Nocturnal reduction in circulating adiponectin concentrations related to hypoxic stress in severe obstructive sleep apnea-hypopnea syndrome

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Nakagawa Y, Kishida K, Kihara S, Sonoda M, Hirata A, Yasui A, Nishizawa H, Nakamura T, Yoshida R, Shimomura I, Funahashi T. Nocturnal reduction in circulating adiponectin concentrations related to hypoxic stress in severe obstructive sleep apnea-hypopnea syndrome. Am J Physiol Endocrinol Metab 294:E778–E784, 2008. First published January 15, 2008; doi:10.1152/ajpendo.00709.2007.—Previous reports demonstrated that adiponectin has antiatherosclerotic properties. Obstructive sleep apnea-hypopnea syndrome (OSAHS) is reported to exacerbate atherosclerotic diseases. We investigated nocturnal alternation of serum adiponectin levels before sleep and after wake-up in OSAHS patients and the effect of sustained hypoxia on adiponectin in vivo and in vitro. We measured serum adiponectin concentrations in 75 OSAHS patients and 18 control subjects before sleep and after wake-up and examined the effect of one-night nasal continuous positive airway pressure (nCPAP) on adiponectin in 24 severe OSAHS patients. We investigated the effects of hypoxia on adiponectin in mice and cultured adipocytes with a sustained hypoxia model. Circulating adiponectin levels before sleep and after wake-up were lower in severe OSAHS patients than in control subjects [before sleep: 5.9 ± 2.9 vs. 8.8 ± 5.6 μg/ml (P < 0.05); after wake-up: 5.2 ± 2.6 vs. 8.5 ± 5.5 μg/ml (P < 0.01), respectively; means ± SD]. Serum adiponectin levels diminished significantly during sleep in severe OSAHS patients (P < 0.0001), but one-night nCPAP improved the drop in serum adiponectin levels [−18.4 ± 13.4% vs. −10.4 ± 12.4% (P < 0.05)]. In C57BL/6J mice and 3T3-L1 adipocytes, hypoxic exposure decreased adiponectin concentrations by inhibiting adiponectin regulatory mechanisms at transcriptional and posttranscriptional levels. The present study demonstrates nocturnal reduction in circulating adiponectin levels in severe OSAHS. Our experimental studies showed that hypoxic stress induced adiponectin dysregulation at transcriptional and posttranscriptional levels. Hypoxic stress is, at least partly, responsible for the reduction of serum adiponectin in severe OSAHS. Nocturnal reduction in adiponectin in severe OSAHS may be an important risk for cardiovascular events or other OSAHS-related diseases during sleep.

nasal continuous positive airway pressure

RECENT STUDIES HAVE DEMONSTRATED that adipose tissue is not only a passive reservoir for energy storage but also produces and secretes a variety of bioactive molecules called adipocytokines, including adiponectin (1a, 20), tumor necrosis factor-α, leptin, and plasminogen activator inhibitor type 1 (PAI-1) (36). Dysregulated production of adipocytokines is associated with the pathophysiology of obesity-related diseases (1a, 9, 27). The biological functions of adiponectin, which we identified as an adipocytokine in the human adipose cDNA library (20), include improvement of glucose (21) and lipid metabolism (26), prevention of inflammation (31) and atherosclerosis (24), and cardiovascular protection (14, 30, 38). Serum adiponectin levels are low in visceral obesity (1a), insulin resistance (10), type 2 diabetes (9), and cardiovascular diseases (29). Previous studies demonstrated the possible association between visceral obesity and obstructive sleep apnea-hypopnea syndrome (OSAHS) (39, 40). More recent studies reported that obese subjects with OSAHS had hypoadiponectinemia (36, 46).

