Relaxin in Obstructive Sleep Apnea: Relationship with Blood Pressure and Inflammatory Mediators

Anna Bonanno^a Loredana Riccobono^a Maria Rosaria Bonsignore^{a, b}
Anna Lo Bue^a Adriana Salvaggio^a Giuseppe Insalaco^a Oreste Marrone^a

^aNational Research Council, Institute of Biomedicine and Molecular Immunology 'A. Monroy', and ^bUniversity of Palermo, DiBiMIS, Palermo, Italy

Key Words

Relaxin · Obstructive sleep apnea · Metalloproteinase · Vascular endothelial growth factor

Abstract

Background: Obstructive sleep apnea (OSA) is associated with nocturnal intermittent hypoxia, which may be responsible for increased circulating levels of vascular endothelial growth factor (VEGF) and inflammatory mediators, such as metalloproteinases (MMPs), and which contributes to the pathogenesis of systemic hypertension. Why some OSA patients remain normotensive is poorly understood. Relaxin-2, a pregnancy hormone, may sometimes circulate in men and could increase in hypoxic conditions. It exerts a vasodilatory activity and can modulate the release of molecules, such as MMPs and VEGF. **Objectives:** The objective of this study was to explore if circulating relaxin-2 in male OSA subjects may be related to OSA severity, to circulating levels of MMPs, of their inhibitors (tissue inhibitors of metalloproteinases; TIMPs), and of VEGF, and if it may protect from hypertension. **Patients and Methods:** Fifty untreated male subjects with suspected OSA were recruited. After nocturnal polysomnography, a morning venous blood sample was withdrawn.

Then, 24-hour ambulatory blood pressure (BP) monitoring was performed. **Results:** The respiratory disturbance index in the sample was 30.4 [interquartile range (IQR) 15.6–55.2]. Relaxin-2 was detectable in 20 subjects. These subjects did not differ in OSA severity or diurnal and nocturnal BP from subjects with undetectable relaxin-2, but they showed lower TIMP-1 (126.8 ± 29.1 vs. 156.9 ± 41.7 pg/ml, respectively; $p = 0.007$) and a marginally higher MMP-9/TIMP-1 molar ratio [0.58 (IQR 0.23–1.35) vs. 0.25 (IQR 0.15–0.56); $p = 0.052$]. **Conclusions:** Relaxin-2 in male subjects was not related to OSA severity, but it was associated with lower TIMP-1. As it was often undetectable, even when BP values were normal, it is unlikely that it plays a role as a major factor protecting from hypertension in OSA.

© 2016 S. Karger AG, Basel

Introduction

Obstructive sleep apnea (OSA) is characterized by nocturnal intermittent hypoxia, which is one of the main factors responsible for its frequent association with sev-

A.B. and L.R. contributed equally to the study.

Table 1. ELISA reagents list

Analyte	Sample type	Vendor	Sensitivity	Catalog
Relaxin-2	plasma	R&D Systems, Minneapolis, Minn., USA	1.0 pg/ml	DRL200
MMP-9	serum	GE Healthcare Ltd., Amersham, UK	0.6 ng/ml	RPN2614
TIMP-1	serum	GE Healthcare Ltd., Amersham, UK	1.25 ng/ml	RPN2611
MMP-2	serum	GE Healthcare Ltd., Amersham, UK	0.37 ng/ml	RPN2617
TIMP-2	serum	GE Healthcare Ltd., Amersham, UK	3.0 ng/ml	RPN2618
VEGF	plasma	R&D Systems, Minneapolis, Minn., USA	9.0 pg/ml	DVE00

Instrument used: Wallac 1420 Victor2 Multilabel Counter (PerkinElmer Life Sciences, Turku, Finland).

eral comorbidities. This detrimental effect is partly due to the stimulation of the synthesis of angiogenetic and inflammatory factors through an increased activation of transcription factors such as hypoxia-inducible factor-1 (HIF-1) and nuclear factor- κ B (NF- κ B) [1]. In fact, increased levels of circulating molecules, such as vascular endothelial growth factor (VEGF) [2–5] and metalloproteinases (MMPs) [6–9], have been reported in OSA. One of the most common OSA comorbidities is systemic hypertension [10]. The mechanisms leading to increased blood pressure (BP) in OSA are very complex and incompletely understood and include sympathetic hyperactivity [11] and endothelial dysfunction [12], with decreased nitric oxide availability [13] and, possibly, increased endothelin release [14].

