Role of leptin as antioxidant in obstructive sleep apnea: an in vitro study using electron paramagnetic resonance method

By: Madalina Macrea, Thomas Martin, Leon Zagrean, Zhenquan Jia, Hara Misra

Macrea, M., Martin, T., Zagrean, L., Jia, Z., Misra, H. (2013). Role of leptin as antioxidant in obstructive sleep apnea: an in vitro study using electron paramagnetic resonance method. *Sleep and Breathing, 17(1),* 105-110. doi: 10.1007/s11325-012-0656-8

The final publication is available at Springer via http://dx.doi.org/10.1007/s11325-012-0656-8.

Abstract:

Introduction: As in obstructive sleep apnea (OSA), the chronic cycles of hypoxia and reoxygenation are thought to be conducive of oxidative stress (OS) with generation of reactive oxygen species, identifying effective mechanisms of protection against oxidant-mediated tissue damage becomes of outmost importance. Leptin's role had been recently extended into that of participant to OS; while its exact role in this process is yet to be defined, elevated leptin levels correlate significantly with several indices of OSA disease severity such as nocturnal hypoxemia, possibly acting as a counteractive mechanism against the chronic intermittent hypoxia-related OS and serving as a marker of future risk of atherosclerotic disease. We therefore investigated leptin's antioxidant mechanism on superoxide ($O_2^{-\bullet}$) anions using spectrophotometry and electron paramagnetic resonance (EPR).

Methods: The $O_2^{-\bullet}$ was generated by oxidation of xanthine (XAN) by xanthine oxidase (XO) in the presence of spin trap 5-diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide with various concentrations of leptin (0.001, 0.01, 0.1, and 1 mg/ml) and without leptin. Signal intensity between 3,440 and 3,540 G was expressed as standard means ± SD. The activity of leptin on XO was determined by monitoring the conversion of XAN to uric acid at 293 nm using a Beckman DU 800 UV–visible spectrophotometer.

Results: Leptin added to aqueous solutions at 0.1 and 1 mg/ml concentrations was associated with a statistically significant decrease in the EPR signal due to leptin's direct scavenging activity towards the $O_2^{-\bullet}$.

Conclusion: Leptin is an antioxidant agent of possible use as a marker of OS and future risk of atherosclerotic disease in OSA.

Abbreviations

ROS Reactive oxygen species

$O_2^{-\bullet}$ Superoxide anion radical

XO Xanthine oxidase

OSA Obstructive sleep apnea

SOD Superoxide dismutase

VEGF Vascular endothelial growth factor

EPR Electron paramagnetic resonance

XAN Xanthine

DEPMPO 5-Diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide

PBS Phosphate buffered saline

SOD Superoxide dismutase

GSH-P Glutathione peroxidase

GSH-R Glutathione reductase

Keywords: Leptin | Antioxidants | Electron paramagnetic resonance

Article:

Introduction

Obstructive sleep apnea (OSA) is a common disorder characterized by repetitive collapse of the pharyngeal airway during sleep resulting in a myriad of adverse vascular risks from insulin resistance to dyslipidemia, elevated diastolic blood pressure, and leptin levels. The mechanism of action by which hyperleptinemia is present in adults with OSA [*1*] is likely multifactorial; these patients have central or peripheral (receptor and post receptor) [*2*] leptin resistance with subsequently high circulating leptin levels, possibly related to an enhanced sympathetic drive due to hypoxia [*3*, *4*]. Another plausible explanation could be that leptin acts as a counter effect to hypoventilation [*5*, *6*], as studies have also demonstrated that leptin levels fall subsequently to continuous positive airway pressure (CPAP) therapy [*7*, *8*]. Recent evidence revealed the multifaceted properties of leptin, a 167-amino acid protein with a structure similar to cytokines and secreted predominantly by the adipocyte cells. Initially discovered as a hormone that regulated food intake and energy balance [*9*], it subsequently proved as having important roles in hypoxia such as inducer of angiogenesis [*10*] and promoter of the hypoxia-inducible gene [*11*]. As in OSA, the chronic cycles of hypoxia and reoxygenation [*12*] are thought to be conducive of

oxidative stress (OS) and generation of reactive oxygen species (ROS) [*13*] by a pathologic mechanism similar to that of ischemia–reperfusion, identifying effective mechanisms of protection against oxidant-mediated tissue damage becomes of outmost importance especially for severe disease due to reduced values of antioxidant capacity [*14*].

