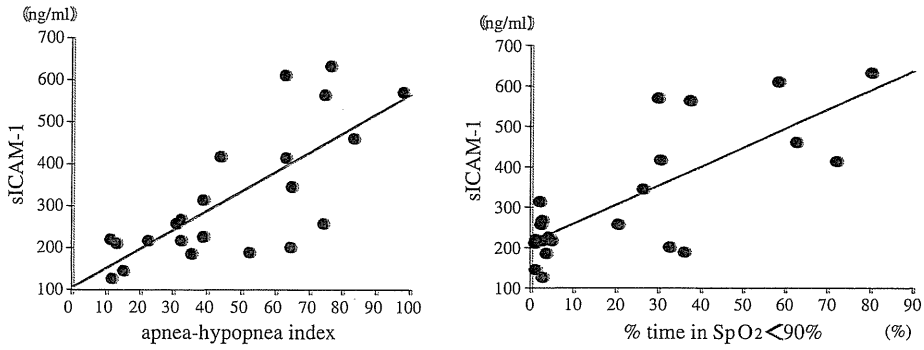

Fig. 1 Relationship between serum concentrations of sICAM-1 and nocturnal hypoxic stress evaluated by the apnea–hypopnea index (AHI) and % time in SpO_2 <90 % in patients with OSAS. Serum concentrations of sICAM-1 correlated significantly with the AHI ($r = 0.747$, $p < 0.0001$) and % time in SpO_2 <90 % ($r = 0.766$, $p < 0.0001$)

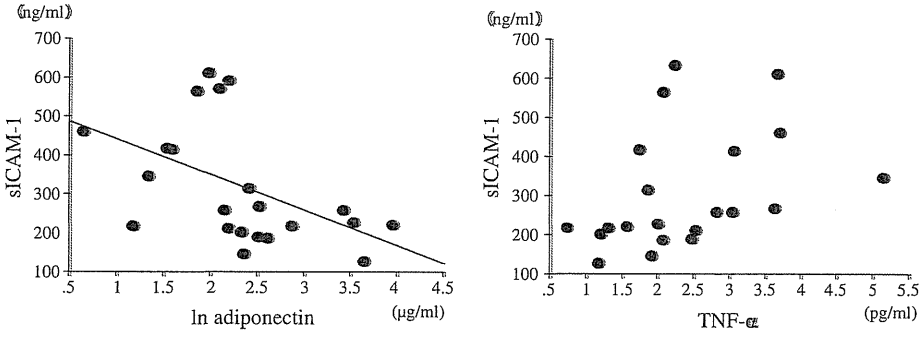

Fig. 2 Relationship between serum concentrations of sICAM-1 and plasma levels of adiponectin (log-transformed) and tumor necrosis factor-α (TNF-α) in patients with OSAS. Serum concentrations of sICAM-1 correlated significantly with adiponectin ($r = -0.476$, $p = 0.024$) but not with TNF-α

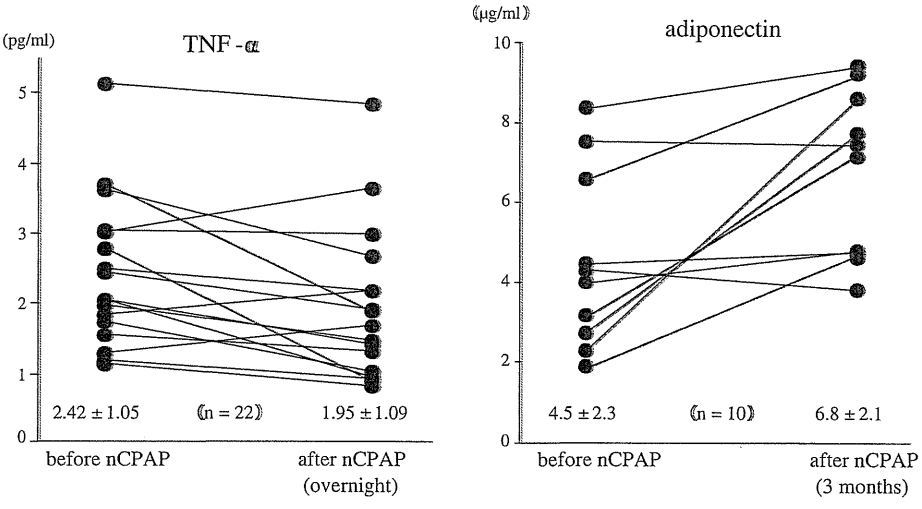

Fig. 3 Effects of nasal continuous positive airway pressure (nCPAP) on plasma levels of tumor necrosis factor-α (TNF-α) and adiponectin. Plasma TNF-α levels were significantly decreased after overnight nCPAP treatment ($p < 0.05$). Plasma adiponectin levels were significantly elevated after long-term (3 months) nCPAP treatment with no significant changes in the body mass index ($p < 0.02$)

adiponectin. We also found that sICAM-1 levels correlated significantly with the AHI and % time in SpO_2 <90 %. Furthermore, they were inversely correlated with plasma adiponectin levels but not with TNF-α levels.

Adiponectin exerts antiatherosclerotic effect by inhibiting the proliferation of the vascular smooth muscle cells and binding to collagen I, III, and V in the vascular intima which might be involved in the repair process of atherosclerotic change. Plasma adiponectin has been reported to suppress the expression of E-selectin, ICAM-1, and vascular cell adhesion molecule-1 on the vascular endothelial cells [9]. Therefore, a reduced adiponectin level may contribute to the pathogenesis of atherosclerosis and become a risk factor for cardiovascular events. Clinically, a reduced adiponectin level is associated with cardiovascular events [9, 14].

Circulating adiponectin levels in patients with OSAS have not been unequivocally determined so far. Lower adiponectin levels [15–18] and comparable or higher levels [19–22] compared with control subjects without OSAS and with similar BMI have been documented. Moreover, the relationship between nocturnal hypoxia evaluated by the

AHI and adiponectin levels in patients with OSAS also is controversial [15, 16, 23]. We found that plasma adiponectin levels were significantly correlated with the severity of nocturnal hypoxia but not with the BMI. Furthermore, plasma adiponectin levels were significantly increased after 3 months of nCPAP treatment which is in line with several previous studies [24]. These data suggest that reduced adiponectin levels are associated with hypoxic stress during sleep in patients with OSAS. Indeed, recent studies have demonstrated that the mRNA degradation of adiponectin is accelerated under hypoxia compared with normoxia [17, 25]. Consequently, reduced adiponectin levels associated with hypoxic stress may explain, in part, the development of atherosclerosis in patients with OSAS.

TNF-α is an inflammatory cytokine, which is regulated by nuclear factor-κB (NF-κB), a master regulator of inflammatory gene expression. We have previously reported monocyte NF-κB activation in patients with OSAS [26]. TNF-α contributes to atherogenesis [6] and its circulating levels are correlated with cardiovascular risk [27]. In the present study, we found a significant positive correlation between plasma TNF-α levels and the AHI, which is in line with several previous studies [28, 29]. This may suggest that hypoxic stress can facilitate the production of TNF-α and that elevated TNF-α levels play an important role in the development of atherosclerosis in OSAS.

Previous studies have provided evidence that atherosclerosis is related to inflammatory processes involving adhesion molecules [6, 8, 9]. In particular, transendothelial migration of monocytes induced by adhesion molecules is a crucial step in the pathogenesis of atherosclerosis. The circulating levels of soluble adhesion molecules are elevated in patients with OSAS [11] and are reduced by nCPAP treatment [30, 31]. We found that sICAM-1 levels significantly correlated not only with the severity of nocturnal hypoxia but also with adiponectin levels in this patient group. TNF-α activates NF-κB by phosphorylation of IκB-α and facilitates the transcription of adhesion molecule genes in the vascular endothelial cells. On the other hand, adiponectin inhibits the activation of NF-κB by suppressing the phosphorylation of IκB-α and subsequent gene transcription [32]. Therefore, decreased adiponectin levels related to hypoxic stress during sleep appear to facilitate atherosclerosis by increased expression of adhesion molecules on the vascular endothelial cells in patients with OSAS. However, we did not observe any correlation between TNF-α and sICAM-1 levels in the present study, which suggests that sICAM-1 levels are modulated mainly by adiponectin. Furthermore, adiponectin may suppress the production of TNF-α. Indeed, it was demonstrated that adiponectin knockout mice showed high levels of TNF-α mRNA in the adipose tissue and high plasma TNF-α levels, which could be reversed by virus-mediated adiponectin expression [33].

