apnea, GSWCs: generalized spike and wave complexes, SWS: slow wave sleep, REM: rapid eye movement sleep, NREM: non rapid eye movement sleep, AHI: apnea hypopnea index, SpO₂: percutaneous oxygen saturation

partial seizures at the age of 13. Her seizure semiology consisted of a loss of awareness and motion arrest lasting for 1 minute; thereafter, the seizure sometimes evolved into a secondarily generalized tonic clonic seizure. Interestingly, a seizure was once induced by hyperventilation. Her developmental milestones were normal, and she had no cognitive impairment. Interictal electroencephalogram (EEG) showed 2-3 Hz generalized spike and wave complexes (GSWCs) that were sometimes maximal at the right frontal area but not on the left side, and intermittent rhythmic slow waves at the right frontal area. Magnetic resonance imaging (MRI) of the head showed no apparent abnormality. Valproic acid, which was the first treatment administered, was not effective, but her seizures were well controlled after switching to carbamazepine (CBZ). Based on these findings, she was diagnosed as having focal epilepsy arising from the right frontal area despite the appearance of GSWCs. We consider that the GSWCs were a result of secondary bilateral synchrony. At least 1 year before and 1 year after the introduction of nCPAP, she was seizure-free and the dose of CBZ (800 mg/day) and its serum concentrations (8.5-13.3 µg/mL) were consistent.

The patient experienced excessive daytime sleepiness and had exhibited loud snoring at the age of 18. She was therefore referred to our clinic. At that time, her height and weight were 159.3 cm and 99.5 kg, respectively, and her

BMI was 39.2 kg/m². The results of an arterial blood gas analysis were pH, 7.39; PaCO₂, 43.5 mmHg; PaO₂, 88.1 mmHg; and HCO₃⁻, 26.2 mmol/L with room air. The Epworth Sleepiness Scale score was 9 (reference value ≤10) points. She was suspected to have OSA and underwent polysomnography (PSG) (Somnostar Pro System, Fukuda Denshi, Tokyo, Japan). The sleep stages were defined according to the criteria of Rechtschaffen and Kales (10). Apnea was defined as the cessation of airflow for ≥10 seconds, and hypopnea was defined as a >50% decrease in a valid measure of airflow in association with oxygen desaturation of >4% or an arousal (11). The apnea and hypopnea index (AHI) was calculated as the number of episodes of apnea and hypopnea per hour over the total sleep time. The number of GSWCs during PSG recording was counted with respect to each sleep stage and the total sleep time. The PSG showed severe OSA with an AHI of 52.2 (Table) and also showed that GSWCs occurred 30 times (6 during stage 1; 14, stage 2; 3, stage 3; 5, stage 4; and twice during rapid eye movement (REM) (Figure).

Two weeks after the first PSG, the patient was introduced to nCPAP. The PSG that was performed on the second night of nCPAP treatment showed a reduction in the AHI to 5.7 (Table). However, the GSWCs increased, to 94 times during the study period, which was more than three times the number detected during the first PSG (9, during stage 1; 66,

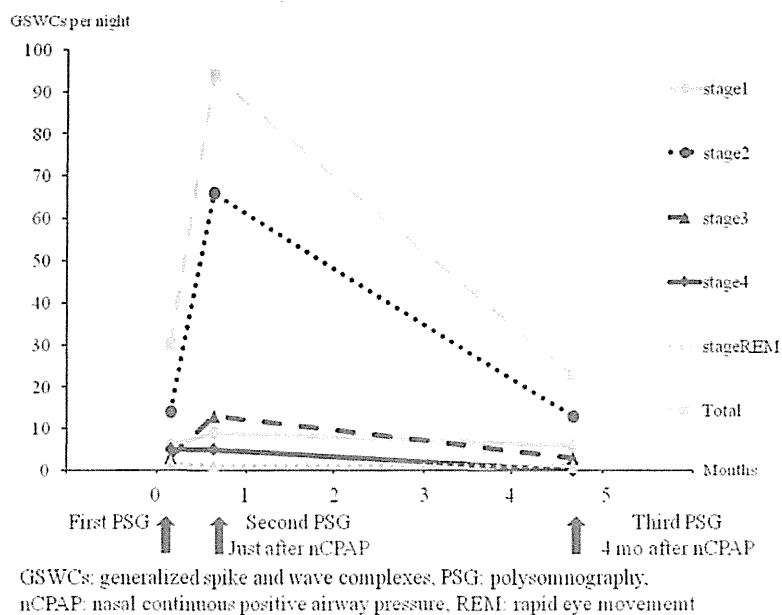


Figure. The number of generalized spike and wave complexes (GSWCs) observed during polysomnography (PSG) recorded before, immediately after and four months after the introduction of nasal continuous positive airway pressure (nCPAP). The gray line indicates the temporal change in the number of GSWCs recorded during stage 1, the black small dotted line the GSWCs during stage 2, the black large dotted line stage 3, black line stage 4, gray small dotted line rapid eye movement (REM), and the gray large dotted line represents the total number of GSWCs recorded during the sleep study. Immediately after the introduction of nCPAP, the GSWCs increased to 94 times (9, stage 1; 66, stage 2; 13, stage 3; 5, stage 4; and once for REM), which was more than three times the number before CPAP (total of 30; 6, stage 1; 14, stage 2; 3, stage 3; 5, stage 4; and twice for REM), and then the value returned to the baseline level (total of 23 times; 6, stage 1; 13, stage 2; 3, stage 3; 0, stage 4; and once for REM).

stage 2; 13, stage 3; 5, stage 4; and once during REM) (Figure). With respect to the patient's sleep architecture, slight decreases in the total sleep time and sleep efficacy, an increase in the arousal index, a decrease in stage 1 and REM and increased slow wave sleep were observed in comparison to findings of the baseline PSG (Table). Despite these findings by PSG, the patient did not experience any clinical seizures and her daytime sleepiness improved.

A follow-up PSG was performed four months after the introduction of nCPAP, which revealed a normalized AHI of 4.3 (Table). There were 23 GSWCs during the sleep study, thus indicating a return to close to the baseline level (6 during stage 1; 13, stage 2; 3, stage 3; 0, stage 4; and once during REM). Regarding her sleep architecture, a slight increase in sleep efficacy, a slight decrease in the arousal index, decreased stage 1 and increased stage 2 were observed in comparison with the baseline PSG (Table).

Discussion

We herein reported a patient who experienced a transient increase in epileptiform discharges after the introduction of nCPAP. With regard to the worsening effect of the treatment for SDB on epilepsy, one previously reported OSA patient

had a new-onset seizure due to frontal lobe epilepsy after the introduction of nCPAP (12). This report, in addition to our experience with our patient, suggests that treatment for OSA can provoke either new-onset seizures or an increase in epileptiform discharges. However, our examination of the literature did not yield any previous reports showing an increase in epileptiform discharges immediately after the introduction of nCPAP.

Although the mechanism responsible for the worsening of epilepsy following SDB treatment remains unknown, several possible mechanisms can be considered. Acute defragmentation in the sleep architecture, acute improvement in the arterial blood gases, and acute reversal of regional cerebral hyper- or hypoperfusion can be caused by the acute release of airway collapse in OSA by nCPAP (13, 14). These drastic changes usually induce favorable clinical results, but they could rarely cause a transient increase in epileptiform discharges, as was the case in our patient. This short-term adverse effect might have been related to the lack of beneficial effects of CPAP on the early mortality rate in patients with central sleep apnea and heart failure (15). In addition, the contribution of instability in slow wave sleep was suggested by analyzing the cyclic alternating pattern in sleep EEGs (12). The authors of that study also mentioned that

nCPAP might force the EEG synchronization, which may trigger the epileptiform discharges. Since REM sleep deactivates generalized seizures and generalized discharges (16), a decreased REM sleep percentage following CPAP introduction is also a possible mechanism underlying the increase in epileptiform discharges.

