Plasma Orexin-A Levels and Body Composition in COPD*

Takeshi Matsumura, MD; Mika Nakayama, MD; Hiroaki Satoh, MD; Asuka Naito, MD; Kazuyuki Kamahara, MD; and Kiyohisa Sekizawa, MD

**Study objective:** To study the role of orexins in regulating body composition in patients with COPD.

**Design:** Prospective study.

**Patients and measurements:** We measured the plasma concentration of orexin-A in 20 patients with COPD and compared the results to those obtained from 10 age-matched control subjects. Patients with COPD were classified into two groups based on their body mass index (BMI): a normal weight (NW) group (BMI > 20) and an underweight (UW) group (BMI < 20).

**Results:** The plasma orexin-A level was significantly lower in patients with COPD than in control subjects. In patients with COPD, the level was significantly lower in the UW group than in the NW group. Plasma orexin-A levels significantly correlated with BMI and fat mass values, but there was no significant relationship between plasma orexin-A levels and the fat-free mass of patients with COPD.

**Conclusion:** These results suggest that orexin-A levels are altered with weight loss and changes in body composition in patients with COPD. (CHEST 2003; 123:1060–1065)

**Key words:** body composition; COPD; fat mass; orexin; weight loss

**Abbreviations:** BMI = body mass index; FFM = fat-free mass; FM = fat mass; NW = normal weight; ODI = oxygen desaturation index; TNF = tumor necrosis factor; UW = underweight; VC = vital capacity

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Weight loss is frequently observed in patients with COPD. Low body weight has been shown to be a poor prognostic factor because an inverse relationship has been established between low body weight and physical performance as well as survival, irrespective of degree of lung function impairment. Weight loss in patients with COPD is thought to result from an imbalance between the intake and energy expenditure, although the exact mechanism responsible for weight loss in patients with COPD is not fully understood.

Previous studies have shown that a substantial number of patients with COPD have increased resting energy expenditure. An increase in energy expenditure of breathing has been suggested as a reason for this observed increase. Animal studies and clinical findings suggest that elevated levels of inflammatory mediators were observed in chronic wasting diseases such as cancer, cystic fibrosis, cardiac cachexia, and COPD.

The applications of molecular and genetic techniques to the study of body weight regulation have given new insight into the physiology of appetite, energy consumption, and metabolic signaling. Kalra et al suggested that body weight is regulated by a feedback loop in which peripheral signals pertaining to nutritional status are integrated within a center in the hypothalamus. They suggest that neuropeptides are the essential effector molecules of the hypothalamus. Recently, a novel group of neuropeptides known as orexins (orexin-A and orexin-B) has been identified within the lateral hypothalamus. Orexins have been shown to stimulate feeding when injected intracerebroventricularly, and the expression of orexin-precursor (prepro-orexin) messenger RNA has been found to increase with food deprivation.

Although orexins are thought to be involved in body weight regulation, the relationship between orexins and body weight in patients with COPD has not been examined.

We therefore investigated the plasma orexin-A levels of patients with COPD with different body

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*From the Department of Respiratory Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba-city, Ibaraki, Japan.

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Correspondence to: Kiyohisa Sekizawa, MD, Department of Respiratory Medicine, Institute of Clinical Medicine, University of Tsukuba, Tsukuba-city, Ibaraki, 305-8575, Japan; e-mail: kiyo-se@md.tsukuba.ac.jp
weights in order to determine a relationship between plasma orexin-A levels and body composition. We also studied the correlation between plasma levels of orexin-A and tumor necrosis factor (TNF-α), which have been shown to relate to body weight loss and cachexia in patients with cancer.\textsuperscript{10,11}