In patients with OSAHS, repetitive nocturnal episodes of apneas elicit hypoxemia, hypercapnia, increased sympathetic activities, surges in blood pressure, increases in cardiac wall stress, and cardiac arrhythmias (19, 35). OSAHS is also associated with hypercoagulability, vascular oxidative stress (44), systemic inflammation, and endothelial dysfunction (18) during sleep. Patients with OSAHS have severe perturbations of autonomic, hemodynamic, humoral, and vascular regulation probably due to hypoxemia (intermittent and sustained), reoxygenation, neurohormonal abnormality, abnormal metabolism, low sleep quality, and other factors during sleep that contrast with the physiology for normal sleep (32). In the present study, we measured serum adiponectin levels before sleep and after wake-up in OSAHS patients and control subjects and also examined the alteration in serum adiponectin levels during one-night sleep. We further examined the effect of one-night nasal continuous positive airway pressure (nCPAP) on the alteration of serum adiponectin levels.

Hypoxia (intermittent and sustained), reoxygenation, neurohormonal abnormality, abnormal metabolism, low sleep quality, and other factors in OSAHS during sleep could explain the nocturnal fall in circulating adiponectin levels (19, 35). The present study focused on hypoxic stress, although other factors could be involved. On the other hand, previous studies reported that adiponectin is regulated by several factors at both transcriptional (22) and posttranscriptional (28) levels. Previous studies demonstrated that exposure to 1% O2 hypoxia results in transcriptional suppression in vitro (3, 8, 41, 45). We therefore focused attention on dysregulation of posttranscriptional levels of adiponectin by exposure to hypoxia, similar to the previous report on the regulation of adiponectin by testosterone (28). We investigated the effect of hypoxia on adiponectin in mice and cultured cells, using the sustained hypoxia stress method.
MATERIALS AND METHODS

Human Studies

Patients. We studied 93 Japanese patients with OSAHS, including 78 men (45.5 ± 13.0 yr, mean ± SD) and 15 women (51.5 ± 13.0 yr) between February 2006 and March 2007, who were newly diagnosed as having OSAHS. The control group consisted of 18 Japanese control subjects who were free of OSAHS, including 15 men (40.4 ± 12.3 yr) and 3 women (45.2 ± 12.4 yr). All participants underwent overnight cardiorespiratory monitoring (Osaka University: Somté; Compumedics, Melbourne, Australia; Yoshida Suimín-kokyu Clinic: Alice 4 Diagnostics Sleep System, Respironics). Each polysomnographic recording was analyzed for the number of apneas and hypopneas during sleep. The oxygen desaturation index (ODI), the lowest oxygen saturation, and the time at desaturation below 90% in minutes of total bed time for the entire night were measured. Apnea was defined as arrest of airflow >10 s. Hypopnea, or partial closure of the airway during sleep, was defined as ≥50% reduction in airflow associated with ≥4% desaturation. An obstructive apnea was defined as the absence of airflow in the presence of rib cage and/or abdominal excursions. The apnea-hypopnea index (AHI) was defined as the total number of apneas and hypopneas per hour of sleep. The diagnosis of OSAHS was based on AHI of ≥15 [control = 18 (13 men and 5 women)] and classified as mild AHI ≥5 to <15 [n = 24 (21 men and 3 women)], moderate AHI ≥15 to <30 [n = 12 (8 men and 4 women)], or severe AHI ≥30 [n = 39 (36 men and 3 women)], according to the guidelines of the American Academy of Sleep Medicine Task Force (1).

Twenty-four of 39 patients who had AHI >30 were titrated with nCPAP during polysomnography (Fuji Respironics) by experienced technicians. The critical pressure was determined by an automatic CPAP device (REM star Auto M series with C-Flex, Respironics). The recording methods were described in detail previously (13, 34).

All subjects were engaged in little or no physical activity, but all had a regular annual health check. Each subject was asked to complete a questionnaire on sleep symptoms, family history, medical history, and medications. Blood pressure was measured with a standard mercury sphygmomanometer on the right arm after the subject had been resting in the supine position for at least 10 min after wake-up. Mean values were determined from two independent measurements taken at 5-min intervals.