The most plausible mechanism in this particular patient is the acute change in arterial blood gases, since she had experienced a seizure induced by hyperventilation in the past. Indeed, upon the introduction of nCPAP treatment, the number of awaking times increased from 5.6/h to 8.4/h, while the numbers of microarousals did not change significantly, from 14.1/h to 13.6/h. In general, awakening from sleep induces relative hyperventilation, which may have evoked epileptiform discharges in this particular patient (Table). A follow-up PSG showed a decrease in the number of awakenings to 2.5/h with stable microarousals. These decreased awakenings resulted in decreased relative hyperventilation, and thus, the GSWC frequency returned to the approximate baseline level. These findings were consistent with the fact that seizures are provoked by hyperventilation in some patients with generalized and focal epilepsy (17).

Physicians should therefore be aware of a possible increase in epileptiform discharges or the new appearance of seizures immediately after treatment for SDB in patients with epilepsy and SDB, although the incidence of such a worsening effect of SDB treatment on epilepsy may be very low.

There is a limitation associated with our report. The variability in the night-to-night GSWC frequency could also be a possible mechanism underlying the transient increase in GSWCs after nCPAP introduction, although the variability of GSWCs in this particular patient were relatively small. Nevertheless, this should be kept in mind by physicians when evaluating epileptic patients being introduced to nCPAP treatment.

The authors state that they have no Conflict of Interest (COI).

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Relationships of Decreased Lung Function with Metabolic Syndrome and Obstructive Sleep Apnea in Japanese Males

Chikara Yoshimura¹, Toru Oga¹, Kazuo Chin¹, Misa Takegami², Ken-ichi Takahashi³, Kensuke Sumi⁴, Takaya Nakamura⁵, Yukiyo Nakayama-Ashida⁶, Itsunari Minami⁶, Sachiko Horita⁷, Yasunori Oka⁸, Tomoko Wakamura⁹, Shunichi Fukuhara², Michiaki Mishima¹⁰ and Hiroshi Kadotani¹¹

Abstract

Objective Decreased lung function as assessed by forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) is shown to be associated with cardiovascular morbidity and mortality. Although the underlying mechanisms for this association remain unknown, metabolic syndrome and obstructive sleep apnea (OSA) may have a role. We analyzed the relationships between metabolic syndrome and OSA in a cross-sectional health survey of middle-aged male employees.

Methods In this secondary analysis, we re-analyzed the relationships of lung function determined by spirometry with metabolic syndrome and OSA based on the respiratory disturbance index (RDI) with a type 3 portable monitor.

Results We analyzed 273 subjects. Independent of age, body mass index (BMI) and smoking, quartiles for lower FVC and FEV₁ were associated with a higher risk of metabolic syndrome compared with quartiles for the highest FVC and FEV₁, respectively. A similar trend was observed regarding the risk associated with waist circumference, and in FVC cases, dyslipidemia. The risk of hyperglycemia was significantly higher in quartiles for the second lowest FVC and FEV₁ than in quartiles for the highest FVC and FEV₁, respectively. A significant trend for an increase in RDI was observed in accordance with quartiles for lower FVC, but not FEV₁.

Conclusion There was a significant relationship between lung function impairment and metabolic syndrome through mainly abdominal obesity, partially through hyperglycemia, and also through dyslipidemia, but only with respect to restrictive lung function. Restrictive lung function was also related to OSA. This epidemiologic evidence may indicate underlying mechanisms between decreased lung function and cardiovascular risk.

Key words: epidemiologic study, lung function, metabolic syndrome, obstructive sleep apnea

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¹Department of Respiratory Care and Sleep Control Medicine, Graduate School of Medicine, Kyoto University, Japan, ²Department of Epidemiology and Healthcare Research, Graduate School of Medicine and Public Health, Kyoto University, Japan, ³Department of Respiratory Medicine, Red Cross Otsu Hospital, Japan, ⁴Department of Respiratory Medicine, National Hospital Organization Minami Kyoto Hospital, Japan, ⁵Department of Respiratory Medicine, Kyoto City Hospital, Japan, ⁶Horizontal Medical Research Organization, Graduate School of Medicine, Kyoto University, Japan, ⁷Department of Human Nursing, Faculty of Human Health, Sonoda Women's University, Japan, ⁸Department of Sleep Medicine, Graduate School of Medicine, Ehime University, Japan, ⁹Department of Environmental Health Nursing, Graduate School of Medicine, Human Health Sciences, Kyoto University, Japan, ¹⁰Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Japan and ¹¹Center for Genomic Medicine, Graduate School of Medicine, Kyoto University, Japan

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Correspondence to Dr. Kazuo Chin, chink@kuhp.kyoto-u.ac.jp

Introduction

Decreased lung function as assessed by forced vital capacity (FVC) and forced expiratory volume in one second (FEV₁) is suggested to be associated with increased cardiovascular morbidity and mortality (1-5). Although the underlying mechanisms remain unknown, significant relationships of lung function impairment with such cardiovascular risk factors as hypertension (6), diabetes mellitus (7), atherosclerosis (8) or obesity (9) have been shown. Metabolic syndrome, which is characterized by the cluster of abdominal obesity, elevated blood pressure, hyperglycemia and dyslipidemia, is also associated with cardiovascular risk. Thus, metabolic syndrome may be the driving force of the association of decreased lung function with cardiovascular disease, as recent several population-based studies indicated (8, 10-14).

In addition, some studies have indicated that decreased lung function as assessed by FVC or FEV₁ is associated with the severity of obstructive sleep apnea (OSA) such as mortality (15), hypercapnia (16, 17), pulmonary hypertension (18) and suspected subclinical lung injury (19, 20). OSA is a well-known risk factor for cardiovascular disease, overlapping with the above-described clusters of its risk factors (21). Although direct comparisons between spirometric measurements and OSA often show inconsistent results, population-based studies to clarify these inconsistencies in findings related to lung function and OSA have been sparse (22).

We hypothesized that impairment of lung function is significantly related to both metabolic syndrome and OSA as supported by findings of an epidemiologic study. Therefore, in the present cross-sectional study, we aimed to simultaneously analyze the relationships of lung function with metabolic syndrome and OSA in the same population sample.

Materials and Methods

Subjects

We previously conducted a cross-sectional health survey in middle-aged male employees at a wholesale company (23) and analyzed the relationship between metabolic syndrome and OSA in 275 subjects (24). In the present study, we reviewed the data in addition to the results of spirometry and re-analyzed the relationships of lung function with metabolic syndrome and OSA. As one subject did not undergo lung function testing and one subject had inadequate lung function data, they were excluded from the analysis. Finally, data on 273 subjects were analyzed. The study protocol was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee. Written informed consent was obtained from all subjects.

Spirometry

Spirometric testing for determining FVC and FEV₁ was performed using a spirometer (Chestgraph HI-101, CHEST, Tokyo, Japan) according to the recommended method (25). Those values were expressed as the percentage of the predicted values, which were based on recommendations of the Japanese Respiratory Society (26).

Anthropometric and biochemical measures

Height and weight were measured in the standing position. Waist circumference and blood pressure were measured by trained research staff at the same time that the subjects were trained in the use of a type 3 portable monitor (PM) and actigraph. Measurements of fasting blood sugar, total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were obtained retrospectively from the company's periodic inspection data (24).

Diagnosis of metabolic syndrome

First, the diagnosis of metabolic syndrome was based on the Japanese criteria (27), namely, if a subject had a waist circumference ≥ 85 cm for men and ≥ 2 of the following risk factors: 1) high blood pressure (systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg); 2) increased fasting plasma glucose ≥ 110 mg/dL; and 3) increased triglycerides ≥ 150 mg/dL or decreased HDL-cholesterol < 40 mg/dL. Twenty-five subjects who took blood pressure-lowering medications, 6 subjects who took medications for hyperglycemia, and 13 subjects who took medications for hyperlipidemia were included as hypertensive, hyperglycemic and hyperlipidemic subjects, respectively.

In addition, to evaluate the consistency of our findings, we also used the National Cholesterol Education Program (NCEP) criteria on the diagnosis of metabolic syndrome (28). Specifically, when a subject had 3 of the following 5 characteristics, a diagnosis of metabolic syndrome was indicated: increased waist circumference (≥ 85 cm for men), systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg, fasting glucose ≥ 110 mg/dL, triglycerides ≥ 150 mg/dL, and HDL-cholesterol < 40 mg/dL. Waist circumference was determined to take into account ethnicity (24).

