Aihara K, Oga T, Harada Y, Chihara Y, Handa T, Tanizawa K, Watanabe K, Tsuboi T, Hitomi T, Mishima M, Chin K.	Comparison of biomarkers of subclinical lung injury in obstructive sleep apnea.	Respir Med	105	939-945	2011
Nagayoshi M, Tanigawa T, Yamagishi K, Sakurai S, Kitamura A, Kiyama M, Okada T, Maeda K, Ohira T, Imano H,	Self-Reported Snoring Frequency and Incidence of Cardiovascular Disease: The Circulatory Risk in Communities Study (CIRCS).	J Epidemiol	In press		2012
Yamauchi M, Tamaki S, Yoshikawa M, Ohnishi Y, Nakano H, Jacono FJ, Loparo KA, Strohl KP, Kimura H.	Differences in breathing patterning during wakefulness in patients with mixed apnea-dominant vs obstructive-dominant sleep apnea.	Chest	140	54-61	2011
山内基雄, 木村　弘	閉塞性睡眠時無呼吸症候群の病態生理と診断	綜合臨床	60	1647-1651	2011
山内基雄, 吉川雅則, 木村 弘	原発性肺胞低換気症候群・肥満低換気症候群	呼吸と循環	59	145-148	2011
太田浩世、木村　弘	睡眠呼吸障害と糖尿病	Medicina	48	1010-1013	2011
中野　博	簡易モニターのピットフォール	睡眠医療	5	189-193	2011
中野　博	症状からアプローチするプライマリケア—いびき	日本医師会雑誌	140	s45-s48（特別号）	2011
淡野桜子　谷川武	睡眠呼吸障害の社会的問題点 わが国における疫学研究を中心に	medicina	48	928-933	2011
淡野桜子　谷川武	睡眠障害と生活習慣病	保健師ジャーナル	67	579-583	2011
淡野桜子　谷川武	睡眠時無呼吸症候群（SAS）対策の現状	公衆衛生	75	760-763	2011
淡野桜子、谷川武	睡眠呼吸障害と合併症　－特に糖尿病との関連での最近の話題－	睡眠医療	6	31-35	2012

Ⅳ. 研究成果の刊行物・別刷

2．酸化ストレス，脂質異常症，耐糖能障害

山内　基雄，木村　　弘

はじめに

米国を中心とした大規模疫学研究において，閉塞性睡眠時無呼吸症候群(obstructive sleep apnea syndrome：OSAS)は生命予後悪化因子であることが明らかにされ，その大きな要因として脳卒中を含む心臓血管疾患が挙げられている[1-4]．なかでも，Youngらは18年間の前向き研究において，心臓血管疾患に関連した死亡は経鼻的持続陽圧呼吸(nasal continuous positive airway pressure：nasal CPAP)療法が施行されていない重症OSASで，睡眠呼吸障害を有さない症例に比較して約5.2倍高かったと報告している[1]．

心臓血管疾患発症の背景因子として，酸化ストレス，脂質異常症，インスリン抵抗性を含む耐糖能障害は重要な位置を占める．近年，睡眠呼吸障害と酸化ストレス，耐糖能障害との関連について，臨床および基礎研究の両面から報告が蓄積されている．本稿では，睡眠呼吸障害がいかにして酸化ストレス，脂質異常症や耐糖能障害を惹起し，心臓血管疾患発症進展に寄与するかについて解説したい．

酸化ストレスと睡眠呼吸障害

酸化ストレスとは，生体の内因性あるいは外因性の原因により生じる活性酸素種(reactive oxygen species：ROS)を生体が十分処理することができない状態をいう．生体はROSを水まで還元する消去系システムを備えているが，その消去系システムに何らかの障害が生じた場合や，消去系システムを凌駕するROSの生成がなされると酸化ストレス状態に陥る．過剰なROSは生体組織傷害を引き起こし，多岐にわたる疾患の発症に寄与することが知られている[5]．循環器領域，とりわけ冠動脈疾患においては虚血再灌流傷害による心筋傷害が注目されてきた．これは心筋梗塞などの心筋虚血(組織低酸素状態)を解除すると，再灌流したことによってさらに心筋傷害が増悪する状態をいう．すなわち，急速再酸素化時に心筋でROSが過剰産生され，心筋傷害が引き起こされるのである．この現象は，まさにOSASで終夜繰り返される無呼吸低呼吸による組織低酸素再酸素化の生理学的変化に類似している．したがって，OSASによる生理学的変化の中で，間歇的低酸素血症は酸化ストレスとの関連が最も強いと考えられる．

ここ約10年で，OSASと酸化ストレスとの有意な関連性について多くのエビデンスが蓄積されてきた．Schulzらは OSAS患者の末梢血好中球の産生するROSが有意に高く，CPAPによりROS産生が低下することを[6]，Dyugovskayaらは OSAS患者の末梢血単球と顆粒球の一部で同様のことを報告している[7]．脂質過酸化を酸化ストレスのマーカーとした研究は多くなされており，チオバルビツール酸反応陽性物質(TBARS)はOSASで有意に高値を示し，CPAPで低下する[8,9]．さらに，OSASでは酸化ストレス防御機能(活性酸素種消去系システム)，すなわち抗酸化能の低下がみられ，CPAPはその機能を改善することも示されている[9-11]．また，われわれの報告ではDNA酸化的

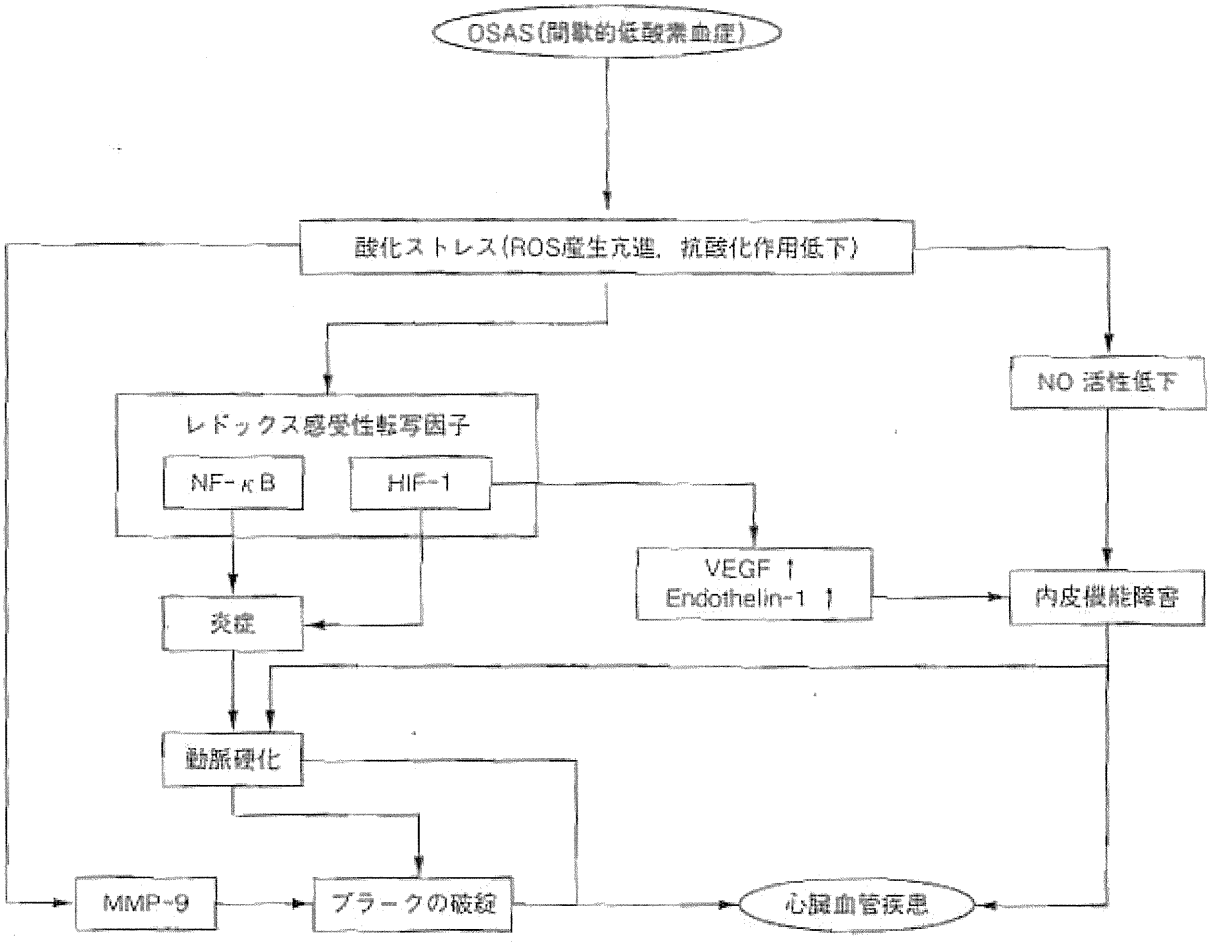

図1　OSASにおける酸化ストレスを介した心臓血管疾患発症進展機序

(文献22より改変)

損傷マーカー，尿中8-OHdGは重症OSAS患者で有意に高く，8-OHdGは肥満や喫煙などの酸化ストレスに影響を及ぼす交絡因子を調整した後においても，OSASの重症度と有意な相関関係を示した．なかでも，酸素飽和度低下指数(oxigen desaturation index：ODI)が無呼吸-低呼吸指数(apnea-hypopnea index：AHI)よりも8-OHdGと強い相関を示した．AHIは低呼吸の定義によっては，脳波上一過性覚醒(arousal)が加味されることがあり，AHIは必ずしも間歇的低酸素状態の重症度を示すとはいえない．したがって，ODIと8-OHdGが最も強い相関を示したことは，OSASの酸化ストレス亢進には間歇的低酸素血症が重要である可能性を示唆する興味深い知見である[12]．

酸化ストレスは肥満や喫煙など多くの因子と関連し，一方でOSASの病態生理学的変化は，無呼吸低呼吸に伴う間歇的低酸素血症，胸腔内圧変動，睡眠分断など多岐にわたる．そのため，OSASと酸化ストレスの関連を検討する際に絶えず，OSAS側ではOSASのどの因子が酸化ストレスに最も寄与するのか，酸化ストレス側では肥満や喫煙など交絡因子の影響の調整が問題となる．しかしながら，動物を用いた研究では，このような交絡因子を除外することが可能であり，間歇的低酸素血症単独の効果を評価することができる．間歇的低酸素に曝露されたラットの心筋では酸化ストレスが亢進しており，心機能障害と関連している可能性が示されている[13,14]．また，間歇的低酸素に曝露されたラットやマウスの脳皮質や脳神経細胞では酸化ストレスが亢進しており，それらは脳神経細胞のアポトーシスや総睡眠時間の延長，睡眠潜時の短縮，さらには空間位置学習能力の低下を引き起こす[15-18]．