The effect of nCPAP treatment on the circulating levels of adiponectin and sICAM-1 has not been fully elucidated. It was documented that reduced adiponectin levels during sleep were attenuated by overnight CPAP treatment in severe OSAS [17]. After 12 months of nCPAP treatment, a significant decrease in plasma sICAM-1 levels was reported [31]. In the present study, no significant effect of overnight nCPAP treatment on the circulating levels of adiponectin and sICAM-1 was observed, while plasma TNF-α levels significantly reduced after the treatment. Although the reason for this discrepancy is unclear, a possible explanation would be the marked difference between baseline plasma concentrations of adiponectin and TNF-α, remarkably short half-life (approximately 6–7 min) of TNF-α [34] and immediate variation in plasma TNF-α levels under hypoxic stress in patients with OSAS [35]. In addition, a previous study demonstrated that etanercept, a TNF-α antagonist, did not decrease adiponectin levels [36]. This suggests that adiponectin is produced and secreted independently of TNF-α. The effect of long-term nCPAP treatment on an increase in plasma adiponectin levels without significant changes in BMI was prominent. Further studies are needed to elucidate the mechanism underlying this phenomenon.

Our study has several limitations. First, because it did not include a control group, the baseline values of adiponectin, sICAM-1, and TNF-α are difficult to interpret. Second, we enrolled a small number of patients with OSAS, especially those undergoing nCPAP treatment. Therefore, a future study with a larger number of subjects is required to validate our findings.

In conclusion, our findings suggest that both reduced adiponectin and elevated TNF-α in plasma are associated with OSAS-induced hypoxic stress. Decreased adiponectin levels are associated with elevated sICAM-1 levels. The effect of nCPAP treatment on adiponectin levels seems to be of clinical importance for preventing cardiovascular events in patients with OSAS.

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Conflict of interest None of the authors have financial conflicts of interest to declare as it relates to the contents of this manuscript.

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Distribution of Bone Mineral Content Is Associated with Body Weight and Exercise Capacity in Patients with Chronic Obstructive Pulmonary Disease

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Key Words
Bone mineral content · Chronic obstructive pulmonary disease · Body mass index · Exercise capacity

Abstract
Background: Although low bone mineral density is highly prevalent in patients with chronic obstructive pulmonary disease (COPD), the distribution of the reduced bone mass has not been fully elucidated. ***Objectives:*** To determine regional bone mass loss in patients with COPD and investigate whether the change in distribution may be associated with body weight loss and functional capacity. ***Methods:*** Body mass index (BMI) was assessed, and height squared indices were derived for the bone mineral content index (BMCI) of the arms, legs and trunk by dual-energy X-ray absorptiometry in 45 male patients with COPD and 12 age- and sex-matched control subjects. Pulmonary function tests were performed, and maximal oxygen uptake ($\dot{V}O_2$max) was measured. ***Results:*** The BMCI was lower in the total bone, legs and trunk of patients with COPD than in control subjects, although the BMCI in the arms was similar between the groups. BMI correlated significantly with the BMCI in all 3 segments. Bone mineral content (BMC) in the trunk, expressed as a percentage of total BMC (BMC trunk/total BMC), correlated significantly with BMI. The BMCI in the trunk was closely related with $\dot{V}O_2$max but not with airflow limitation. ***Conclusions:*** There was a regional difference in BMC reduction, but a predominant reduction of bone mass in the trunk was not associated with the severity of airflow limitation but rather with body weight loss and exercise intolerance. These data suggest that body weight loss and exercise intolerance are important risk factors for vertebral fracture in patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a usually progressive airflow limitation that is not fully reversible [1]. COPD has been recognized as a systemic disease with extrapulmonary manifestations such as cachexia, skeletal muscle wasting, cardiovascular disease, metabolic syndrome and depression [2]. Moreover, patients with COPD are at a higher risk of developing osteoporosis than are healthy subjects [3]. Osteoporotic fractures remarkably reduce quality of life and are associated with mortality [4]. In addition, pulmonary function impairment caused by vertebral fractures is an important problem in patients with COPD [5].

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Osteoporosis is a systemic skeletal disease characterized by low bone mineral density (BMD) and microarchitectural changes in bone tissue that increases an individual's susceptibility to fractures [6]. The World Health Organization definition of osteoporosis is based on BMD measurements [7]. Dual-energy X-ray absorptiometry (DXA) is the gold standard for taking these measurements [8]. Osteopenia is defined as a BMD that is 1–2.5 standard deviations (SDs) below the mean for young adults (i.e. the T score), while osteoporosis is defined as a BMD >2.5 SDs below the mean for young adults [7]. Many studies have demonstrated lower BMD in patients with COPD [9–12].

Multiple sites can be used to measure BMD by DXA. The sites most frequently used are the hip, lumbar spine, forearm and whole body. The International Society for Clinical Densitometry advocates measurement of BMD of the lumbar spine and the hip and to base the diagnosis of osteoporosis on the lowest T score of the measured locations [13]. Indeed, a higher prevalence of osteoporosis was found using local (hip and lumbar spine) compared to whole-body DXA scanning in patients with clinically stable COPD [14]. Accordingly, we hypothesize that nutritional status, airflow limitation and exercise capacity would have different effects on bone mass in each subregion, including the arms, legs and trunk.

Our objectives were to determine the distribution of reductions in bone mineral content (BMC) as a measurement of bone mass and investigate whether the BMC distribution is associated with body weight loss and maximal exercise capacity.

Materials and Methods

Subjects

We enrolled 45 male outpatients with COPD diagnosed according to the definition of the Global Initiative for Chronic Obstructive Lung Disease [1] and 12 age-matched male control subjects. Female patients were not included in this study because of their remarkable differences from male patients with regard to the prevalence of osteoporosis and bone metabolism. In addition, subjects were excluded from the study if they were receiving oral corticosteroid therapy or had known heart disease, malignancy, cor pulmonale or any other inflammatory or metabolic condition. All subjects gave written, informed consent, and the study had local research ethics committee approval.

Pulmonary Function Tests

All patients underwent pulmonary function testing. Vital capacity (VC), forced vital capacity (FVC), forced expiratory volume in 1 s (FEV_1), residual volume (RV) and total lung capacity were measured by a pulmonary function instrument using computer processing (FUDAC 70, Fukuda Denshi, Tokyo, Japan), and the FEV_1/FVC ratio was calculated. Lung volumes were determined by the helium gas dilution method, and diffusing capacity for carbon monoxide (DLCO) was measured by the single-breath method. The values obtained were expressed as a percentage of the predicted values [15]. Arterial blood samples obtained in room air were analyzed by a standard blood gas analyzer (ABL800, Radiometer Corp., Copenhagen, Denmark).

Body Mass Index and Body Composition Analysis

Body mass index (BMI) was calculated as weight divided by height squared. BMC and fat-free mass (FFM) were measured by DXA using a total-body scanner (Lunar DPX, Lunar Radiation Corp., Madison, Wisc., USA). BMC and FFM in the subregions, including the trunk, arms and legs, can be determined separately or together as the whole body. Height squared indices were derived for BMC (BMCI) and FFM (FFMI) of the arms, legs and trunk. BMC in each subregion, expressed as a percentage of total BMC, i.e. BMC arms/total BMC, BMC legs/total BMC and BMC trunk/total BMC, was defined as the BMC distribution.

Exercise Performance

All patients underwent maximal exercise tests on a cycle ergometer (STB-1350, Nihon Kohden, Tokyo, Japan). After 1 min of unloaded pedaling, the workload was increased by 10 W every minute in a ramp protocol until exhaustion. Gas exchange was monitored during the exercise test with a computerized metabolic cart (Vmax 229, SensorMedics Corp., Yorba Linda, Calif., USA). Minute ventilation, oxygen uptake ($\dot{V}O_2$) and carbon dioxide output were measured by the breath-by-breath method. Arterial oxygen saturation was also monitored by a pulse oximeter (BSM-8500, Nihon Kohden).