Examinations and home monitoring

Each subject wore a type 3 PM (Morpheus: Teijin, Tokyo, Japan, which is the same as Somt : Compumedics, Victoria, Australia) for 2 consecutive nights and an actigraphy (Actiwatch AWLight: Mini Mitter, OR, USA) to estimate sleep/wake time at home for 7 days. The respiratory disturbance index (RDI: number of apnea and hypopnea episodes per hour of the analyzed sleep time length) was calculated from PM and actigraphy data (23, 24). PM records were visually inspected and scored by two or more medical doctors specialized in respiratory medicine. Apnea (cessation of breathing for ≥ 10 seconds) and hypopnea ($> 50\%$ reduction in the

Table 1. Characteristics of the 273 Male Subjects

	Mean \pm SD
Age (years)	44 \pm 8
BMI (kg/m ²)	23.9 \pm 3.1
Waist circumference (cm)	83.6 \pm 8.5
Smokers (current / ex / never) (%)	56.0 / 24.2 / 19.8
Smoking (pack years)	24.7 \pm 21.5
Systolic blood pressure (mmHg)	129 \pm 14
Diastolic blood pressure (mmHg)	81 \pm 11
RDI (/h)	10.2 \pm 10.8
Blood parameters	
Total cholesterol (mg/dL)	203 \pm 32
Triglycerides (mg/dL)	122 \pm 81
HDL-cholesterol (mg/dL)	57 \pm 14
Blood glucose (mg/dL)	104 \pm 22
FVC (L)	4.00 \pm 0.64
FVC (%predicted)	92.4 \pm 11.4
FEV ₁ (L)	3.21 \pm 0.54
FEV ₁ (%predicted)	86.1 \pm 11.2

BMI: body mass index, RDI: respiratory disturbance index, HDL: high-density lipoprotein, FVC: forced vital capacity, FEV₁: forced expiratory volume in one second

amplitude of nasal pressure or respiratory effort associated with more than 3% reduction in oxyhemoglobin saturation \geq 10 seconds) were scored blind to other information, except sleep/wake time estimated by the actigraphy. Data without oxygen saturation signals or indecipherable recordings were excluded from analysis. When data from both recorded nights were available, records from the second night were analyzed further. Subjects with an RDI of 5-14.9, 15-29.9, and \geq 30 were considered to have mild, moderate and severe sleep disordered breathing, respectively. The high reliability of the RDI was demonstrated in our preceding study (23).

Statistics

Results are expressed as mean \pm SD unless otherwise stated. Subjects were divided into FVC (%predicted) and FEV₁ (%predicted) quartiles, and the highest quartile (quartile 1) was used as a reference group in the following regression analyses. Based on the FVC (%predicted), the resultant four categories were as follows: quartile 1 (n=68), \geq 101.0%; quartile 2 (n=69), \geq 92.5%, <101.0%; quartile 3 (n=68), \geq 83.0%, <92.5%; and quartile 4 (n=68), <83.0%. Similarly, based on the FEV₁ (%predicted), four categories were as follows: quartile 1 (n=68), \geq 94.0%; quartile 2 (n=69), \geq 85.0%, <94.0%; quartile 3 (n=68), \geq 78.5%, <85.0%; and quartile 4 (n=68), <78.5%. Chi-square tests were used to analyze the relationship of FVC and FEV₁ quartiles with the prevalence of metabolic syndrome. Multivariate logistic regression analyses were performed to assess the relationships of the presence of metabolic syndrome or of each of its components to lung function after adjustment for patients' age, body mass index (BMI) and smoking, which are all cardiovascular risk factors. Results of these regression analyses are presented in terms of the relative risks (RRs) with corresponding 95% confidence intervals (CIs). The trends of RDI levels across FVC and FEV₁ quartiles were

examined using a linear model with RDI as a dependent variable and with quartiles as independent variables. Multiple regression analyses were performed after adjustment for those confounders. P values less than 0.05 were considered to be statistically significant.

Results

Characteristics of the 273 subjects are shown in Table 1. A total of 160 subjects (58.6%) had OSA; 16 subjects had severe, 42 subjects had moderate and 102 subjects had mild OSA. Based on the Japanese criteria and NCEP criteria, 57 (20.9%) and 66 (24.2%), respectively, of the 273 subjects had metabolic syndrome. Regarding each component of metabolic syndrome, abdominal obesity, hypertension, hyperglycemia and dyslipidemia were present in 115 subjects (42.1%), 155 subjects (56.8%), 53 subjects (19.4%) and 89 subjects (32.6%), respectively.

Relationships of lung function with metabolic syndrome and its components

FVC and FEV₁ quartiles were significantly related to the prevalence of metabolic syndrome (p=0.048 and 0.0033, respectively). These relations were similar when using the NCEP criteria (p=0.010 and 0.0002, respectively).

In the multivariate analyses, the risk of metabolic syndrome increased in quartiles for lower FVC (Table 2). Subjects in FVC quartiles 4, 3 and 2 had a significantly higher risk of metabolic syndrome compared with FVC quartile 1 both on the Japanese and NCEP criteria. Regarding each component of metabolic syndrome, the risk of increased waist circumference as defined for metabolic syndrome was highest in FVC quartile 4 (RR=3.21, p=0.043) than in FVC quartile 1 and the risk of hyperglycemia was significantly higher only in FVC quartile 3 (RR=2.71, p=0.046) compared with FVC quartile 1. The risk of dyslipidemia was significantly higher in FVC quartiles 4 and 3 (RR=2.53, p=0.021; RR=2.40, p=0.030, respectively) compared to FVC quartile 1.

Regarding the FEV₁ quartiles, subjects in FEV₁ quartiles 4 (quartile for lowest FEV₁) and 3 had a significantly higher risk of metabolic syndrome compared with FEV₁ quartile 1 (quartile for highest FEV₁) both using the Japanese and NCEP criteria (Table 3). A similar trend was observed for the risk of increased waist circumference as defined for metabolic syndrome. Subjects in FEV₁ quartiles 4 and 3 had significantly higher risk compared with FEV₁ quartile 1 (RR=3.75, p=0.044; RR=6.10, p=0.0033, respectively). The risk of hyperglycemia was significantly higher only in the FEV₁ quartile 3 (RR=2.69, p=0.043) compared with FEV₁ quartile 1.

Relationships of lung function with OSA

A significant trend for an increase in RDI was observed in accordance with quartiles for lower FVC (p=0.038) (Figure A). This trend remained significant after adjustment for

Table 2. Relative Risk of Metabolic Syndrome and Each Component Across FVC Quartiles

	FVC quartile			
	4	3	2	1
Mean FVC, %predicted	77.9	88.0	96.7	107.0
Metabolic syndrome ^a	3.59 (1.24-10.42)*	3.21 (1.08-9.58)*	3.05 (1.01-9.24)*	1.0
Prevalence, %	29.4	23.5	20.3	10.3
Metabolic syndrome ^b	4.40 (1.55-12.55)*	5.25 (1.83-15.12)*	3.83 (1.30-11.25)*	1.0
Prevalence, %	32.4	30.9	23.2	10.3
Waist circumference	3.21 (1.04-9.90)*	2.81 (0.98-8.08)	1.90 (0.67-5.34)	1.0
Hypertension	0.83 (0.41-1.67)	1.37 (0.68-2.79)	0.98 (0.49-1.96)	1.0
Hyperglycemia	1.56 (0.57-4.22)	2.71 (1.02-7.24)*	2.17 (0.79-5.94)	1.0
Dyslipidemia	2.53 (1.15-5.57)*	2.40 (1.09-5.29)*	1.92 (0.86-4.30)	1.0

Data are presented as relative risk (95% confidence interval) and have been adjusted for age, BMI and smoking. In all analyses, Quartile 1 is the referent. * $p < 0.05$. ^a Japanese criteria, ^b National Cholesterol Education Program (NCEP) criteria.

Abbreviations: the same as in Table 1.