The severity of respiratory disorders, in addition to common risk factors such as obesity and advanced age, is correlated with the occurrence of hypertension. However, why some OSA patients do not develop hypertension despite severe respiratory disorders remains unexplained. Possibly, unknown protective mechanisms play important roles and effectively counteract factors causing hypertension. Actually, an increase in natriuretic factors has been described in OSA [15, 16], although their protective role from hypertension in this condition has not been demonstrated.

One factor protecting from increased BP could be relaxin-2 (hereafter defined as ‘relaxin’), a hormone which exerts important functions particularly during pregnancy and which, among other effects, has a vasodilatory action [17, 18]. Besides, it modulates the release of other molecules, such as VEGF [19], or some MMPs and their inhibitors (tissue inhibitors of metalloproteinases; TIMPs) [20, 21], and through this mechanism, it may participate in other functions, such as vascular remodeling [22]. In men, the prostate is the main source of relaxin, which may

exert a paracrine action in the surrounding tissues [23]. However, some adult males may also show detectable levels of circulating relaxin [24], particularly if they have systemic hypertension [25] or cardiac failure [26], and it has been hypothesized that relaxin increases in hypoxic conditions together with other molecules, such as VEGF, due to effects of HIF-1 [27].

So far, no study has explored relaxin in OSA. We asked if relaxin could be found in male patients with OSA and be related to OSA severity. Besides, we investigated if it could be related to circulating levels of MMPs and their inhibitors (TIMPs) and of VEGF, and if it could operate as a factor maintaining normal BP levels.

Patients and Methods

Consecutive subjects referred to the sleep laboratory of our institution for suspected OSA were screened for recruitment in this study starting in March 2012. Criteria for recruitment were male gender, age between 30 and 65 years, body mass index (BMI) <40, no current pharmacologic treatment, and no clinical history of cardiovascular disease or other major comorbidity. Systemic hypertension was not an exclusion criterion, provided that patients were free of pharmacologic treatment at the time of the study. The protocol was approved by the ethical committee of our institution (protocol No. 9902). All patients meeting inclusion criteria were asked to participate until December 2013, when 50 patients had been recruited. Patients who accepted gave written informed consent.

Subjective sleepiness was assessed by the Epworth sleepiness scale with scores >10 indicating excessive sleepiness. All subjects underwent an attended nocturnal full polysomnographic study (SOMNOmedics, SOMNOscreen, Germany). In the morning, within 30 min of the end of the polysomnography, a venous blood sample was withdrawn. Immediately afterwards, 24-hour ambulatory BP monitoring was initiated. A cuff of appropriate size was applied on the nondominant arm. The automatic BP recorder (model 90207, Space-Labs Medical, Inc., Issaquah, Wash., USA) was programmed to perform one BP reading and a heart rate measurement every 20 min throughout the whole application period.

Table 2. Anthropometric and polysomnographic characteristics of the subjects under study

Age, years	49.7±8.6
BMI	30.2±3.7
ESS score	9.1±5.0
TST, min	359.0±70.0
SE, %	77.1±14.8
N1, %	33.9±13.6
N2, %	39.6±10.7
N3, %	12.7±7.5
R, %	13.9±4.6
Ar/AwI	41.6±19.2
RDI, n/h	30.4 (15.6–55.2)
AHI, n/h	24.6 (11.8–54.7)
T90, %	2.25 (0.1–15.5)
Lowest SaO ₂ , %	81 (75–87)

Data are presented as means ± SD or as medians (IQR). ESS = Epworth sleepiness scale; TST = total sleep time; SE = sleep efficiency; N1 = sleep stage non-REM 1; N2 = sleep stage non-REM 2; N3 = sleep stage non-REM 3; R = sleep stage REM; Ar/AwI = arousal/awakening index; AHI = apnea/hypopnea index; T90 = sleep time spent at SaO₂ <90%.