Statistical Analysis

Values are expressed as means ± SD. The differences among measured parameters in the two groups were determined by unpaired Student's t tests. Pearson's correlation coefficients between static lung function, body composition measurements and maximal $\dot{V}O_2$ ($\dot{V}O_2$max) were calculated. Differences of $p < 0.05$ were considered statistically significant.

Results

Anthropometric and Pulmonary Function Data

The study was conducted from November 2011 to December 2012. Patient characteristics are summarized in table 1. There was no difference in age between the control and patient groups (71 ± 6 vs. 70 ± 6 years). BMI was significantly lower in the patient group than in the controls (18.5 ± 2.6 vs. 22.3 ± 1.9; $p < 0.0001$). There was no difference in smoking status between the two groups. FEV_1% predicted, FEV_1/FVC ratio and %VC were significantly lower in the patient group than in the control group (45.4 ± 22.5 vs. 93.0 ± 3.9%, 41.4 ± 12.2 vs. 84.0 ± 2.3% and 84.5 ± 21.3 vs. 95.7 ± 2.0%, respectively; $p < 0.0001$).

Table 1. Characteristics of COPD patients

Number	45
Age, years	70±6
BMI, kg/m^2	18.5±2.6
Current smokers/ex-smokers, %	12/88
GOLD stage, n	
I	5
II	11
III	17
IV	12
FEV_1, % predicted	45.4±22.5
FEV_1/FVC, %	41.4±12.2
VC, % predicted	84.5±21.3
RV/TLC, %	57.8±10.0
DLCO, % predicted	42.8±23.5
PaO_2, mm Hg	72.2±8.4
$PaCO_2$, mm Hg	44.1±5.0
$\dot{V}O_2$max	593.4±273.1
$\dot{V}E$max	31.7±10.8
ΔSpO_2	6.4±5.1

Values are means ± SD. GOLD = Global Initiative for Chronic Obstructive Lung Disease; VC = vital capacity; RV = residual volume; TLC = total lung capacity; PaO_2 = arterial oxygen tension; $PaCO_2$ = arterial carbon dioxide tension; $\dot{V}E$max = maximum minute ventilation; ΔSpO2 = difference in oxygen saturation as measured by pulse oximetry between before and after exercise testing.

Table 2. BMCI and FFMI of subregions in COPD patients and controls

	BMCI, kg/m^2		FFMI, kg/m^2	
	controls (n = 12)	COPD (n = 45)	controls (n = 12)	COPD (n = 45)
Total	0.92±0.17	0.79±0.14**	17.0±1.6	15.2±1.7^{++}
Arms	0.13±0.03	0.11±0.02	1.69±0.30	1.43±0.35^{+}
Legs	0.33±0.06	0.29±0.05*	5.53±0.84	5.14±0.80
Trunk	0.26±0.06	0.21±0.06*	8.15±0.68	7.21±0.75^{+++}

Values are means ± SD. * $p < 0.05$, ** $p < 0.01$: difference in BMCI compared to controls. $^{+}$ $p < 0.05$, $^{++}$ $p < 0.01$, $^{+++}$ $p < 0.005$: difference in FFMI compared to controls.

Body Composition Analysis

The BMCI and FFMI values of the patients and control subjects for both the total and subregions are shown in table 2. Total BMCI and FFMI values of the patients were significantly lower than those of the controls ($p < 0.01$ for both). BMCI in the legs and trunk was significantly reduced in the patients compared with the control subjects ($p < 0.05$ for both). However, there was no significant difference in arm BMCI between the two groups (fig. 1). In contrast, the FFMI in the arms and trunk was significantly lower in the patients than in the control subjects ($p < 0.05$ for both), and there was no significant difference in FFM in the legs between the two groups. In addition, there was a significant relationship between total BMCI and total FFMI in patients with COPD ($r = 0.418$, $p < 0.005$).

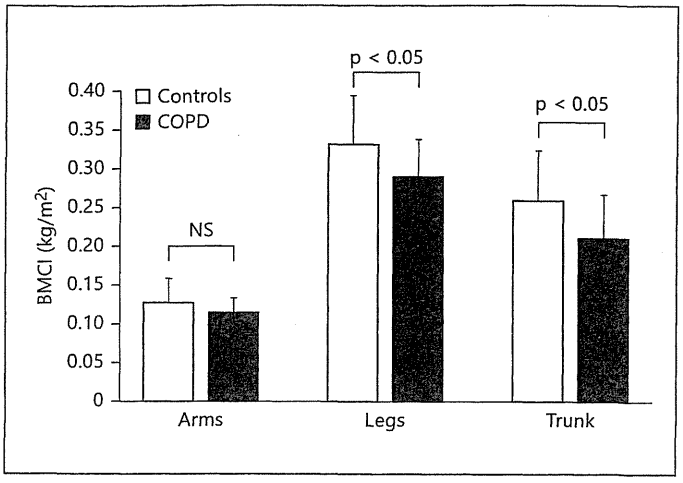

Fig. 1. BMCI in the arms, legs and trunk in patients with COPD. Values are means ± SD of 45 patients with COPD and 12 controls.

Correlations between BMC and BMI in Patients with COPD

Total and subregion BMCI correlated significantly with BMI (table 3). BMC trunk/total BMC correlated significantly with BMI (fig. 2), whereas BMC arms/total BMC and BMC legs/total BMC did not correlate with BMI.

Correlation between BMC and Functional Capacity

Total BMCI and BMCI for each subregion did not correlate with FEV_1% predicted, and nor did the distribution of BMC (table 3). Total BMCI did not correlate with other pulmonary function parameters, including %RV, arterial oxygen tension and arterial carbon dioxide tension, but did correlate with %DLCO ($r = 0.339$, $p < 0.05$; data not shown). Total and subregion BMCI correlated significantly with $\dot{V}O_2$max (table 3). BMC trunk/total BMC correlated significantly with $\dot{V}O_2$max ($p = 0.0099$), while BMC arms/total BMC and BMC legs/total BMC did not correlate with $\dot{V}O_2$max.

Table 3. Relationship between BMI and functional capacity and BMC in subregions or distribution of BMC

	BMI, kg/m^2		%FEV$_1$		$\dot{V}O_2$max	
	r	p value	r	p value	r	p value
BMCI						
Total	0.496	0.0004	0.176	0.2502	0.414	0.0048
Arms	0.465	0.0011	0.105	0.4958	0.344	0.0217
Legs	0.466	0.0011	0.157	0.3063	0.467	0.0012
Trunk	0.542	0.0001	0.243	0.1087	0.453	0.0018
BMC arms/total BMC	0.002	0.9916	−0.199	0.4367	−0.104	0.5043
BMC legs/total BMC	−0.054	0.7282	−0.082	0.5961	0.120	0.4399
BMC trunk/total BMC	0.401	0.0059	0.284	0.0584	0.382	0.0099

r = Pearson's correlation coefficient.

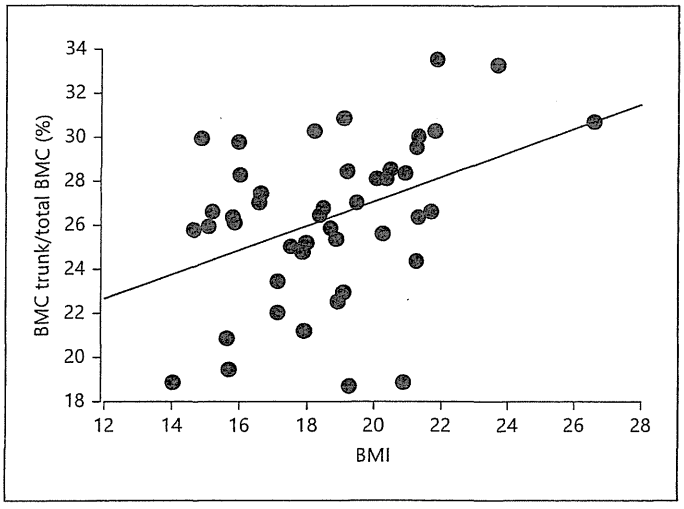

Fig. 2. Relationship between BMI and BMC trunk/total BMC.