Diabetes mellitus was defined according to World Health Organization criteria and/or treatment for diabetes mellitus. Dyslipidemia was defined as a total cholesterol concentration of >220 mg/dl, triglyceride concentration >150 mg/dl, HDL-cholesterol concentration <40 mg/dl, and/or treatment for dyslipidemia. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg, or treatment for hypertension. Patients with a previous diagnosis of dyslipidemia, hypertension, or diabetes mellitus and receiving drugs for any of these conditions were also included in this study. The numbers of patients on medications known to increase adiponectin, 5'-GATGGCAGAGATGGCACTCC-3' and 5'-CTTGCACGTGCCGGCCCTCAT-3'; 36B4, 5'-AAGCGCTCTGGCATTGTCT-3' and 5'-CCCGGGAGCCATGGTGT-3'.

Pulse-chase studies. Pulse-chase studies were performed to analyze the secretion steps of the newly synthesized adiponectin proteins, according to the procedure described previously (11, 28). 3T3-L1 adipocytes (day 7) were plated onto six-well plates and were incubated with fetal calf serum (FCS)-free complete Dulbecco’s modified Eagle’s medium (DMEM) for 12 h. For metabolic labeling, cells were subjected to measurement of serum and mRNA [with real-time quantitative polymerase chain reaction (rt-PCR) as described previously (1, 7)] and for measurement of serum and mRNA with real-time quantitative polymerase chain reaction (rt-PCR) as described previously (7, 28). Briefly, total RNAs were extracted by using RNA STAT-60 (Tel-Test, Friendswood, TX). First-strand cDNA was synthesized from 320 ng of total RNA with the Thermoscript reverse transcription-polymerase chain reaction system (Invitrogen, Carlsbad, CA). rt-PCR amplification was conducted with the ABI PRISM 7900HT Sequence Detection system and the SDS Enterprise Database (Applied Biosystems, Foster City, CA) using SYBR Green polymerase chain reaction Master Mix (Applied Biosystems). The final result for each sample was normalized to the respective 36B4 in consideration for its stability, as reported previously (8). We also investigated 18s ribosomal RNA and cyclophilin as other internal standards in this study. The sequences of the primers used for rt-PCR were as follows: adiponectin, 5'-GATGGCAGAGATGGCACTCC-3' and 5'-CTTGCACGTGCCGGCCCTCAT-3'; 36B4, 5'-AAGCGCTCTGGCATTGTCT-3' and 5'-CCCGGGAGCCATGGTGT-3'.

Measurement of serum adiponectin concentrations. In each sleep study, venous blood samples were obtained before sleep and after wake-up while the subject was in the supine position. For the purpose of the present study, serum samples that were obtained at baseline from each study participant and stored at −20°C were thawed and assayed for adiponectin levels by sandwich enzyme-linked immunosorbent assay (ELISA) (Otsuka, Japan) (1a, 7, 11, 15, 28). The Medical Ethics Committee of Osaka University approved this study. All subjects enrolled in this study were Japanese, and each gave written informed consent.

Animal and Cell Culture Studies

Animals and exposure to hypoxia. Male C57BL/6J mice (each group n = 5 or 6) were obtained from Clea Japan (Tokyo, Japan) and kept under a 12:12-h light-dark cycle (lights on 8:00 AM to 8:00 PM) and constant temperature (22°C) with free access to food (Oriental Yeast, Osaka, Japan) and water. Male mice were housed in cages exposed to room air (ambient atmosphere) or in hypoxia chambers (Tel-Test, Osaka, Japan) at −10% O2 concentration for hypoxia stress study in vivo (25).