On the next morning, the patients were asked about the time they had gone to bed the previous night and the time they had got up in the morning, so that wake and sleep data could be separately analyzed.

The polysomnographic recordings were manually analyzed. Sleep was scored in three non-REM stages (from stage N1 to N3) and one REM stage (stage R), and arousals and awakenings were counted [28]. An arousal/awakening index was calculated as (number of arousals + awakenings)/hours of sleep time. Sleep efficiency was calculated as percentage of sleep time in the total recording time. Apneas were identified on the airflow signal and were classified as obstructive, central, or mixed according to the behavior of thoraco-abdominal movements. Hypopneas were scored when a ≥30% reduction in the airflow signal was detected in association with a ≥4% oxyhemoglobin saturation (SaO₂). Respiratory effort-related arousals were identified as sequences of breaths lasting ≥10 s characterized by inspiratory flattening of the flow signal, which were followed by an arousal and an SaO₂ fall <4% [28]. The apnea/hypopnea index was calculated as the (number of apneas + hypopneas)/hours of sleep time. The respiratory disturbance index (RDI) was calculated as the (number of apneas + hypopneas + respiratory effort-related arousals)/hours of sleep time. The lowest SaO₂ was manually identified. The percent of sleep time with SaO₂ <90% was automatically calculated by the software of the device. OSA was considered as an RDI ≥5, moderate OSA as an RDI between 15 and 30, and severe OSA as an RDI >30 [29].

The blood samples were drawn under fasting conditions, immediately centrifuged for plasma/serum collection, and frozen at –80°C until enzyme-linked immunosorbent assay (ELISA) analyses were performed. Biochemical markers included relaxin-2, MMP-9, MMP-2, TIMP-1, TIMP-2, and VEGF. Molar ratios between MMP-9 and its inhibitor TIMP-1, as well as between MMP-

Fig. 1. Correlation between MMP-9 and VEGF in the patients under study. Different symbols identify subjects with RDI <30 and >30.

2 and its inhibitor TIMP-2, were calculated. All commercial reagents were used according to the manufacturers' specifications. Reagents are described in table 1. All samples were analyzed in duplicate and averaged. The intraassay coefficient of variation for these measurements was <6%.

Data are shown as means ± standard deviations (SD) or medians with interquartile ranges (IQR) in parentheses as appropriate. Comparisons between groups of patients were done by unpaired Student's t test or Mann-Whitney U test for normally and nonnormally distributed variables, respectively. Relationships between variables were evaluated by simple linear regression analysis. p values <0.05 were considered significant.

Results

The characteristics of the enrolled subjects are shown in table 2. Four subjects had no OSA, 7 had mild, 14 had moderate, and 25 had severe OSA. Three subjects had a high systolic BP (≥130 mm Hg), 4 subjects a high diastolic BP (≥80 mm Hg), and 10 subjects had both high systolic and diastolic BP in the 24 h.

The results of biochemical markers in the patients without and with severe OSA are shown in table 3. Relaxin, MMP-9, TIMP-1, and TIMP-2 did not differ significantly between the groups. MMP-2 was lower, and VEGF higher, in the patients with severe OSA. VEGF was positively correlated with MMP-9 ($r = 0.41$, $p = 0.0034$; fig. 1). None of the studied molecules was correlated with nocturnal hypoxemia or with either diurnal or nocturnal BP.

