Increase in Bilirubin Levels of Patients with Obstructive Sleep Apnea in the Morning—A Possible Explanation of Induced Heme Oxygenase-1

Kazuo Chin MD, PhD,[1] Motoharu Ohi MD, PhD,[3] Kouichi Shimizu MD,[2] Takaya Nakamura MD,[2] Fumiyo Miyaoka MD,[1] Michiaki Mishima MD, PhD,[1] and Takashi Nakamura MD, PhD[1]

[1]Department of Physical Therapeutics, Kyoto University Hospital of Medicine; [2]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University; [3]Osaka Kaisei Hospital

Study Objectives: In the absence of heme oxygenase-1 (HO-1), which catalyzes the oxidation of heme to generate carbon monoxide and indirect bilirubin, hypoxia induces severe right ventricular dilation and infarction. Despite severe hypoxemia during sleep, patients with obstructive sleep apnea-hypopnea syndrome (OSAHS) rarely die during sleep. We hypothesized that apnea-related hypoxemia would induce HO-1 and increase bilirubin levels in the morning in OSAHS patients. Therefore, bilirubin levels in OSAHS patients were analyzed before and after nasal continuous positive airway pressure (nCPAP) therapy.

Design: Bilirubin levels in the afternoon before sleep and in the morning immediately after sleep were determined before and after nCPAP treatment.

Setting: University Hospital in Kyoto, Japan.

Patients: The subjects were 22 patients with OSAHS (mean (SEM) apnea and hypopnea index of 60 (5)) who were treated with nCPAP and 13 controls.

Interventions: N/A

Measurements and Results: Before nCPAP treatment, total after-sleep bilirubin level was significantly higher than the pre-sleep level ($p<0.0001$). The difference between the serum indirect bilirubin levels in the morning versus in the previous afternoon [D-(M-A)-IB] decreased significantly with nCPAP treatment ($p<0.01$). The magnitude of decrease in D-(M-A)-IB after nCPAP treatment correlated significantly with changes in the percent time spent with arterial O_2 saturation below 90% ($r=0.44$; $p=0.04$) and 85% ($r=0.49$; $p=0.02$), respectively, during sleep after nCPAP treatment.

Conclusions: The increase in bilirubin level by HO-1 might protect OSAHS patients from disorders related to hypoxemia.

Key words: Heme oxygenase-1 (HO-1); bilirubin; nCPAP

INTRODUCTION

RECENTLY, IT HAS BEEN REPORTED THAT HYPOXIA INDUCES SEVERE RIGHT VENTRICULAR DILATION AND INFARCTION IN HEME OXYGENASE-1 (HO-1) null mice.[1] Heme oxygenase-1 (HO-1), also known as heat shock protein 32,[2,3] is a 32-kDa protein. Expression of HO-1,[4,5] which is the major enzyme involved in heme degradation, is dramatically induced by heme and by a variety of stress conditions, including exposure to hypoxia, hyperoxia, hydrogen peroxide, irradiation, heavy metals, chemotherapeutic agents, microbial products such as endotoxin, hormones, and various cytokines. HO-1, induced by various factors, protects against tissue injury by metabolizing heme; fostering the synthesis of the Fe-sequestering protein ferritin; producing carbon monoxide (CO), which is vasodilatory; and promoting the production of indirect bilirubin, an anti-oxidant (Figure 1).

Obstructive sleep apnea-hypopnea syndrome (OSAHS) causes hypoxemia, sleep fragmentation, elevated sympathetic nerve activity, hypertension[6] and cerebral ischemia[7] during sleep. Studies suggest that OSAHS is a risk factor for hypertension,[8,9] stroke[10] and myocardial infarction.[11] However, the increase in the mortality rate in untreated patients with OSAHS due to cerebrovascular and cardiovascular diseases[12,13] is controversial.[14] In addition, patients with OSAHS seldom die in their sleep.[15] Therefore, there might be some protective responses in OSAHS patients to the adverse effects of sleep apnea. Recently, it has been reported that OSAHS has significant effects on plasma cytokine levels, such as tumor necrosis factor-α (TNFα) and interleukin-6 (IL-6).[16] OSAHS also has significant effects on the levels of heat shock protein (HSP), which is one of the heat shock (stress) proteins.[17] We hypothesized that apnea-related hypoxemia would induce HO-1 and that the bilirubin levels in the morning in OSAHS patients are higher than those before sleep and that these relationships are changed by nasal continuous positive airway pressure (nCPAP) treatment. We determined the bilirubin levels in the afternoon before sleep and in the morning