Table 3. Relative Risk of Metabolic Syndrome and Each Component Across FEV₁ Quartiles

	FEV ₁ quartile			
	4	3	2	1
Mean FEV ₁ , %predicted	72.4	82.0	89.1	100.8
Metabolic syndrome ^a	3.43 (1.13-10.43)*	6.98 (2.30-21.14)*	2.08 (0.64-6.83)	1.0
Prevalence, %	25.0	33.8	14.5	10.3
Metabolic syndrome ^b	3.40 (1.19-9.69)*	8.30 (2.92-23.58)*	1.99 (0.65-6.06)	1.0
Prevalence, %	27.9	41.2	15.9	11.8
Waist circumference	3.75 (1.03-13.58)*	6.10 (1.83-20.33)*	2.02 (0.67-6.08)	1.0
Hypertension	0.79 (0.39-1.60)	1.20 (0.59-2.46)	1.01 (0.50-2.03)	1.0
Hyperglycemia	1.42 (0.53-3.79)	2.69 (1.03-7.01)*	1.45 (0.52-4.08)	1.0
Dyslipidemia	0.58 (0.26-1.28)	0.85 (0.39-1.82)	1.52 (0.74-3.13)	1.0

Data are presented as relative risk (95% confidence interval) and have been adjusted for age, BMI and smoking. In all analyses, Quartile 1 is the referent. * $p < 0.05$. ^a Japanese criteria, ^b National Cholesterol Education Program (NCEP) criteria.

Abbreviations: the same as in Table 1.

age, BMI and smoking ($p=0.035$). In contrast, there was no significant trend between RDI and FEV₁ quartiles ($p=0.54$) (Figure B).

Discussion

In the present study, we simultaneously analyzed the relationships of lung function impairment to metabolic syndrome and OSA in an epidemiologic study of middle-aged males in Japan. Our main findings were: 1) both lower FVC and lower FEV₁ were significantly associated with metabolic syndrome independent of age, BMI and smoking; 2) regarding separate components of metabolic syndrome, lower FVC and lower FEV₁ were related mainly to increased waist circumference and partially to hyperglycemia, with only lower FVC being significantly associated with dyslipidemia; and 3) lower FVC, not lower FEV₁, was significantly associated with higher RDI.

Both low FVC and low FEV₁ are associated with cardiovascular risk and mortality (1-5). The present results indicate that metabolic syndrome may be the driving force in these relationships. Recently, several population-based cross-sectional studies indicated a positive relationship between metabolic syndrome and lung function impairment in

Asia (8, 10-13) and Europe (14), although the underlying mechanisms are not well understood. The opinion as to whether a restrictive or obstructive pattern is more closely related to metabolic syndrome is inconsistent, although a restrictive pattern seems to be dominant (11). Our study also indicated that even a small decline in FVC [from a mean of 107.0% of predicted (quartile 1) to a mean of 96.7% of predicted (quartile 2)] was associated with a 3-fold risk of metabolic syndrome. In contrast, although a decline in FEV₁ from a mean of 100.8% of predicted (quartile 1) to a mean of 89.1% of predicted (quartile 2) was not significantly associated with such risk, unexpectedly, a 7-fold increased risk in FEV₁ quartile 3 (mean of 82.0% of predicted) was observed. This may be due in part to an exaggeration caused by sampling bias in the small number of patients involved, which is a limitation of the present study. Despite this, the present results indicated that we should pay more attention to lung function levels even when within normal limits or when reductions are small.

We found an independent relationship between lung function impairment in both FVC and FEV₁ and abdominal obesity as a metabolic component. Although this is consistent with recent findings (14), the present results indicated that this association is applicable to Japanese people whose BMI

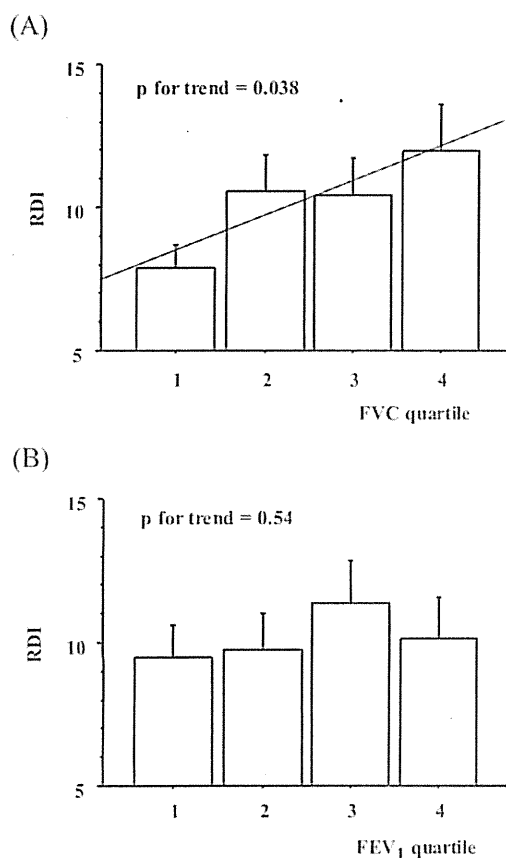


Figure. Relationships of lung function as assessed by forced vital capacity (FVC) quartiles (A) and forced expiratory volume in one second (FEV₁) quartiles (B) with obstructive sleep apnea (OSA). Quartile 1 is the quartile for highest FVC and FEV₁.

is lower than in western populations. There are two possible mechanisms for the association between abdominal obesity and lung function. Firstly, abdominal obesity mechanically affects the diaphragm and chest wall compliance with decreased lung volume, which will result in decreased FVC. Lower levels of ventilation may also cause the early closure of peripheral airways, leading to decreased FEV₁. Secondly, abdominal obesity is associated with systemic inflammation such as shown by elevated C-reactive protein and interleukin-6 values, and systemic inflammation and adipocytokines are risk factors for decreased lung function (13, 29, 30). Thus, mechanical effects and chronic systemic inflammation due to visceral adiposity may be links between lung function impairment and metabolic syndrome.

Incidentally, as in an obese status, systemic inflammation can also be increased during abnormal weight loss in a variety of diseases, possibly related to respiratory and peripheral muscle weakness and impaired lung function (31, 32). This paradoxical feature of systemic inflammation may in part be associated with its dual opposing roles of protectively removing injurious stimuli and initiating the healing process and of negatively impacting disease progression, although further study is needed.

The risk of hyperglycemia was significantly higher in quartile 3, but not in quartile 4, compared to quartile 1 in both FVC and FEV₁. Cross-sectional studies indicate that there is an association between lung function impairment and a glycemic state or diabetes (7, 33, 34). Although this may be due to multiple factors such as microangiopathy, chronic inflammation, autonomic neuropathy, loss of elastic recoil secondary to collagen glycosylation of lung parenchyma and hypoxia-induced insulin resistance, the magnitude of such lung function impairment is likely to cause only subclinical rather than clinically evident abnormalities (34). In addition, in the present context, there might be a small number of subjects with severe or prolonged diabetes as possibly the cause of severely decreased lung function. Therefore, we might not have observed a significant association between the lowest and highest quartiles regarding the risk of hyperglycemia.

In addition, regarding the components of metabolic syndrome, decreased FVC was significantly related to dyslipidemia while decreased FEV₁ was not. Recent studies reported that both FVC and FEV₁ are associated with dyslipidemia and coronary atherosclerosis (8) and triglyceride levels (13). As a recent topic, airflow limitation tends to be associated with atherosclerosis, as shown in the relationship between chronic obstructive pulmonary disease (COPD) and increased mortality due to cardiovascular disease (35, 36). Although the mechanisms are not clearly defined, some remodeling processes may progress simultaneously in both the lung and arteries through hyperlipidemia. Thus, although the results are not necessarily consistent with regard to FVC and FEV₁, the relationship between low lung function and dyslipidemia is noted.

As a novel finding, decreased FVC was significantly related to the RDI independent of age, obesity and smoking. Regarding the relationships between lung function impairment and OSA, population-based studies have been rarely performed, and, in addition, obesity, which is an important risk factor for OSA, might have been a confounding effect. Previous studies showed that low FVC or VC is associated with severity of OSA as shown by hypercapnia (16, 17) or pulmonary hypertension (18). In addition, circulating KL-6, which is a mucin-like glycoprotein mainly expressed on alveolar type II and bronchiolar epithelial cells in human lungs, is considered to be a marker of subclinical lung injury due to OSA through endothelial dysfunction (19). Subsequently, we reported that circulating KL-6 is significantly associated with restrictive lung function and gas exchange derangement (20). Thus, although we did not measure arterial partial tension of carbon dioxide, pulmonary artery pressure or KL-6 in this population-based study, decreased FVC may represent some severe effects of OSA.