Measurement of serum adiponectin concentrations and adipose adiponectin mRNA expression in mice. Mice were used at 10–13 wk of age in this study, because serum adiponectin levels decrease gradually in younger mice and can be influenced by body weight gain in older mice. Mice were killed under pentobarbital sodium anesthesia (50 mg/kg body wt) at the indicated times under each condition, and then various tissues and blood samples were collected. Each sample was subjected to measurement of serum and mRNA [with real-time quantitative polymerase chain reaction (rt-PCR) as described previously (7, 28)]. Briefly, total RNAs were extracted by using RNA STAT-60 (Tel-Test, Friendswood, TX). First-strand cDNA was synthesized from 320 ng of total RNA with the Thermoscript reverse transcription-polymerase chain reaction system (Invitrogen, Carlsbad, CA). rt-PCR amplification was conducted with the ABI PRISM 7900HT Sequence Detection system and the SDS Enterprise Database (Applied Biosystems, Foster City, CA) using SYBR Green polymerase chain reaction Master Mix (Applied Biosystems). The final result for each sample was normalized to the respective 36B4 in consideration for its stability, as reported previously (8). We also investigated 18s ribosomal RNA and cyclophilin as other internal standards in this study. The sequences of the primers used for rt-PCR were as follows: adiponectin, 5'-GATGGCAGAGATGGCACTCC-3' and 5'-CTTGCACGTGCCGGCCCTCAT-3'; 36B4, 5'-AAGCGCTCTGGCATTGTCT-3' and 5'-CCCGGGAGCCATGGTGT-3'.

Pulse-chase studies. Pulse-chase studies were performed to analyze the secretion steps of the newly synthesized adiponectin proteins, according to the procedure described previously (11, 28). 3T3-L1 adipocytes (day 7) were plated onto six-well plates and were incubated with fetal calf serum (FCS)-free complete Dulbecco’s modified Eagle’s medium (DMEM) for 12 h. For metabolic labeling, cells were washed with PBS and incubated with methionine- and cysteine-free DMEM without serum for 30 min to deplete the intracellular pools. The medium was subjected to measurement of adiponectin (by ELISA), and the cells were harvested for mRNA (rt-PCR) as described previously (7, 28).

Measurement of adiponectin secretion into medium and mRNA expression in cell cultures. 3T3-L1 cells were maintained and differentiated as described previously (7, 28). On day 7, the cells were cultured for 12 h under 10% or 15% O2 hypoxia or control conditions (18–21% O2-5% CO2; each group n = 6). An aliquot of the culture medium was subjected to measurement of adiponectin (by ELISA), and the cells were harvested for mRNA (rt-PCR) as described previously (7, 28). Briefly, total RNAs were extracted by using RNA STAT-60 (Tel-Test, Friendswood, TX). First-strand cDNA was synthesized from 320 ng of total RNA with the Thermoscript reverse transcription-polymerase chain reaction system (Invitrogen, Carlsbad, CA). rt-PCR amplification was conducted with the ABI PRISM 7900HT Sequence Detection system and the SDS Enterprise Database (Applied Biosystems, Foster City, CA) using SYBR Green polymerase chain reaction Master Mix (Applied Biosystems). The final result for each sample was normalized to the respective 36B4 in consideration for its stability, as reported previously (8). We also investigated 18s ribosomal RNA and cyclophilin as other internal standards in this study. The sequences of the primers used for rt-PCR were as follows: adiponectin, 5'-GATGGCAGAGATGGCACTCC-3' and 5'-CTTGCACGTGCCGGCCCTCAT-3'; 36B4, 5'-AAGCGCTCTGGCATTGTCT-3' and 5'-CCCGGGAGCCATGGTGT-3'.
beled adiponectin in medium and cell lysates, metabolic labeling was performed for 2 h. The labeling medium was then replaced with 1 ml of FCS-free DMEM and set into an incubator under control condition or hypoxia (15% O2-5% CO2 atmosphere) for 1, 2, 4, 8, or 12 h. The medium and cell lysates were collected at indicated times for immunoprecipitation assays. At each time point, the medium was collected, and the cells were washed with PBS without calcium and magnesium ions, suspended in 300 μl of disruption buffer [mmol/l: 10 Tris-HCl (pH 7.5), 150 NaCl, 5 EDTA, 10 benzamidine, and 1 PMSF, with 1% Nonidet P-40], and lysed with three repetitive freeze-thaw cycles. The cell lysates were adjusted to equal protein concentration with disruption buffer and subjected to immunoprecipitation. A total of 500 μl of cell lysates (100 μg of total protein) was mixed with an equal volume of disruption buffer lacking EDTA and Nonidet P-40 and immunoprecipitated with 5 μl of rabbit polyclonal antibody against mouse adiponectin (OCT12202) overnight at 4°C, followed by incubation with 40 μl of protein G beads for 2 h at 4°C. For the medium, aliquots (300 μl) of metabolically labeled culture medium were added to 200 μl of 2.5× immunoprecipitation buffer [IPB; 1× IPB = (mmol/l) 10 Tris-HCl (pH 7.5), 150 NaCl, 1 EDTA, 10 benzamidine, and 1 PMSF, with 1% Nonidet P-40] and immunoprecipitated as described above. The immunoprecipitates were then washed three times with 1× IPB, followed by solubilization with a sample buffer, and subjected to SDS-PAGE. After electrophoresis the gel was dried, and radiolabeled proteins were analyzed by autoradiography. The band intensities were quantified by densitometry.