Discussion

In the present study, we found that BMCI was lower in the whole body, legs and trunk in patients with COPD than in control subjects, although BMCI in the arms was similar between the groups. In addition, BMCI in each subregion correlated significantly with BMI, while only the ratio of trunk BMC to total BMC correlated significantly with BMI. We also demonstrated that BMCI was not significantly correlated with the severity of airflow limitation but was correlated with maximal exercise performance.

Multiple sites, including the hip, lumbar spine, forearm and whole body, can be used to measure BMD by DXA, and significant differences among various skeletal sites have been found in patients with osteoporosis [16]. A recent study demonstrated that BMD of the hip and lumbar spine may be more sensitive than whole-body BMD for the diagnosis of osteoporosis in patients with COPD [14]. However, BMD of the upper extremities was not evaluated in that study.

We found that BMCI in the arms of patients with COPD was comparable to that of control subjects, while BMCI was lower in the whole body, legs and trunk in patients with COPD. Although the mechanism of BMC preservation in the arms is unclear, a possible explanation may be mechanical stresses on the bones in the arms in daily life. The metabolic and ventilatory requirements as well as dyspnea during unsupported arm exercise are greater in patients with COPD than in healthy subjects [17]. However, the arms are recruited for many activities of daily living such as lifting, bathing and dressing in patients with COPD as well as healthy subjects, whereas patients with COPD tend to refrain from walking and standing in daily life [18].

Previous studies have demonstrated that a lower BMD is associated with low BMI [11, 19–21]. However, a relationship between regional change in BMC and BMI has not been examined. Our data demonstrated that BMCI in each subregion correlated significantly with BMI as well as total BMCI. In particular, BMCI in the trunk was closely related with BMI compared with that in the arms and legs. With regard to the distribution of BMC reduction, BMC trunk/total BMC correlated significantly with BMI, while BMC arms/total BMC and BMC legs/total BMC did not. A high prevalence of vertebral fracture in patients with COPD has been documented [3, 22–24]. The risk of vertebral fractures is related to disease severity [23, 24] and systemic corticosteroid use [22, 23]. Our

data suggest that body weight loss leads to a disproportional decrease in BMC in the trunk and raises the possibility that vertebral fractures may be common in underweight patients with COPD. It is known that the bones in the trunk, including the vertebrae and ribs, consist predominantly of cancellous bone, which has enhanced bone metabolism. Thus, BMC in the trunk may be more susceptible to malnutrition than that in the extremities.

The deterioration of the microarchitecture and bone remodeling are also important risk factors for bone fractures in patients with COPD. Microarchitectural changes can be assessed by histomorphometric analysis or microcomputed tomographic analysis of bone biopsy samples [25], which are too invasive to be considered in a routine clinical setting. Therefore, these analyses were not performed in the present study. Bone turnover markers represent bone remodeling and are commonly used as independent predictors of fracture risk [26]. In patients with COPD, these markers can be significant predictors of fractures independent of BMD, but we did not determine them in the present study.

Several studies have reported a strong correlation between the prevalence of osteoporosis and reduced FFM in patients with COPD [23, 24, 27]. In line with these studies, a significant correlation between total BMCI and total FFMI was found in our study, while the distribution of BMC differed from that of FFMI.

A higher Global Initiative for Chronic Obstructive Lung Disease stage and/or a lower FEV_1 have been shown to be correlated with osteoporosis and/or a low BMD [20, 28, 29]. Moreover, a significant correlation between FEV_1 and BMD in subjects without COPD [30, 31] has been reported. On the other hand, several studies have demonstrated no significant relationship between $\%FEV_1$ and BMD in patients with COPD [3, 10, 27, 32]. These relationships between pulmonary function parameters and BMD are complex and not yet clear. In the present study, we found no significant relationship between total or subregional BMCI and $\%FEV_1$. However, BMCI was significantly correlated with %DLCO in accordance with other studies [33, 34].

Reduced physical activity due to impaired pulmonary function may cause osteoporosis [35]. Patients with COPD have been shown to be physically inactive compared with age-matched healthy subjects [36]. Although an association between physical activity and BMD in healthy women has been demonstrated [37, 38], the effect of exercise capacity on BMD has not been fully elucidated in patients with COPD. A significant relationship between osteoporosis and 6-min walk distance was reported in patients with COPD [3]. In a longitudinal study, the change in total BMC was shown to be significantly correlated with 12-min walk distance [39]. In the present study, a significant relationship between BMCI and $\dot{V}O_2max$ was demonstrated. This result indicated that the reduction of bone mass was related to exercise intolerance, not to the severity of airflow limitation.

Furthermore, systemic inflammation has been considered a major cause of osteoporosis in COPD. Several studies have demonstrated that physical inactivity deteriorates exercise-induced oxygen desaturation [40] and systemic inflammation and results in higher serum levels of interlekin-6 and tumor necrosis factor-α [41, 42], which can contribute to osteoporosis [43]. These findings suggest that active patients with higher $\dot{V}O_2max$ levels may have lower levels of these cytokines, resulting in preserved bone mass. In addition, $\dot{V}O_2max$ is known to be determined by circulatory and ventilatory capacity and exercise muscle performance. We hypothesize that oxygen delivery to bone tissue may be better in patients with higher $\dot{V}O_2max$ than in those with lower $\dot{V}O_2max$.

Moreover, we demonstrated that BMC trunk/total BMC correlated significantly with $\dot{V}O_2max$, while BMC arms/total BMC and BMC legs/total BMC did not. Although the precise mechanism is unclear, several possible explanations for the significant relationship between BMC trunk/total BMC and $\dot{V}O_2max$ can be offered. The effects of cytokines on bone metabolism may differ between the extremities and the vertebrae. The relationship between cytokine production by peripheral blood mononuclear cells and the rate of annual change in BMD has shown significant differences in the lumbar spine and the femoral neck, possibly reflecting differences in the proportion of trabecular and cortical bone at these sites [44]. Accordingly, we speculate that inactive patients with systemic inflammation may have lower BMD in the lumbar spine than in the extremities.

Decreased BMC in the trunk is a risk factor for vertebral fractures, which were not evaluated in the present study. Kyphosis due to vertebral fractures may reduce pulmonary function and ventilatory capacity [45]. In the present study, BMC trunk/BMC total was significantly correlated with maximal voluntary ventilation, which is a significant determinant of $\dot{V}O_2max$ [46] (data not shown). This may partly explain the significant relationship between BMC trunk/BMC total and $\dot{V}O_2max$.

Our study has several limitations. Firstly, we did not determine pulmonary function parameters, except for spirometric data and $\dot{V}O_2max$ in the controls. Accord-

ingly, it is unknown whether BMCI is significantly correlated with %DLCO and $\dot{V}O_2$max in both patients with COPD and control subjects. Secondly, despite evidence of statistical significance, a direct cause-effect relationship between $\dot{V}O_2$max and BMC trunk/BMC total could not be established. An interventional study is required that includes exercise training and/or antiosteoporotic medication. Thirdly, our study included a small number of patients with COPD and control subjects. Therefore, a future study with a larger number of subjects is required to validate our findings.

In conclusion, the current study demonstrated a regional difference in BMC reduction in patients with COPD and that BMC reduction is not associated with the severity of airflow limitation but rather with body weight loss and exercise intolerance. Body weight loss and exercise intolerance are more closely related to BMC reduction in the trunk than in the extremities. These data suggest that weight loss and exercise intolerance are important risk factors for vertebral fractures in patients with COPD.

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Financial Disclosure and Conflicts of Interest

All authors acknowledge that there are no conflicts of interest involving any companies/organizations whose products or services may have influenced this study.