Decreased lung function is reported to be associated with cardiovascular morbidity and mortality (1-5). The significant relationships of decreased lung function with metabolic syndrome and OSA shown in the present study indicate a possible key role in this association. Among components of meta-

bolic syndrome, abdominal obesity and, partially, hyperglycemia seemed to be important, both of which cause systemic inflammatory conditions leading to cardiovascular diseases. Restrictive lung function was associated with dyslipidemia, which may lead to a systemic atherosclerotic process that includes the heart and arteries. It was also associated with OSA. Proposed mechanisms by which OSA predisposes to cardiovascular diseases include sympathetic excitation, vascular endothelial dysfunction and metabolic dysregulation, as well as oxidative stress and inflammation induced by cyclical intermittent hypoxia (37). Thus, complicated mechanisms seem to be involved in the association between decreased lung function and cardiovascular risk.

The importance of spirometry is recognized from the point of early detection of COPD whose prevalence and consequent burden are rapidly increasing globally (38), and measurements by spirometry tend to be included in general medical checkups. However, as shown in the present study, these measurements might not be used only as a screening tool assessing airflow limitation but for broader severity indices.

The present study has some limitations. First, the sample size was relatively small due to the small number of employees. Second, we did not perform polysomnography. However, an unattended type 3 PM has been reported to provide a valid RDI when manually scored (39), to be capable of accurately measuring a wide range of apnea/hypopnea indices and to have a high specificity and sensitivity in comparison with polysomnography (23).

In conclusion, we showed a relationship between lung function impairment and metabolic syndrome independent of cardiovascular risk factors such as age, obesity and smoking. Regarding metabolic components, the association was shown to be mainly through abdominal obesity, and partially through hyperglycemia; only in restrictive lung function was there an association with dyslipidemia. Restrictive lung function was also related to OSA. This epidemiologic evidence may indicate the underlying mechanism for the relationship between decreased lung function and cardiovascular risk and help us to better stratify patients at risk for cardiovascular disease using lung function data.

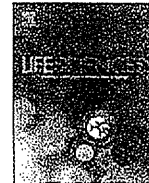
The authors state that they have no Conflict of Interest (COI).

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Attenuation of glucose-induced insulin secretion by intermittent hypoxia via down-regulation of CD38

Hiroyo Ota^a, Shinji Tamaki^a, Asako Itaya-Hironaka^b, Akiyo Yamauchi^b, Sumiyo Sakuramoto-Tsuchida^b, Takashi Morioka^a, Shin Takasawa^b, Hiroshi Kimura^{a,*}

^a Second Department of Internal Medicine, Nara Medical University, Kashihara 634-8521, Japan

^b Department of Biochemistry, Nara Medical University, Kashihara 634-8521, Japan

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ABSTRACT

Aims: Sleep apnea syndrome (SAS) is characterized by recurrent episodes of oxygen desaturation during sleep, the development of daytime sleepiness, and deterioration in the quality of life. Accumulating evidence suggests the association of intermittent hypoxia (IH), a hallmark of SAS, and type 2 diabetes independently on body mass index and waist circumference. In addition to insulin resistance, the progression to type 2 diabetes is dependent on the impairment of glucose-induced insulin secretion (GIS) from pancreatic β -cells. However, the direct effects of IH on GIS are elusive.

Main methods: HIT-T15 hamster β -cells and isolated rat islets were exposed to 64 cycles/24 h of IH (5 min hypoxia/10 min normoxia) or normoxia for 24 h. Changes of GIS and gene expression in IH-treated β -cells were analyzed by ELISA and real-time RT-PCR, respectively.

Key findings: After IH treatment, GIS both from IH-treated HIT-T15 cells and isolated rat islets were significantly attenuated. The level of insulin mRNA was unchanged by IH. The mRNA levels of *glucose transporter 2 (Glut2)*, *glucokinase (GK)*, *sulfonylurea receptor1 (SUR1)*, and *L-type Ca²⁺ channel1.2 (Cav1.2)* in IH-treated-islets were similar to those in normoxia-treated islets. In contrast, the mRNA level of *CD38* in IH-treated islets was significantly lower than that in normoxia-treated islets. The reporter gene assay revealed that the transcription of *CD38* was attenuated by IH, and the transfection of *CD38* expression vector recovered the attenuation of GIS by IH.

Significance: These results indicate that IH stress directly attenuates GIS from β -cells via the down-regulation of *CD38*.

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Introduction

Sleep apnea syndrome (SAS) is characterized by recurrent episodes of oxygen desaturation during sleep, the development of daytime sleepiness, and deterioration in the quality of life. Recently, it has been suggested that SAS may contribute to the development of metabolic syndrome (Coughlin et al., 2004; Tasali and Ip, 2008). Recently, multiple epidemiological studies have provided evidence implicating the presence of SAS as a risk factor of insulin resistance and type 2 diabetes (Tasali et al., 2008). Punjabi et al. (2004) reported that type 2 diabetes is associated with SAS independently of age, sex, and body habitus. Muraki et al. (2010) prospectively showed the association between nocturnal intermittent hypoxia (IH) and the risk of developing type 2 diabetes among community-dwelling Japanese participants. Bortos et al. showed sleep apnea retained a statistically significant association

with diabetes, whereas the use of positive airway pressure, treatment for SAS, independently decreased the incidence and severity after adjusting age, race, baseline fasting blood glucose, and change in body mass index (Bortos et al., 2009). The progression to type 2 diabetes depends on both the impairment of glucose-induced insulin secretion (GIS) from pancreatic β -cells and the presence of insulin resistance. One of the postulated mechanisms for the metabolic alterations associated with SAS might be attributed to IH during sleep, which leads to substantial alterations in both pancreatic β -cell function and organ glucose homeostasis. However, the direct effect of IH stress on β -cell function remains elusive. In this study, we investigated direct effects of IH on GIS and the changes of gene expression by IH.

Materials and methods

Cell culture

Hamster insulinoma HIT-T15 cells (ATCC number: CRL-1777) were purchased from American Type Culture Collection (Manassas, VA, USA) and were grown in RPMI1640 medium (Sigma, St. Louis,

* Corresponding author at: Second Department of Internal Medicine, Nara Medical University, 840 Shijo-cho, Kashihara 634-8522, Japan. Tel.: +81 744 29 8854; fax: +81 744 29 0907.

E-mail address: kimura@nmu-gw.naramed-u.ac.jp (H. Kimura).

MO, USA) containing 5.5 mM glucose, 10% (v/v) FCS, 100 units/ml penicillin G (Wako Pure Chemical Industries, Ltd., Osaka, Japan), and 100 µg/ml streptomycin (Wako Pure Chemical Industries). Cells were either exposed to normoxia (21% O₂, 5% CO₂, and balance N₂) or to intermittent hypoxia (IH: 64 cycles of 5 min of 1% O₂, 5% CO₂ and balance N₂/10 min normoxia) using a custom-designed, computer-controlled incubation chamber attached to an external O₂–CO₂–N₂ computer-driven controller (O₂ programmable control, 9200E SP, Wakenyaku Co., Ltd, Kyoto, Japan). This condition is almost similar with that reported in patients with severe degree of SAS: In severe degree of SAS, patients are repeatedly exposed to severe hypoxemia following by mild hypoxemia or normoxic condition, i.e., IH. We previously reported that the magnitude of IH expressed by SpO₂ was fluctuated between 75–98% and 50–80% in SAS (Nijijima et al., 1999), which was almost equivalent to the medium condition in the present study.

GIS from isolated rat islets or HIT-T15 cells

We isolated islets of Langerhans from Wistar male rats (250–350 g) (Kiwa Laboratory Animals Co., Ltd., Kimino, Japan) as described previously (Takasawa et al., 1993a, 1998, 2010). Islets (20–25 islets/2 ml in 12-well plate) and HIT-T15 cells (2.5 × 10⁴ cells/100 µl in 96-well plate) were incubated in RPMI 1640 (5.5 mM glucose) + 10% FCS at 37 °C under normoxia or IH for 24 h. Following the normoxia/IH exposure, the medium was discarded, and islets and HIT-T15 cells were incubated either in 1 ml (islets) or 0.1 ml (HIT-T15) of RPMI1640 containing 5.5 mM or 22 mM glucose in normoxia for 30–60 min. After glucose stimulation for 30 min (60 min in HIT-T15), the medium was recovered. Insulin concentration in the islet medium was determined by a rat insulin ELISA kit (Morinaga Institute of Biological Science, Inc., Yokohama, Japan) as described (Noguchi et al., 2008). Insulin concentration in the HIT-15 medium was determined by an insulin ELISA kit (Shibayagi, Shibukawa, Japan) using hamster insulin as a standard. Total protein of HIT-T15 cells was quantified using the Coomassie protein assay reagent kit (Pierce, Rockford, IL, USA).