### Statistical Analysis

Continuous variables are presented as means ± SD and were compared by one-way or two-way analysis of variance (ANOVA) with Fisher’s protected least significant difference test for multiple-group analysis or unpaired Student’s t-test for experiments with only two groups. In all cases, P values <0.05 were considered statistically significant. All analyses were performed with the STATVIEW 5.0 system (HULINKS, Tokyo, Japan). Animal and cell experiments were performed at least three times. We used power analysis to set the minimum requirement of case numbers required to obtain “statistically significant” results for validation of our hypothesis.

### RESULTS

#### Human Studies

**Serum adiponectin levels in patients with OSAHS and control subjects.** The characteristics of the subjects enrolled in this study are presented in Table 1. AHI, ODI 4%, and the percentage of arterial O2 saturation from pulse oximetry (SpO2)<90% were significantly higher and the lowest SpO2 were significantly lower in OSAHS patients than in the control subjects (Table 1). Serum adiponectin concentrations at 7:00 AM in patients with severe OSAHS (5.2 ± 2.6 μg/ml, mean ± SD) were significantly lower than in control subjects (8.5 ± 5.5 μg/ml; Fig. 1) (P < 0.01).

Next, we focused on nocturnal alternation in serum adiponectin levels. The mean serum adiponectin concentrations before sleep (at 8:00 PM) in patients with severe OSAHS (5.9 ± 2.9 μg/ml) were significantly lower than in control subjects (8.8 ± 5.6 μg/ml, P < 0.05; Fig. 1). Furthermore, in patients with severe OSAHS, adiponectin levels were significantly lower after wake-up (5.2 ± 2.6 μg/ml) than before sleep (5.9 ± 2.9 μg/ml, P < 0.0001; Fig. 1). However, there were no significant differences in circulating adiponectin levels between the two samples obtained at 8:00 PM and 7:00 AM in moderate OSAHS, mild OSAHS, and control groups. There was no significant difference in the form of adiponectin multimers between before sleep and after wake-up in patients with severe OSAHS (data not shown).

**Effects of one-night nCPAP treatment on serum adiponectin levels.** nCPAP is the gold standard treatment for OSAHS (23). We investigated the effect of one-night nCPAP treatment