Accepted for publication November 2000

Address correspondence to: Kazuo Chin MD, Department of Physical Therapeutics, Kyoto University Hospital of Medicine, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan; Tel: +81-75-751-3852; Fax: +81-75-751-3854; E-mail: chink@kuhp.kyoto-u.ac.jp

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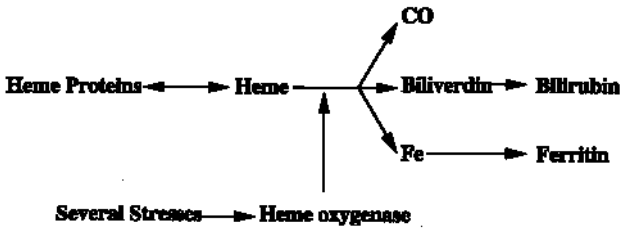

Figure 1—Pathway of heme breakdown.

immediately after sleep in OSAHS patients before and after nCPAP was started and in an age-matched control group.

METHODS

Subjects

The patients were 22 males (age: mean [SEM] 47.0 [2.9] yr, body mass index [BMI]: 29.4 [0.7] kg/m^2) with OSAHS (apnea and hypopnea index: AHI>20 episodes/h) who underwent nCPAP. Polysomnography was performed[17,18] before nCPAP was started and again on the first night of nCPAP. The period between the two polysomnographic examinations was one week. OSAHS was established on the basis of clinical and polysomnographic criteria. In addition to clinical symptoms, an AHI of more than 20 events per hour was also used as a selection criterion because these patients were candidates for nCPAP therapy (Table 1). Normal age-matched subjects consisted of 13 men (age: 43.6 [1.9] yr, BMI: 24.2 [0.52] kg/m^2). They were healthy doctors or health care workers who did not snore heavily. BMI in the control group was significantly lower than that in the OSAHS group ($p<0.01$). This study was approved by the Institutional Committee for the Protection of Human Subjects, and all patients gave their informed consent prior to the study.

Polysomnography

Polysomnography was performed before the nCPAP therapy and again on the first night of nCPAP therapy. Surface electrodes were applied using standard techniques to obtain an electroencephalogram, an electromyogram of the chin, an electrocardiogram, and an electrooculogram. Sleep was defined according to the criteria of Rechtscaffen and Kales.[19] Ventilation was monitored by inductive plethysmography (Respitrace; Ambulatory Monitoring, Ardsley, NY). Airflow was monitored by thermistors (Nihon Kohden, Tokyo) placed at the nose and mouth, and arterial O_2 saturation (SaO_2) was monitored continuously with a pulse oximeter (Minolta-Pulsox 7, Tokyo). A polygraph (Nihon Kohden Polygraph System RM-6000, Tokyo) was run continu-

Table 1—Polysomnographic characteristics and serum variables

		nCPAP(-)(n=22)	nCPAP(+)(n=22)	controls(n=13)	p
AHI or DI	(1/h)	59.5(4.5)[50.2-68.7]*	3.9(0.7)[2.4-5.4]	1.9(0.5)[0.7-3.1]	<0.0001
SaO2<90%	(%)	36.2(4.4)[45.2-27.0]*	0.6(0.2)[0.1-1.1]	0(0)[0-0]	<0.0001
SaO2<85%	(%)	22.1(3.9)[14.0-30.2]*	0.2(0.1)[0.0-0.4]	0(0)[0-0]	<0.0001
GOT(m)	(IU/L)	32.6(4.7)[22.8-42.5]	28.0(3.2)[21.3-34.6]	23.4(3.1)[15.9-30.9]	0.42
GPT(m)	(IU/L)	55.6(13.7)[27.1-84.2]	47.5(8.7)[29.4-65.7]	26.9(8.2)[7.4-46.4]	0.41
LDH(m)	(IU/L)	306.0(14.0)[277-335]	284(16.1)[261-307]	300(11.7)[273-328]	0.42
TB(a)	(mg/dl)	0.44(0.04)[0.36-0.51]	0.44(0.05)[0.34-0.53]	0.45(0.03)[0.40-0.51]	0.94
TB(m)	(mg/dl)	0.65(0.04)[0.57-0.73]	0.56(0.05)[0.46-0.65]	0.48(0.06)[0.35-0.60]	0.10
DB(a)	(mg/dl)	0.14(0.01)[0.12-0.17]	0.14(0.02)[0.10-0.19]	0.19(0.02)[0.15-0.24]	0.14
DB(m)	(mg/dl)	0.21(0.01)[0.18-0.24]	0.19(0.02)[0.16-0.23]	0.19(0.03)[0.12-0.26]	0.73
IB(a)	(mg/dl)	0.29(0.03)[0.23-0.35]	0.29(0.03)[0.23-0.36]	0.28(0.02)[0.23-0.32]	0.92
IB(m)	(mg/dl)	0.44(0.03)[0.38-0.51]*	0.36(0.04)[0.29-0.44]	0.29(0.03)[0.22-0.35]	0.01
D-(M-A) IB	(mg/dl)	0.15(0.02)[0.11-0.19]*	0.07(0.03)[0.02-0.12]	0.008(0.04)[-0.07-0.09]	0.002