Relaxin was undetectable in 30 subjects (16 with RDI <30 and 14 with RDI >30). Subjects with detectable re-

Table 3. Biochemical markers in patients without and with severe OSA

	RDI <30 (n = 25)	RDI >30 (n = 25)	p value
Relaxin-2, pg/ml	0 (0–0.76)	0 (0–0.94)	0.503
MMP-9, ng/ml	149.8 (93.4–222.2)	231.4 (94.2–346.7)	0.290
TIMP-1, ng/ml	143.9±45.5	145.9±33.9	0.858
MMP-9/TIMP-1 molar ratio	0.26 (0.18–0.63)	0.43 (0.18–0.76)	0.467
MMP-2, ng/ml	1,181±162.0	1,092.8±131.8	0.043
TIMP-2, ng/ml	70.9±20.2	71.1±28.8	0.970
MMP-2/TIMP-2 molar ratio	4.33 (3.87–6.29)	4.33 (3.53–5.21)	0.327
VEGF, pg/ml	45.4 (38.6–62.9)	61.5 (46.5–96.0)	0.047

Data are presented as means ± SD or as medians (IQR).

Table 4. Polysomnography scores and biochemical markers in subjects with undetectable and with detectable relaxin

	Undetectable relaxin	Detectable relaxin	p value
RDI, n/h	28.9 (12.6–60.6)	33.2 (16.2–54.8)	0.642
AHI, n/h	23.3 (10.0–59.0)	27.0 (13.9–52.5)	0.736
Ar/AwI, n/h	38.5 (24.4–52.7)	41.3 (27.9–48.9)	0.961
Lowest SaO ₂ , %	80.8±8.6	80.0±7.4	0.735
T90, %	1.0 (0.1–19.2)	3.3 (0.7–13.0)	0.621
Ps wake, mm Hg	130.1±9.6	126.7±9.2	0.218
Ps sleep, mm Hg	113.6±9.0	112.2±8.2	0.581
Pd wake, mm Hg	82.0±7.3	78.1±6.4	0.055
Pd sleep, mm Hg	68.0±6.0	66.4±5.7	0.355
MMP-2, ng/ml	1,114.3±141.5	1,171.9±166.2	0.199
TIMP-2, ng/ml	69.3±23.8	73.5±26.0	0.565
MMP-2/TIMP-2 molar ratio	4.33 (3.88–5.66)	4.50 (3.60–6.38)	0.983
MMP-9, ng/ml	151.6 (84.7–279.5)	227.1 (103.8–525.7)	0.085
TIMP-1, ng/ml	156.9±41.7	126.8±29.1	0.007
MMP-9/TIMP-1 molar ratio	0.25 (0.15–0.56)	0.58 (0.23–1.35)	0.052
VEGF, pg/ml	52.4 (39.1–63.7)	61.1 (37.2–84.8)	0.352

Data are presented as means ± SD or as medians (IQR). AHI = Apnea/hypopnea index; Ar/AwI = arousal/awakening index; T90 = sleep time spent with SaO₂ <90%; Ps = systolic BP; Pd = diastolic BP.

laxin levels showed lower TIMP-1 values and a marginally higher MMP-9/TIMP-1 molar ratio in comparison to subjects with undetectable relaxin, but no significant differences in other biochemical markers, in polysomnographic scores, or in BP levels were identified (table 4).

Then, since hypertensive subjects showed slightly worse nocturnal sleep respiratory disorders, we matched 13 normotensive and 13 hypertensive subjects in a case-control fashion based on a difference in RDI ≤2. These subjects also showed similar age, BMI, and nocturnal SaO₂. No difference in any biochemical marker was found

between the groups (table 5). In the case-control group, relaxin was detectable in 6 normotensive and in 5 hypertensive subjects.