Recently, leptin's role had been extended into that of participant to OS by upregulating transcript levels of superoxide dismutase (SOD) 1, glutathione peroxidase, and glutathione S-transferase in ob/ob mice [*15*] and stimulation of the mRNA expression of SOD 2 in the serum-deprived cardiomyocytes [*16*, *17*]. Given leptin's strong correlation with the degree of obesity [*18*] and implicitly obesity-induced, inflammation-related OS [*19*], its exact role in this process is yet to be defined. However, as case–control studies have demonstrated increased serum leptin in OSA, despite similar levels of obesity [*20*], and these levels have correlated significantly with the degree of nocturnal hypoxemia independent of the degree of obesity in these patients [*14*, *21*, *22*], we thought to further assess leptin's ability and mechanism as a counteractive agent [*23*] against chronic intermittent hypoxia (CIH)-related OS.

Oxidative stress is defined as the imbalanced redox state in which prooxidants overwhelm the antioxidant capacity, resulting in an increased production of ROS, ultimately leading to oxidative damage of cellular macromolecules [*15*]. Perhaps the most important ROS, given its long half life and ability to move to locations where it can undergo interactions with other molecules, is superoxide anion radical ($O_2^{-\bullet}$) [*24*]; its corresponding antioxidant defense includes direct-acting enzymes, such as SOD and catalase, and supporting enzymes, such as xanthine oxidase (XO). The univalent reduction of molecular oxygen by XO action on xanthine (XAN)/hypoxanthine generating $O_2^{-\bullet}$ is known to play an important role in the oxidative tissue injury process. Activation of XO, which in turn may produce increased amounts of $O_2^{-\bullet}$, may result in the disruption of the macromolecules by acting both directly and/or indirectly via the formation of secondary oxidants [*25*]. The therapeutic effects derived from the inhibition of XO/$O_2^{-\bullet}$ have been successfully demonstrated for gout, tumor lysis syndrome, cardiovascular disease, stroke, and diabetes. On the other hand, studies looking for a similar therapeutic effect in the lungs have been extremely few [*26*] without conclusive results. In OSA disease, however, an important role of XO had been detailed by demonstrating that allopurinol, an XO inhibitor, reduced the level of OS as quantified by malonaldehyde, an oxidative damage marker, probably by decreasing free radical generation through inhibition of the XO system [*12*]. Therefore, due to the proven clinical efficacy of decreasing systemic OS through the XO system and the known leptin safe profile [*27*] and convenience of the intranasal route administration [*28*], we hypothesized that one of leptin's protective mechanisms against hypoxia/reoxygenation with ROS formation is due to its ability to inhibit XO and subsequently $O_2^{-\bullet}$ formation and we tested this hypothesis using spectrophotometry and electron paramagnetic resonance (EPR) spectroscopy in combination with spin trapping, the most sensitive and specific technique of detecting free radicals.

Materials and methods

Chemicals and materials

Purified 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline N-oxide (DEPMPO) was purchased from Alexis (Carlsbad, CA, USA). Leptin, XAN, XO, and all other chemicals and reagents were obtained from Sigma Chemicals (St. Louis, MO, USA).

EPR study of the scavenging activity of leptin towards $O_2^{-\bullet}$

EPR is an excellent approach for the detection of radicals [*14*]. The $O_2^{-\bullet}$ was generated by oxidation of XAN by XO in the presence of spin trap DEPMPO and with or without leptin as previously described with slight modifications [*29*].