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Original Article

Branched-chain amino acid-rich diet improves skeletal muscle wasting caused by cigarette smoke in rats

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ABSTRACT — Cigarette smoke induces skeletal muscle wasting by a mechanism not yet fully elucidated. Branched-chain amino acids (BCAA) in the skeletal muscles are useful energy sources during exercise or systemic stresses. We investigated the relationship between skeletal muscle wasting caused by cigarette smoke and changes in BCAA levels in the plasma and skeletal muscles of rats. Furthermore, the effects of BCAA-rich diet on muscle wasting caused by cigarette smoke were also investigated. Wistar Kyoto (WKY) rats that were fed with a control or a BCAA-rich diet were exposed to cigarette smoke for four weeks. After the exposure, the skeletal muscle weight and BCAA levels in plasma and the skeletal muscles were measured. Cigarette smoke significantly decreased the skeletal muscle weight and BCAA levels in both plasma and skeletal muscles, while a BCAA-rich diet increased the skeletal muscle weight and BCAA levels in both plasma and skeletal muscles that had decreased by cigarette smoke exposure. In conclusion, skeletal muscle wasting caused by cigarette smoke was related to the decrease of BCAA levels in the skeletal muscles, while a BCAA-rich diet may improve cases of cigarette smoke-induced skeletal muscle wasting.

Key words: Cigarette smoke, Branched-chain amino acids, Skeletal muscle wasting, Negative energy balance

INTRODUCTION

Cigarette smoke induces skeletal muscle wasting (Barreiro *et al.*, 2010; Liu *et al.*, 2011; Nakatani *et al.*, 2003; Rinaldi *et al.*, 2012; Rom *et al.*, 2012). Systemic effects caused by cigarette smoke such as negative energy balance, oxidative stress, and systemic inflammation contribute to skeletal muscle wasting (Liu *et al.*, 2011; Rom *et al.*, 2012). However, the precise mechanism by which cigarette smoke induces muscle wasting has not been fully elucidated.

Valine (Val), leucine (Leu), and isoleucine (Ile) are classified as branched-chain amino acids (BCAAs). BCAAs are metabolized mainly in skeletal muscles and are utilized as energy sources during exercise (Michael, 1996; Platell *et al.*, 2000). In patients with surgical stresses, advanced malignant diseases (Choudry *et al.*, 2006) and chronic diseases such as chronic obstructive pulmonary disease (COPD) (Jagoe and Engelen, 2003) and liver cirrhosis (Fischer *et al.*, 1976), plasma BCAA levels reduce.

In COPD, which is mainly caused by cigarette smoke, muscle wasting is one of the important comorbidities. The lowered BCAA levels are presumably related to an accelerated BCAA degradation in skeletal muscles caused by negative energy balance. In COPD, the degradation of BCAAs in skeletal muscles is thought to partially contribute to the degradation of muscle protein and skeletal muscle wasting (Engelen and Schols, 2003). A BCAA-rich diet has been reported to be useful for nutritional support in the rehabilitation of underweight patients with COPD (Kubo *et al.*, 2006).

Therefore, we hypothesized that cigarette smoke exposure alone would decrease plasma and muscle BCAA lev-

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els and that the decreases of BCAA levels related to the muscle wasting caused by cigarette smoke. In the present study, to test this hypothesis we investigated the relationship between cigarette smoke-induced skeletal muscle wasting and changes in BCAA levels in both plasma and skeletal muscles in rats. Furthermore, the effects of a BCAA-rich diet on cigarette smoke-induced skeletal muscle wasting were also investigated.

MATERIALS AND METHODS

Animals and diets

All procedures performed during these animal experiments were approved by our Institutional Ethics Committee in accordance with the Guidelines for Animal Experiments of the Nara Medical University School of Medicine and the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society.

Experimental animals and cigarette smoke exposure

Ten-week-old, male Wistar Kyoto (WKY/Izm) rats were purchased from Japan SLC, Inc. (Shizuoka, Japan) and were fed with AIN-93G (control diet; Oriental Yeast Co., Ltd., Tokyo, Japan) or a BCAA-rich diet and water *ad libitum* throughout the preconditioning and experimental periods in the Laboratory Animal Research Center at Nara Medical University. Animals were kept in limited-access barrier housing at a room temperature of 22°C ± 1°C, with a humidity level of 55% ± 10%, and a 12-hr light/dark cycle, with illumination from 08:00 to 20:00 hr.

As a control diet, AIN-93G was prepared. For the BCAA-rich diet, 2.5% BCAAs (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) were added to the AIN-93G content. The composition of the control diet and the BCAA-rich diet are shown in Table 1.

Animals were compulsively exposed to cigarette smoke using a tobacco smoke exposure apparatus (MIPS, Inc., Osaka, Japan) based on the method by Tomoda *et al.* (2011, 2012). The animals were provided the following 4 groups depending on whether they were exposed to the cigarette smoke and the diets they received: non-smoking/control diet, non-smoking/BCAA-rich diet, smoking/control diet, and smoking/BCAA-rich diet. Eight rats fed with control diet (n = 4) or BCAA-rich diet (n = 4) were exposed to smoke from 30 cigarettes for 20 min between 08:00 and 10:00 A.M. for five days a week, (Monday to Friday) for four weeks. At the same time eight rats fed with a control diet (n = 4) or BCAA-rich diet (n = 4) in the non-smoking group were also kept for 20 min in the apparatus holders. Smoke was generated by inhaling the fired cigarette with compressor in the apparatus. The generated smoke was mixed with 7 volumes of air and the mixture was used as exposed smoke. The smoke was moved via a soft flexible tube from the apparatus to a chamber connected with holders in which 8 animals were kept separately. 2 sec of the smoke, followed by 2 sec of air was inhaled to each animal via their noses at a rate of 15 puffs per minute.

Body weight was recorded during the experimen-

Table 1. Components of control (AIN-93G) and BCAA-rich diets.

Ingredient	Control (AIN-93G)	BCAA-rich
Cornstarch	132.000	132.000
α-Corn Starch	397.486	397.486
Casein	200.000	175.000
BCAA mix*	0.000	25.000
Sucrose	100.000	100.000
Soybean oil	70.000	70.000
Dietary Fiber	50.000	50.000
Mineral mix	35.000	35.000
Vitamin mix	10.000	10.000
L-Cystine	3.000	3.000
Choline bitartrate	2.500	2.500
Tert-butylhydroquinone	0.014	0.014

*Isoleucine:Leucine:Valine = 1:2:1 (g/kg)

tal period. The animals were decapitated under anesthesia using intraperitoneal sodium pentobarbital injection (50 mg/kg) within 12 hr after the last cigarette smoke exposure, and BCAA levels in the plasma and skeletal muscles were evaluated.

Measurement of BCAA levels in plasma and skeletal muscles

BCAA levels in plasma and skeletal muscles were measured according to the method by Deyl *et al.* (1986). Twelve hours after the final cigarette smoke exposure, a whole blood sample was collected from the abdominal artery of each animal per group, under anesthesia with pentobarbital sodium (50 mg/kg i.p.). Plasma was separated by centrifugation and stored at -80°C. After merciful death, the bilateral soleus and gastrocnemius skeletal muscles were dissected and weighed; the right gastrocnemius muscles were stored at -80°C until measurement of BCAA levels. Each muscle per animal was homogenized by suspending the material in saline solution. The specimens were centrifuged at 3,000 rpm for 20 min at 4°C. The supernatant fraction was used for amino acid analysis. A 500 µl aliquot of the supernatant and plasma was mixed with 750 µl of 5% sulfosalicylic acid and then centrifuged at 3,000 rpm for 20 min at 4°C. The BCAA levels in the muscle and plasma were measured from the supernatants using high-performance liquid chromatography (HPLC) (Hitachi, Ltd.,Tokyo, Japan).

Statistical analysis

Parameters between the two groups were compared using the Mann-Whitney U test. Independent correlations of skeletal muscle BCAA levels with muscle weight were examined using multivariable regression analyses. Comparisons of food intake and body weight between the two groups were performed with two-way analysis of variance. The level of statistical significance was set as $p < 0.05$.

RESULTS

Changes in food intake of each group during the entire experimental period

Fig. 1 shows changes in food intake during the entire experimental period. Cigarette smoke significantly decreased food intake in both control diet and BCAA-rich diet groups. Food intake was significantly decreased in BCAA-rich diet groups at the first and second week in both non-smoking and smoking groups, whereas not at the third and last week.