Discussion

The results of this study suggest that in male OSA patients, although relaxin is unrelated to the severity of respiratory disorders and of nocturnal hypoxemia, it could influence circulating levels of some inflammatory media-

Table 5. Biochemical markers in subjects with normal and high BP who had been matched for RDI

	Normotensive subjects (n = 13)	Hypertensive subjects (n = 13)	p value
Age, years	48.3±10.2	51.5±8.4	0.398
BMI	30.5±3.5	30.5±3.0	0.701
RDI, n/h	33.0±17.9	33.0±17.6	0.967
24-hour Ps, mm Hg	119.4±5.2	133.3±5.0	<0.001
24-hour Pd, mm Hg	71.8±5.3	82.3±5.2	<0.001
T90, %	5.3 (0.1–11.9)	2.0 (0.2–9.0)	0.522
Lowest SaO ₂ , %	81.2±8.1	78.9±9.2	0.538
Relaxin, pg/ml	0.0 (0.0–8.2)	0.0 (0.0–0.76)	0.898
MMP-2, ng/ml	1,117.0±141.3	1,136.9±145.9	0.732
TIMP-2, ng/ml	84.6±28.0	69.8±25.0	0.178
MMP-9, ng/ml	134.9 (94.2–436.6)	231.4 (105.6–320.7)	0.980
TIMP-1, ng/ml	141.1±34.4	141.5±56.3	0.984
VEGF, pg/ml	58.9 (43.7–91.9)	56.5 (39.6–68.0)	0.270

Data are presented as means ± SD or as medians (IQR). Ps = Systolic BP; Pd = diastolic BP; T90 = sleep time spent with SaO₂ <90%.

tors. In fact, its presence was associated with higher MMP-9/TIMP-1 molar ratios and with lower TIMP-1, in agreement with previous observations in other experimental contexts [20, 21]. However, in our sample, relaxin did not differ between normotensive and hypertensive subjects, and BP levels did not differ between subjects with detectable and undetectable relaxin. Therefore, these findings do not support a role of relaxin as a protective factor from hypertension in OSA. However, the lower levels of TIMP-1, a molecule inhibiting MMP-9 activity, in patients with detectable relaxin levels could suggest that relaxin may influence atherogenic and vascular remodeling processes in OSA, in which MMP-9 actively participates.

In this study, we hypothesized that circulating relaxin in OSA could be related to the severity of respiratory disorders and, in particular, of nocturnal hypoxemia. Instead, we found that relaxin was not detectable in the plasma of most patients, similar to previously reported results in normal male subjects [24]. Besides, nocturnal SaO₂ values did not differ between patients with detectable and undetectable relaxin, despite a wide range of hypoxic levels. To our knowledge, little information is available in the literature about a relationship between relaxin and hypoxia. Our data do not support an influence of hypoxia in OSA on circulating relaxin levels. However, this does not exclude that when relaxin is present, it may play a role in biochemical and pathophysiological mechanisms of OSA.

The findings of lower TIMP-1 and a higher MMP-9/TIMP-1 ratio in the subjects with detectable circulating relaxin suggest that the presence of relaxin may influence circulating levels of these substances in OSA. However, MMP-9 and TIMP-1 did not significantly differ between patients with and without severe OSA. Actually, although most authors reported increased MMP-9 in OSA [6–9], other authors did not [30, 31]. Our data show a trend to increased MMP-9 in the severe OSA patients, which did not reach statistical significance. However, the group of patients without severe OSA included a substantial number of subjects with a moderate degree of the disease. Possibly, a relationship between OSA and MMP-9 would have been revealed when comparing the severe OSA patients with a healthier group of control subjects. Unexpectedly, MMP-2 was lower in the severe OSA patients. Few data are available on MMP-2 in OSA. One study reported no relationship between OSA severity and MMP-2 [8], while another study described increased MMP-2 levels in OSA [9]. Further studies would be useful to better understand the behavior of this molecule in OSA. Another field of interest could be the role of MMPs in the development of arterial stiffness and hypertension in OSA [32]. In fact, these molecules are involved in the metabolism of the extracellular matrix, and arterial stiffness and hypertension may be associated with an increase in their levels [33, 34]. In our sample, MMPs were not related to patients' BP. A better understanding of the relationship between MMPs and BP in OSA would require

measurements of arterial stiffness and a larger range of BP levels in the patients, which was not among the aims of this study.