Briefly, the reaction mixture in phosphate buffered saline (PBS) contained 36 μM XAN, 32 mU/ml XO, and 10 mM DEPMPO in the absence (control samples) or presence of various concentrations of leptin (0.001, 0.01, 0.1, and 1 mg/ml) in a final volume of 0.1 ml in a test tube. After 10 min of reaction, the reaction mixture was then transferred to a capillary tube for EPR spectral analysis. EPR spectra were recorded at room temperature using a spectrometer (Bruker D-200 ER, IBM Bruker), operating at the X-band with a TM cavity and capillary tube. The EPR spectrometer settings were modulation frequency, 100 KHz; X-band microwave frequency, 9.5 GHz; microwave power, 20 mW; modulation amplitude, 6.3 G; time constant, 160 s; scan time, 200 s; and receiver gain, 4×10^5. The effect was estimated by the percent age of variation in the EPR signal intensity of the DEMPO–$O_2^{-\bullet}$ adduct compared with that of the controls without leptin (representing 100%).

Measurement of uric acid formation by xanthine oxidase reaction

The activity of XO was determined by monitoring the conversion of XAN to uric acid at 293 nm using a Beckman DU 800 UV–visible spectrophotometer. The reaction mixture contained 1.0 ml of 50 mM PBS, pH 7.4, 0.1 mM DTPA, 36 μM XAN, and 32 mU/ml XO in the absence or presence of various concentrations of leptin (0.001, 0.01, 0.1, and 1 mg/ml). The reaction mixture was incubated for 5 min at 37°C prior to measurement of uric acid production at 293 nm and prior to the addition of XO to start the reaction.

Statistical analysis

The EPR signal intensity ($n = 8$) between 3,440 and 3,540 G was reported as standard means ± SD. The Mann–Whitney test was used for identifying the effect of various leptin concentrations (0.001, 0.01, 0.1, and 1 mg/ml) on $O_2^{-\bullet}$ and uric acid formation by the XAN/XO system as compared with the control. Results were reported as p value and confidence interval; $p < 0.05$ was considered statistically significant.

Results

In order to confirm the precise $O_2^{-\bullet}$ scavenging activity, a steady-state enzymatic XAN/XO system was used. At all final concentrations, leptin added to the aqueous solution resulted in a decrease in EPR signal that was concentration dependent. The EPR signal intensities of leptin-containing solutions compared with controls were as follows: 85 ± 23% for the low concentration leptin solution (LL, i.e., 0.001 mg/ml); 83 ± 23% for the intermediate concentration leptin solution (IL, i.e., 0.01 mg/ml); 71 ± 17% for the high concentration leptin solution (HL, i.e., 0.1 mg/ml); and 50 ± 14% for the very high leptin concentration solution (VHL, i.e., 1 mg/ml). As shown in Fig. 1, the intensity of the signal decreased, from top (control) to bottom (VHL), with increasing leptin concentrations of 0.001 mg/ml (Fig. 1, LL), 0.01 mg/ml (Fig. 1, IL), 0.1 mg/ml (Fig. 1, HL), and 1 mg/ml (Fig. 1, VHL). Compared with controls, leptin in both HL ($p = 0.0003$, CI 15.7–41.79) and VHL ($p < 0.0001$, CI 39.38–60.62) solutions was found to inhibit the $O_2^{-\bullet}$ statistically significantly (Fig. 2).