さて，酸化ストレスの亢進がどのようにOSASの心臓血管疾患発症進展に寄与するのであろうか(図1)．ROSは，NF-κBをはじめとしたレドッ

クス感受性転写因子を活性化させることが知られている．OSAS患者では，末梢血単球中のNF-κBが活性化されており，その転写産物であるTNF-α，MCP-1，MMP-9など炎症性サイトカイン，ケモカインの産生が亢進し，単球・マクロファージの血管内皮への浸潤能が亢進し，血管内皮局所の炎症を引き起こすことが一連の研究で示されている[19-21]．さらに，NF-κBはその他全身性炎症に関わる様々な蛋白を産生し，血管内皮傷害を経て心臓血管疾患の基盤となる動脈硬化を引き起こすのである[22]．また，hypoxia inducible factor-1（HIF-1）は同じくレドックス感受性転写因子であり，HIF-1はVEGFやエンドセリンなどを産生し，血管内皮機能障害を引き起こす．ただ，持続的低酸素曝露がHIF-1を活性化させることは明らかにされているものの，間歇的低酸素曝露がHIF-1の活性化を誘導するかどうかは完全には明らかではない[23]．また，一酸化窒素（NO）は血管内皮機能を維持する上で非常に重要な因子であるが，ROSはNOを速やかに失活させることが知られており，間歇的低酸素により亢進した酸化ストレスはNOの失活を介して，血管内皮機能障害を惹起する可能性も考えられている[22]．

脂質異常症と睡眠呼吸障害

OSASと脂質異常症の関連についての報告は意外に少なく，また結果は相反している．すなわち，臨床研究ではOSASと脂質異常症は関連し，CPAPは脂質異常症を改善するというエビデンスは希薄である[23]．おそらく併存する肥満因子が大きく影響しているものと考えられる．一方で，ラットやマウスを用いた研究では，脂質異常症は間歇的低酸素曝露の期間に依存し，4日間程度の間歇的低酸素曝露では脂質代謝に影響はないが，3～4週間間歇的低酸素に曝露させることでLDLコレステロールの上昇などの脂質異常症が観察される[24-26]．

糖代謝異常と睡眠呼吸障害

近年，OSASと脳卒中を含む心臓血管疾患との密接な関連に次いで，OSASと耐糖能障害との関連が注目されてきている．米国における代表的な大規模疫学研究，Wisconsin Sleep Cohort StudyおよびSleep Heart Health Studyは，AHI15回/hr以上の患者では，AHI 5回/hr未満の患者に比較して約1.4～2.3倍耐糖能障害が観察されると報告している[27,28]．またアジアからの報告では，香港でIpらがAHIとインスリン抵抗性指数（HOMA-IR）や空腹時インスリン値は独立した相関関係を示すことを報告した[29]．このように，横断的研究においてはOSASと耐糖能障害との有意な関連性を示唆する報告が多い．しかしながら，前向き研究や治療介入研究では事情が少し異なる．スウェーデンと米国からは，それぞれ2,688人，69,852人のNurses' Health Study Cohortを10年間観察したところ，睡眠呼吸障害のサロゲートマーカーであるいびきの有無は糖尿病発症と独立した関連を示したものの[30,31]，終夜睡眠ポリグラフでOSASと診断した症例の前向き研究（Wisconsin Sleep Cohort Study）では，4年間の観察期間においてOSASの存在は糖尿病発症には寄与していなかった[28]．さらに，CPAP療法の糖代謝改善効果については相反した結果が報告されており[32]，依然この領域についてはさらなる研究報告の蓄積が待たれる．

糖尿病の病型には人種差が指摘されている．つまり，日本人はインスリン分泌障害型，欧米人はインスリン抵抗性型といわれる．最近の遺伝子解析で，この人種差を説明するいくつかの責任遺伝子が同定されつつある．したがって，わが国で耐糖能障害とOSASの関連を議論する場合，欧米からの大規模疫学研究をそのままわが国に当てはめてよいかどうかは重要な問題であるとともに，耐糖能障害の評価方法を適切に選択しなければならないと考える．つまり，インスリン分泌障害型を特徴とするわが国で，インスリン抵抗性指数が果たしてOSASとの関連を正しく評価するかどうかは疑問が残る．近年，横断的研究および前向き研究で，OSASと耐糖能障害は有意な関連があること，また夜間酸素飽和度低下指数は有意な糖尿病発症危険因子であるとのわが国の報告が散見されるが[33,34]，人種差が明らかになった現在，OSASと耐糖能障害に関するわが国からの報告が盛んに

世界に向かって発信されることを期待したい．

OSASが耐糖能障害を引き起こす機序については，従来から無呼吸低呼吸に伴う交感神経活動の亢進，コルチゾールの過剰分泌などが考えられてきた．近年，睡眠時間そのものの剥奪や，深睡眠（徐波睡眠）の剥奪により耐糖能異常が惹起されるとの報告もあり[35-37]，OSASに関連した睡眠分断や睡眠構築の悪化もその原因かもしれない．また，間歇的低酸素曝露による酸化ストレスを介したレドックス感受性転写因子が膵臓β細胞においても変調を来し，膵臓β細胞増殖あるいはアポトーシス，分泌障害を引き起こし糖代謝障害に寄与する可能性も考えられる．

おわりに

以上，睡眠呼吸障害と酸化ストレス，脂質異常症，および耐糖能障害との関連について概説した．OSASでは，酸化ストレスが亢進していることはほぼ間違いないと思われる．酸化ストレスを起点とした炎症性プロセスは，心臓血管疾患発症に重要な役割を果たすと考えられる．OSASの脂質異常症との関連については，さらなるエビデンスの蓄積が待たれる．耐糖能障害については，いまだ完全にその有意な関連が証明されているとは言い難い．耐糖能障害のphenotype（インスリン抵抗性型，インスリン分泌障害型）について人種差の存在が明らかにされている中で，インスリン分泌障害型といわれる日本人を対象としたOSASと耐糖能障害に関する研究の蓄積が必要であると考える．また，OSASと耐糖能障害の有意な関連を確実にするためには，耐糖能障害を引き起こすメカニズムのさらなる研究も不可欠である．

文　献

1）Young T, Finn L, Peppard PE, et al：Sleep disordered breathing and mortality：eighteen-year follow-up of the Wisconsin sleep cohort. Sleep 2008；31：1071-1078.

2）Marin JM, Carrizo SJ, Vicente E, et al：Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure：an observational study. Lancet 2005；365：1046-1053.

3）Yaggi HK, Concato J, Kernan WN, et al：Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 2005；353：2034-2041.

4）Lavie P, Lavie L, Herer P：All-cause mortality in males with sleep apnoea syndrome：declining mortality rates with age. Eur Respir J 2005；25：514-520.

5）谷口直之，淀井淳司：酸化ストレス・レドックスの生化学．共立出版株式会社，東京，2000.

6）Schulz R, Mahmoudi S, Hattar K, et al：Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea Impact of continuous positive airway pressure therapy. Am J Respir Crit Care Med 2000；162：566-570.

7）Dyugovskaya L, Lavie P, Lavie L：Increased adhesion molecules expression and production of reactive oxygen species in leukocytes of sleep apnea patients. Am J Respir Crit Care Med 2002；165：934-939.

8）Barcelo A, Miralles C, Barbe F, et al：Abnormal lipid peroxidation in patients with sleep apnoea. Eur Respir J 2000；16：644-647.

9）Lavie L, Vishnevsky A, Lavie P：Evidence for lipid peroxidation in obstructive sleep apnea. Sleep 2004；27：123-128.

10）Christou K, Moulas AN, Pastaka C, et al：Antioxidant capacity in obstructive sleep apnea patients. Sleep Med 2003；4：225-228.

11）Barcelo A, Barbe F, de la Pena M, et al：Antioxidant status in patients with sleep apnoea and impact of continuous positive airway pressure treatment. Eur Respir J 2006；27：756-760

12）Yamauchi M, Nakano H, Maekawa J, et al：Oxidative stress in obstructive sleep apnea. Chest 2005；127：1674-1679.

13）Liu JN, Zhang JX, Lu G, et al：The effect of oxidative stress in myocardial cell injury in mice exposed to chronic intermittent hypoxia. Chin Med J (Engl) 2010；123：74-78.

14）Chen L, Einbinder E, Zhang Q, et al：Oxidative stress and left ventricular function with chronic intermittent hypoxia in rats. Am J Respir Crit Care Med 2005；172：915-920.

15）Veasey SC, Davis CW, Fenik P, et al：Long-term intermittent hypoxia in mice：protracted hypersomnolence with oxidative injury to sleep-wake brain regions. Sleep 2004；27：194-201.

16）Zhan G, Serrano F, Fenik P, et al：NADPH oxidase mediates hypersomnolence and brain oxidative injury in a murine model of sleep apnea. Am J Respir Crit Care Med 2005；172：921-929.