Measurement of apoptosis

HIT-T15 cells (2.5 × 10⁴ cells/100 µl in 96-well plate) were incubated 37 °C over night and the medium was replaced with RPMI1640 ± 1% FCS containing 22 mM glucose. After a 24-h treatment of normoxia or IH, apoptosis was detected by the TUNEL method using an apoptosis screening kit (Wako Pure Chemical Industries) as described (Kobayashi et al., 2000).

Transient transfection and luciferase reporter assay

The reporter construct was prepared by inserting the fragment of human CD38 gene promoter (–136/+30) (Nata et al., 1997) upstream of a firefly luciferase reporter gene in pGL4.17-Basic vector (Promega, Madison, WI, USA). HIT-T15 cells were grown in 24-well plates to 70–80% confluency and were transfected with the reporter plasmid by lipofection using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). Briefly, the cells were transfected with 0.8 µg of reporter plasmid and 0.1 µg of pCMV-SPORT-βgal plasmid (Invitrogen), as an internal control, as described previously (Nakazawa et al., 2005). After exposure to IH for 24 h, the transfected cells were harvested and processed for the luciferase assay as described (Akiyama et al., 2001; Takasawa et al., 2006).

GIS from HIT-T15 cells transfected with CD38 expression vector

pCineo (Promega) and hCD38/pCineo, in which human CD38 cDNA (encoding amino acids 1–300) (Takasawa et al., 1993b) was inserted in *Xho* I/*Xba* I sites of pCineo vector, were transfected into

HIT-T15 cells grown in 96-well plates using Lipofectamine™ 2000 reagent. After transfection, the cells were exposed to normoxia or IH for 24 h, and the cells were incubated in 0.1 ml of RPMI1640 medium containing 5.5 mM or 22 mM glucose in normoxia for 60 min. After glucose stimulation, the medium was recovered. Insulin concentration in the medium was determined by an Insulin ELISA kit (Shibayagi) using hamster insulin as a standard.

Real-time RT-PCR

Total RNA was isolated using a RNA protect cell mini kit (Qiagen, Hilden, Germany) and a FastPure RNA kit (Takara Bio, Otsu, Japan) from HIT-T15 cells and rat islets, respectively. The cDNA was synthesized from total RNA as template using a High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA) for template of real-time PCR. Real-time PCR was performed using Fast SYBR® Green Master Mix (Applied Biosystems). All the PCR primers were synthesized by NGRL (Sendai, Japan), and the primer sequences for each primer set are described in Table 1. The mRNA expression levels were normalized to the mRNA level of *HPRT* in rat or *β-actin* (*BACT*) in hamster.

Data analysis

Results are expressed as mean ± SE. Statistical significance was determined by Student's *t*-test using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA).

Results

Attenuation of GIS from IH-treated β-cells

As shown in Fig. 1A, GIS from IH-treated HIT-T15 β-cells was significantly attenuated, whereas that from the cells treated with normoxia was increased (*P* < 0.05). In order to see whether IH contributed to β-cell death which subsequently decreases insulin secretion from

Table 1
Primers used for real-time RT-PCR.

Primer (position)
Rat <i>HPRT</i>
Forward 5'-CTCATGGACTGATTATGGACAGGAC-3' (179–203 of NM_012583.2)
Reverse 5'-GCAGGTCAGCAAAGAACTTATAGCC-3' (277–301 of NM_012583.2)
Rat insulin 1 (<i>Ins1</i>)
Forward 5'-GGAACCTGGTTCTCTACAC-3' (176–196 of NM_019129)
Reverse 5'-GGGAGTGGTGGACTCAG-3' (374–390 of NM_019129)
Rat insulin 2 (<i>Ins2</i>)
Forward 5'-GACCAGCTACAGTCGGAAC-3' (10–29 of J04807)
Reverse 5'-TCCACGATCCCGCTCTCTG-3' (304–323 of J04807)
Rat glucose transporter 2 (<i>Glt2</i>)
Forward 5'-TAAGGGCCACTGAGGACATC-3' (890–909 of J03145)
Reverse 5'-TGCCAGCTCTGAAAATC-3' (1086–1105 of J03145)
Rat glucokinase (<i>GK</i>)
Forward 5'-AAGGGAACAACATCGTAGGA-3' (621–640 of NM_012565)
Reverse 5'-CATTCGGCTCTCACTA-3' (731–750 of NM_012565)
Rat <i>SUR1</i>
Forward 5'-CAGGACCAAGAGCTGGAGAAGGA-3' (2803–2825 of NM_013039)
Reverse 5'-CATCCAGCAGAAGCCATCTCTT-3' (2886–2908 of NM_013039)
Rat <i>Cav1.2</i>
Forward 5'-GAAATTCAGAAGCGAAAAG-3' (5162–5181 of NM_012517)
Reverse 5'-CCTGCTGCTACTCTGGTAGTAG-3' (5399–5420 of NM_012517)
Rat <i>CD38</i>
Forward 5'-GAAAGGGAAGCCTACCACGA-3' (166–186 of NM_013127)
Reverse 5'-GCCGAGGATTTGACTATAGATCA-3' (219–242 of NM_013127)
Hamster <i>β-actin</i>
Forward 5'-GAAGTACCCCATGAACACG-3' (201–220 of AJ3120092)
Reverse 5'-GGTCTCAAACATGATCTGGG-3' (359–378 of AJ3120092)
Hamster <i>insulin</i>
Forward 5'-TGGTGTGGGGAGCGTGG-3' (195–213 of NM_008387)
Reverse 5'-CCAGTCCAGTTTGCCACT-3' (259–278 of NM_008387)