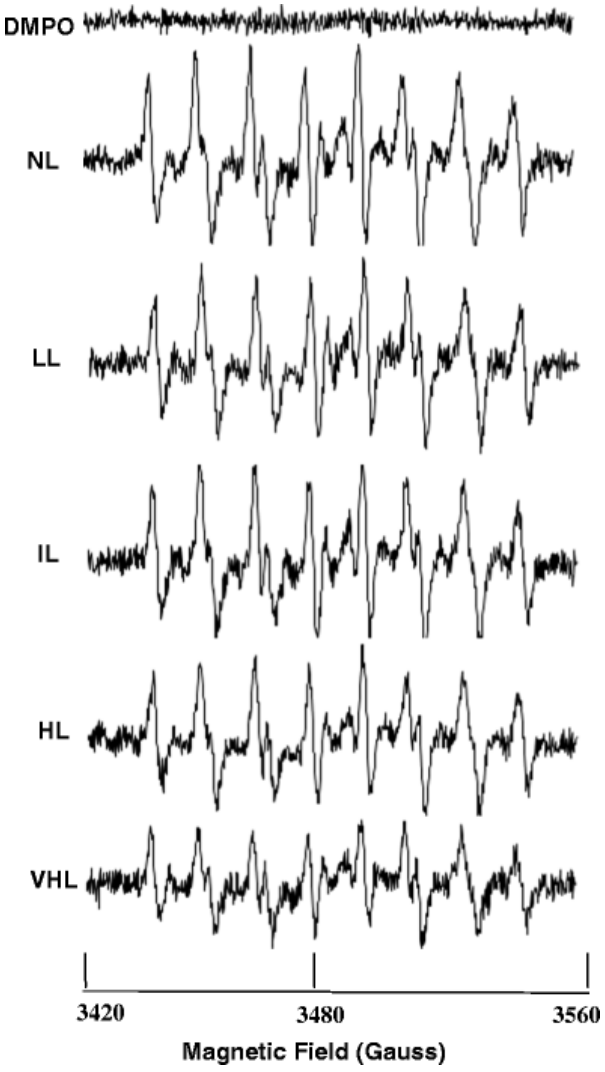

Fig. 1 EPR spectroscopy in combination with the spin probe DEPMPO was used to examine the superoxide-scavenging capability of leptin. From *top* to *bottom*: NL (no leptin) or control; LL,

i.e., 0.001 mg/ml leptin concentration; IL, i.e., 0.01 mg/ml leptin concentration; HL, i.e., 0.1 mg/ml leptin concentration; and VHL, 1 mg/ml leptin concentration

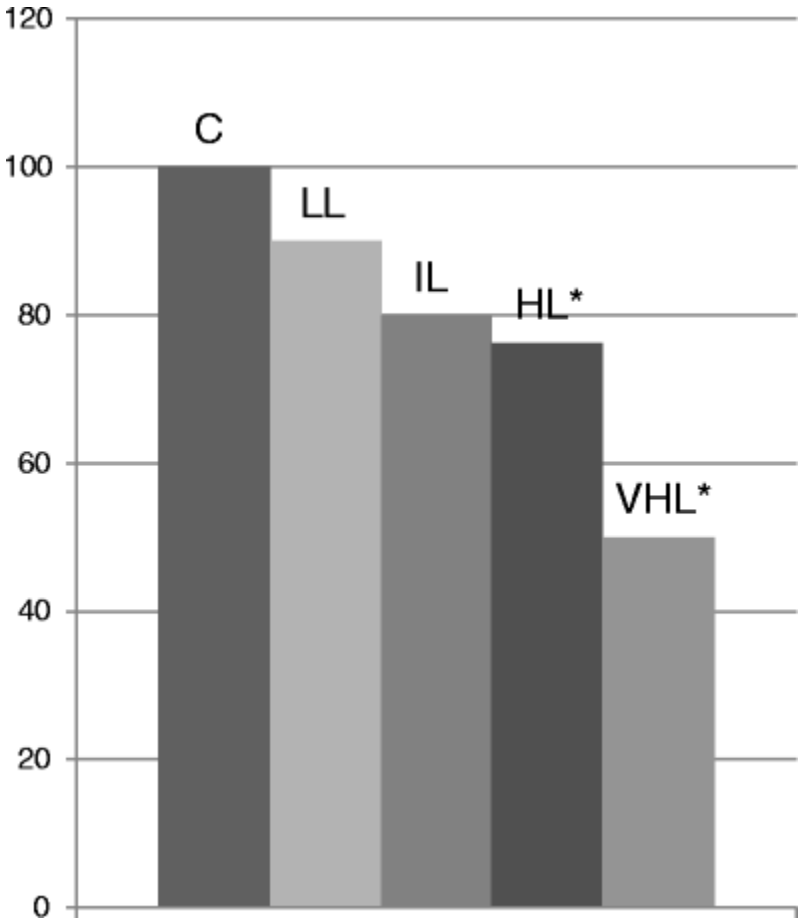

Fig. 2 Scavenging of the superoxide anion by leptin. Effect of leptin concentrations on the EPR signal corresponding to the $O_2^{-\bullet}$ adduct with DEPMPO. The 100% reference value corresponds to the level of the DEPMPO–$O_2^{-\bullet}$ adduct produced in the corresponding control sample without leptin. *NL* no leptin; *LL* low leptin concentration solution, i.e., 0.001 mg/ml; *IL* intermediate leptin concentration solution, i.e., 0.01 mg/ml; *HL* high leptin concentration solution, i.e., 0.1 mg/ml; and *VHL* very high leptin concentration solution, i.e., 1 mg/ml. Mann–Whitney test for each treatment showed differences ($p < 0.05$); *asterisk*, significant differences compared to control