17) Xu W, Chi L, Row BW, et al : Increased oxidative stress is associated with chronic intermittent hypoxia-mediated brain cortical neuronal cell apoptosis in a mouse model of sleep apnea. Neuroscience 2004 ; 126 : 313-323.
18) Row BW, Liu R, Xu W, et al : Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. Am J Respir Crit Care Med 2003 ; 167 : 1548-1553.
19) Yamauchi M, Tamaki S, Tomoda K, et al : Evidence for activation of nuclear factor kappaB in obstructive sleep apnea. Sleep Breath 2006 ; 10 : 189-193.
20) Tamaki S, Yamauchi M, Fukuoka A, et al : Production of inflammatory mediators by monocytes in patients with obstructive sleep apnea syndrome. Intern Med 2009 ; 48 : 1255-1262.
21) Tamaki S, Yamauchi M, Fukuoka A, et al : Nocturnal hypoxic stress activates invasive ability of monocytes in patients with obstructive sleep apnoea syndrome. Respirology 2009 ; 14 : 689-694.
22) Yamauchi M, Kimura H : Oxidative stress in obstructive sleep apnea : putative pathways to the cardiovascular complications. Antioxid Redox Signal 2008 ; 10 : 755-768.
23) Drager LF, Jun J, Polotsky VY : Obstructive sleep apnea and dyslipidemia : implications for atherosclerosis. Curr Opin Endocrinol Diabetes Obes 2010 ; 17 : 161-165.
24) Li J, Savransky V, Nanayakkara A, et al : Hyperlipidemia and lipid peroxidation are dependent on the severity of chronic intermittent hypoxia. J Appl Physiol 2007 ; 102 : 557-563.
25) Li J, Thorne LN, Punjabi NM, et al : Intermittent hypoxia induces hyperlipidemia in lean mice. Circ Res 2005 ; 97 : 698-706.
26) Perry JC, D'Almeida V, Souza FG, et al : Consequences of subchronic and chronic exposure to intermittent hypoxia and sleep deprivation on cardiovascular risk factors in rats. Respir Physiol Neurobiol 2007 ; 156 : 250-258.
27) Punjabi NM, Shahar E, Redline S, et al : Sleep-disordered breathing, glucose intolerance, and insulin resistance : the Sleep Heart Health Study. Am J Epidemiol 2004 ; 160 : 521-530.
28) Reichmuth KJ, Austin D, Skatrud JB, et al : Association of sleep apnea and type II diabetes : a population-based study. Am J Respir Crit Care Med 2005 ; 172 : 1590-1595.
29) Ip MS, Lam B, Ng MM, et al : Obstructive sleep apnea is independently associated with insulin resistance. Am J Respir Crit Care Med 2002 ; 165 : 670-676.
30) Al-Delaimy WK, Manson JE, Willett WC, et al : Snoring as a risk factor for type II diabetes mellitus : a prospective study. Am J Epidemiol 2002 ; 155 : 387-393.
31) Elmasry A, Janson C, Lindberg E, et al : The role of habitual snoring and obesity in the development of diabetes : a 10-year follow-up study in a male population. J Intern Med 2000 ; 248 : 13-20.
32) Tasali E, Ip MS : Obstructive sleep apnea and metabolic syndrome : alterations in glucose metabolism and inflammation. Proc Am Thorac Soc 2008 ; 5 : 207-217.
33) Muraki I, Tanigawa T, Yamagishi K, et al : Nocturnal intermittent hypoxia and the development of type 2 diabetes : the Circulatory Risk in Communities Study (CIRCS). Diabetologia 2010 ; 53 : 481-488.
34) Otake K, Sasanabe R, Hasegawa R, et al : Glucose intolerance in Japanese patients with obstructive sleep apnea. Intern Med 2009 ; 48 : 1863-1868.
35) Spiegel K, Knutson K, Leproult R, et al : Sleep loss : a novel risk factor for insulin resistance and Type 2 diabetes. J Appl Physiol 2005 ; 99 : 2008-2019.
36) Spiegel K, Leproult R, Van Cauter E : Impact of sleep debt on metabolic and endocrine function. Lancet 1999 ; 354 : 1435-1439.
37) Tasali E, Leproult R, Ehrmann DA, et al : Slow-wave sleep and the risk of type 2 diabetes in humans. Proc Natl Acad Sci USA 2008 ; 105 : 1044-1049.

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Differences in relationships among sleep apnoea, glucose level, sleep duration and sleepiness between persons with and without type 2 diabetes

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SUMMARY

Obstructive sleep apnoea is common in patients with diabetes. Recently, it was reported that short sleep duration and sleepiness had deleterious effects on glucose metabolism. Thereafter, several reports showed relationships between glucose metabolism and obstructive sleep apnoea, sleep duration or sleepiness. But the interrelationships among those factors based on recent epidemiological data have not been examined. We analysed data on 275 male employees (age, 44 ± 8 years; body mass index, 23.9 ± 3.1 kg m^{-2}) who underwent a cross-sectional health examination in Japan. We measured fasting plasma glucose, sleep duration using a sleep diary and an actigraph for 7 days, and respiratory disturbance index with a type 3 portable monitor for two nights. Fifty-four subjects (19.6%) had impaired glucose metabolism, with 21 having diabetes. Of those 21 (body mass index, 25.9 ± 3.8 kg m^{-2}), 17 (81.0%) had obstructive sleep apnoea (respiratory disturbance index ≥ 5). Regarding the severity of obstructive sleep apnoea, 10, four and three had mild, moderate and severe obstructive sleep apnoea, respectively. The prevalence of obstructive sleep apnoea was greater in those with than without diabetes ($P = 0.037$). Multiple regression analyses showed that the respiratory disturbance index independently related to fasting plasma glucose only in the diabetic subjects. In patients with diabetes, after adjustment for age, waist circumference, etc. sleep fragmentation had a greater correlation with fasting plasma glucose than sleep duration, but without significance ($P = 0.10$). Because the prevalence of obstructive sleep apnoea is extremely high in patients with diabetes, sufficient sleep duration with treatment for obstructive sleep apnoea, which ameliorates sleep fragmentation, might improve fasting plasma glucose.

INTRODUCTION

Sleep and glucose metabolism have long been known to have a complex interrelationship (Spiegel *et al.*, 2009). As the prevalence of obesity, diabetes, sleep apnoea and habitual sleep restriction continue to rise together, accumulating laboratory and epidemiological evidence suggests that sleep restriction and sleep apnoea may be partially responsible for the epidemic of obesity and diabetes (Spiegel *et al.*, 2009). In addition, sleepiness is said to be a marker of insulin resistance, independent of obesity (Barceló *et al.*, 2008).

Diabetes is now a global health priority, as the International Diabetes Federation (2010) estimates that 285 million people worldwide have diabetes and that this total will rise to 438 million in 20 years. However, the epidemiology and pathophysiology of diabetes are apparently different among races. A recent report indicated that diabetes in Asia is characterized by rapid rates of increase over short periods, onset at a relatively young age and a low body mass index (BMI), and that specific strategies for the prevention and control of diabetes in Asia are essential (Chan *et al.*, 2009).

Obstructive sleep apnoea (OSA) is a highly prevalent sleep disturbance, with its severity increasing with the degree of obesity. The prevalence of OSA among patients with diabetes reported in the four studies that used the gold standard method of full polysomnography (PSG) for OSA assessment and that defined OSA as an apnoea–hypopnoea index (AHI) >5 events h^{-1} was consistently higher than 50% (average 73%; Aronsohn *et al.*, 2010; Einhorn *et al.*, 2007; Foster *et al.*, 2009; Resnick *et al.*, 2003). Thus, studies in Western populations revealed very high rates of OSA among individuals with diabetes, but diabetic individuals in Asian populations have a lower average BMI than those in Western populations. Because obesity is a strong risk factor for OSA, it is important to examine whether there are similar associations between the respiratory disturbance index (RDI) and diabetes in Asian populations. However, there have been few reports of population-based studies from Asia, where the prevalence of diabetes as well as that of OSA is interestingly compatible to that in the West (Chan *et al.*, 2009; Ramachandran *et al.*, 2010).

In addition to the relationship between diabetes and OSA, the relationship between diabetes and sleep duration is gaining considerable attention. However, no previous studies have simultaneously examined the inter-relationship among diabetes, sleep duration and OSA using objective assessments of sleep duration rather than self-reported assessments. Thus, we hypothesized that the prevalence of diabetes was high in patients with OSA in Japan despite the lower BMI in Japan as well as in other East Asian countries. The hypothesis that sleep duration has a significant association with fasting plasma glucose (FPG) in patients with OSA was also considered. Moreover, we not only tested the impacts of sleep duration on FPG, but also those of sleep quality assessed by sleep fragmentation. To test these hypotheses, we used information from a cross-sectional epidemiological health survey of 275 middle-aged male employees in Japan (Chin *et al.*, 2010; Nakayama-Ashida *et al.*, 2008). Although most epidemiological studies used subjective self-reported sleep duration, such self-reports may be inaccurate and cause misclassification of sleep duration (Van Den Berg *et al.*, 2008). The actigraphic measurements used in this study would ensure greater accuracy of sleep duration and sleep quality than in previous reports.

MATERIALS AND METHODS

Subjects

We performed a cross-sectional epidemiological health examination of male employees of an urban wholesale company in Japan. Male subjects only were chosen for the study, as the number of female employees was very small. Of the 322 male employees who recorded their breathing during sleep using a home monitoring system (Nakayama-Ashida *et al.*, 2008), 275 were investigated further by determining their clinical characteristics and comorbidities (Chin *et al.*, 2010). Forty-seven of the 322 employees were excluded because blood parameters or waist circumferences were not measured. The relative proportions of the degrees of severity of OSA were similar in these 47 excluded subjects as in the 275 subjects. The study protocol was approved by the IRB Committee, which is associated with the Kyoto University Graduate School and Faculty of the Medicine Ethics Committee. The committee follows the principles of the Declaration of Helsinki. The study approval number was E-37. Written informed consent was obtained from all subjects.

Measurements of body weight and waist circumference, and diagnosis of diabetes

Trained research staff measured body weight and waist circumference. Fasting blood samples were taken in the morning after an overnight sleep. Measurements of FPG levels were obtained retrospectively from the company's periodic examination data (Chin *et al.*, 2010). For these measurements, all subjects were told not to eat and drink anything after 20:00 hours except water, and their blood was sampled the following morning after 09:00 hours. Thus, all subjects had undergone a more than 12-h fast. In accordance with guidelines of the American Diabetes Association and World Health Organization, diabetes was considered present when the FPG level was 126 mg dL^{-1} (7.0 mM) or greater, or hypoglycaemic medication (insulin or oral hypoglycaemic agent) was being administered. Impaired fasting glucose (IFG) was considered present when the FPG level was 110 mg dL^{-1} (6.1 mM) or greater but lower than 126 mg dL^{-1} (7.0 mM; Hayashino *et al.*, 2008).

Analysis of data from actigraph and a portable monitor

Each participant was asked to wear an actigraph (Morgenthaler *et al.*, 2007; Actiwatch AW-Light: Mini Mitter, Bend, OR,

USA) for seven consecutive days (five workdays and two weekend days) to estimate sleep/wake time and a type 3 portable monitor (PM; Morpheus; Teijin, Tokyo, Japan, which is the same as Somté; Compumedics, Vic., Australia; Chesson *et al.*, 2003), an alternative to PSG in the diagnosis of OSA (Kushida *et al.*, 2005), for two nights at home. The PM recorded chest and abdominal respiratory movements, nasal pressure, oxygen saturation, heart rate and body position.