Values are means (SEM)[95%CI]. nCPAP(-) indicates before nCPAP treatment; nCPAP (+), after nCPAP treatment; AHI, apnea and hypopnea index; DI, desaturation index; S_aO_2, arterial O_2 saturation; (m), in the morning; (a), in the afternoon; GOT, glutamic oxaloacetic transaminase; GPT, glutamic pyruvic transaminase; LDH, lactic dehydrogenase; TB, total bilirubin; DB, direct bilirubin; IB, indirect bilirubin; D-(M-A) IB, difference between the indirect bilirubin levels in the morning versus levels in the previous afternoon;
*$p<0.01$ vs after nCPAP treatment and controls.

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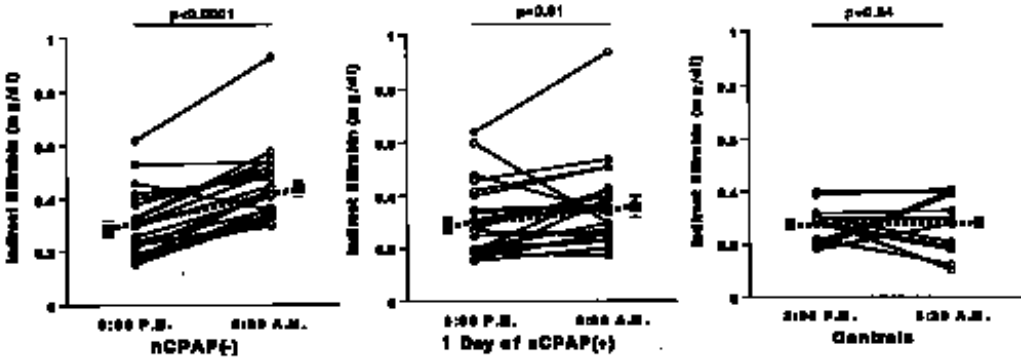

Figure 2—Indirect bilirubin levels in the sera of patients (n=22) with obstructive sleep apnea-hypopnea syndrome in the afternoon and the next morning with (-) and without (+) nasal continuous positive airway pressure treatment (nCPAP) treatment. The data of the controls (n=13) are also shown. Dotted line represents the mean (SEM).

ously at 10 mm/s to simultaneously record all of the above physiological data throughout the course of the experiment. All of the parameters were stored in a data recorder (Sony 621A, Tokyo) for subsequent analysis.

Apnea was defined as the cessation of airflow at the nose and mouth lasting for more than 10 seconds.[20] Hypopnea was defined as a decrease of 50% or more in thoracoabdominal motion associated with a fall in baseline oxygen saturation of 4% or more.[21] The AHI was calculated to express the number of episodes of apnea and hypopnea per hour of total sleep time.

Normal control subjects were monitored for their SaO_2 during sleep by a pulse oximeter (Minolta-Pulsox 5). Desaturation during sleep was defined as a fall in baseline oxygen saturation of 4% or more. The desaturation index was calculated to express the number of episodes of desaturation per hour of total sleep time.

Protocol (Blood Samples)

Bilirubin was reported to have a circadian variation in which the bilirubin decreased significantly around 3:00 P.M.[22] Therefore, blood samples were drawn at 3:00 P.M. following three hours of fasting before polysomnography, and again at 8:30 A.M. after polysomnography. Blood samples were also drawn at 8:30 A.M. after one month of nCPAP treatment (n=19). Blood samples were drawn within one minute of venostasis from an antecubital vein into vacuum-collection tubes using a 21-gauge needle.