At all final concentrations (0.001, 0.01, 0.1, and 1 mg/ml), leptin failed to inhibit uric acid formation by the XAN/XO system as compared with control (Fig. 3), indicating that leptin does not inhibit XO, the source of $O_2^{-\bullet}$ production. Therefore, the decrease in EPR signal was due to leptin's direct effect on the $O_2^{-\bullet}$.

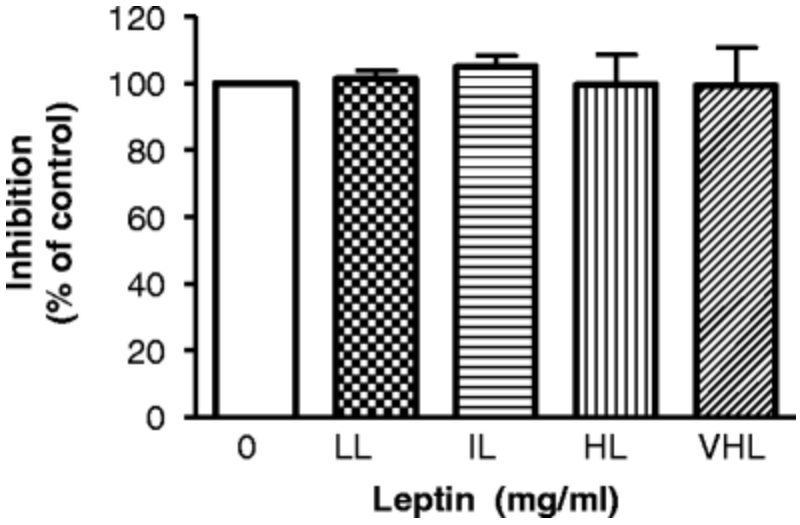

Fig. 3 The effects of leptin on the formation of uric acid by the xanthine/xanthine oxidase system. The reaction mixture contained 36 μM xanthine and 32 mU/ml xanthine oxidase in PBS. Indicated various concentrations of leptin were added to the reaction mixture. After incubation for 5 min at 37°C, the formation of uric acid was measured at 293 nm

Discussion

Our results demonstrate that leptin added to aqueous solutions at 0.1 and 1 mg/ml concentrations was associated with a statistically significant decrease in the EPR signal due to leptin's direct scavenging activity towards the $O_2^{-\bullet}$, the highest scavenging activity being seen at the final concentration of 1 mg/ml.

The formation of toxic-free oxygen radicals, such as $O_2^{-\bullet}$, has been implicated in several major disease processes, both of acute (post-hypoxic neuronal damage [*9*], sepsis, and acute lung injury [*30*]) and chronic pathology (atherosclerosis [*31*], immunity [*32*], and ischemic and myopathic heart disease [*33*]). In OSA disease, the increased ROS [*34*] due to intermittent hypoxia/reoxygenation phenomenon are thought to participate to the endothelial damage, linking the OS to the important concept of future risk of atherosclerotic disease; several markers of such processes, including $O_2^{-\bullet}$ [*35*], hydrogen peroxide [*36*], nitric oxide [*37*], and ROS in CD11c monocytes [*38*], have been found to be altered in this disease and restored subsequent to CPAP therapy.

While our study is the first to specifically address a potential role of leptin in the OSA-associated redox balance, mounting general evidence supports our findings, reporting antioxidant properties of leptin in several other non-OSA conditions. Patients with leptin gene mutation had defective antioxidant defense as evidenced by reduced levels of glutathione peroxidase (GSH-P) and erythrocyte copper–zinc superoxide dismutase [*23*]. In vitro, leptin resulted in SOD activation in the ob/ob mice [*15*] and primary cultured cardiomyocytes [*16*], and in vivo, leptin acted as a powerful antioxidant in the rat bladder after ischemia–reperfusion injury [*39*]. The above

findings, along with reports of leptin administration to the leptin-deficient ob/ob mouse resulting in the rebuild of the antioxidant enzymes catalase, GSH-P, and GSH-R [*30*], bring into question the potential research and clinical role of leptin as an antioxidant agent by mechanisms yet to be defined.