We estimated sleep duration at night by analysing the actigraph tracings in conjunction with information from a sleep diary kept by the subjects (Nakayama-Ashida *et al.*, 2008). Sleep duration was assessed separately for an entire week, which included both workdays and weekend days. The RDI (number of apnoea and hypopnoea episodes per hour of the analysed time length) was calculated from PM data using the sleep durations obtained from the actigraph. PM records were visually inspected and scored by at least two medical doctors specialized in respiratory medicine. Apnoea is defined as cessation of breathing for at least 10 s, and hypopnoea as a more than 50% reduction in the amplitude of nasal pressure or respiratory effort associated with a more than 3% reduction in oxyhaemoglobin saturation for at least 10 s. Apnoea and hypopnoea were scored blinded to other information except for the sleep/wake time estimated by actigraphy. Data without oxygen saturation values, illegible recordings and data for <2 h were excluded from the analysis. When data from both recorded nights were available, PM records from the second night were analysed. Subjects with an RDI of 5–14.9, 15–29.9 and ≥30 were considered to have mild, moderate and severe OSA, respectively. We also assessed average sleep fragmentation from data obtained by the actigraph for seven consecutive nights. Sleep fragmentation, an index of restlessness during sleep expressed as a percentage, was calculated by summing two percentages: (i) the percentage of the sleep period spent moving (epoch with >2 activity counts is considered moving); and (ii) the percentage of immobile phases (consecutive epochs with no movement) that are only ≤1 min long (Knutson *et al.*, 2011).

Assessment of sleepiness

The modified Japanese version of the Epworth Sleepiness Scale (ESS) was used to assess subjective sleepiness (Takegami *et al.*, 2009). Subjects also filled out a sleep diary during the survey period.

Statistical analysis

Results are expressed as mean ± SD. Unpaired *t*-tests or exact chi-square tests were used to compare the backgrounds of participants. One-sample Kolmogorov–Smirnov tests were used to check whether the data had normal distribution. The relationships between two sets of data were analysed by Pearson correlation coefficient tests. *P*-values <0.05 were considered to be statistically significant. Stepwise multiple regression analyses were performed to identify those variables that could predict the FPG level and ESS scores. Specifically, age, waist circumference, RDI, average sleep fragmentation, ESS scores and sleep duration were analysed to identify those variables that could predict FPG independently. In the same way, age, waist circumference, RDI, average sleep fragmentation, sleep duration and FPG were analysed to identify those variables that could predict ESS scores independently. Statistical analyses were performed using Statview 5.0 (SAS Institute, Cary, NC, USA).

RESULTS

Characteristics of the subjects

Table 1 shows the characteristics of the subjects. Of the 275 subjects, 21 (7.6%) had diabetes; among these, six (2.2%) were being treated with hypoglycaemic medication. Further examination proved that all 21 had type 2 diabetes. Subjects with diabetes were older, more obese, had a larger waist circumference and waist-to-hip ratio, and were more likely to have OSA than those with normal fasting glucose ($P = 0.036$) and than those without diabetes ($P = 0.037$). Thirty-three subjects (12.0%) had IFG. Subjects with IFG were also older, more obese, and had a larger waist circumference and waist-to-hip ratio than those with normal fasting glucose. But the prevalence of OSA in those with IFG did not differ significantly from those with normal fasting glucose ($P = 0.71$).

Among the 21 subjects with diabetes, 17 (81.0%) had OSA (RDI ≥ 5). Regarding the severity of OSA, 10 (47.6%), four (19.0%) and three (14.3%) had mild, moderate and severe OSA, respectively. Among the 33 subjects with IFG, 20 (60.7%) had OSA, with nine (27.3%), nine (27.3%) and two (6.1%) having mild, moderate and severe OSA, respectively. When considering the 221 subjects with normal fasting glucose, 124 (56.1%) had OSA, with 84 (38.0%), 29 (13.1%), and 11 (5.0%) having mild, moderate and severe OSA, respectively.

Association between diabetes, sleep apnoea and sleep duration

Firstly, we analysed the relationships between FPG and OSA. There was a significant relationship between FPG and RDI in the entire subject population [correlation coefficient (r) = 0.19, $P = 0.0019$]. Then, when that population was divided into two groups, that is, those with and without diabetes, FPG and RDI had a significant relationship in both groups before adjustment; however, the relationship was only significant in subjects with diabetes after adjustment (subjects with diabetes: $r = 0.45$, $P = 0.042$, and $r = 0.49$, $P = 0.040$ after adjustment for age, waist circumference, which was a marker of visceral adiposity, average sleep fragmentation, ESS scores and sleep duration; subjects without diabetes: $r = 0.14$, $P = 0.030$ and $r = 0.009$, $P = 0.89$

Table 1 Characteristics of the subjects

	All subjects	*Subjects with diabetes*	*Subjects with NFG*	P-*value*	*Subjects with IFG*	*Subjects with NFG*	P-*value*
Number of subjects (%)	275 (100.0)	21 (7.6)	221 (80.4)		33 (12.0)	221 (80.4)	
Age (years)	44 ± 8	50 ± 5	43 ± 8	<0.001	47 ± 6	43 ± 8	0.0039
BMI (kg m^{-2})	23.9 ± 3.1	25.9 ± 3.8	23.5 ± 2.9	<0.001	25.5 ± 3.5	23.5 ± 2.9	<0.001
Waist circumference (cm)	83.6 ± 8.5	89.7 ± 8.8	82.4 ± 7.9	<0.001	88.3 ± 9.2	82.4 ± 7.9	<0.001
Waist-to-hip ratio	0.88 ± 0.05	0.92 ± 0.05	0.87 ± 0.05	<0.001	0.91 ± 0.06	0.87 ± 0.05	<0.001
RDI (h^{-1})	10.2 ± 10.7	13.9 ± 10.5	9.4 ± 10.2	0.054	12.8 ± 13.6	9.4 ± 10.2	0.090
OSA* *n*	161	17	124	0.036	20	124	0.71
Average sleep fragmentation (%)	32.0 ± 10.4	31.8 ± 9.4	32.3 ± 10.2	0.81	30.2 ± 11.9	32.3 ± 10.2	0.27
ESS score	8.2 ± 4.3	8.2 ± 4.9	8.0 ± 4.3	0.84	9.1 ± 3.7	8.0 ± 4.3	0.21
Average sleep duration (h)	6.0 ± 0.8	5.7 ± 0.9	6.0 ± 0.8	0.068	6.0 ± 0.7	6.0 ± 0.8	0.60
Workday sleep duration (h)	5.6 ± 0.9	5.4 ± 0.9	5.7 ± 0.9	0.26	5.7 ± 0.9	5.7 ± 0.9	0.85
Weekend sleep duration (h)	6.9 ± 1.5	6.2 ± 1.3	6.9 ± 1.5	0.040	6.8 ± 1.0	6.9 ± 1.5	0.55
FPG (mg dL^{-1})	103.7 ± 22.0	156.5 ± 48.1	96.8 ± 7.1	<0.001	116.0 ± 4.8	96.8 ± 7.1	<0.001

Values are presented as mean ± SD or *n* (%) unless stated otherwise.
*OSA was defined as having RDI of more than 5 h^{-1}.
BMI, body mass index; ESS, Epworth Sleepiness Scale; FPG, fasting plasma glucose; IFG, impaired fasting glucose; NFG, normal fasting glucose; OSA, obstructive sleep apnoea; RDI, respiratory disturbance index.

after adjustment for age, waist circumference, average sleep fragmentation, ESS scores and sleep duration; Fig. 1a,b). When we divided the subjects without diabetes into two groups, that is, those with IFG and those with normal fasting glucose, to analyse the relationship between FPG and RDI, we found no significant correlation (subjects with IFG: $r = 0.14$, $P = 0.45$; subjects with normal fasting glucose: $r = 0.10$, $P = 0.15$; Fig. 1c,d).

Secondly, as to the relationship between FPG and sleep duration, we found a significant inverse relationship between FPG and sleep duration in the entire subject group ($r = -0.13$, $P = 0.041$). However, when separately analysing subjects with and without diabetes, FPG was not related to sleep duration (subjects with diabetes: $r = -0.24$, $P = 0.30$; subjects without diabetes: $r = 0.005$, $P = 0.93$). After adjustment for age, waist circumference, RDI, average sleep

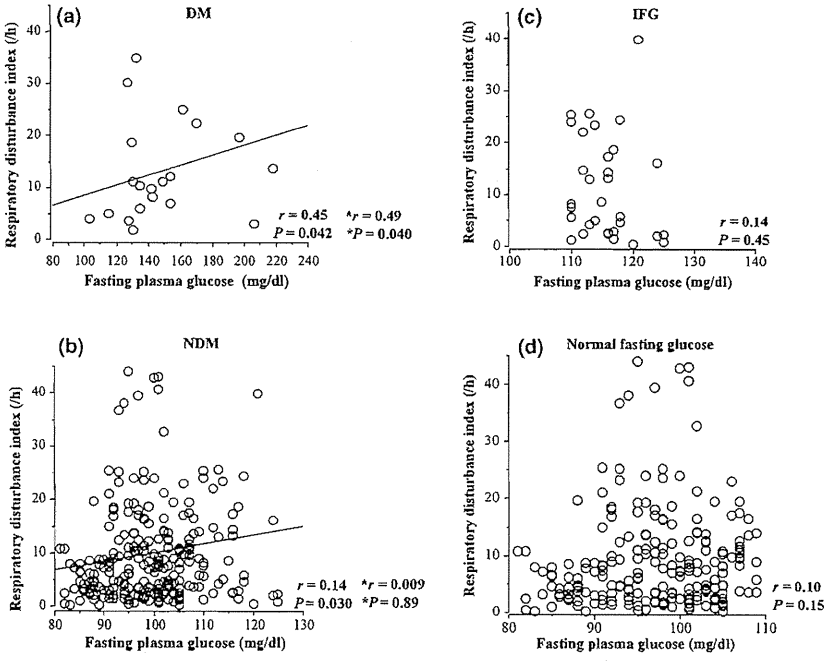

Figure 1. Relationship between FPG and RDI in the subjects with diabetes (DM) (a), those without diabetes (NDM) (b), those with impaired fasting glucose (IFG) (c) and those with normal fasting glucose (d). *r and *P were values after adjustment for age, waist circumference, average sleep fragmentation, ESS scores and sleep duration.

fragmentation and ESS scores, FPG also was not related to sleep duration (subjects with diabetes: $r = -0.30$, $P = 0.27$; subjects without diabetes: $r = 0.009$, $P = 0.88$). Although the relationships were not significant, both before and after adjustment, the correlations between FPG and sleep duration tended to be stronger in the subjects with diabetes than in those without diabetes.