The blood samples were analyzed for total and direct bilirubin, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and lactic dehydrogenase (LDH) levels. Serum K^+ levels were also measured before nCPAP treatment. Indirect bilirubin level was calculated from total and direct bilirubin.

Statistical Analysis

All variables were normally distributed and are quoted as means with standard errors. Differences among the values obtained from those patients with and without nCPAP and controls (Table 1) and before and after 1 day and 1 month of nCPAP treatment (Figure 5) were tested by analysis of variance (ANOVA). If there was a significant difference by ANOVA, the difference between two values was tested by a multiple comparison method (Fisher's protected least significant differences) with a Bonferroni correction. Two-tailed paired or unpaired t tests were used to compare the data between the two conditions. Regression analysis was performed between pairs of variables. A p value of <0.05 was considered to be significant.

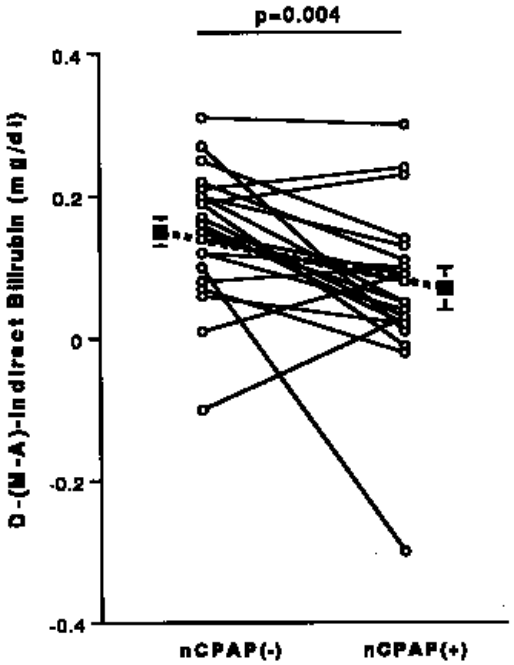

Figure 3—Difference (n=22) between the serum indirect bilirubin levels in the morning vs. in the previous afternoon [D-(M-A)-indirect bilirubin] with (+) and without (-) nasal continuous positive airway pressure treatment (nCPAP) treatment. Dotted line represents the mean (SEM).

RESULTS

Bilirubin levels in OSAHS patients

nCPAP treatment improved the OSAHS group's mean AHI from 59.5 (4.5) to 3.9 (0.7) (Table 1). Serum total bilirubin levels in the morning significantly increased before nCPAP treatment (from 0.44 [0.04] mg/dl in the afternoon to 0.65 [0.04] mg/dl the next morning, $p<0.0001$) (Table 1). After the first night of nCPAP treatment, the indirect bilirubin levels in the morning decreased significantly (before nCPAP: 0.44 (0.03) mg/dl vs. after nCPAP

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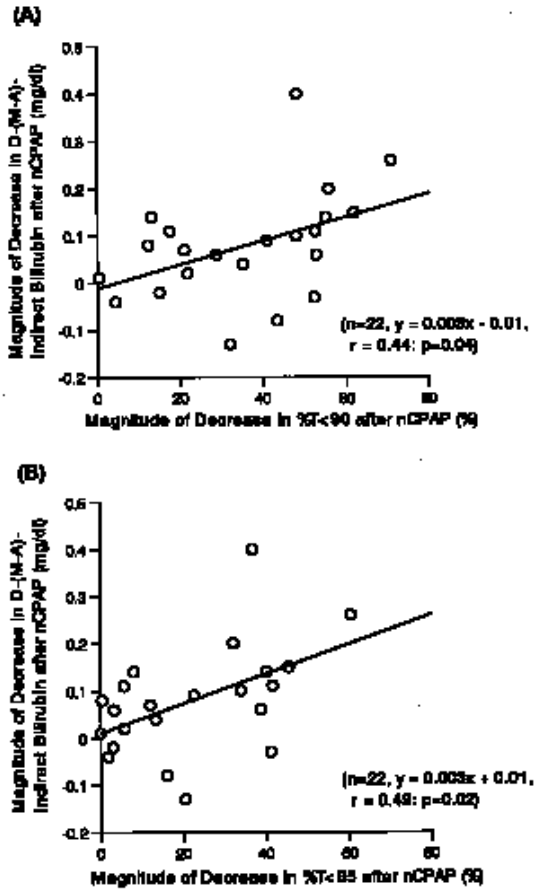

Figure 4—The relationship between the magnitude of decrease in the percentage of time spent with an arterial O_2 saturation below 90% (%T< 90) (A) and 85% (%T< 85) (B) during sleep after nCPAP treatment and the magnitude of decrease in D-(M-A) indirect bilirubin after nCPAP treatment. nCPAP: nasal continuous positive airway pressure treatment, D-(M-A) indirect bilirubin: difference between the indirect bilirubin levels in the morning vs. levels on the previous afternoon.