Few of the studies with focus on leptin and its oxidant/antioxidant properties contradict our results; in these studies, leptin administration to the non-HIV human monocytes, heart endothelial cells, and rat hepatic stellate cells resulted in stimulation of the oxidative burst and ROS production [*40–42*]. However, in none of these studies, the antioxidant response had been quantified and therefore we consider that a rather beneficial effect of leptin could have been missed due to lack of evidence of the increased antioxidant component in excess to that of ROS production, leading to an overall improved redox balance [*30*].

To the best of our knowledge, this is the first study to evaluate leptin's scavenging properties on $O_2^{-\bullet}$ using the EPR spin trapping method, establishing evidence towards its antioxidant mechanisms and weighing the clinical value of these new findings in the context of OSA disease.

The main weakness of our study is related to concentrating our efforts on using a high concentration of leptin solution. As the few available studies on leptin's protective role against OS report contradicting results for the low or moderate concentration solutions [*17*, *43*, *44*], we instead opted for using high and very high leptin concentration solutions given current evidence reporting leptin administration by intranasal or peripheral route as being safe [*27*] and efficient [*45*, *46*]. We also opted on studying a single ROS, the $O_2^{-\bullet}$, due to (1) its involvement in oxygenation–reoxygenation phenomenon, such as seen in CIH induced by OSA [*47*]; (2) known interaction with nitric oxide radicals yielding peroxynitrite, another major ROS; and (3) previous evidence demonstrating potent $O_2^{-\bullet}$ scavengers (i.e., manganese (III) tetrakis (1-methyl-4-pyridyl) porphyrin pentachloride) being successful in preventing the intermittent hypoxia-evoked changes in the cardiorespiratory system as well as gene expression [*48*].

Our study has several strengths: first, it benefited from the use of the EPR spin trapping method [*49*], a very accurate and elegant method for the detection of radicals. Second, it looked into detailing a possible antioxidant pathway for leptin, rather than evidencing its endpoint properties only as antioxidant for $O_2^{-\bullet}$. Lastly, it expanded the important concept of OSA-related redox balance into clinical significance by eloquently demonstrating leptin's properties as antioxidant and marker of OS.

In conclusion, our study establishes evidence towards leptin's potential as an antioxidant agent and suggests its possible clinical significance as a marker of OS and future risk of atherosclerotic disease in OSA.

References

1. Ip M, Lam K, Ho C, Tsang KW, Lam W (2000) Serum leptin and vascular risk factors in obstructive sleep apnea. Chest 118:580–586

2. Wang M, Orci L, Ravazzola M, Unger RH (2005) Fat storage in adipocyte requires inactivation of leptin's paracrine activity: implications for treatment of human obesity. P N A S 120:18011–18016

3. Zwillich C (1998) Sleep apnea and autonomic function. Thorax 53:S20–S24

4. Somers VK, Dyken ME, Clary MP, Abboud FM (1995) Sympathetic neural mechanisms in obstructive sleep apnea. J Clin Invest 96:1897–1904

5. Phipps PR, Starritt E, Caterson GRR (2002) Association of serum leptin and hypoventilation in human obesity. Thorax 57:75–76

6. O'Donnell CP, Schaub CD, Haines AS, Berkowitz DE, Tankersley CG, Schwartz AR, Smith PL (1999) Leptin prevents respiratory depression in obesity. Am J Respir Crit Care Med 159:1477–1484

7. Chin K, Shimizu K, Nakamura T, Narai N, Masuzaki H, Ogawa Y, Mishima M, Nakamura T, Nakao K, Ohi M (1999) Changes in intraabdominal visceral fat and serum leptin levels in patients with obstructive sleep apnea syndrome following nasal continuous positive airway pressure therapy. Circulation 100:706–712

8. Harsch IA, Konturek PC, Koebnick C (2003) Leptin and ghrelin levels in patients with obstructive sleep apnoea: effect of CPAP treatment. Eur Respir J 22:251–257

9. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM (1994) Positional cloning of the mouse obese gene and its human homologue. Nature 372:425–432

10. Park HY, Kwon HM, Lim HJ, Hong BK, Lee JY, Park BE, Jang Y, Cho SY, Kim HS (2001) Potential role of leptin in angiogenesis: leptin induces endothelial cell proliferation and expression of matrix metalloproteinases in vivo and in vitro. Exp Mol Med 33:95–102

11. Guerre-Millo M, Grosfeld A, Isaad T (2002) Leptin is a hypoxia-inducible gene. Obes Res 8:856

12. El Solh AA, Saliba R, Bobinski T, Grant BJ, Berbary E, Miller N (2006) Allopurinol improves endothelial function in sleep apnea: a randomized controlled study. Eur Resp J 4:997–1002

13. Lee-Chiong T (2006) Sleep: a comprehensive handbook. Wiley-Liss, New York

14. Christou K, Moulas AN, Pastaka C, Gourgoulianis KI (2003) Antioxidant capacity in obstructive sleep apnea patients. Sleep Med 4:225–228

15. Sainz N, Rodriguez A, Catalan V, Becerril S, Ramírez B, Gómez-Ambrosi J, Frühbeck G (2010) Leptin administration downregulates the increased expression levels of genes related to oxidative stress and inflammation in the skeletal muscle of ob/ob mice. Mediators Inflamm 2010:784343

16. Eguchi M, Liu YI, Shin EJ, Sweeney G (2008) Leptin protects H9c2 rat cardiomyocytes from H_2O_2-induced apoptosis. FASEB J 75:3136–3144

17. Zheng J, Fang J, Yin YJ, Wang X-C, Ren An-Jing, Bai J, Sun X-J, Yuan W-J, Lin L (2010) Leptin protects cardiomyocytes from serum deprivation induced apoptosis by increasing antioxidant defense. Clin Exp Pharmacol Physiol 37:955–962

18. Sanchez-de-la-Torre M, Mediano O, Barcelo A, Pierola J, de la Pena M, Esquinas C et al (2012) The influence of obesity and obstructive sleep apnea on metabolic hormones. Sleep Breath (in press)

19. Lee SD, Ju G, Choi JA, Kim JW, Yoon IY (2012) The association of oxidative stress with central obesity in obstructive sleep apnea. PMID: 21614576

20. Phillips BG, Kato M, Narkiewicz K, Chloe I, Somers VK (2000) Increases in leptin levels, sympathetic drive, and weight gain in obstructive sleep apnea. Am J Physiol Heart Circ Physiol 279:234–237

21. Tatsumi K, Kasahara Y, Kurosu K, Tanabe N, Takiguchi Y, Kuriyama T (2005) Sleep oxygen desaturations and circulating leptin in obstructive sleep apnea–hypopnea syndrome. Chest 127:716–721

22. McArdle N, Hillman D, Beilin L, Watts G (2007) Metabolic risk factors for vascular disease in obstructive sleep apnea. AJRCCM 175:190–195

23. Ozata M, Uckaya G, Aydin A, Isimer A, Ozdemir IC (2000) Defective antioxidant defense system in patients with a human leptin mutation. Horm Metab Res 37:269–272

24. Kohen R, Nyska A (2002) Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions and methods for their quantification. Toxicol Pathol 30:620–650

25. Floyd RA (1990) Role of oxygen free radicals in carcinogenesis and brain ischemia. FASEB J 4:2587–2597

26. Pacher P, Nivorozhkin A, Szabo C (2006) Therapeutic effects of xanthine-oxidase inhibitors: Renaissance half a century after the discovery of allopurinol. Pharmacol Rev 58:87–114

27. Mackintosh RM, Hirsch J (2001) The effects of leptin administration in non-obese human subjects. Obes Res 8:462–469

28. Schulz C, Paulus K, Lehnert H (2004) Central nervous and metabolic effects of intranasally applied leptin. Endocrinology 6:2696–2701

29. Fridrich I (1970) Quantitative aspects of the production of superoxide anion radical by milk xanthine oxidase. J Biol Chem 245:4053–4057