With regard to the relationship between FPG and average sleep fragmentation, we did not find a significant relationship in the entire subject group ($r = 0.036$, $P = 0.57$). However, a tendency toward a significant relationship in the subjects with diabetes ($r = 0.37$, $P = 0.10$) was found. In the subjects without diabetes, FPG was not related to sleep fragmentation ($r = -0.050$, $P = 0.44$). After adjustment for age, waist circumference, RDI, ESS scores and sleep duration, those results did not differ (subjects with diabetes: $r = 0.38$, $P = 0.10$; subjects without diabetes: $r = -0.057$, $P = 0.34$). Although the relationships were not significant, the correlations between FPG and average sleep fragmentation tended to be stronger in the subjects with than without diabetes. Moreover, in the subjects with diabetes, correlations with FPG and average sleep fragmentation after adjustment tended to be stronger than those with FPG and sleep duration.

Lastly, multiple regression analyses to predict FPG were performed in the entire subject group and in subjects with and without diabetes adjusted for age, waist circumference, RDI, average sleep fragmentation, ESS scores and sleep duration. In the entire subject group, age and waist circumference independently explained FPG [contribution rate (R^2) = 4.5 and 3.5%, respectively; Table 2a]. The rates of the contribution were small, which in part was because the subjects were considered as a single group regardless of the presence of diabetes. We then divided the subjects into two groups (with and without diabetes) to examine whether the effects of the RDI would differ between the two groups. These analyses revealed that in the subjects with diabetes, only RDI independently explained FPG ($R^2 = 19.9\%$; Table 2b). In contrast, age and waist circumference, but not RDI, independently explained FPG in the subjects without diabetes ($R^2 = 7.1$ and 3.5%, respectively; Table 2a,b). When considering subjects with IFG and those with normal fasting glucose, only age independently explained FPG ($R^2 = 6.3\%$, both). Those results did not differ when we replaced sleep fragmentation by sleep duration in the analysis (Table 2a,b).

Furthermore, we performed an additional analysis replacing waist circumference by BMI. Pearson correlation coefficients tests showed that there was no significant relationship between FPG and BMI in the subjects with diabetes ($r = -0.037$, $P = 0.88$), while there was a significant relationship between FPG and BMI in subjects without diabetes ($r = 0.15$, $P = 0.019$). Multiple regression analyses showed that after adjustment for age, BMI, RDI, average sleep fragmentation, ESS scores and sleep duration, only the RDI independently explained FPG in the subjects with diabetes ($R^2 = 19.9\%$), a result similar to that with the use of waist circumference. In the subjects without diabetes, age and BMI (alternative to waist circumference) independently explained FPG ($R^2 = 8.2$ and 2.9%, respectively).

Association between diabetes and sleepiness

As to relationships between FPG and sleepiness and between RDI and sleepiness in the subjects with and without diabetes, as well as in all subjects taken together, no significant relationships were found. Only sleep duration was significantly related to sleepiness in subjects with and without diabetes (subjects with diabetes: $r = -0.67$, $P < 0.001$; subjects without diabetes: $r = -0.17$, $P = 0.0067$). Multiple regression analyses adjusted for age, waist circumference, RDI, average sleep fragmentation, sleep duration and FPG showed that in the subjects with diabetes, only sleep duration independently contributed to sleepiness ($R^2 = 44.9\%$), while in the subjects without diabetes, although sleep duration also independently contributed to sleepiness, the contribution rate was much lower ($R^2 = 3.0\%$) than in those with diabetes.

DISCUSSION

Among the 275 male workers who underwent a health examination in an urban company in Japan, the prevalence of diabetes was 7.6%, which is the same as in the general population in that age group in Japan as reported in 2008 (Ministry of Health, Labor and Welfare, 2008). The prevalence of OSA in patients with diabetes is high in Japan as well as in Western countries. In this study, RDI and sleep duration were significantly related to FPG, as has already been reported (Aronsohn *et al.*, 2010). However, after adjustment for the confounders of age, waist circumference, average sleep fragmentation, sleepiness and sleep duration, RDI was significantly related to FPG only in subjects with diabetes. Sleep fragmentation was more closely correlated with FPG than sleep duration in patients with diabetes, but without significance ($P = 0.10$).

In this study, OSA was highly prevalent in subjects with diabetes, which is compatible with findings of recent Western studies (77 and 86%; Aronsohn *et al.*, 2010; Foster *et al.*, 2009) and a Japanese study (78%; Kashine *et al.*, 2010); however, subjects in the Japanese study were not from the general population. Although the mean BMI (25.9 kg m^{-2}) in subjects with diabetes in the present study was lower in comparison with subjects in the Western studies (33.8 and 36.5 kg m^{-2}; Aronsohn *et al.*, 2010; Foster *et al.*, 2009) and in the Japanese study (28.3 kg m^{-2}; Kashine *et al.*, 2010), a low BMI is characteristic of persons with diabetes in Asia (Chan *et al.*, 2009). This finding is similar to those showing that the prevalence of OSA is as high in Asian populations as in Western populations even though Asian subjects with OSA have a lower BMI than Western subjects. The mean BMI in our study was

Table 2 (a) Relationships with FPG; (b) stepwise multiple regression analyses to predict FPG

	r	P-*value*	*95% CI*	
(a)				
In the entire subject group				
Age (years)	0.23	<0.001	0.11–0.34	
Waist circumference (cm)	0.20	<0.001	0.084–0.32	
RDI (h^{-1})	0.19	0.0019	0.072–0.31	
Average sleep fragmentation (%)	0.036	0.57	−0.086 to 0.16	
ESS scores	0.043	0.49	−0.079 to 0.16	
Sleep duration (h)	−0.13	0.041	−0.25 to 0.005	
In the subjects with diabetes				
Age (years)	−0.23	0.32	−0.60 to 0.22	
Waist circumference (cm)	0.016	0.95	−0.42 to 0.45	
RDI (h^{-1})	0.45	0.042	0.017–0.74	
Average sleep fragmentation (%)	0.37	0.10	−0.075 to 0.69	
ESS scores	−0.10	0.66	−0.51 to 0.35	
Sleep duration (h)	−0.24	0.30	−0.61 to 0.22	
In the subjects without diabetes				
Age (years)	0.28	<0.001	0.16–0.39	
Waist circumference (cm)	0.21	0.0011	0.083–0.32	
RDI (h^{-1})	0.14	0.030	0.013–0.26	
Average sleep fragmentation (%)	−0.050	0.44	−0.17 to 0.076	
ESS scores	0.066	0.30	−0.060 to 0.19	
Sleep duration (h)	0.005	0.93	−0.12 to 0.13	
	Model 1		Model 2	
	β	R^2 (%)	β	R^2 (%)
(b)				
In the entire subject group				
Age (years)	0.20	4.5	0.20	4.5
Waist circumference (cm)	0.17	3.5	0.17	3.5
RDI (h^{-1})	NA	NA	NA	NA
Average sleep fragmentation (%)	NA	NA	–	–
ESS scores	NA	NA	NA	NA
Sleep duration (h)	–	–	NA	NA
Cumulative R^2		8.0		8.0
In the subjects with diabetes				
Age (years)	NA	NA	NA	NA
Waist circumference (cm)	NA	NA	NA	NA
RDI (h^{-1})	0.45	19.9	0.45	19.9
Average sleep fragmentation (%)	NA	NA	–	–
ESS scores	NA	NA	NA	NA
Sleep duration (h)	–	–	NA	NA
Cumulative R^2		19.9		19.9
In the subjects without diabetes				
Age (years)	0.26	7.1	0.26	7.1
Waist circumference (cm)	0.17	3.5	0.17	3.5
RDI (h^{-1})	NA	NA	NA	NA
Average sleep fragmentation (%)	NA	NA	–	–
ESS scores	NA	NA	NA	NA
Sleep duration (h)	–	–	NA	NA
Cumulative R^2		10.6		10.6

CI, confidence interval; ESS, Epworth Sleepiness Scale; FPG, fasting plasma glucose; RDI, respiratory disturbance index; β, standard regression coefficient; r, correlation coefficient; R^2, contribution rate; NA, not applicable.
Model 1 was adjusted for age, waist circumference, RDI, average sleep fragmentation and ESS scores.
Model 2 was adjusted for age, waist circumference, RDI, ESS scores and sleep duration.

comparable to that in normal Japanese males. Also the prevalence of diabetes and IFG in Japanese males in their 40 s was reported to be 7.6 and 11.0%, respectively, in 2007 (Ministry of Health, Labor and Welfare, 2008), similar to findings of this study (7.6 and 12%, respectively). Therefore, our data that reflect the current background in Japan might be applicable to Western populations, although further studies are required to confirm this.

The proportion of OSA among our subjects, especially of mild OSA, was higher than that previously reported (Young *et al.*, 1993). We suspect as one reason that we defined hypopnoea as a more than 50% reduction in the amplitude of nasal pressure or respiratory effort associated with a more than 3% reduction in oxyhaemoglobin saturation, while Young *et al.* (1993) defined hypopnoea as a discernible reduction in respiratory airflow accompanied by a decrease of 4% or more in oxyhaemoglobin saturation. In addition, to detect hypopnoea, we used a nasal pressure sensor, while Young *et al.* (1993) used a thermo sensor. The American Academy of Sleep Medicine (1999) reported that a pressure sensor was much more effective and sensitive than a thermo sensor to detect hypopnoea, especially slight hypopnoea. In addition, the sampling time of the percutaneous arterial saturation was 1 s in our study. The sampling time, which means constant intervals to convert the amplitude of analogue signals to numbers, might have become shorter and more accurate in the 2000s than in the 1990s owing to advances in technology. However, Young *et al.* (1993) did not describe the sampling time in their methods. Also, our findings might have been influenced by the fact that we could make assessments under usual circumstances, where more than half of the subjects took alcohol before sleep (Nakayama-Ashida *et al.*, 2008).

Some relationship between diabetes and OSA was indicated by the following findings: (i) subjects with diabetes had a higher prevalence of OSA than those without diabetes ($P = 0.037$); (ii) there was a significant relationship between FPG and RDI ($P = 0.042$); and (iii) multiple regression analyses showed a significant association between FPG and RDI only in persons with diabetes. Diabetes has been linked to OSA in many studies. However, obesity, which is a common condition predisposing persons to both OSA and diabetes, is a main confounder, and causality remains to be determined (Tasali *et al.*, 2009). Among our study subjects with diabetes, waist circumference and BMI were not independently related to FPG, but RDI was. Cross-sectional analyses in an American cohort (Punjabi *et al.*, 2004) and an Australian cohort (Marshall *et al.*, 2009) also showed a significant relationship between diabetes and OSA. However, the Australian study showed that after adjustments for patient characteristics, the association was not significant. Thus, the independent relationship between OSA and diabetes needs further study.