**Materials and Methods**

**Subjects**

Twenty patients with smoking-related COPD were recruited from the Respiratory Outpatient Department at Tsukuba University Hospital. COPD was diagnosed on the basis of history, physical examination, and spirometric data, according to American Thoracic Society guidelines.\textsuperscript{12} Vital capacity (VC), FVC, and FEV\textsubscript{1} were measured using a standard spirometer (AutoSpirometer System 55; Minato; Osaka, Japan). Chronic airflow obstruction was defined as follows: (1) FEV\textsubscript{1}/FVC > 70%, and (2) FEV\textsubscript{1} > 80% of predicted values. Predicted values were calculated in accordance with the Japanese Thoracic Society statement on standardized lung function testing.\textsuperscript{13} Subjects were excluded if they received systemic corticosteroids. Patients with a history of malignancy, cardiac failure, diabetes mellitus, or other endocrine diseases were also excluded. This study was approved by the University of Tsukuba Ethics Committee, and informed consent was obtained from each subject. The control group consisted of 10 subjects shown to have normal pulmonary function (FEV\textsubscript{1}/FVC > 70%).

**Measurement of Plasma Orexin-A and TNF-α**

Blood was obtained from fasting subjects by vein puncture at 9 AM. Blood was collected in evacuated blood collection tubes containing ethylenediamine-tetraacetic acid. The plasma was separated from the blood cells by centrifugation at 1,000 g for 10 min at 4°C within 30 min of collection. Plasma samples were stored at −80°C until analysis. Plasma orexin-A was measured using a radioimmunoassay kit (Peninsula Laboratories; San Carlos, CA), as previously described.\textsuperscript{8,14} Briefly, the sample and the standard peptide were incubated with rabbit antiserum against

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**Table 1—Characteristics of the Subjects**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Control Subjects (n = 10)</th>
<th>COPD</th>
<th>NW (n = 13)</th>
<th>UW (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>62.0 ± 0.5</td>
<td>63.6 ± 2.7</td>
<td>63.7 ± 2.8</td>
<td></td>
</tr>
<tr>
<td>Height, m</td>
<td>1.63 ± 0.02</td>
<td>1.62 ± 0.02</td>
<td>1.61 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68.7 ± 2.7</td>
<td>58.0 ± 1.9</td>
<td>45.9 ± 2.0*</td>
<td></td>
</tr>
<tr>
<td>VC, L</td>
<td>3.44 ± 0.20</td>
<td>3.15 ± 0.19</td>
<td>2.60 ± 0.24†</td>
<td></td>
</tr>
<tr>
<td>VC, % of predicted</td>
<td>101.5 ± 5.1</td>
<td>96.0 ± 5.4</td>
<td>78.0 ± 5.2‡</td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1}, L</td>
<td>2.91 ± 0.19</td>
<td>1.22 ± 0.12</td>
<td>0.76 ± 0.13‡</td>
<td></td>
</tr>
<tr>
<td>FEV\textsubscript{1}, % of predicted</td>
<td>100.0 ± 5.5</td>
<td>42.6 ± 4.31</td>
<td>26.8 ± 3.91‡</td>
<td></td>
</tr>
<tr>
<td>PaO\textsubscript{2}, mm Hg</td>
<td>N.D.</td>
<td>76.8 ± 2.21</td>
<td>84.7 ± 2.80</td>
<td></td>
</tr>
<tr>
<td>PaCO\textsubscript{2}, mm Hg</td>
<td>N.D.</td>
<td>43.3 ± 1.69</td>
<td>38.6 ± 2.73</td>
<td></td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>101.1 ± 2.5</td>
<td>96.4 ± 1.7</td>
<td>93.0 ± 2.5</td>
<td></td>
</tr>
<tr>
<td>Orexin-A, pg/mL</td>
<td>23.5 ± 0.5</td>
<td>17.5 ± 0.9</td>
<td>14.1 ± 0.5‡</td>
<td></td>
</tr>
<tr>
<td>Pack-years</td>
<td>26.4 ± 10.7</td>
<td>67.7 ± 16.4</td>
<td>65.7 ± 9.7†</td>
<td></td>
</tr>
<tr>
<td>ODI, 4% dips/h</td>
<td>N.D.</td>
<td>0.94 ± 0.28</td>
<td>0.50 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SE.

\*p < 0.05 vs control subjects.

\*p < 0.01 vs control subjects.