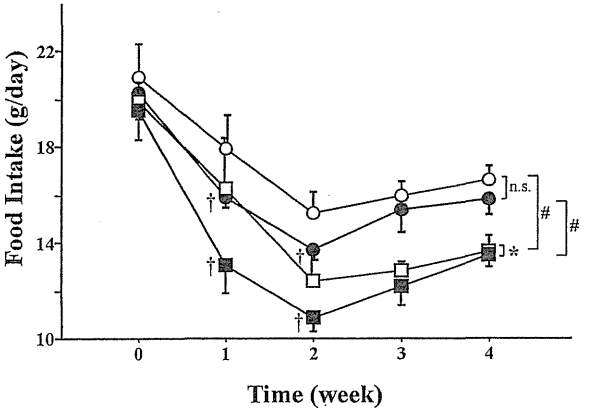

Fig. 1. Changes in food intake of each group during the entire experimental period. Each point indicates the mean ± S.D. of 4 animals. Symbols; Group A ○: non-smoking/control diet (n = 4), Group B ●: non-smoking/BCAA-rich diet (n = 4), Group C □: smoking/control diet (n = 4), Group D ■: smoking/BCAA-rich diet (n = 4). $^{\#}p < 0.01$: Group A vs. Group C, and Group B vs. Group D. $^{*}p < 0.05$: Group C vs. Group D. †$p < 0.05$: Group A vs. Group B and Group C vs. Group D at the first and the second week. n.s., not significant Data were statistically analyzed by the Mann-Whitney U test and two-way analysis of variance (ANOVA).

Changes in body weight of each group during the entire experimental period

Fig. 2 shows changes in body weight during the entire experimental period. Cigarette smoke significantly inhibited body weight gain in both BCAA-rich diet and control diet groups. However there were no significant differences in body weight between BCAA-rich diet and control diet groups in both non-smoking and smoking groups.

Effect of a BCAA-rich diet on skeletal muscle weight

Fig. 3 shows changes in skeletal muscle weight after the last cigarette smoke exposure. In rats fed with the control diet, cigarette smoke significantly decreased muscle weight (4.6 ± 0.2 vs. 4.2 ± 0.1 g, respectively, $p = 0.028$). Conversely, in both the non-smoking and smoking groups, rats fed with the BCAA-rich diet showed significant increases in the muscle weight (4.6 ± 0.2 vs. 5.0 ± 0.2 g, respectively, $p = 0.038$) (4.2 ± 0.1 vs. 4.5 ± 0.3 g, respectively, $p = 0.047$). Cigarette smoke decreased the skeletal muscle weight but a BCAA-rich diet increased the muscle weight that had reduced due to cigarette smoke exposure.

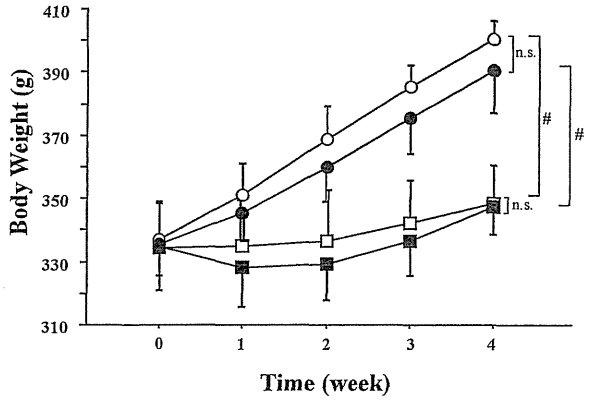

Fig. 2. Changes in body weight of each group during the entire experimental period. Each point indicates the mean ± S.D. of 4 animals. Symbols; Group A ○: non-smoking/control diet (n = 4), Group B ●: non-smoking/BCAA-rich diet (n = 4), Group C □: smoking/control diet (n = 4), Group D ■: smoking/BCAA-rich diet (n = 4). $^{\#}p < 0.01$: Group A vs. Group C, and Group B vs. Group D. Data were statistically analyzed by two-way analysis of variance (ANOVA).

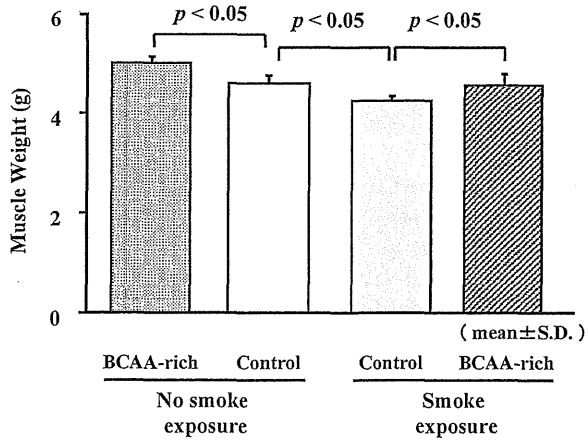

Fig. 3. The effect of the BCAA-rich diet on changes in soleus and gastrocnemius muscle weight. Cigarette smoke exposure significantly decreased muscle weight in the control diet groups. The BCAA-rich diet significantly increased skeletal muscle weight that had reduced due to cigarette smoke exposure, while in the non-smoking groups, the BCAA-rich diet significantly increased the muscle weight.

Effect of a BCAA-rich diet on changes in plasma BCAA levels

Fig. 4 shows changes in plasma BCAA levels after the last cigarette smoke exposure. Changes in each plasma BCAA levels are shown in Table 2. In the control diet groups, cigarette smoke decreased plasma BCAA levels (439.6 ± 15.6 vs. 392.1 ± 41.7 nmol/ml, respectively, $p = 0.083$). Val and Ile levels had significantly decreased. On the other hand, in both the non-smoking and smoking groups, BCAA-rich diet significantly increased plasma BCAA levels (non-smoking: 439.6 ± 15.6 vs. 495.2 ± 14.3 nmol/ml, respectively, $p = 0.021$) (smoking; 392.1 ± 41.7 vs. 453.8 ± 20.3 nmol/ml, respectively, $p = 0.043$). All plasma BCAAs (Val, Leu, and Ile) had significantly increased. Cigarette smoke decreased plasma BCAA levels but a BCAA-rich diet increased plasma BCAA levels that had decreased due to cigarette smoke exposure.

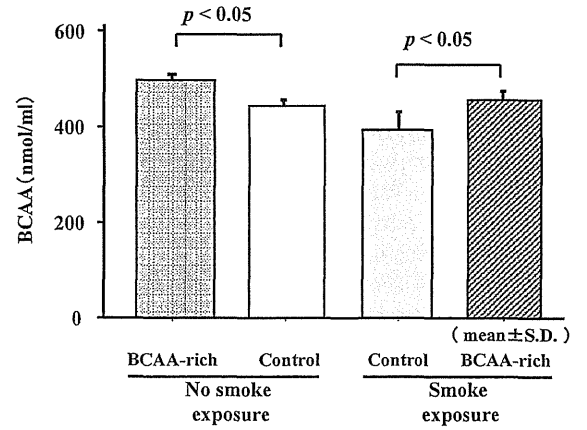

Fig. 4. The effect of the BCAA-rich diet on changes in plasma BCAA levels. Cigarette smoke exposure decreased plasma BCAA levels in the control diet groups. The BCAA-rich diet significantly increased the plasma BCAA level that had reduced due to cigarette smoke, while in the non-smoking groups, the BCAA-rich diet significantly increased the plasma BCAA level.

Effect of a BCAA-rich diet on changes in muscle BCAA levels

Fig. 5 shows changes in skeletal muscle BCAA levels after the last cigarette smoke exposure. Changes in BCAA levels in the muscle are shown in Table 3. In rats fed with the control diet, cigarette smoke significantly decreased muscle BCAA levels (544.2 ± 22.3 vs. 466.0 ± 47.5 nmol/ml, respectively, $p = 0.021$). Among the BCAAs, Val levels had significantly decreased. In smoking groups, the BCAA-rich diet had significantly increased muscle BCAA levels (466.0 ± 47.5 vs. 533.7 ± 43.4 nmol/ml, respectively, $p = 0.021$). Among the BCAAs, Val and Ile lev-

Table 2. The effect of BCAA-rich diet on changes in plasma BCAA levels

	No smoke exposure		Smoke exposure	
	BCAA-rich	Control	Control	BCAA-rich
Val (nmol/ml)	[c]209.3 ± 5.0	190.9 ± 7.0	[a]166.0 ± 16.1	[b]189.1 ± 6.9
Leu (nmol/ml)	[c]166.0 ± 6.4	142.7 ± 6.6	133.4 ± 13.1	[b]155.0 ± 13.1
Ile (nmol/ml)	[c]119.9 ± 4.0	106.0 ± 2.7	92.7 ± 12.7	[b]109.7 ± 5.7

(mean ± S.D.)