As it was said that short sleep duration induced poor glucose metabolism (Spiegel *et al.*, 2009), in our study, a significant relationship between FPG and sleep duration was noted when the entire subject group was considered, although that relationship disappeared when subjects were divided according to having and not having diabetes. One reason might be the small number of subjects in each group after the division. The use of the actigraph might be another reason. Most previous epidemiological studies examined the relationships between diabetes and sleep duration as assessed by subjective self-reported measures (Tasali *et al.*, 2009). Objective actigraph-measured sleep duration is more accurate than self-reported sleep duration (Van Den Berg *et al.*, 2008). Moreover, total sleep time and sleep efficiency do not always significantly differ from those determined by PSG data and the combined data from actigraphy and subjective reports (Kushida *et al.*, 2005). Thus, our study design differed from that of previous studies on this issue, and sleep duration could have been assessed more accurately.

Associations between FPG and sleep fragmentation were reported (Knutson *et al.*, 2011). In this study, we found only a tendency toward a significant relationship between FPG and sleep fragmentation in the subjects with diabetes. One reason might be the small number of subjects, another might be that we used average sleep fragmentation for 7 days, and the PM was attached to the subjects for two of the 7 days. Sleep fragmentation on the days that PM recordings were made might have been overestimated as the PM might have disturbed sleep. In addition, in subjects with OSA, sleep fragmentation might fluctuate widely day by day, and determining sleep fragmentation might be more difficult than in those without OSA. Although sleep fragmentation might significantly affect FPG in subjects with OSA as well as the recent report (Knutson *et al.*, 2011), further studies are needed.

Previously, we reported the effects of the presence of hypertension on the relationship between OSA and sleepiness. We showed that sleepiness was related to the severity of OSA in hypertensive subjects but not in non-hypertensive subjects, although sleepiness was related to short sleep duration in both groups (Harada *et al.*, 2011). Thus, we thought that the effects of sleepiness would differ between subjects with and without diabetes. Then we assessed the effects of the presence of diabetes on the relationship between OSA and sleepiness. Barceló *et al.* (2008) reported that sleepiness in OSA was associated with higher FPG and insulin resistance and might be a risk factor for diabetes. However, in this study, we did not find such an association. Perhaps this was because the ESS scores used in our study were subjective assessments of sleepiness and might differ from objective measurements. In addition, our study subjects in this study had short sleep durations, which might have significant effects on the relationships between sleepiness and FPG. Although only sleep duration was independently related to sleepiness in both study groups, the influence of short sleep duration on sleepiness was much greater in the subjects with diabetes ($R^2 = 44.9\%$) than in those without diabetes ($R^2 = 3.0\%$). Therefore, ensuring sufficient

sleep duration could ameliorate sleepiness in subjects with diabetes.

The present study has some limitations. First, this is a cross-sectional study and it was difficult to completely exclude the influence of confounders such as age, obesity, medication, etc. Second, the number of subjects was small, and further studies with a larger number of subjects are needed to clarify this issue. Third, we did not perform PSG, partly because we wanted to assess sleep under usual lifestyle conditions. However, the non-attached type 3 monitoring was reported to be reliable under the specific conditions in which our study was conducted (Collop *et al.*, 2007). In addition, as to the reliability of the PM used in this study, Cunnington *et al.* (2003) stated AHI measured by PSG and by the PM highly correlated ($r = 0.94$), and also stated the median difference (AHI by PSG–AHI by the PM) was -0.5 events h^{-1} (95% confidence intervals -4.4 to 5.4, $P = 0.83$). Further, they indicated that the same PM as we used could correctly evaluate OSA that had been defined by PSG with a sensitivity of 96% and specificity of 83% (Cunnington *et al.*, 2009). In addition, as we previously reported, inter-scorer and night-to-night reliability of RDI were excellent (interclass correlation coefficient of 0.98 and 0.95, respectively; Nakayama-Ashida *et al.*, 2008). Also, laboratory tests on blood samples of the study subjects were performed yearly in accordance with the rules of the company. Therefore, subjects were advised yearly not to eat and drink anything after 20:00 hours except water and, of course, they were given the same instructions when this study was performed. Although the blood studies were performed after a longer than 12-h fast, we did not assess the exact time when the last meal or drink was consumed. Therefore, some subjects might have taken food or drink after 20:00 hours. We did not measure haemoglobin A1c values and we could not examine insulin resistance as we did not measure fasting insulin. Also, actigraphy seemed to have some limitations as it could not identify specific sleep stages like rapid eye movement sleep, although we did not consider this a problem because we only assessed awake time and sleep time during the night. We did not administer the Multiple Sleep Latency Test to estimate sleepiness objectively. ESS scores used in our study reflected subjective sleepiness and might differ from objectively measured sleepiness. Lastly, our participants did not include women because there were few female employees in the company.

In conclusion, OSA and sleep duration were related to FPG, and RDI was independently related to FPG in the subjects with diabetes. From results of this study, in addition to those of other studies (Aronsohn *et al.*, 2010; Punjabi *et al.*, 2004; Spiegel *et al.*, 2009), patients with diabetes should be examined for the presence of OSA. If OSA is present, it should be treated intensively, which will possibly have a favourable effect on plasma glucose control. Although the association between FPG and sleep duration in this study was weak, patients with diabetes should be advised to get sufficient sleep with less sleep fragmentation through the treatment of OSA.

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CONFLICTS OF INTEREST

No authors have indicated any financial conflicts of interest.

REFERENCES

American Academy of Sleep Medicine Task Force. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. *Sleep*, 1999, 22: 667–689.

Aronsohn, R. S., Whitmore, H., Van Cauter, E. and Tasali, E. Impact of untreated obstructive sleep apnea on glucose control in type 2 diabetes. *Am. J. Respir. Crit. Care Med.*, 2010, 181: 507–513.

Barceló, A., Barbé, F., de la Peña, M. *et al.* Insulin resistance and daytime sleepiness in patients with sleep apnoea. *Thorax*, 2008, 63: 946–950.

Chan, J. C., Malik, V., Jia, W. *et al.* Diabetes in Asia: epidemiology, risk factors, and pathophysiology. *JAMA*, 2009, 301: 2129–2140.

Chesson, A. L., Jr, Berry, R. B. and Pack, A. American Academy of Sleep Medicine; American Thoracic Society; American College of Chest Physicians. Practice parameters for the use of portable monitoring devices in the investigation of suspected obstructive sleep apnea in adults. *Sleep*, 2003, 26: 907–913.

Chin, K., Oga, T., Takahashi, K. *et al.* Associations between obstructive sleep apnea, metabolic syndrome and sleep duration, as measured with an actigraph, in an urban male working population in Japan. *Sleep*, 2010, 33: 89–95.

Collop, N. A., Anderson, W. M. and Boehlecke, B. *et al.* Portable Monitoring Task Force of the American Academy of Sleep Medicine. Clinical guidelines for the use of unattended portable monitors in the diagnosis of obstructive sleep apnea in adult patients. Portable Monitoring Task Force of the American Academy of Sleep Medicine. *J. Clin. Sleep Med.*, 2007, 3: 737–747.

Cunnington, D., Menagh, J., Cherry, G. and Teichtahl, H. Comparison of full in laboratory polysomnography to a portable sleep data acquisition device. *Am. J. Respir. Crit. Care Med.*, 2003, 167: A405.

Cunnington, D., Garg, H. and Teichtahl, H. Accuracy of an ambulatory device for the diagnosis of sleep disordered breathing. *Indian J. Sleep Med.*, 2009, 4: 143–148.

Einhorn, D., Stewart, D. A., Erman, M. K., Gordon, N., Philis-Tsimikas, A. and Casal, E. Prevalence of sleep apnea in a population of adults with type 2 diabetes mellitus. *Endocr. Pract.*, 2007, 13: 355–362.

Foster, G. D., Sanders, M. H., Millman, R. *et al.* Sleep AHEAD Research Group. Obstructive sleep apnea among obese patients with type 2 diabetes. *Diabetes Care*, 2009, 32: 1017–1019.

Harada, Y., Oga, T., Chin, K. *et al.* Effects of the presence of hypertension on the relationship between obstructive sleep apnoea and sleepiness. *J. Sleep Res.*, 2011, 20: 538–543.

Hayashino, Y., Fukuhara, S., Okamura, T. *et al.* HIPOP-OHP Research Group. A prospective study of passive smoking and risk of diabetes in a cohort of workers: the High-Risk and Population Strategy for Occupational Health Promotion (HIPOP-OHP) study. *Diabetes Care*, 2008, 31: 732–734.

International Diabetes Federation. Sleep apnoea and type 2 diabetes. 2010. http://www.idf.org/sleep-apnoea-and-type-2-diabetes. [accessed 25 November 2011].

Kashine, S., Kishida, K., Funahashi, T. *et al.* Characteristics of sleep-disordered breathing in Japanese patients with type 2 diabetes mellitus. *Metabolism*, 2010, 59: 690–696.

Knutson, K. L., Van Cauter, E., Zee, P., Liu, K. and Lauderdale, D. S. Cross-sectional associations between measures of sleep and markers of glucose metabolism among subjects with and without diabetes: the Coronary Artery Risk Development in Young Adults (CARDIA) Sleep Study. *Diabetes Care*, 2011, 34: 1171–1176.

Kushida, C. A., Littner, M. R., Morgenthaler, T. *et al.* Practice parameters for the indications for polysomnography and related procedures: an update for 2005. *Sleep*, 2005, 28: 499–521.

Marshall, N. S., Wong, K. K., Phillips, C. L., Liu, P. Y., Knuiman, M. W. and Grunstein, R. R. Is sleep apnea an independent risk factor for prevalent and incident diabetes in the Busselton Health Study? *J. Clin. Sleep Med.*, 2009, 5: 15–20.

Ministry of Health, Labor and Welfare. Report of national health and nutrition survey 2007. 2008. http://www.mhlw.go.jp/houdou/2008/12/dl/h1225-5d.pdf. [accessed 25 November 2011].