\*p < 0.05 vs NW group patients.
Human orexin-A at 4°C for 16 to 24 h and 125I-orexin-A was added. After further incubation at 4°C for 16 to 24 h, antirabbit IgG raised in goat and normal rabbit serum was added and incubated at room temperature for 90 min. Radioimmunoassay buffer was added, the mixture was centrifuged at 1,700 g for 20 min, and the supernatant (sample) was separated. The amount of bound antibody in the sample was counted using a γ counter. The sensitivity of the assay was 10 pg/mL. We used an average value of replicate samples (n = 3) from same subject for analysis of plasma orexin-A. Serum levels of glucose were also measured. Plasma TNF-α was measured using an enzyme-linked immunosorbent assay kit (Japan Immunoresearch Laboratories, Gumma, Japan). The sensitivity of the assay was 5 pg/mL.

**Sleeping Status**

Sleeping status was measured as 4% oxygen desaturation index (ODI), the number of desaturation events ≥ 4%/h, using pulse oximetry (PULSOX M-24A; Teijin; Tokyo, Japan) in patients with COPD.

**Body Composition**

Body mass index (BMI) was calculated, and patients with COPD were categorized into two groups: normal weight (NW) patients (BMI > 20), or underweight (UW) patients (BMI < 20). Fat-free mass (FFM) and fat mass (FM) were measured using a bioelectrical impedance method,16–18 using a body fat analyzer (TBF-210; Tanita; Tokyo, Japan). Whole-body resistance was measured by standing barefoot on two electrodes.17 Age, sex, height, weight, and resistance were used as independent variables in the prediction of FFM and FM.17 A wide range of FFM values were obtained using the bioelectrical impedance method, all of which were in close agreement with FFM values measured by dual-energy radiograph absorptiometry.17

**Statistical Analysis**

Results are reported as means ± SE. Statistical analysis was performed using the Mann-Whitney U test in order to analyze differences between the two groups. The relationship between several continuous variables was evaluated using the Spearman rank correlation technique. A p value < 0.05 was considered significant.

**RESULTS**

Clinical characteristics and plasma orexin-A levels of patients with COPD and control subjects are shown in Table 1. Of 20 patients with COPD, 13 patients were considered to be of NW (NW group) and 7 patients were categorized as UW (UW group).

There were no statistical differences among the control subjects, NW patients with COPD, and UW patients with COPD with respect to age and height. There were no statistical differences among the NW patients and UW patients with sleeping status. Serum TNF-α level was undetectably low in all 20 patients. FEV₁ percentage of predicted was significantly lower in UW patients than in NW patients (p = 0.04), so the UW group had significantly greater degrees of airflow limitation. VC percentage of predicted was significantly lower in UW patients than in control subjects (p = 0.04).

Figure 1 shows the body composition and plasma orexin-A levels of control subjects and patients with COPD. BMI, FM, and FFM values were highest in control subjects and lowest in UW patients (Fig 1, top left, A; top right, B; and bottom left, C). Likewise, levels of plasma orexin-A were highest in control subjects and lowest in UW patients (Fig 1, bottom right, D).

![Figure 1](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21992/)

**Figure 2.** Relationship between BMI and plasma orexin-A levels (top, A), FM and plasma orexin-A levels (center, B), and FFM and plasma orexin-A levels (bottom, C) in patients with COPD. In patients with COPD, plasma orexin-A levels significantly correlated with BMI (r = 0.49, p = 0.03) (top, A) and FM (r = 0.53, p = 0.03) (center, B) values, whereas a significant relationship was not observed between FFM values and plasma orexin-A levels (r = 0.22, p = 0.35) (bottom, C).
In patients with COPD, plasma orexin-A levels significantly correlated with BMI (r = 0.49, p = 0.03) [Fig 2, top, A] and FM (r = 0.53, p = 0.02) [Fig 2, center, B] values, whereas a significant relationship was not observed between FFM values and plasma orexin-A levels (p = 0.35) [Fig 2, bottom, C]. In patients with COPD, neither BMI, FM, FFM, nor plasma orexin-A levels significantly correlated with FEV1 percentage of predicted in patients with COPD (Fig 3), and a significant relationship was not observed between ODI and plasma orexin-A levels (p = 0.77).

