Rapid Effect of nCPAP Therapy on Circulating Plasma Leptin in OSAS Patients

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Obstructive sleep apnea syndrome (OSAS) is a problem of obese patients. Leptin prevents fat deposition in adipose tissue and insulin reduces plasma glucose, but levels of these hormones are sometimes high in obese patients with OSAS. Thus, leptin and insulin do not function properly in OSAS, and increase resistance to leptin and insulin may cause paradoxical state, that is, leptin and insulin resistance. The present study investigates the rapid effect of nasal continuous positive airway pressure (nCPAP) ventilation on serum leptin and insulin levels in OSAS. Levels of plasma leptin and insulin at four sampling points a day decreased in OSAS patients (*p<0.05, n=10) by nCPAP. Nocturnal mean-nadir arterial oxygen desaturation (nmSaO₂) correlated with plasma leptin levels in OSAS patients (*p<0.05, n=10). Nasal CPAP did not affect circulating soluble leptin receptor levels in OSAS patients. These findings indicate that leptin and insulin are related to nocturnal hypoxia in OSAS patients, and that nCPAP have a possibility of improving leptin and insulin resistance. (Kitakanto Med J 2005; 55: 29-35)

Key Words: leptin, soluble leptin receptor, obstructive sleep apnea syndrome, hypoxia, nasal CPAP

Introduction

Obstructive sleep apnea syndrome (OSAS) is a complication of obesity. Fifty percent of obese men have OSAS. Clinical features of OSAS are excessive daytime sleepiness, altered sleep behavior, cardiac arrhythmia and pulmonary hypertension, which lead to diabetes mellitus and cardiac dysfunction with chronic congestive heart failure. Recently, there have been reported about relationship between OSAS and plasma hormone levels.

Leptin plays an important role in obesity. Leptin prevents fat deposition in adipose tissue, nonetheless, plasma leptin levels are paradoxically higher in obese than in non-obese. Leptin resistance has been raised to explanation for this paradox. Several report suggested that soluble leptin receptor and glucocorticoid levels (counter-regulatory substances for leptin) affect leptin resistance. Increased circulating soluble leptin receptor levels in sera impaired transport of leptin across the blood-brain barrier, and increased glucocorticoid levels, are implicated in leptin resistance. Furthermore, the concentrations of insulin and cortisol levels regulate plasma leptin levels. Leptin plays an important role not only obesity, but also respiration. Deficiency of leptin activity or decreased leptin levels in the central nervous system (CNS) induced hypoventilation in C57BL/6j-Leploo mice. Furthermore, sleep apneic men have higher concentrations of leptin than nonapneic men. These studies suggest that leptin is associated with respiratory system. Reports show that about 15% OSAS patients have glucose intolerance. These data suggested that some OSAS patients have insulin resistance.

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Fig. 1 Study protocol. All patients received a first polysomnogram within a day after admission. When patients were diagnosed as having OSAS in the first polysomnogram, they received nCPAP therapy for two days, then, a second polysomnogram was applied with installation of nCPAP machine. Blood samples were drawn at 12:00 (12:00- I), 21:00 on the day of polysomnogram, and at 06:00, 12:00 (12:00- II) on next day both first and second polysomnograms.

Table 1 Patients characteristics

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>Control</th>
<th>OSAS before nCPAP</th>
<th>OSAS After nCPAP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No of Pts</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Age, yr</td>
<td>48.0 (4.6)</td>
<td>47.9 (4.4)</td>
<td>47.9 (4.4)</td>
</tr>
<tr>
<td></td>
<td>Epworth scale, point</td>
<td>5.6 (1.0)</td>
<td>10.3 (1.0)*</td>
<td>7.7 (0.9)*</td>
</tr>
<tr>
<td></td>
<td>BMI, kg/m²</td>
<td>23.1 (0.7)</td>
<td>30.0 (1.5)*</td>
<td>29.2 (1.5)</td>
</tr>
<tr>
<td></td>
<td>AHI, episodes/hr</td>
<td>5.7 (1.6)</td>
<td>51.5 (9.1)*</td>
<td>11.6 (2.4)*</td>
</tr>
<tr>
<td></td>
<td>mean with desaturation (mmSaO₂), %</td>
<td>93.8 (0.5)</td>
<td>81.9 (2.6)*</td>
<td>90.9 (1.3)*</td>
</tr>
<tr>
<td></td>
<td>T-cho, mg/dl</td>
<td>173.4 (14.5)</td>
<td>183.6 (20.4)</td>
<td>180.7 (19.3)</td>
</tr>
<tr>
<td></td>
<td>TG, mg/dl</td>
<td>172.3 (32.7)</td>
<td>212.6 (38.1)</td>
<td>200.1 (35.9)</td>
</tr>
<tr>
<td></td>
<td>HDL-cho, mg/dl</td>
<td>45.0 (2.8)</td>
<td>45.4 (5.1)</td>
<td>42.7 (6.7)</td>
</tr>
</tbody>
</table>

Data are presented as means (±SEM). *p<0.05 shows control group (n=10) vs OSAS group (n=10). *p<0.05 shows before CPAP (n=10) vs After CPAP (n=10).