0.36 (0.04) mg/dl: p=0.0004) (Table 1 and Fig. 2). The difference between the indirect bilirubin levels in the morning vs. levels in the previous afternoon (D-[M-A] indirect bilirubin) decreased significantly with nCPAP treatment (from 0.15 [0.02] to 0.07 [0.03] mg/dl; p<0.01) (Table 1 and Fig. 3). The magnitude of decrease in D-(M-A) indirect bilirubin after nCPAP treatment correlated significantly with the magnitude of changes in the percentage of time spent with an arterial O_2 saturation below 90% and 85%, respectively, during sleep after nCPAP treatment (Figure 4), but not with the magnitude of improvement in AHI (r=0.07; p=0.24). Before nCPAP treatment, the difference between K^+ (P.M. 3.97 [0.07] mEq/L; A.M. 3.95 [0.05] mEq/L; p=0.28) and LDH (P.M. 322 [11] IU/L; A.M. 306 [14] IU/L; p=0.16) levels before and after sleep did not significantly change.

After one month of nCPAP treatment, the indirect bilirubin levels (n=19) decreased more significantly according to the duration of treatment, while the direct bilirubin levels remained the same (Figure 5). BMI of the subjects did not change significantly during this period (BMI: before, 29.3 [0.8] kg/m^2, after one month of nCPAP, 29.1 [0.8] kg/m^2, p=0.14) The six patients who were randomly checked for use of nCPAP used the treatment 5.0 (0.8) hours per day.[23]

Bilirubin Levels in Control Subjects

The controls were doctors or other health care workers who were familiar with the procedure for SaO_2 measurement. All of the control subjects said that the nocturnal SaO_2 monitoring did not disturb their sleep and the duration of monitoring SaO_2 was 415[17] minutes. The desaturation index of the control subjects during sleep was 1.9 (0.5) (Table 1). The total bilirubin levels in the afternoon and the next morning and D-(M-A) indirect bilirubin levels in control subjects were 0.45 (0.03) mg/dl, 0.48 (0.06) mg/dl, and 0.008 (0.04) mg/dl, respectively. These data were not significantly different with such data in OSAHS patients with nCPAP treatment (Table 1 and Fig. 2).

DISCUSSION

This study showed that the indirect bilirubin levels in OSAHS patients increased significantly in the morning after sleep without nCPAP treatment, and that this increased value was decreased significantly after nCPAP treatment (Table 1 and Figs. 2, 3, and 5) The difference of K^+ and LDH levels before and after sleep did not significantly change before nCPAP treatment. Therefore, the significant increase in indirect bilirubin levels in the morning vs. levels in the previous afternoon without nCPAP treatment would not be due to hemolysis, but was possibly induced by HO-1 with repetitive sleep apnea. This is the first report of the existence of a protective factor (bilirubin) in pro-

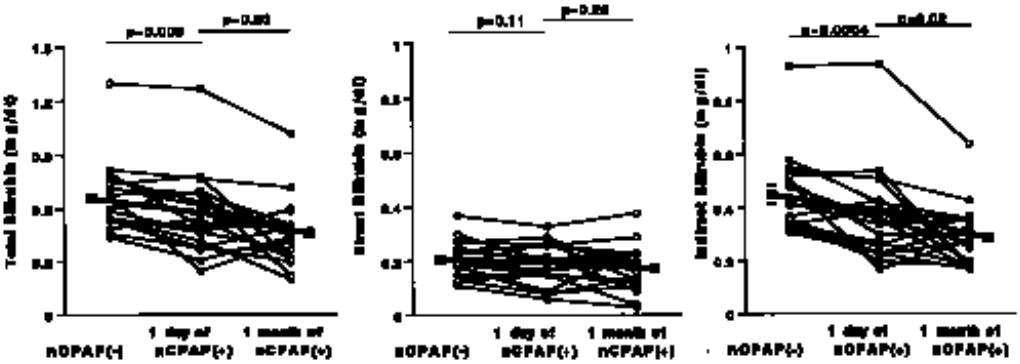

Figure 5—Levels of total, direct and indirect bilirubin in the sera of patients (n=19) with obstructive sleep apnea-hypopnea syndrome before, after 1 day and after 1 month of nasal continuous positive airway pressure (CPAP) treatment. Dotted line represents the mean (SEM).