Morgenthaler, T., Alessi, C., Friedman, L. *et al.* Standards of Practice Committee; American Academy of Sleep Medicine. Practice parameters for the use of actigraphy in the assessment of sleep and sleep disorders: an update for 2007. *Sleep*, 2007, 30: 519–529.

Nakayama-Ashida, Y., Takegami, M., Chin, K. *et al.* Sleep-disordered breathing in the usual lifestyle setting as detected with home monitoring in a population of working men in Japan. *Sleep*, 2008, 31: 419–425.

Punjabi, N. M., Shahar, E., Redline, S., Gottlieb, D. J., Givelber, R. and Resnick, H. E. Sleep Heart Health Study Investigators. Sleep-disordered breathing, glucose intolerance, and insulin resistance: the Sleep Heart Health Study. *Am. J. Epidemiol.*, 2004, 160: 521–530.

Ramachandran, A., Wan Ma, R. C. and Snehalatha, C. Diabetes in Asia. *Lancet*, 2010, 375: 408–418.

Resnick, H. E., Redline, S., Shahar, E. *et al.* Sleep Heart Health Study. Diabetes and sleep disturbances: findings from the Sleep Heart Health Study. *Diabetes Care*, 2003, 26: 702–709.

Spiegel, K., Tasali, E., Leproult, R. and Van Cauter, E. Effects of poor and short sleep on glucose metabolism and obesity risk. *Nat. Rev. Endocrinol.*, 2009, 5: 253–261.

Takegami, M., Suzukamo, Y., Wakita, T. *et al.* Development of a Japanese version of the Epworth Sleepiness Scale (JESS) based on item response theory. *Sleep Med.*, 2009, 10: 556–565.

Tasali, E., Leproult, R. and Spiegel, K. Reduced sleep duration or quality: relationships with insulin resistance and type 2 diabetes. *Prog. Cardiovasc. Dis.*, 2009, 51: 381–391.

Van Den Berg, J. F., Van Rooij, F. J., Vos, H. *et al.* Disagreement between subjective and actigraphic measures of sleep duration in a population-based study of elderly persons. *J. Sleep Res.*, 2008, 17: 295–302.

Young, T., Palta, M., Dempsey, J., Skatrud, J., Weber, S. and Badr, S. The occurrence of sleep-disordered breathing among middle-aged adults. *N. Engl. J. Med.*, 1993, 328: 1230–1235.

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CASE REPORT

Impact of nasal continuous positive airway pressure for congenital adrenal hyperplasia with obstructive sleep apnea and bruxism

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Abstract
Introduction Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders in humans. The most frequent CAH variant is 21-hydroxylase deficiency. Patients with 21-hydroxylasedeficiency require long-term glucocorticoid replacement treatment. Although sleep disturbance is frequently observed under glucocorticoid replacement treatment, a case of obstructive sleep apnea (OSA) in patients with CAH has not been reported.
Case report A 43-year-old man with CAH who complained about sleep disturbance and sleep bruxism was diagnosed as obstructive sleep apnea (OSA) by polysomnography (PSG). Following the introduction of nasal continuous positive airway pressure (nCPAP), his sleep disturbance with symptoms also improved; in addition, he was able to reduce the dose of glucocorticoid replacementtherapy without any adverse consequences on his sleep pattern.
Conclusions Physicians who treat a patient with CAH should know the possibility in the existence of OSA in their patients. Because symptoms with OSA in CAH patient may increase the dosage of long-term glucocorticoid treatment for the patient, which may induce several adverse effects including body weight gain on the patient.

Keywords congenital adrenal hyperplasia · obstructive sleep apnea · nasal continuous positive airway pressure · glucocorticoid replacement therapy

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Introduction

Congenital adrenal hyperplasia (CAH) is a group of autosomal recessive disorders in humans, with an estimated worldwide incidence of 1 in 15,000 live births [1]. The most frequent CAH variant, accounting for 95% of all affected patients, is 21-hydroxylase deficiency. This deficiency is caused by inactivating mutations in the *CYP21* gene, presenting as impaired cortisol synthesis and increased corticotrophin secretion. The resulting adrenal stimulation leads to increased production of androgens. In healthy individuals, it is well known that there is a marked circadian rhythm in cortisol release with the lowest levels occurring shortly after midnight and rising between 0200 and 0300 h, and peaking around 0600 to 0800 h (after waking). Patients with 21-hydroxylase deficiency require long-term glucocorticoid treatment to inhibit excessive secretion of corticotoropin-releasing hormone and corticotropin by the hypothalamus and pituitary, respectively, and to reduce elevated levels of adrenal sex steroids [2]. Glucocorticoid replacement therapy in adults has to be adjusted according to individual goals; there is no perfect regimen [3]. The dosage of glucocorticoid replacement therapy must be carefully adjusted to mimic this circadian rhythm. Inadequate levels of glucocorticoid replacement

Table 1 Polysomnogram analysis of the effect of introducing CPAP on OSA

	Before CPAP	After CPAP
Sleep duration (min)	478	431.5
Awakenings/h	17.1	9.9
Micro-arousals/h	9.3	6.5
Arousal index/h	26.4	16.4
Sleep onset (min)	3	4
Sleep efficacy (%)	86.8	86.1
Stage I (%)	6.5	12.6
Stage II (%)	66.1	63.5
SWS (%)	9	0.7
REM (%)	18.4	23.2
AHI/h	26.2	1.8
Obstructive apnea/h	2	0
Mixed apnea/h	0	0
Central apnea/h	0.1	1.5
Hypopnea/h	24.1	0.3
Minimum SpO_2 (%)	80	89

Descriptive statistics were calculated and for measurements of duration of sleep bruxism before and after nCPAP and compared by unpaired *t* tests. Values are ± standard deviation

CPAP Continuous positive airway pressure, *SWS* Slow wave sleep, *REM* Rapid eye movement, *AHI* Apnea hypopnea index, *SpO_2* Percutaneous oxygen saturation

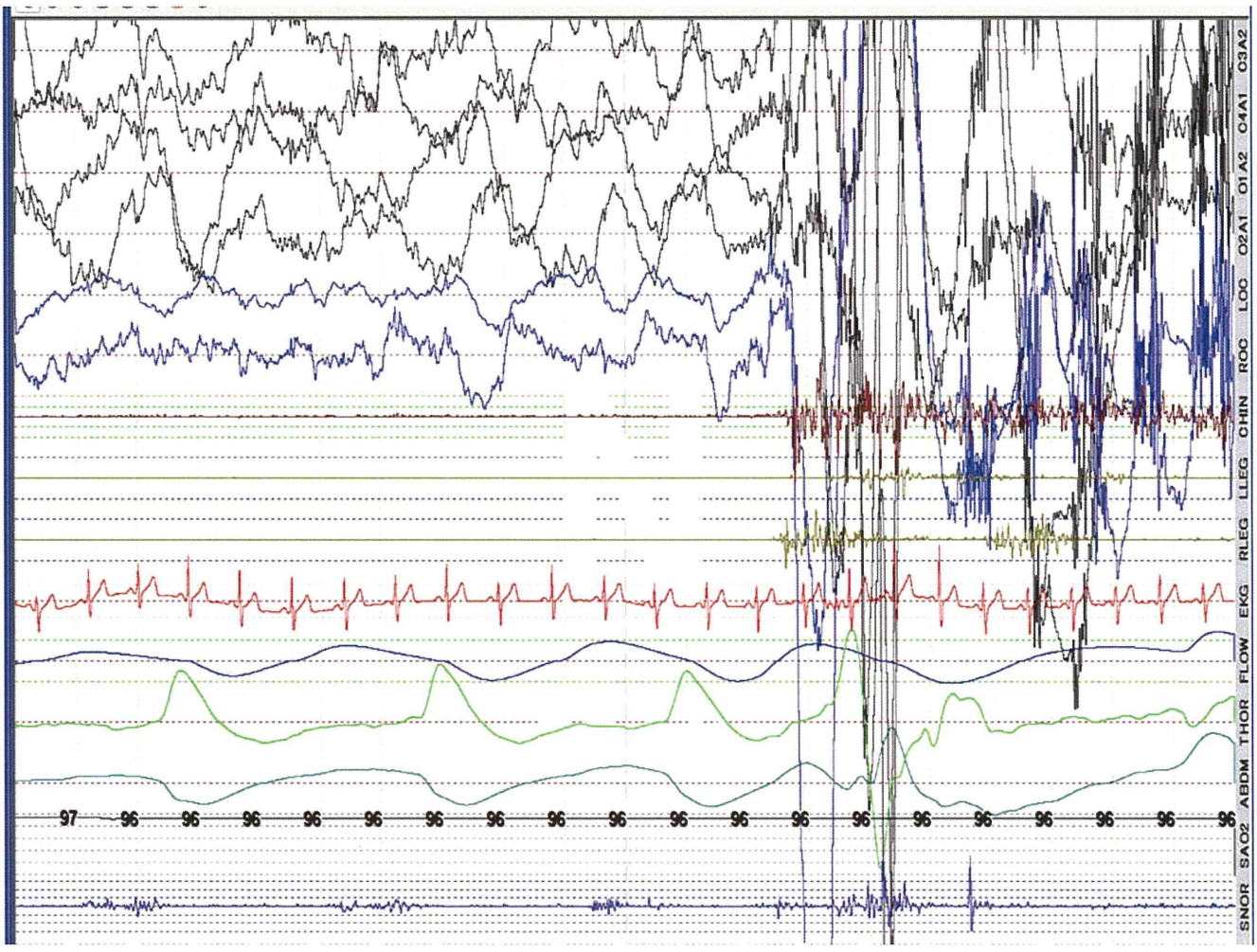

Fig. 1 A 30-s epoch of a diagnostic polysomnogram. Sleep bruxism was present with preceding micro-arousal. Depicted derivations include (from *top* to *bottom*) four electroencephalographic derivations; left and right electrooculogram; chin and both extremities electromyograms; electrocardiogram; thermal sensor; combined, thoracic, and abdominal movements by piezoelectric belts; oxygen saturation; snore

therapy may lead to many adverse effects such as sleep disturbance, elevated body mass index (BMI), glucose tolerance, osteoporosis and increased cardiovascular risk [4–7]. Increase in BMI is a main risk factor for obstructive sleep apnea (OSA) [8], which often induces sleep disturbance. We report here the first case of a CAH patient who complained of sleep disturbance and was diagnosed to have OSA and bruxism via polysomnography (PSG), and for whom nCPAP was prescribed.