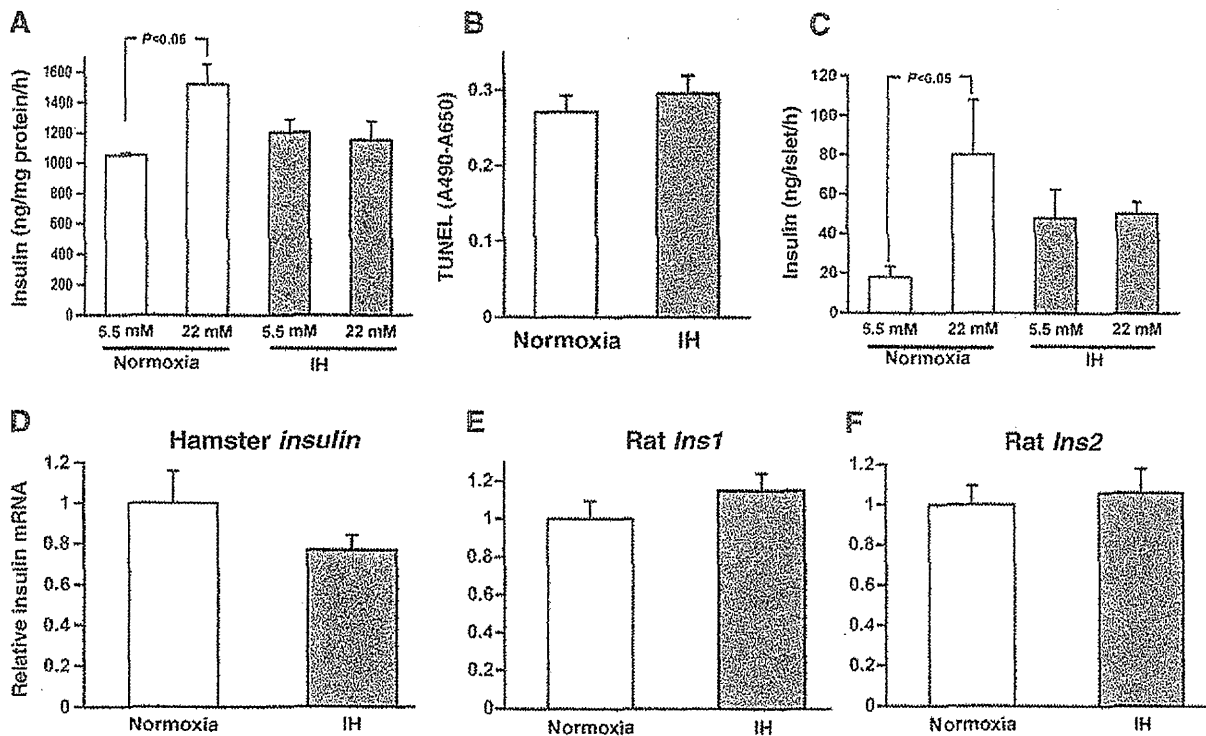


Fig. 1. Attenuation of GIS from HIT-T15 insulinoma cells and rat islets by IH. HIT-T15 cells and isolated rat islets were exposed to normoxia or IH for 24 h, and then stimulated by 5.5 mM or 22 mM glucose. GIS from HIT-T15 cells (A) and from rat islets (C) were measured by ELISA using hamster or rat insulin as control, respectively. HIT-T15 cell apoptosis (B) was detected by the TUNEL method. The mRNA levels of hamster *insulin* (D), rat *insulin1* (E), and rat *insulin2* (F) were measured by real-time RT-PCR using β -actin (for hamster) and *HPRT* (for rat) as endogenous control. The mRNA level of HIT-T15 cells or islets exposed to normoxia was set to 1. Data are expressed as mean \pm SE for each group (A: n=8, B: n=8, C: n=4, D: n=8, E and F: n=7).

remaining β -cells, we measured HIT-T15 cell apoptosis and found that IH did not change apoptosis of HIT-T15 β -cells (Fig. 1B). GIS of rat islets was also abolished by the treatment of IH (Fig. 1C). The levels of insulin mRNAs in HIT-T15 cells and rat islets were unchanged by IH treatment (Fig. 1D–F).

Down-regulation of CD38 mRNA

We then examined the mRNA levels of several genes involved in GIS in the islets and found that the mRNA levels of *glucose transporter 2* (*Glut2*), *glucokinase* (*GK*), *sulfonylurea receptor 1* (*SUR1*), and α 1c subunit of voltage-dependent L-type calcium channels (*Cav1.2*) were unchanged between IH-treated and normoxia-treated islets (Fig. 2). These results suggest that IH makes no difference in the gene expression concerning Ca^{2+} influx from extracellular sources for GIS. We then analyzed genes involved in Ca^{2+} mobilization from intracellular pools such as CD38 and found that the mRNA level of CD38 was significantly lower in IH-treated islets than in normoxia-treated islets (39% of the control) (Fig. 3A). To investigate whether IH inhibits CD38 transcription, HIT-T15 cells were transiently transfected with the reporter plasmid consisting of a luciferase reporter gene under the control of human CD38 promoter. After exposure to IH for 24 h, we measured luciferase activities of normoxia- and IH-treated cells and found that the transcriptional activity of CD38 was attenuated by IH ($P<0.001$) (Fig. 3B).

Recovery of GIS from IH-treated β -cells by the expression of CD38

CD38 was reported to play an essential role in GIS (Ikehata et al., 1998; Yagui et al., 1998; Kato et al., 1999; Pupilli et al., 1999). We found that IH suppressed CD38 transcription (Fig. 3) as well as GIS (Fig. 1). Therefore, it is quite possible that the IH-induced GIS

attenuation is mediated by the suppression of CD38 expression in β -cells. To verify this possibility, we prepared CD38 expression vector (hCD38/pCneo) and transfected it into HIT-T15 cells. After the exposure to normoxia or IH for 24 h, the media were changed to fresh media containing 5.5 mM and 22 mM glucose and incubated in normoxia for further 60 min. After the glucose stimulation, we measured insulin concentrations in the media and found that GIS of HIT-T15 cells transfected with control vector (pCneo) was abolished by the treatment of IH (Fig. 4) as was seen in non-transfected cells (Fig. 1A). In contrast, HIT-T15 cells transfected with CD38 expression vector showed an increased insulin secretion by glucose stimulation ($P<0.05$) (Fig. 4), although the insulin secretion induced by 22 mM glucose did not reach the levels of normoxia-treated control β -cells. This may result from the low transfection efficiency of HIT-T15 cells (about 20%). These results suggest that the suppression of CD38 transcription in the IH-treated β -cells is crucial for the attenuation of GIS.

Discussion

Recently, there has been great interest in the interaction between SAS and metabolic dysfunction. SAS is commonly found in patients with type 2 diabetes. Recent research demonstrates a possible correlation between the two conditions, SAS and type 2 diabetes, independent of obesity. Punjabi et al. (2004) showed a significant association between oxygen desaturation during sleep and glucose intolerance. Reichmuth and co-workers (2005) and Botros et al. (2009) showed a significant cross-sectional association between SAS and type 2 diabetes. In addition to the development of glucose intolerance and insulin resistance, the progression to type 2 diabetes is dependent on the impairment of GIS from pancreatic β -cells (Gerich, 1998). However the direct effects of SAS on β -cells have been obscured. In the present study, we exposed hamster insulin-secreting HIT-T15 cells and isolated

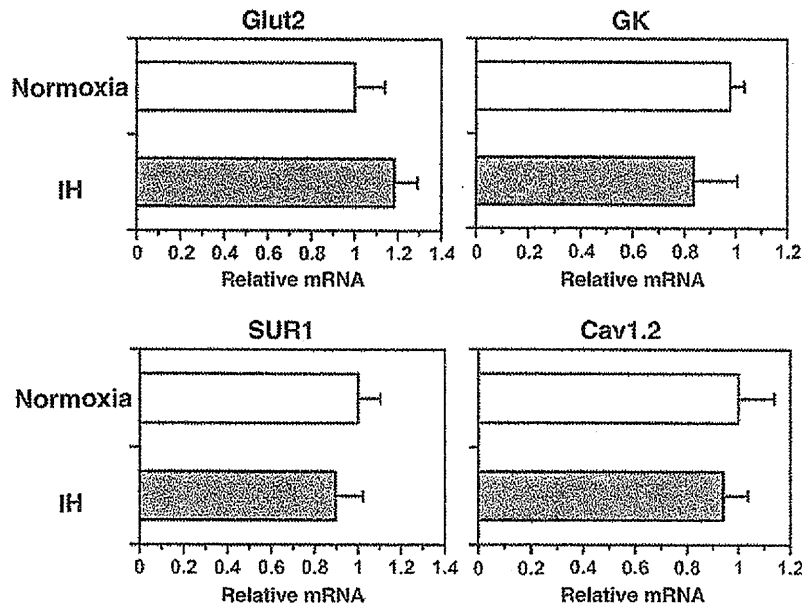


Fig. 2. IH did not change the mRNA levels of *Glut2*, *GK*, *SUR1*, and *Cav1.2* in rat islets. Pancreatic islets were treated with normoxia or IH for 24 h. The mRNA levels of *Glut2*, *GK*, *SUR1*, and *Cav1.2* were measured by real time RT-PCR using *HPRT* as an endogenous control and the mRNA level in islets exposed to normoxia was set to 1.0 for each gene. Results are expressed as mean \pm SE (n = 7).

rat islets to IH for 24 h, mimicking SAS patients' β -cells, in order to clarify whether IH causes attenuation of GIS and found that the exposure to IH attenuated GIS from both islets and HIT-T15 cells whereas GIS from

the cells treated with normoxia was increased by 22 mM glucose. Since the expression of insulin mRNAs was unchanged by IH exposure, impaired GIS from the IH-exposed β -cells was attributed to a defect in the signaling of glucose to insulin secretion. Glucose signaling to insulin secretion is initiated by the uptake of glucose and subsequent metabolism of the sugar in β -cells is essential to insulin secretion (Guillam et al., 2000). We therefore examined gene expression of glucose transporter 2 (*Glut2*), which is responsible for glucose uptake in β -cells (Thorens et al., 1992), and glucokinase (*GK*), which constitutes a rate-limiting step in glucose metabolism and thus determines glucose utilization and insulin secretion in β -cells (Van Schaftingen et al., 1994), using real-time quantitative RT-PCR. In fact, decreased expression of *Glut2* occurred simultaneously with the loss of GIS in numerous animal models of type 2 diabetes (Unger, 1991) and *Glut2* knockout mouse