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portion to the severity of the hypoxemia during sleep in OSAHS patients.

Bilirubin, a product of heme catabolism, inhibits protoporphyrinogen oxidase, providing an additional mechanism for hypoxic adaptation mediated through feedback inhibition of the heme biosynthetic pathway.[24,25] It was reported that bilirubin levels showed small variations between morning (7:00—8:00 A.M.) and evening (5:00—6:00 P.M.).[26] Another report found a downward trend in bilirubin levels throughout the afternoon.[22] However, the latter report also showed that bilirubin levels at 9:00 A.M. and 2:00 P.M. were almost the same. The bilirubin levels in our controls at 3:00 P.M. (0.45 [0.03] mg/dl) and at 8:30 A.M. (0.48 [0.06] mg/dl) were almost the same as shown in previous reports.[22,26] Therefore, normal circadian variations of serum bilirubin levels would not have a significant effect on this study. Bilirubin is itself a potent antioxidant.[27-29] In vitro, Stocker and coworkers[28] showed that bilirubin, at concentrations found in normal human plasma, protects albumin from oxidation. This antioxidant effect of bilirubin also has been demonstrated in vivo.[29] It is said that the measurement of bilirubin may be a useful index of in vivo oxidative stress, although no big differences in bilirubin levels should be expected, because bilirubin is destroyed by the same molecules that induce its production.[30] Due to this rapid metabolism of bilirubin, the correlation between the degree of arterial desaturation and the magnitude of increase in D-(M-A) indirect bilirubin might be weak (r=0.44, 0.49) , although it was statistically significant (p=0.04 or 0.02) (Figure 4).

As mentioned above, the origin of the elevation of bilirubin levels in the morning in OSAHS patients would hardly be caused by hemolysis because K^+ and LDH levels before and after sleep did not change significantly before nCPAP treatment. Therefore, high levels of bilirubin in the morning in OSAHS patients before nCPAP treatment would be induced by the degradation of heme through HO. Indeed, the magnitude of decrease in D-(M-A) indirect bilirubin after nCPAP treatment correlated significantly with the magnitude of changes in the percentage of time spent with an arterial O_2 saturation below 90% and 85%, respectively, during sleep after nCPAP treatment (Fig. 4).

One HO isoenzyme (HO-2) is expressed constitutively, and the other (HO-1) is induced by an extraordinary array of stimuli.[4,5] Recently, it has been reported that HO-1 plays an important protective role in the adaptation of the cardiovascular system to hypoxia. It also has been suggested that HO-1 may play a central role in cardiac physiology by protecting cardiomyocytes from pressure-induced injury and secondary oxidative damage.[1] Thus, bilirubin, CO and ferritin induced by HO-1 might protect OSAHS patients with apnea-related hypoxemia, although CO and ferritin were not measured in this study.

If HO-1 levels have significant effects on the cardiovascular system under hypoxic conditions,[1] OSAHS patients whose HO-1 levels are low would have cardiovascular diseases due to OSAHS. Therefore, to understand the direct role of HO-1 in OSAHS patients, HO-1 levels, such as in peripheral blood mononuclear cells,[17] should be measured before, during, and after sleep in OSAHS patients with and without nCPAP treatment. In addition to the lack of HO-1 measurements, one of the limitations of this study was that controls were not BMI-matched with the OSAHS patients. However, the BMI of OSAHS patients before and after one month of nCPAP therapy did not change significantly. Therefore, BMI and BMI-related hepatic metabolism would have little effect on the results of this study.

Although we did not measure HO-1 levels in OSAHS patients before and after nCPAP treatment, the increase in indirect bilirubin levels during sleep in OSAHS patients before nCPAP treatment might be the result of increased HO-1 activity induced by hypoxemia but not AHI. Although the health effects of hypoxemia due to obstructive sleep apnea are not overt at present,[14] this increase in bilirubin level might protect OSAHS patients from disorders related to hypoxemia induced by repetitive sleep apnea.

AKNOWLEDGMENTS

This work was funded by the Japanese Ministry of Health and Welfare.

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