30. Chabot F, Mitchell JA, Gutteridge JMC (1998) Reactive oxygen species in acute lung injury. Eur Respir J 11:745–757

31. Prasad K, Kalra J (1993) Oxygen free radicals and hypercholesterolemic atherosclerosis: effect of vitamin E. Am Heart J 125:958–973

32. Knight JA (2000) Review: free radicals, antioxidants, and the immune system. Ann Clin Lab Sci 30:145–158

33. Chen TI, Lai CJ, Hsieh CJ, Tsai KL, Yang KT (2011) Differences in left ventricular cardiomyocytes loss induced by chronic intermittent hypoxia between spontaneously hypertensive and Wistar–Kyoto rats. Seep Breath 15:845–854

34. Christou K, Markoulis N, Moulas A, Pastaka C, Gourgoulianis KI (2003) Reactive oxygen metabolites (ROMs) as index of oxidative stress in obstructive sleep apnea patients. Sleep Breath 7:105–110

35. Schultz R, Mahmoudi S, Hattar K, Sibelius U, Olschewski H, Mayer K, Seeger W, Grimminger F (2000) Enhanced release of superoxide from polymorphonuclear neutrophils in obstructive sleep apnea. Am J Respir Crit Care Med 162:566–570

36. Murri M, Garcia-Delgado R, Alcazar-Ramirez L, Fernández-Ramos A, Alcaide J, Cardona F, Tinahones F (2011) Continuous positive airway pressure treatment effect on oxidative stress and circulating progenitor cell levels in sleep patients with apnea–hypopnea syndrome. Respir Care, May, 20

37. Ip MSM, Lam B, Chan LY, Zheng L, Tsang KWT, Fung PC, Lam WK (2000) Circulating nitric oxide is suppressed in obstructive sleep apnea and is reversed by nasal continuous positive airway pressure. Am J Respir Crit Care Med 162:2166–2171

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

39. Hamarat M, Yenilmez A, Erkasap N, Burhanettin I, Erinc A, Tulay K, Cavit C, Canan BD (2010) Protective effects of leptin on ischemia/reperfusion injury in rat bladder. Chin J Physiol 43:145–150

40. Sanchez-Pozo C, Rodriguez-Bano J, Dominiguez-Castellano A (2003) Leptin stimulates the oxidative burst in control monocytes but attenuates the oxidative burst in monocytes from HIV-infected patients. Clin Exp Immunol 134:464–469

41. He WH, Li B, Zhu X (2010) The role and mechanism of NADPH oxidase in leptin-induced reactive oxygen species production in hepatic stellate cells. Zhonghua Gan Zang Bing Za Zhi 18:849–854

42. Jian-Xiong C, Yu H , Tuo Q, Zeng H, Aschner J (2007) Leptin stimulates angiogenesis in myocardial endothelial cells: Involvement of p47phox NADPH oxidase. FASEB J 21. 372.10

43. Xu FP, Chen MS, Wang YZ (2004) Leptin induces hypertrophy via endothelin-1–reactive oxygen species pathway in cultured neonatal rat cardiomyocytes. Circulation 110:1269–1275

44. Konstantinidis D, Paletas K, Koliakos G, Kaloyianni M (2009) The ambiguous role of the Na^+–H^+ exchanger isoform 1 (NHE1) in leptin-induced oxidative stress in human monocytes. Cell Stress and Chaperones 14:591–601

45. Schulz C, Paulus K, Lehnert H (2004) Central nervous and metabolic effects of intranasally applied leptin. Endocrinology 145:2696–2701

46. Fliedner S, Schulz C, Lehnert H (2006) Brain uptake of intranasally applied radioiodinated leptin in Wistar rats. Endocrinology 147:2088–2094

47. Prabhakar NR (2002) Sleep apnea. An oxidative stress? Am J Respir Crit Care Med 165:859–860

48. Prabhakar NR (2001) Oxygen sensing during intermittent hypoxia: cellular and molecular mechanisms. J Appl Physiol 90:1986–1994

49. Jia Z, Zhu H, Misra BR, Mahaney JE, Li Y, Misra HP (2008) EPR studies on the superoxide-scavenging capacity of the nutraceutical resveratrol. Mol Cell Biochem 313:187–194