### Table 1. Clinical and biochemical characteristics of subjects

<table>
<thead>
<tr>
<th></th>
<th>Control (AHI &lt; 5)</th>
<th>Mild (5 ≤ AHI &lt; 15)</th>
<th>Moderate (15 ≤ AHI &lt; 30)</th>
<th>Severe (30 ≤ AHI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number</strong></td>
<td>18</td>
<td>24</td>
<td>12</td>
<td>39</td>
</tr>
<tr>
<td><strong>Sex, male/female</strong></td>
<td>13/5</td>
<td>21/3</td>
<td>8/4</td>
<td>36/3</td>
</tr>
<tr>
<td><strong>Age, yr</strong></td>
<td>43.8 ± 12.2</td>
<td>45.9 ± 12.6</td>
<td>52.1 ± 14.1</td>
<td>46.4 ± 14.3</td>
</tr>
<tr>
<td><strong>BMI, kg/m²</strong></td>
<td>66.9 ± 14.8</td>
<td>77.1 ± 19.9</td>
<td>77.5 ± 17.3</td>
<td>87.5 ± 19.0*</td>
</tr>
<tr>
<td><strong>Waist circumference, cm</strong></td>
<td>86.7 ± 10.9</td>
<td>92.2 ± 13.7</td>
<td>98.9 ± 10.5*</td>
<td>100.7 ± 12.8**</td>
</tr>
<tr>
<td><strong>AHI, events/h</strong></td>
<td>2.4 ± 1.5</td>
<td>8.9 ± 2.7†</td>
<td>19.4 ± 4.3††</td>
<td>57.8 ± 18.6**</td>
</tr>
<tr>
<td><strong>ODI 4%, events/h time in bed</strong></td>
<td>2.0 ± 2.0</td>
<td>6.7 ± 4.6*</td>
<td>16.6 ± 6.9*†</td>
<td>52.5 ± 22.0**</td>
</tr>
<tr>
<td><strong>Baseline SpO₂, %</strong></td>
<td>96.8 ± 1.4</td>
<td>95.6 ± 1.9</td>
<td>95.2 ± 2.1††</td>
<td>94.4 ± 2.0††</td>
</tr>
<tr>
<td><strong>Lowest SpO₂, %</strong></td>
<td>88.3 ± 3.9</td>
<td>84.1 ± 5.6*</td>
<td>78.8 ± 3.3*†</td>
<td>67.6 ± 10.7**†</td>
</tr>
<tr>
<td><strong>SpO₂&lt;90%, % time in bed</strong></td>
<td>0.1 ± 0.1</td>
<td>1.6 ± 2.9</td>
<td>6.7 ± 7.6*†</td>
<td>30.3 ± 21.9**†</td>
</tr>
<tr>
<td><strong>Fasting glucose, mg/dl</strong></td>
<td>112 ± 26</td>
<td>117 ± 43</td>
<td>17.3 ± 14</td>
<td>113 ± 19**</td>
</tr>
<tr>
<td><strong>IRI, μU/ml</strong></td>
<td>9.1 ± 5.7</td>
<td>12.5 ± 7.4</td>
<td>14.1 ± 7.5</td>
<td>19.1 ± 13.8*</td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>2.4 ± 2.1</td>
<td>3.2 ± 2.4</td>
<td>3.7 ± 2.7</td>
<td>5.3 ± 4.1*</td>
</tr>
<tr>
<td><strong>Total cholesterol, mg/dl</strong></td>
<td>208 ± 40</td>
<td>202 ± 29</td>
<td>207 ± 58</td>
<td>202 ± 25</td>
</tr>
<tr>
<td><strong>Triglyceride, mg/dl</strong></td>
<td>187 ± 146</td>
<td>155 ± 79</td>
<td>167 ± 92</td>
<td>156 ± 80†</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mg/dl</strong></td>
<td>56 ± 17</td>
<td>44 ± 8*</td>
<td>41 ± 12*</td>
<td>45 ± 11*</td>
</tr>
<tr>
<td><strong>Systolic blood pressure, mmHg</strong></td>
<td>113 ± 18</td>
<td>125 ± 15</td>
<td>125 ± 11</td>
<td>129 ± 15*</td>
</tr>
<tr>
<td><strong>Diastolic blood pressure, mmHg</strong></td>
<td>72 ± 11</td>
<td>75 ± 11</td>
<td>79 ± 11</td>
<td>85 ± 14*</td>
</tr>
<tr>
<td><strong>Serum adiponectin before sleep, μg/ml</strong></td>
<td>8.8 ± 5.6</td>
<td>7.9 ± 5.4</td>
<td>6.0 ± 3.0</td>
<td>5.9 ± 2.9†</td>
</tr>
<tr>
<td><strong>Serum adiponectin after wake-up, μg/ml</strong></td>
<td>8.5 ± 5.5</td>
<td>7.9 ± 5.6</td>
<td>5.8 ± 3.0</td>
<td>5.2 ± 2.6*</td>
</tr>
</tbody>
</table>