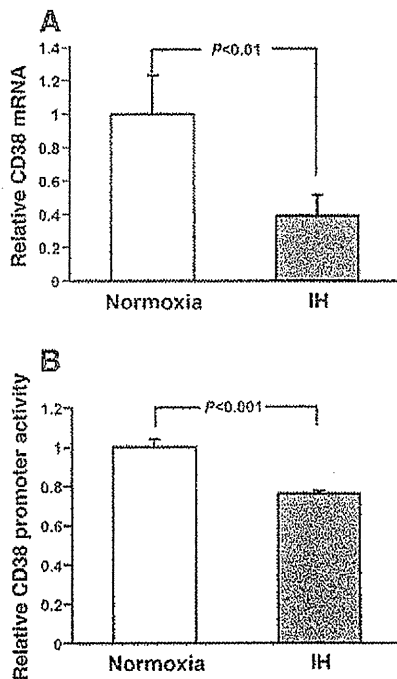


Fig. 3. IH exposure decreased *CD38* mRNA in islets via the inhibition of *CD38* promoter activity. (A) Isolated rat islets were treated with normoxia or IH for 24 h. The level of *CD38* mRNA was measured by real time RT-PCR using *HPRT* as an endogenous control and the mRNA level in islets exposed to normoxia was set to 1. Results are expressed as mean \pm SE (n = 7). (B) Reporter plasmid for human *CD38* promoter (–136–+30) was transfected into HIT-T15 cells with pCMV-SPORT- β gal plasmid (8:1) and the cells were exposed to normoxia or IH for 24 h. After normoxia/IH treatment, luciferase activity was measured as *CD38* transcription activity. Luciferase activity was normalized by β -galactosidase activity and the promoter activity of HIT-T15 cells exposed to normoxia was set to 1.0. Data are expressed as mean \pm SE for each group (n = 6).

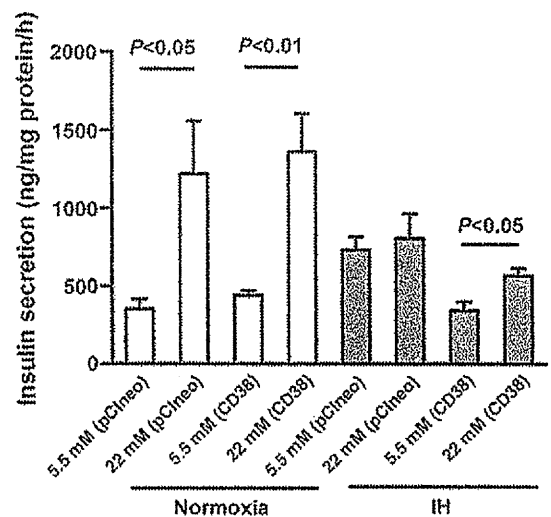


Fig. 4. Recovery of GIS by the expression of *CD38*. HIT-T15 cells were transfected with pCneo (control vector) or hCD38/pCneo (*CD38* expression vector) and exposed to normoxia or IH for 24 h. GIS after normoxia/IH was determined. Results are expressed as mean \pm SE for each group (n = 5).

islets showed the loss of the first phase of insulin secretion (Guillam et al., 1997, 2000). GK acts as a glucose-sensor and its defect/mutation causes MODY2 (OMIM, #12581). Targeted disruption of GK caused diabetes due to defective insulin secretion to glucose (Terauchi et al., 1995). In IH-treated islets, mRNA levels of *Glut2* and *GK* were never decreased compared with those in normoxia-treated islets (Fig. 2), indicating that a defect in glucose metabolism is not likely to be the cause of impaired GIS in IH-treated islets. The signaling defect is therefore considered to lie in the downstream of ATP generation. In this respect, we next examined the mRNA levels of several genes involved in Ca^{2+} influx from extracellular sources (Ashcroft et al., 1984) and found that there was no difference in the mRNA levels of *Cav1.2*, *Kir6.2*, and *SUR1*. Cyclic ADP-ribose (cADPR) acts as a second messenger for Ca^{2+} mobilization from an intracellular Ca^{2+} pool, for GIS (Takasawa et al., 1993a, 1998; Okamoto and Takasawa, 2002) and CD38 has both ADP-ribosyl cyclase and cADPR hydrolase activities for cADPR synthesis and hydrolysis, respectively (Takasawa et al., 1993b; Okamoto and Takasawa, 2002). We therefore analyzed genes involved in Ca^{2+} mobilization from intracellular pools, and found that the mRNA level of CD38 and the CD38 transcription were significantly lower in IH-treated β -cells than in normoxia-treated cells, suggesting that IH attenuates GIS via the down-regulation of CD38. This was confirmed by the fact that the over-expression of CD38 recovered GIS from IH-treated β -cells (Fig. 4). Several lines of evidence such as CD38 knockout mice (Kato et al., 1999), CD38 missense mutation (Yagui et al., 1998), and autoantibodies against CD38 (Ikehata et al., 1998; Pupilli et al., 1999; Antonelli et al., 2002; Marchetti et al., 2002; Mallone et al., 2002; Pupilli et al., 2005) indicate that the down-regulation/dysfunction of CD38 in β -cells results in attenuation of GIS. Therefore, the association between nocturnal intermittent hypoxia and the risk of developing type 2 diabetes among community-dwelling Japanese participants (Muraki et al., 2010) may be mediated by the down-regulation of CD38 by IH in the participants. In addition to the recovery of GIS, over-expression of CD38 normalized basal insulin secretion in 5.5 mM glucose (Fig. 4). CD38 was reported to interact CD31 to form CD38/CD31 complex (Deaglio et al., 2010). CD38/CD31 complex may stabilize β -cell membrane from damages induced by IH.

There is repeated occurrence of apnea/hypoventilation and resumption of ventilation in SAS patients that can increase production of reactive oxygen species (ROS). At the stage of hypoxia, cells adjust to the hypoxic environment through autoregulation. At the stage of re-oxygenation, oxygen in cells sharply increases, resulting in the increased production of ROS including oxygen free radicals such as superoxide anion (O_2^-) and hydroxyl radicals ($\cdot\text{OH}$) that promote oxidative stress reactions. IH model used in this study mimics this condition. In fact, the basal insulin secretion in 5.5 mM glucose was rather enhanced in IH (Fig. 1). Superoxide anion and hydroxyl radicals produced by IH may damage pancreatic β -cell membranes to leak insulin into culture medium. Recently, CD38 knockout mice are reported to be resistant to oxidative stresses through inhibition of reactive oxygen species production and Ca^{2+} overload (Ge et al., 2010). The CD38-cADPR signal system is considered to be involved in inflammation/hypersensitivity of lung/airway system (Ebihara et al., 1997; Sasamori et al., 2004; Guedes et al., 2006, 2008). In this study, the level of CD38 mRNA was significantly decreased (Fig. 3A) and GIS response was markedly attenuated (Fig. 1). The down-regulation of CD38 in β -cells by IH may inhibit ROS production and Ca^{2+} overload to survive in the IH condition. In fact, our data (Fig. 1B) suggest that apoptosis of IH-treated β -cells were unchanged comparing to that of the normoxia-treated control cells.

CD38 were also reported to play a critical role in maternal nurturing behavior and social recognition, which are frequently affected in autism spectrum disorders, by regulating oxytocin secretion (Jin et al., 2007). Malow et al. (2006) reported that identification and treatment of sleep disorders, including SAS, improved daytime behavior in children with autism spectrum disorders. In this study, we found that

IH attenuated the CD38 expression in pancreatic β -cells. Therefore, it is quite possible that the down-regulation of CD38 by IH occurs not only in β -cells but also in hypothalamus/neuro-pituitary cells. If it occurs, SAS may be involved in the development of autism spectrum disorders by down-regulation of CD38.

Conclusion

Our studies indicate that the cyclic change of hypoxia-reoxygenation, which occurs in SAS patients, attenuates glucose-induced insulin secretion from pancreatic β -cells via CD38 down-regulation, resulting in glucose intolerance and type 2 diabetes.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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