**Discussion**

This is the first clinical study to demonstrate the relationship between plasma orexin-A levels and body composition in patients with COPD. In the present study, plasma orexin-A levels were reduced in patients with COPD, compared to the control group. This was especially notable in UW patients with COPD. Furthermore, plasma orexin-A levels correlated significantly with the BMI and FM values of patients with COPD. These results suggest that orexin-A might be altered with weight loss in patients with COPD. Since patients with diabetes mellitus were excluded from the present study, these results were probably not influenced by elevated serum glucose levels.19,20

Food deprivation in animals increased abundance of orexin-precursor messenger RNA.8,9 Therefore, increased levels in depleted patients as a compensatory mechanism would be expected. However, in UW patients with COPD, decreased plasma levels of orexin-A were observed. One of the explanations why this paradoxical phenomenon might have occurred is because orexin-A may play an important role not only in the promotion of body weight change but also in the regulation of energy metabolism. Another explanation may be that what happens nutritionally in patients with COPD is quite different from that in subjects who are merely depleted.

Orexins are newly discovered neuropeptides that act as endogenous ligands for an orphan G-protein–coupled receptor.8 Orexin-A consists of 33 amino acids, and its sequence shares a 46% identity with that of orexin-B. Orexins are produced from a single protein precursor encoded by orexin-precursor messenger RNA. Orexin-containing neurons are located both within, and adjacent to, the lateral hypothalamus. These areas have been implicated in the regulation of food intake, body weight, and energy homeostasis.9,21 It has been shown that food intake is stimulated on administration of synthetic orexins into brain ventricles,8,22 or the lateral hypothalamus.23 A dose-dependent suppression of food intake has been achieved by central injection of anti-orexin antibody.

**Figure 3.** Relationship between BMI and FEV1 percentage of predicted (% pred) (top left, A), FM and FEV1 percentage of predicted (top right, B), FFM and FEV1 percentage of predicted (bottom left, C), and plasma orexin-A levels and FEV1 percentage of predicted (bottom right, D) in patients with COPD. Neither BMI (r = 0.39, p = 0.09) [top left, A], FM (r = 0.38, p = 0.10) [top right, B], FFM (r = 0.16, p = 0.48) [bottom left, C], nor plasma orexin-A levels (r = 0.07, p = 0.77) [bottom right, D] were significantly correlated with FEV1 percentage of predicted in patients with COPD.
to fasted rats, indicating that orexins may have physiologically relevant action on feeding behavior.

Plasma orexin-A levels and BMI, FFM, and FM values were highest in the control group and lowest in UW patients with COPD, indicating that plasma orexin-A levels might influence body composition in patients with COPD. However, a reduction in plasma orexin-A levels and BMI, FFM, and FM values may not be a reflection of impaired pulmonary function because none of these parameters were found to be related to FEV1 percentage of predicted in patients with COPD. Although a significant relationship was found between plasma orexin-A levels and BMI and FM values, no relationship was found between orexin-A levels and FFM (a measure of functional tissue depletion). These findings suggest that orexin-A might influence the body composition of patients with COPD through regulation of FM, rather than by directly altering their global body composition. The mechanism by which orexin-A regulates fat distribution in patients with COPD is still uncertain. Leptin, a hormone produced mainly by adipocytes, has been found to play an important role in the regulation of energy metabolism, and has been shown to participate in the regulation of orexin levels in animals; and it has been suggested that leptin might provide negative feedback in the regulation of FM in patients with COPD.

Plasma leptin levels have been shown to relate to BMI and percentage of fat in patients with COPD, as well as to the FM values of patients with emphysema. Leptin, orexin-A, and TNF-α are well-known and important factors relevant to body weight loss. However, the relationship among these factors in patients with COPD is still unknown. In the present study, we showed that there was no correlation between plasma levels of orexin-A and those of TNF-α. Although plasma leptin levels were not measured in the present study, it is possible that interactions between orexin-A and leptin might be responsible for alterations in the body composition in patients with COPD. The interaction of these two factors will be clarified in the near future.

The present study shows that orexin-A levels may be associated with alteration of body composition in patients with COPD. Studies on the orexin system may provide new insight into the control of body composition in patients with COPD.

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