Therapy with nasal continuous positive airway pressure (nCPAP) is globally accepted as the treatment of choice for OSAS. However, there are only a few reports about the effect of nCPAP on leptin and soluble leptin receptor in OSAS patients. The present study investigates whether nCPAP affects plasma leptin, soluble leptin receptor, insulin, cortisol and glucose levels in obese OSAS patients.

Patients and Methods

Twenty subjects were admitted to our hospital mainly with complaint of snoring or daytime sleepiness. The diagnosis of OSAS and candidates for nCPAP therapy were determined based on the clinical and polysomnographic criteria as follows. Clinical symptoms were assessed using the Epworth Sleepiness Scale. An apnea plus hypopnea index (AHI) of more than 20 events per hour and nocturnal mean-nadir arterial oxygen desaturation (mmSaO₂) <80%, or lowest SaO₂ <70% were diagnosed as OSAS. All subjects underwent polysomnogram (Alice 3, version 1.19, Chest M.I. Inc. Co.Ltd.,) to diagnosis SAS, and blood sampling. The study was conducted according to the guidelines of The Declaration of Helsinki. All patients gave written informed consent. Approval for study was given by the Ethical Committee of Gunma University Institution.

The study protocol is shown in Fig.1. All subjects underwent an initial polysomnogram within a day after admission to our hospital. We designed the whole study was completed within a week. Subjects diagnosed as having OSAS on first polysomnogram received nCPAP therapy (Sleep Mate, SLV-VP, ResMed Ltd., Australia) following for two days, then, they received a second polysomnogram. The subjects were divided into two groups; control group was 10 patients (ten men) who did not have OSAS. OSAS group was 10 patients (nine men and one woman), and they received nCPAP therapy. The clinical features and parameters of polysomnogram are shown in Table 1.

Measurement of plasma leptin, soluble leptin receptor, cortisol, insulin, glucose levels and Biochemical parameters

All patients took only hospital meals three times of a day. Blood samples were collected at 12:00 (12:00- I), 21:00 on the day of the polysomnogram, at 06:00, and 12:00 (12:00- II) on the following day of the polysomnogram. Plasma leptin, soluble leptin receptor, cortisol, insulin, and glucose levels were determined in samples that were centrifuged immediately at 3,000 rpm for 15 min at 4°C, and stored at −80°C. Plasma leptin levels were measured by a
radioimmunoassay (RIA) (Linco Research, Inc., St Charles, MO, U.S.A) according to the method as described.20 Plasma soluble form of the leptin receptor was quantified using an in vitro sandwich Enzyme-Linked immunosorbent assay (BioVender Laboratory Medicine, Inc. Brno, Czech) as described.21 Absorbance was determined using a micro plate reader (Vmax Kinetic Microplate Reader, Molecular Devices Corporation, Menlo Park, California U.S.A) at 450nm. Plasma insulin and cortisol levels were assessed by RIA. Plasma glucose and biochemical parameters (T-cho, TG, and HDL-cho) were assayed by enzyme-linked immunosorbent assay (ELISA).

**Statistical analysis**

All values are shown as means ±SEM. Comparisons between two groups were achieved using Wilcoxon’s signed-ranks test. Relationships among sleep variables and hormonal values were calculated using Pearson’s correlation analysis. Statistically critical confidence levels selected for all analyses were established at \( P < 0.05 \).

**Results**

**Patients characteristics**

Age did not significantly differ between control and OSAS groups (Table 1). Epworth scale, BMI and A.H.I of OSAS group were significantly higher than control (\( ^* P < 0.05 \)). Nocturnal mean-nadir arterial oxygen desaturation of OSAS group was significantly lower than control (\( ^* P < 0.05 \)). Epworth scale, A.H.I and mmSaO\(_2\) were significantly improved by nCPAP in OSAS group (\( ^* P < 0.05 \)). BMI did not changed significantly during before and after nCPAP therapy.