[a]$p < 0.05$: Smoke exposure v.s. No smoke exposure in the control diet groups.

[b]$p < 0.05$: BCAA v.s. control diet in the smoking groups.

[c]$p < 0.05$: BCAA rich diet v.s. control diet in the non-smoking groups.

Cigarette smoke exposure significantly decreased plasma Val levels in the control diet groups. The BCAA-rich diet significantly increased plasma BCAA levels in both the non-smoking and smoking groups.

Table 3. The effect of BCAA-rich diet on changes in BCAA levels of gastrocnemius muscles

	No smoke exposure		Smoke exposure	
	BCAA-rich	Control	Control	BCAA-rich
Val (nmol/g)	237.1 ± 15.5	229.8 ± 6.4	[a]191.7 ± 13.1	[b]221.7 ± 13.6
Leu (nmol/g)	191.5 ± 10.3	188.8 ± 18.1	162.1 ± 10.3	177.4 ± 22.7
Ile (nmol/g)	138.6 ± 8.2	125.7 ± 9.6	112.2 ± 8.4	[b]134.6 ± 17.9

(mean ± S.D.)

[a]$p < 0.05$: Smoke exposure v.s. No smoke exposure in the control diet groups.

[b]$p < 0.05$: BCAA-rich diet v.s. control diet in the smoking groups.

Cigarette smoke exposure significantly decreased skeletal muscle Val levels in the control diet groups. The BCAA-rich diet significantly increased skeletal muscle Val and Ile levels in the smoking groups. However, the BCAA-rich diet did not change BCAA levels in the non-smoking groups.

els had significantly increased. On the other hand, in the non-smoking group, the BCAA-rich diet did not change the muscle BCAA levels. Neither muscle Val, Leu, nor Ile levels had changed.

Cigarette smoke decreased the muscle BCAA levels as well as plasma BCAA levels. A BCAA-rich diet increased the muscle BCAA levels decreased by cigarette smoke, while it did not change the levels in the non-smoking groups.

Relationship between muscle BCAA levels and muscle weight

As shown in Fig. 6, muscle BCAA levels were significantly correlated to the muscle weight in all rats in this study ($Y = 2.528 + 0.004X$; $r = 0.616$, $p = 0.011$).

DISCUSSION

Systemic inflammation, oxidative stresses, and negative energy balance may also contribute to the muscle wasting caused by cigarette smoke. (Liu *et al.*, 2011; Rom *et al.*, 2012). However, the mechanisms by which muscle wasting occur have not been fully elucidated. In the present study, cigarette smoke induced skeletal muscle wasting with decreased BCAA levels in both plasma and skeletal muscles in rats. Furthermore, the BCAA-rich diet improved the skeletal muscle wasting by cigarette smoke with increase in the BCAA levels in both plasma and skeletal muscle and a correlation between skeletal muscle weight and BCAA levels in the skeletal muscle were demonstrated. These results suggest that the decrease in skeletal BCAA levels may relate with the muscle wasting by cigarette smoke.

However the present study did not make clear how cigarette smoke decrease the BCAA levels. One of the important mechanisms is a decrease in food intake and body weight caused by cigarette smoke. This study demonstrated that BCAA-rich diet improved the muscle wasting caused by cigarette smoke without improvement of food intake or of body weight. These results suggest that, besides the decrease in body weight gain and in food intake, there were other factors contribute to the muscle wasting. BCAAs in skeletal muscles are metabolized to glutamine or alanine (Goodman *et al.*, 1981). Alanine is delivered to the liver and used for glycogenesis, while glutamine is delivered to the small intestine where it is used as an energy source for regeneration of the intestinal mucosa. We had previously demonstrated that cigarette

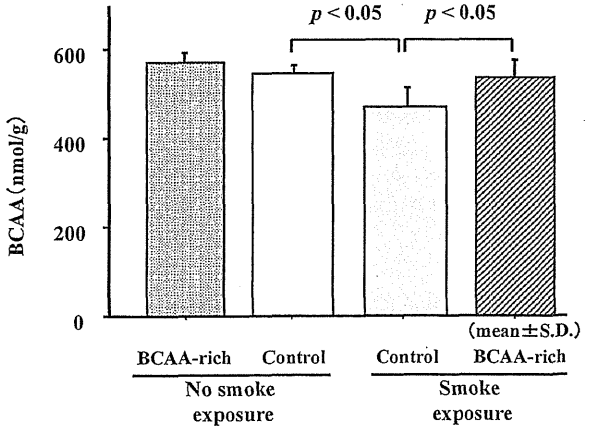

Fig. 5. The effect of the BCAA-rich diet on changes in gastrocnemius muscle BCAA levels. Cigarette smoke exposure significantly decreased skeletal muscle BCAA levels in the control diet groups. The BCAA-rich diet significantly increased the skeletal muscle BCAA level that had reduced due to cigarette smoke exposure. However, in the non-smoking groups, the BCAA-rich diet did not change the skeletal muscle BCAA level.

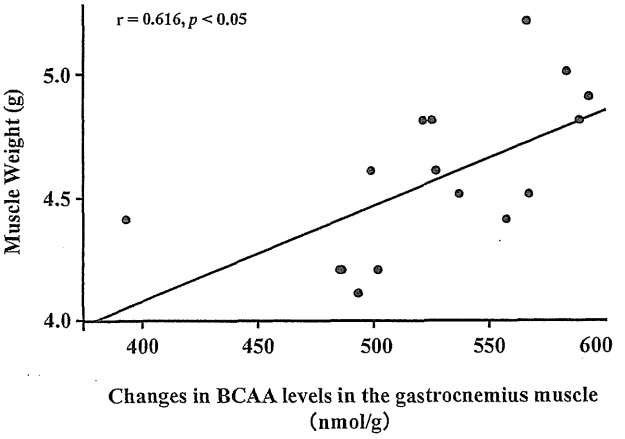

Fig. 6. The relationship between gastrocnemius muscle BCAA levels and muscle weight. The skeletal muscle BCAA levels were significantly correlated with muscle weight in all the rats ($y = 2.528 + 0.004x$; $r = 0.616$, $p < 0.05$).

smoke altered the gut environment (Tomoda *et al.*, 2011). The increase in glutamine usage related to the alteration in the gut environment caused by cigarette smoke that may partially contribute to the decrease in skeletal muscle BCAA levels. Further investigations about how cigarette smoke decreases the BCAA levels are needed.

A BCAA-rich diet improves skeletal muscle weight that is reduced due to cigarette smoke exposure; this increase is accompanied with the increase in BCAA levels in both the plasma and muscles. Increased BCAA levels in the skeletal muscle, achieved by increasing BCAA supply, may reduce muscle wasting caused by cigarette smoke exposure. BCAA has a problem of possessing a bitter taste and a characteristic smell of amino acids. Therefore adding BCAA to a food likely makes it difficult to intake the same amount of food as without it. At the first and second week, food intake was significantly decreased in BCAA-rich diet groups in both non-smoking and smoking groups. However at the third and last week there were no significant differences between control and BCAA-rich diet groups. In body weight there was no significant differences between control diet and BCAA-rich diet in both non-smoking and smoking groups. These results in this study suggests that decrease in food intake by BCAA-rich diet at the first and second week may have only few effect on the improvement of muscle wasting caused by BCAA-rich diet. If the taste and smell of BCAA-rich diet would be improved, we could expect more effects of BCAA on cigarette smoke-induced skeletal muscle wasting.