Data are means ± SD. OSAHS, obstructive sleep apnea-hypopnea syndrome; BW, body weight; BMI, body mass index; AHI, apnea-hypopnea index; ODI, oxygen desaturation index; SpO₂, arterial O₂ saturation from pulse oximetry; IRI, immunoreactive insulin; HOMA-IR, homeostasis model assessment-insulin resistance. *P < 0.01 (control vs. severe); †P < 0.05 (control vs. severe); ‡P < 0.01 (mild vs. severe); 4P < 0.05 (mild vs. severe); 4P < 0.01 (moderate vs. severe); ‡P < 0.01 (control vs. moderate); 5P < 0.05 (control vs. moderate); 6P < 0.01 (control vs. mild); 7P < 0.05 (control vs. mild).
on serum adiponectin levels in 24 patients with severe OSAHS (AHI ≥ 30). One-night nCPAP treatment significantly decreased AHI, ODI 4%, and the time spent at SpO2 < 90% (data not shown). Individual data are shown in Fig. 2A. The percent change in serum adiponectin level [Δadiponectin: (serum adiponectin concentrations after wake-up − before sleep)/before sleep (%)] before one-night nCPAP treatment was −19.1 ± 13.1%, whereas that after nCPAP treatment significantly improved to −10.9 ± 11.8% (P < 0.05; Fig. 2B).

**Animal and Cell Culture Studies**

The present study focused on the effect of hypoxia on adiponectin, which is at least partly a pathophysiological factor in severe OSAHS, although other OSAHS-related factors could be involved. We investigated the effect of exposure to sustained hypoxia on adiponectin in C57BL/6J mice and cultured 3T3-L1 adipocytes, using the sustained hypoxia stress method. Exposure to hypoxia for 4 days resulted in significant suppression of serum adiponectin concentrations and significant change in adipose adiponectin mRNA expression compared with control (P < 0.01). Furthermore, exposure to hypoxia for 2 days suppressed serum adiponectin levels, with no apparent change in adipose mRNA expressions (Fig. 3A). Exposure to hypoxia inhibited adiponectin secretion from cultured 3T3-L1 adipocytes at both transcriptional (10% O2 hypoxia) and posttranscriptional (15% O2 hypoxia) levels, compared with control (Fig. 3B). For further analysis of the posttranscriptional dysregulation of adiponectin, we performed pulse-chase experiments to examine the inhibitory effects of 15% O2 hypoxia on the secretion of newly synthesized adiponectin protein in 3T3-L1 adipocytes. The secretion of radiolabeled adiponectin was continuously inhibited with exposure to 15% O2 hypoxia and from 1 to 12 h of chasing period (Fig. 4A). However, adipocytes exposed to hypoxia showed retention of the labeled adiponectin intracellularly (Fig. 4B).

**DISCUSSION**

We found significantly lower levels of serum adiponectin in patients with severe OSAHS, similar to previous reports in OSAHS patients (36, 46). The alternation of serum adiponectin levels during one-night sleep in severe OSAHS has not been reported. In the present study, we found nocturnal reduction in serum adiponectin levels in patients with severe OSAHS. In addition, such reductions were ameliorated by one-night nCPAP treatment. These results indicate that one-night nCPAP treatment attenuates the nocturnal reduction of serum adiponectin levels. Although high-molecular-weight adiponectin is significantly low in patients with coronary artery disease or obesity (11, 12), in the present study there was no significant difference in the form of adiponectin multimers between before sleep and after wake-up in patients with severe OSAHS (data not shown). In mice and cultured 3T3-L1 adipocytes, exposure to hypoxia decreased adiponectin concentrations by inhibiting adiponectin regulatory mechanisms at both secretion and transcriptional levels.