Detectable relaxin was not associated with higher VEGF levels. VEGF was positively correlated with MMP-9 and was higher in patients with severe OSA, in agreement with the studies showing an increase in this molecule in OSA [2–5].

OSA is now recognized by cardiologists as a factor deserving clinical attention due to its possible pathogenetic role in arterial hypertension [35]. The mechanisms through which OSA may steadily increase daytime BP are not clear. In particular, although several factors which may cause hypertension are commonly observed in OSA, no relationship between the expression of these factors and BP levels has been demonstrated. For example, sympathetic activity is usually increased in OSA, irrespective of the coexistence of hypertension [36]. This suggests that protective mechanisms could be present in some OSA subjects, which mask the relationship between some factors predisposing to increased BP and BP levels, or even prevent the onset of hypertension. Such possible mechanisms are poorly known. In this study, we hypothesized that relaxin in OSA could counteract hypertensive mechanisms, in agreement with current knowledge [17, 18]. This hypothesis was not confirmed. In fact, relaxin was related neither to diurnal nor nocturnal BP. The narrow range of BP values in our sample might be partly responsible for the lack of correlation between relaxin and BP. However, since most patients were normotensive and circulating relaxin was not found in most of them, we believe it unlikely that relaxin plays a significant role as a protective factor from hypertension in OSA.

One limitation of this study was that most subjects were affected by OSA with at least a moderate degree of

the disease. However, their characteristics could hardly be related to the lack of relationship between relaxin and OSA severity due to the high number of subjects with undetectable relaxin levels even among the severe OSA patients. Furthermore, our sample included only mildly hypertensive subjects. This was due to our choice to select only untreated patients in order to avoid any possible confounding effect of pharmacological treatments on the results. The inclusion of more patients with hypertension would have allowed us to better study the relationship between relaxin and BP in OSA. However, the data indicate that even among OSA patients with a narrow range of BP values, mostly representing normotensive levels, relaxin levels may vary widely. Finally, our results cannot be applied to women, who were not included in the study.

In conclusion, circulating relaxin may not be detected in a large part of male subjects with OSA, irrespective of OSA severity. Its presence is associated with a lower TIMP-1. Even among OSA subjects with normal BP values, relaxin is often undetectable, making it unlikely that it plays a major role as a factor protecting from the appearance of hypertension in OSA.

Acknowledgements

This study was supported by the Italian National Research Council, order No. ME.P01.014.002. The authors are grateful to Mr. Giovanni Sciortino for technical assistance during the nocturnal polysomnographic recordings.

Financial Disclosure and Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

- 1 Dewan NA, Nieto FJ, Somers VK: Intermittent hypoxemia and OSA. Implications for comorbidities. *Chest* 2015;147:266–274.
- 2 Imagawa S, Yamaguchi Y, Higuchi M, Neichi T, Hasegawa Y, Mukai HY, Suzuki N, Yamamoto M, Nagasawa T: Levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea – hypopnea syndrome. *Blood* 2001;98:1255–1257.
- 3 Schulz R, Hummel C, Heinemann S, Seeger W, Grimminger F: Serum levels of vascular endothelial growth factor are elevated in patients with obstructive sleep apnea and severe nighttime hypoxia. *Am J Respir Crit Care Med* 2002;165:67–70.
- 4 de la Peña M, Barceló A, Barbe F, Piérola J, Pons J, Rimbau E, Ayllón O, Agustí AG: Endothelial function and circulating endothelial progenitor cells in patients with sleep apnea syndrome. *Respiration* 2008;76:28–32.
- 5 Lavie L, Kraiczi H, Hefetz A, Ghandour H, Perelman A, Hedner J, Lavie P: Plasma vascular endothelial growth factor in sleep apnea syndrome: effects of nasal continuous positive air pressure treatment. *Am J Respir Crit Care Med* 2002;165:1624–1628.
- 6 Tazaki T, Minoguchi K, Yokoe T, Samson KTR, Minoguchi H, Tanaka A, Watanabe Y, Adachi M: Increased levels and activity of matrix metalloproteinase-9 in obstructive sleep apnea syndrome. *Am J Respir Crit Care Med* 2004;170:1354–1359.
- 7 Ye J, Liu H, Li Y, Liu X, Zhu J: Increased serum levels of C-reactive protein and matrix metalloproteinase-9 in obstructive sleep apnea syndrome. *Chin Med J* 2007;120:1482–1486.