Interestingly in this study in non-smoking group the BCAA-rich diet increased the muscle weight. BCAA levels in plasma were increased while those in muscles were not changed. These results suggested that, besides the supply of BCAA, other factors to increase the skeletal muscle weight would exist. Among BCAAs, Leu stimulates protein synthesis through the activation of a key mediator of protein synthesis, mammalian target of rapamycin (mTOR), found in human skeletal muscle (Greiwe *et al.*, 2001). Anabolic effects by BCAA-rich diet may partially contribute to the decrease in the muscle weight in the non-smoking rats. Further investigation about some markers of the mTOR and myofibrillar content should be needed.

One of the limitations of this study is the small size of the sample. However, our analysis showed a statistically significant difference between groups, and we feel that sample size of this study, although limited, is enough to gain some understanding of relationship between BCAA and cigarette smoke -induced skeletal muscle wasting.

In conclusion, cigarette smoke exposure reduced skeletal muscle weight and body weight gain and also reduced BCAA levels in both plasma and skeletal muscles. Furthermore, a BCAA-rich diet counteracted the decrease in skeletal muscle weight that was caused by cigarette smoke

exposure; this increase occurred by increasing BCAA levels in both the plasma and muscles.

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RESPIRATORY RESEARCH

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Analysis of comorbid factors that increase the COPD assessment test scores

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Abstract

Background: The chronic obstructive pulmonary disease (COPD) Assessment Test (CAT) is a concise health status measure for COPD. COPD patients have a variety of comorbidities, but little is known about their impact on quality of life. This study was designed to investigate comorbid factors that may contribute to high CAT scores.

Methods: An observational study at Keio University and affiliated hospitals enrolled 336 COPD patients and 67 non-COPD subjects. Health status was assessed by the CAT, the St. Georges Respiratory Questionnaire (SGRQ), and all components of the Medical Outcomes Study Short-Form 36-Item (SF-36) version 2, which is a generic measure of health. Comorbidities were identified based on patients' reports, physicians' records, and questionnaires, including the Frequency Scale for the Symptoms of Gastro-esophageal reflux disease (GERD) and the Hospital Anxiety and Depression Scale. Dual X-ray absorptiometry measurements of bone mineral density were performed.

Results: The CAT showed moderate-good correlations with the SGRQ and all components of the SF-36. The presence of GERD, depression, arrhythmia, and anxiety was significantly associated with a high CAT score in the COPD patients.

Conclusions: Symptomatic COPD patients have a high prevalence of comorbidities. A high CAT score should alert the clinician to a higher likelihood of certain comorbidities such as GERD and depression, because these diseases may co-exist unrecognized.

Trial registration: Clinical trial registered with UMIN (UMIN000003470).

Keywords: Chronic obstructive pulmonary disease, Health status, Depression, Gastro-esophageal reflux, Comorbidity, Osteoporosis

Background

Chronic obstructive pulmonary disease (COPD) is characterized by progressive and partially reversible airflow limitation, and it is among the leading causes of mortality worldwide [1]. COPD patients manifest a range of comorbidities, some of which may worsen quality of life (QOL) [2], and others may increase the risk of death [3].

According to the latest version of the Global Initiative for Chronic Obstructive Lung Disease (GOLD) guideline, assessment of COPD should be based on the patient's level of symptoms, future risk of exacerbations, and the severity of spirometric abnormalities [4]. A number of questionnaires are available that assess COPD-specific health status, including the St. Georges Respiratory Questionnaire (SGRQ) [5] and the Chronic Respiratory Questionnaire [6]. These are validated and widely used for clinical trials, but they are complex and require special software or licenses to use, limiting their routine applicability in clinical practice. A newly developed questionnaire, the COPD Assessment Test (CAT), offers an alternative to those complex tools [7]. It consists of eight items, each presented as a 6-point semantic differential scale, providing a score out of 40, indicating the impact of the disease.

The usefulness of CAT has recently been reported in a variety of clinical settings, such as for evaluating the

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severity of COPD exacerbations and the effects of rehabilitation [8-10]. However, relatively little is known about the impact of comorbidities on the CAT score of individuals with COPD. A recent study reported that the presence of cardiovascular comorbidity did not significantly affect the CAT score [11]. In another study, the CAT score appeared unaffected by potentially confounding comorbidities including renal failure, obesity, and sleep disorder [8]. A recent study has shown that metabolic and cardiovascular comorbidities may increase in frequency in worse GOLD groups [12]. However, the differential impact of other major comorbidities remains to be investigated.

We hypothesized that symptomatic COPD patients exhibiting high CAT scores have unrecognized comorbidities. Therefore, comorbid factors that might have an impact on increasing the CAT score in COPD patients enrolled in a well-characterized cohort study, called the Keio COPD Comorbidity Research (K-CCR), were evaluated.

Materials and methods

Study populations

Keio University and affiliated hospitals have established an observational COPD cohort designed to prospectively investigate the management of COPD comorbidities. A total of 572 subjects were enrolled between April 2010 and December 2012, including patients who had been diagnosed as having COPD and at risk for COPD (non-COPD) by pulmonary physicians. Inclusion criteria consisted of (1) age≧40 years old, (2) forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) < 0.7, (3) presence of emphysematous changes on chest computed tomography (CT) scans, and (4) chronic respiratory symptoms with significant smoking history (≧30 pack-years). Pulmonary function tests and chest CT scan were performed in all participants, and the COPD group fulfilled the criteria (1) and (2), while the non-COPD group met the criteria (1) and either (3) or (4) without airflow limitation (FEV_1/FVC≧0.7). Excluded were patients who had a history of lung resection surgery or serious complications such as unstable cardiovascular or cerebral diseases and malignant tumors under treatment. For the purpose of this study, only subjects with complete data available for comorbidities (n = 403) were enrolled. All patients were clinically stable and without exacerbations for at least one month prior to recruitment. The protocol was approved by the ethics committees of Keio University and the affiliated hospitals, and written, informed consent was obtained from each patient.

Assessment of clinical parameters

Spirometry was performed in all patients in a stable condition using an electronic spirometer in accordance with the guidelines of the American Thoracic Society [13]. Predicted values of spirometric measurements were derived from the guidelines for pulmonary function tests issued by the Japanese Respiratory Society [14]. Regular treatment was not changed prior to spirometric testing.

At enrollment, a full medical and smoking history and information about current pharmacological treatment were obtained, and clinical examinations were performed. Comorbid diagnoses were established using clinical history and examination findings, supported by a review of available medical records. All of the following questionnaires were completed by the patients themselves at home, when in the stable state.

Questionnaires on QOL

The Japanese version of the CAT was applied for the assessment of COPD-specific health status, together with the SGRQ in Japanese [5,15,16]. The Medical Outcomes Study Short-Form 36-Item (SF-36) version 2 was used to assess general health status [17].

Evaluation of gastro-esophageal reflux disease (GERD)

GERD symptoms were evaluated using a self-reported Frequency Scale for the Symptoms of GERD (FSSG) questionnaire, consisting of 12 items. This is known to reflect the severity of the endoscopic findings of GERD [18], with a cut-off score of 8 points for GERD [19].

Evaluation of anxiety and depression

Depression and anxiety were assessed at baseline using the Hospital Anxiety and Depression Scale (HADS) [20]. This is a validated screening tool for cases of depression and anxiety in both hospitalized and primary care patients with chronic diseases, including COPD [21]. The HADS consists of seven items for anxiety (HAD-A) and seven items for depression (HAD-D). The scores range from 0 to 21 for each subscale, with a score of 0–7 denoting a non-case, 8–10 a possible case, and 11 or higher a probable case, which may guide referral for psychological support [20].

Dual X-Ray Absorptiometry (DXA)

DXA measurements of bone mineral density (BMD) were performed at the hip and lumbar spine using a Hologic 4500A Discovery bone densitometer (Hologic, Bedford, MA) for 248 of the 336 COPD patients. The T-score was used for the evaluation of osteoporosis, in which a T-score greater than −1 is considered normal, -1 to −2.5 osteopenia, and less than −2.5 is diagnostic of osteoporosis [22,23].

Statistical analyses

Data are presented as means ± standard deviation (SD). Univariate associations between CAT scores and other