Case presentation

The patient was a 43-year-old man who had been diagnosed with CAH when he was 4 years old. After the diagnosis, glucocorticoid replacement therapy was started. He suffered from diabetes mellitus, osteoporosis, hyperlipidemia and lumbar hernia; there was no family history of congenital metabolic disorder. His growth was arrested when he was 12 years old. Mutations of the CYP21 gene were detected in 2001. In 2005, when the patient was 39 years old, he complained of daytime fatigue, sleep disturbances such as insomnia with daytime sleepiness, lack of sensation taking a good sleep the feeling that he was unable to have a "good sleep", and sleep bruxism. At that time, his height and weight were 152.6 cm and 55.0 kg, respectively, and his BMI was 23.6 kg/m^2.

The overnight monitoring of his oxygen saturation by pulse oximetry was investigated. The oxygen desaturation index (ODI), defined as the number of 3% oxygen desaturations per hour of sleep, was 10/h, which indicated diagnosis of mild to moderate OSA. Oral appliance therapy was prescribed and his ODI improved to 0.57/h. However, the patient continued to complain of sleep disturbance.

Four years passed. His sleep disturbance continued. He gained approximately 2 kg within 1 month when he

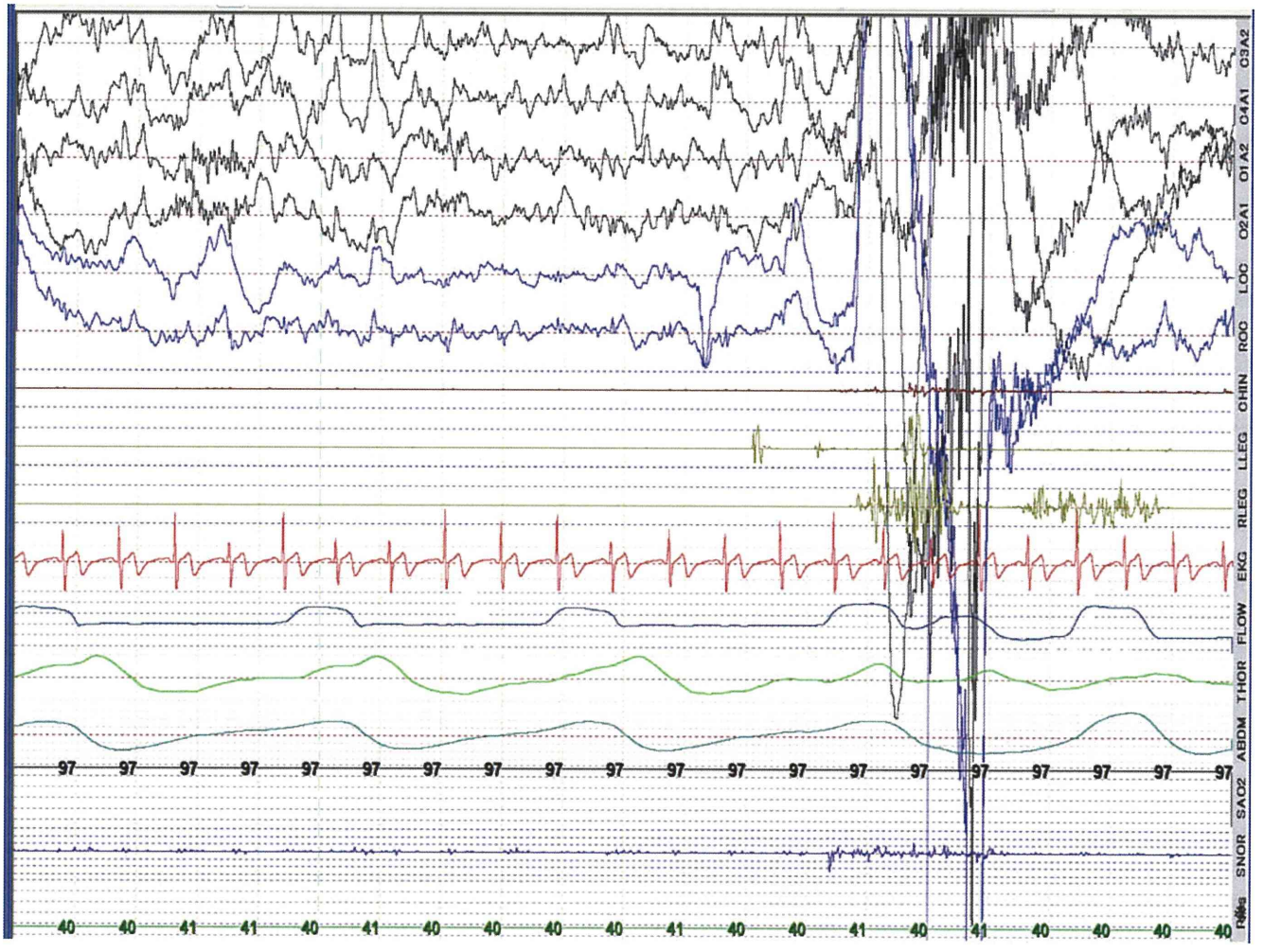

Fig. 2 A 30-s epoch of a therapeutic polysomnogram, with continuous airway pressure set. The duration and amplitude of sleep bruxism was improved. Depicted derivations include (from *top* to *bottom*) four electroencephalographic derivations; left and right electrooculogram; chin and both extremities electromyograms; electrocardiogram; CPAP flow; thoracic and abdominal movements by piezoelectric belts; oxygen saturation; snore; CPAP pressure

received oral 5.0 mg hydrocortisone at 1200, 1500, and 1800 hours, and oral 0.375 mg dexamethasone at 2200 hours as glucocorticoid replacement therapy. His BMI increased from 23.6 to 24.6 kg/m^2. His blood test analysed at 0800 hours revealed that adrenocorticotropic hormone (ACTH) and eosinophil levels were 11 (reference value: 7–56) pg/ml and 300 (reference value: 96–480)/μl, respectively. The Epworth Sleepiness Scale (ESS) score was 12 (reference value ≦10) points.

PSG (Somnostar Pro System, Fukuda Denshi, Tokyo, Japan) was performed without the oral appliance and the apnea and hypopnea index (AHI) was 26.2/h (Table 1 and Fig. 1). Apnea was defined as the cessation of airflow ≧10 s and hypopnea was defined as a >50% decrease in a valid measure of airflow in association with oxygen desaturation of >3% or an arousal. Nasal continuous positive airway pressure (nCPAP) therapy was prescribed and his AHI improved to 1.8/h (Table 1). Bruxism was measured by activation on an electromyogram, together with sounds of bruxism that could be heard by the attendant sleep technician. A comparison between the PSGs recording without nCPAP and with showed an improvement of in mean durations of bruxism from 13.4±7.1 to 9.7±6.0 s ($p<0.05$ 1 Figs. 1 and 2); however, the number of bruxism events was not changed significantly, from 40 to 37.

About 10 days following the introduction of nCPAP, the dose of dexamethasone was reduced from 0.375 to 0.25 mg because his insomnia and daytime sleepiness improved. He subsequently continued nCPAP and the level of glucocorticoid replacement therapy was reduced, resulting to improvements in symptoms of sleep disturbance without exacerbation of the other symptoms.

Discussion

This study describes a CAH patient with moderate OSA, whose symptoms were thought to be symptoms of CAH, which were relieved by nCPAP treatment and which enabled the dose of glucocorticoids to be reduced. To our knowledge, this case represents the first report of OSA in a CAH patient with glucocorticoid replacement treatment.

There has been no report about a CAH patient with OSA. Too high a level of HRT therapy may worsen the severity of OSA by cortisol-induced obesity. Our patient gained 2 kg within 1 month of glucocorticoid therapy. It has been reported many patients with adrenal insufficiency such as CAH have indefinite complaints such as daytime fatigue because of inadequate controlled dosing of glucocorticoid replacement treatment [9, 10]. Therefore, CAH patients who suffer from undiagnosed sleep apnea may be prescribed increasing doses of corticosteroids because of daytime fatigue. The patient in this study was able to reduce his dose of dexamethasone after the introduction of nCPAP treatment. In this way, he might have avoided higher risks of a worsening condition because of elevated BMI due to increased use of corticosteroids in addition to other side effects of corticosteroids, such as diabetes mellitus and osteoporosis.

Our patient also complained sleep bruxism in addition to daytime fatigue and sleep disturbance. The prevalence of sleep bruxism is approximately 8% in the general population. There is no difference in prevalence rates between men and women, but it decreases with age [11]. In an epidemiological study, it has been found that OSA was the highest risk factor for sleep bruxism [12]. Regarding sleep bruxism, the duration improved after nCPAP treatment, although the number of bruxism events did not significantly change. It has been also reported that sleep bruxism is strongly linked to transient arousal episodes [13] and follows an increase in autonomic nervous system [14]. Patients with OSA have a high sympathetic activity when awake, with further increases in sympathetic activity during sleep and these increases are attenuated by treatment with CPAP [15]. In this case, we considered that the attenuated lowered overactivities of sympathetic nerve activity by introducing nCPAP might improve the duration and amplitude of sleep bruxism.

Physicians who treat patients with CAH should be aware of the possibility of OSA in their patients, because symptoms with OSA in CAH patients may lead to increased dosage of long-term glucocorticoid treatment for these patients, which in turn could induce several adverse effects including body weight gain.

References

1. Merke DP, Bornstein SR (2005) Congenital adrenal hyperplasia. Lancet 365:2125–2136
2. Merke DP, Bornstein SR (2003) Congenital adrenal hyperplasia. N Engl J Med 349:776–788
3. Ogilvie CM, Crouch NS, Rumsby G, Creightont SM, Liao LM, Conway GS (2006) Congenital adrenal hyperplasia in adults: a review of medical, surgical and psychological issues. Clin Endocrinol 64:2–11
4. Volkl TMK, Simm D, Beier C, Dorr HG (2006) Obesity among children and adolescents with classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Pediatrics 117: e98–e105
5. al Scoumer KA, Beshyah SA, Niththyananthan R, Johnston DG (1995) Effect of glucocorticoid replacement therapy on glucose tolerance and intermediary metabolites in hypopituitary adults. Clin Endocrinol 42:85–90
6. Zelissen PM, Croughs RJ, van Rijk PP, Raymakers JA (1994) Effect of glucocorticoid replacement therapy on bone mineral density in patients with Addison disease. Ann Int Med 120:207–210
7. Sartorato P, Zulian E, Benedini S, Mariniello B, Schiavi F, Bilora F, Pozzan G, Greggio N, Pagnan A, Mantero F, Scaroni C (2007)