**Plasma hormone levels on control subjects and before and after nCPAP therapy on OSAS subjects**

The ammount of plasma leptin and insulin in OSAS group were higher than those of the control group at all sampling points (\( P < 0.05 \), Fig.2a, b). Cortisol and glucose in OSAS group did not show differences from control group at all sampling points (Fig.2c, d). Plasma leptin and insulin levels after nCPAP treatment in OSAS group were significantly decreased at all sampling points (\( P < 0.05 \), Fig.3a, b). Cortisol and glucose levels in OSAS group were not affected by nCPAP therapy (data not shown). Plasma soluble leptin receptor did not show differences among groups, and also did not show differences between 21:00 and 6:00. Nasal CPAP did not affect the levels of soluble leptin receptor in OSAS group (Fig.4).
Fig. 3 Changes of plasma leptin (panels a) and insulin (panel b) levels before and after nCPAP therapy at four sampling points (12:00-1, 21:00, 06:00 and 12:00-II) in OSAS group (n=10). Blank box indicates before nCPAP and slashed box indicate after nCPAP. Statistically confidence for all analyses expressed *P<0.05.

Correlation between plasma hormone levels and nocturnal mean-nadir arterial oxygen desaturation (nmSaO2)

Plasma leptin levels correlated well with nmSaO2 at all sampling points in OSAS group before nCPAP (p<0.05, Fig.5a, b, c and d). Plasma insulin levels correlated with nmSaO2 only at 12:00–I, and not correlated at other sampling points in OSAS group before nCPAP (p<0.05, Fig.5e, f, g, and h). Cortisol and glucose did not correlated with nmSaO2 at all sampling points (data not shown).

Discussion

The main findings of this study were that nCPAP therapy rapidly reduces plasma leptin and insulin levels in OSAS patients. We speculated the mechanism about underlying relationship between nCPAP therapy and plasma leptin change. The first, several reports showed BMI affect leptin levels. However, we demonstrated BMI did not change during before and after nCPAP treatment, and plasma leptin levels were significantly decreased by nCPAP treatment, in addition, nmSaO2 was correlated plasma leptin levels. Taken together these results, leptin levels are affected by changes of night respiration status independently BMI. The second, the parameter of plasma insulin level significantly decreased after nCPAP treatment. Since insulin stimulates leptin secretion, the mechanisms involved in changes of plasma leptin levels are possibly induced by reduction in plasma insulin. The third, the changes of nmSaO2 could directly affect plasma leptin levels via the Central nervous system. In our study, leptin correlated with nmSaO2 in OSAS group. Indeed, studies have shown that leptin replacement in ob/ob mice (leptin deficient) also increases the ventilatory response to CO2 challenge and that leptin receptors are present in the nucleus of the tractis solitarius and the reticular activating system. These studies suggest that a respiratory control system operates leptin via CNS, and feedback mechanism works for response to change of SaO2 between leptin and the CNS system. Leptin circulates in serum as free form and as bound form to a carrier protein. Since leptin binds its receptor, the balance between free and bound leptin is a potential regulator of leptin bioavailability. In present study, we expected the circulating soluble leptin receptor affect plasma leptin levels, however, circulating soluble leptin receptor levels did not show differences between control and OSAS subjects, and were not affected by nCPAP therapy. A Leptin not only binds to circulating soluble receptor, but also binds to tissue receptors which were expressed lung and several organs. It is needed to evaluate changes of expressed tissue receptor numbers. More precise study is needed. Skeletal muscle is the primary site of whole-body in insulin-mediated glucose uptake. Therefore, the release of skeletal muscle from hypoxia by nCPAP, and increased skeletal muscle microcirculation have possibly contributed to a decrease in insulin resistance in OSAS.
Fig. 5  Correlation between plasma leptin and nocturnal mean-nadir arterial oxygen desaturation (nmSaO$_2$), and correlation between plasma insulin and nmSaO$_2$ in OSAS group (n = 10). Panels a, b, c and d show plasma leptin levels at four sampling points (12:00–I, 21:00, 06:00 and 12:00–II), respectively. Panels e, f, g and h show plasma insulin levels at four sampling points (12:00–I, 21:00, 06:00 and 12:00–II), respectively. All values are shown as means±SEM. Relationships were calculated using Pearson’s correlation analysis. Critical statistical confidence level selected for all analyses was *P < 0.05.
patients.

In conclusion, we demonstrated that plasma leptin, insulin and hypoxia are related, and treating hypoxia with nCPAP rapidly improves high concentration of circulating leptin and insulin. Although short-term nCPAP therapy improves leptin and insulin level, it might be temporary. A rebound or adoption of these changes could occur by long-term nCPAP therapy. Further investigations are needed to define the mechanisms of hypoxia-induced leptin and insulin resistance in OSAS.

References


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