- 8 Volná J, Kemlink D, Kalousová M, Vávrová J, Majerová V, Mestek O, Švarcová J, Šonka K, Zima T: Biochemical oxidative stress-related markers in patients with obstructive sleep apnea. *Med Sci Monit* 2011;17:CR491–CR497.
- 9 Hopps E, Canino B, Montana M, Calandrino V, Urso C, Lo Presti R, Caimi G: Gelatinases and their tissue inhibitors in a group of subjects with obstructive sleep apnea syndrome. *Clin Hemorheol Microcirc* 2015, DOI: 10.3233/CH-151928.
- 10 Budhiraja R, Budhiraja P, Quan SF: Sleep-disordered breathing and cardiovascular disorders. *Respir Care* 2010;55:1322–1330.
- 11 Mansukhani MP, Kara T, Caples SM, Somers VK: Chemoreflexes, sleep apnea, and sympathetic dysregulation. *Curr Hypertens Rep* 2014;16:476.
- 12 Lurie A: Endothelial dysfunction in adults with obstructive sleep apnea. *Adv Cardiol* 2011;46:139–170.
- 13 Weiss J, Liu Y, Li X, Ji E: Nitric oxide and obstructive sleep apnea. *Respir Physiol Neurobiol* 2012;184:192–196.
- 14 Belaidi E, Joyeux-Faure M, Ribuot C, Launois SH, Levy P, Godin-Ribuot D: Major role for hypoxia inducible factor-1 and the endothelin system in promoting myocardial infarction and hypertension in an animal model of obstructive sleep apnea. *J Am Coll Cardiol* 2009;53:1309–1317.
- 15 Krieger J, Laks L, Wilcox I, Grunstein RR, Costas LJ, McDougall JG, Sullivan CE: Atrial natriuretic peptide release during sleep in patients with obstructive sleep apnoea before and during treatment with nasal continuous positive airway pressure. *Clin Sci* 1989;77:407–411.
- 16 Svatikova A, Shamsuzzaman AS, Wolk R, Phillips BG, Olson LJ, Somers VK: Plasma brain natriuretic peptide in obstructive sleep apnea. *Am J Cardiol* 2004;94:529–532.
- 17 Cernaro V, Lacquaniti A, Lupica R, Buemi A, Trimboli D, Giorgianni G, Bolignano D, Buemi M: Relaxin: new pathophysiological aspects and pharmacological perspectives for an old protein. *Med Res Rev* 2014;34:77–105.
- 18 Du XJ, Bathgate RA, Samuel CS, Dart AM, Summers RJ: Cardiovascular effects of relaxin: from basic science to clinical therapy. *Nat Rev Cardiol* 2010;7:48–58.
- 19 Unemori EN, Lewis M, Constant J, Arnold G, Grove BH, Normand J, Deshpande U, Salles A, Pickford LB, Erikson ME, Hunt TK, Huang X: Relaxin induces vascular endothelial growth factor expression and angiogenesis selectively at wound sites. *Wound Repair Regen* 2000;8:361–370.
- 20 Maruo N, Nakabayashi K, Wakahashi S, Yata A, Maruo T: Effects of recombinant H2 relaxin on the expression of matrix metalloproteinases and tissue inhibitor metalloproteinase in cultured early placental extravillous trophoblasts. *Endocrine* 2007;32:303–310.
- 21 Lenhart JA, Ryan PL, Ohleth KM, Palmer SS, Bagnell CA: Relaxin increases secretion of matrix metalloproteinase-2 and matrix metalloproteinase-9 during uterine and cervical growth and remodeling in the pig. *Endocrinology* 2001;142:3941–3949.
- 22 Conrad KP, Shroff SG: Effects of relaxin on arterial dilation, remodeling, and mechanical properties. *Curr Hypertens Rep* 2011;13:409–420.