nCPAP reduces the risk of fatal and nonfatal cardiovascular outcomes (23). In the present study, one-night nCPAP treatment reduced the nocturnal reduction in serum adiponectin, levels.
suggesting that cyclical hypoxemia can have a short-term effect. The effects of CPAP treatment may be related to improvement of sleep quality, metabolism, and other factors; therefore, improvement of the drop in adiponectin levels with one-night nCPAP may be due not only to removing hypoxia partly but also to alteration of other OSAHS-related factors. Although we did not investigate the long-term effect of nCPAP treatment in the present study, several reports found no significant changes in serum levels of adiponectin after 3 mo of long-term nCPAP treatment (6, 42), suggesting that the lack of a long-lasting change in adiponectin can be explained by the influence of body mass on adiponectin secretion, which was unchanged during nCPAP treatment. Considered together, the results of one-night nCPAP seem different from those of long-term nCPAP treatment. Longitudinal and interventional studies are required to compare the long- and short-term effects of nCPAP.

Hypoxia (intermittent and sustained), reoxygenation, neurohormonal abnormality, abnormal metabolism, low sleep quality, and other factors in OSAHS during sleep could explain the nocturnal fall in circulating adiponectin levels (19, 35). The present study focused on hypoxic stress (intermittent and sustained), although other factors could be involved. Nocturnal reduction of adiponectin may be an important risk for OSAHS-related diseases in patients with severe OSAHS.

Fig. 3. A: mouse studies. Dysregulation of adiponectin in hypoxic mice. Mice were housed in chambers under control (C; n = 5) or hypoxic (H; n = 6) conditions for the indicated time periods. Levels of serum adiponectin were measured by ELISA (1a). Total mRNA was extracted from the tissue of individual mice and subjected to real-time quantitative PCR analysis to determine the mRNA levels of adiponectin in white adipose tissue (WAT; epididymal fat tissues). Data were normalized against 36B4 mRNA. The values of mice at 2 days were arbitrarily set as 1.0. Data are means ± SD. Similar results were obtained in 2 other independent experiments. B: cultured cell studies on secretion and adiponectin protein and mRNA levels in 3T3-L1 adipocytes. 3T3-L1 cells were cultured on day 7 under control (n = 6) or hypoxic (15% or 10% O2; n = 6) conditions for the indicated time periods. Levels of adiponectin secreted into the culture medium for the indicated time intervals were analyzed by ELISA. Adiponectin mRNA expression levels in adipose tissues were measured as described in MATERIALS AND METHODS. The values of the 12-h control group were arbitrarily set as 1.0. Data are means ± SD. This experiment was performed 3 times with similar results.

Fig. 4. Pulse-chase experiments of 35S-labeled adiponectin under control or hypoxic (15% O2 hypoxia) conditions in 3T3-L1 adipocytes. Representative autoradiographic data of adiponectin secretion (A) and adipose adiponectin proteins (B) are shown. Band intensities were quantified by densitometry in the media and cell lysates at the indicated time intervals (n = 5). Data are means ± SD. *P < 0.01 vs. control. Similar results were obtained in 3 other independent experiments.

Fig. 5. Schematic presentation of the nocturnal reduction of adiponectin in patients with severe OSAHS. Profound hypoxemia suppresses adiponectin mRNA level in adipose tissue, as reported previously (3, 8, 41, 45). In addition, hypoxia inhibits the secretion of adiponectin. Hypoxic stress of local adipose tissue during sleep seems to play, at least in part, a role in dysregulation of adiponectin production, although other OSAHS-related factors could be involved. Nocturnal reduction of adiponectin may be an important risk for OSAHS-related diseases in patients with severe OSAHS.
DYSREGULATION OF ADIPONECTIN IN OSAHS


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