- 23 Ivell R, Kotula-Balak M, Glynn D, Heng K, Anand-Ivell R: Relaxin family peptides in the male reproductive system – a critical appraisal. *Mol Hum Reprod* 2011;17:71–84.
- 24 Wolf JM, Cameron KL, Clifton KB, Owens BD: Serum relaxin levels in young athletic men are comparable with those in women. *Orthopedics* 2013;36:128–131.
- 25 Gedikli O, Yilmaz H, Kiris A, Karaman K, Ozturk S, Baykan M, Ucar U, Durmus I, Karahan C, Celik S: Circulating levels of relaxin and its relation to cardiovascular function in patients with hypertension. *Blood Press* 2009;18:68–73.
- 26 Dschietzig T, Richter C, Bartsch C, Laule M, Armbruster FP, Baumann G, Stangl K: The pregnancy hormone relaxin is a player in human heart failure. *FASEB J* 2001;15:2187–2195.
- 27 Koos RD, Kazi AA, Roberson MS, Jones JM: New insight into the transcriptional regulation of vascular endothelial growth factor expression in the endometrium by estrogen and relaxin. *Ann NY Acad Sci* 2005;1041:233–247.
- 28 Iber C, Ancoli-Israel S, Chesson AL Jr, Quan SF; American Academy of Sleep Medicine: The AASM Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Westchester, American Academy of Sleep Medicine, 2007.
- 29 Epstein LJ, Kristo D, Strollo PJ, Friedman N, Malhotra A, Patil SP, Ramar K, Rogers R, Schwab RJ, Weaver EM, Weinstein MD: Clinical guideline for the evaluation, management and long-term care of obstructive sleep apnea in adults. *J Clin Sleep Med* 2009;5:263–276.
- 30 Maeder M, Strobel W, Christ M, Todd J, Estis J, Wildi K, Thalmann G, Hilti J, Brutsche M, Twerenbold R, Rickli H, Mueller C: Comprehensive biomarker profiling in patients with obstructive sleep apnea. *Clin Biochem* 2015;48:340–346.
- 31 Kaditis AG, Alexopoulos EI, Karathanasi A, Ntamagka G, Oikonomidi S, Kiriopoulou TS, Zintzaras E, Gourgoulis K: Adiposity and low-grade systemic inflammation modulate matrix metalloproteinase-9 levels in Greek children with sleep apnea. *Pediatr Pulmonol* 2010;45:693–699.
- 32 Kim T, Lee CS, Lee SD, Kang SH, Han JW, Malhotra A, Kim KW, Yoon IY: Impacts of comorbidities on the association between arterial stiffness and obstructive sleep apnea in the elderly. *Respiration* 2015;89:304–311.
- 33 Yasmin, McEniery CM, Wallace S, Dakham Z, Pulsalkar P, Maki-Petaja K, Ashby MJ, Cockcroft JR, Wilkinson IB: Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. *Arterioscler Thromb Vasc Biol* 2005;25:372.
- 34 Tayebjee MH, Nadar S, Blann AD, Gareth Beavers D, MacFadyen RJ, Lip GY: Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in hypertension and their relationship to cardiovascular risk and treatment: a substudy of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT). *Am J Hypertens* 2004;17:764–769.
- 35 Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, Christiaens T, et al: ESH/ESC guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Eur Heart J* 2013;34:2159–2219.
- 36 Marrone O, Riccobono L, Salvaggio A, Mirabella A, Bonanno A, Bonsignore MR: Catecholamines and blood pressure in obstructive sleep apnea syndrome. *Chest* 1993;103:722–727.