Circulating Lipopolysaccharide-Binding Protein and Carotid Intima-Media Thickness in Obstructive Sleep Apnoea

Ivana Trojova[1], Miriam Kozarova[2], Darina Petrasova[3], Zuzana Malachovska[2], Ivana Paranicova[1], Pavol Joppa[1], Ruzena Tkacova[1]

[1]Department of Respiratory Medicine and Tuberculosis, P. J. Safarik University in Kosice, Medical Faculty and L. Pasteur University Hospital, Kosice, Slovakia

[2]4th Department of Internal Medicine, P. J. Safarik University in Kosice, Medical Faculty and L. Pasteur University Hospital, Kosice, Slovakia

[3]Laboratory of Research Biomodels, P. J. Safarik University in Kosice, Medical Faculty, Kosice, Slovakia

Corresponding author:

Prof. Ruzena Tkacova MD, PhD; Department of Respiratory Medicine, P. J. Safarik University, Medical Faculty and L. Pasteur University Hospital; Rastislavova 43, 041 90 Kosice, Slovakia; Telephone number: +421 55 615 2642, Fax number: +421 55 615 2664

Email: ruzena.tkacova@upjs.sk

Short Title:

Endotoxemia and subclinical atherosclerosis in OSA

Source of Support:

This work was supported by the Slovak Research and Development Agency of the Ministry of Education, Slovakia (APVV-0134-11), and grants VEGA 1/0863/15, VEGA 1/0208/16.

Abstract

Circulating lipopolysaccharide-binding protein (LBP), a metabolic endotoxemia marker, was identified as an independent predictor of atherosclerosis. Although increases in carotid intima-media thickness (CIMT) were repeatedly reported in obstructive sleep apnoea (OSA), neither the role of OSA in metabolic endotoxemia nor of LBP in early atherosclerosis were explored in patients with OSA. At a tertiary university hospital we investigated the relationships between OSA, LBP and CIMT in 117 men who underwent full polysomnography and CIMT assessment by B-mode ultrasound. Circulating LBP concentrations and average CIMT increased from patients without OSA to those with mild-moderate and severe OSA [from 32.1±10.3 to 32.3±10.9 to 38.1±10.3 $\mu g.ml^{-1}$, P=0.015; from 0.52±0.09 to 0.58±0.06 to 0.62±0.10 mm, P=0.004, respectively]. Oxygen desaturation index (ODI) was a predictor of serum LBP levels independent of age, waist-to hip ratio (WHR), smoking, hypertension, HDL cholesterol, triglycerides and fasting glucose [P *(ANOVA)*=0.002, R^2=0.154], with no independent effect of the ODI*WHR interaction term on LBP. Furthermore, serum LBP predicted CIMT independently of known risk factors of atherosclerosis including obesity (P<0.001 R^2=0.321). Our results suggest that OSA severity contributes to metabolic endotoxemia in patients with OSA independently of obesity, and that LBP might represent a contributing factor promoting early atherosclerosis in such patients.

Abstract word count: 200

Keywords: obstructive sleep apnoea; carotid atherosclerosis; intima-media thickness; lipopolysaccharide-binding protein; endotoxemia

Introduction

Obstructive sleep apnoea (OSA) is characterized by repeated episodes of upper airway occlusion during sleep which are associated with hypoxia and arousals from sleep. Acutely, repetitive apnoeas and hypopnoeas trigger surges in sympathetic nervous system activity, blood pressure and heart rate. Chronically, patients with OSA are at increased risk for arterial hypertension, stroke, and myocardial ischemia and have an increased cardiovascular morbidity and mortality (Shah *et al*., 2010, Punjabi *et al*. 2009, Marin *et al*. 2005). In addition, signs of subclinical atherosclerosis as evidenced by increased carotid intima-media thickness (CIMT) were reported in OSA including patients with no overt cardiovascular disease (Monneret *et al*. 2010, Fox et al. 2014, Damiani *et al*. 2015, Drager *et al*. 2005).

Metabolic endotoxemia reflected by serum lipopolysaccharide-binding protein (LBP) levels was identified to represent an independent predictor of coronary atherosclerosis in the LURIC study (Lepper *et al.* 2014) thus extending the list of recognized pathogenetic mechanisms of atherosclerosis such as aging, obesity-related metabolic disturbances and inflammatory processes (Libby 2002). Originally, Cani et al. (2008) identified bacterial lipopolysaccharide (LPS) as a triggering factor of insulin resistance and weight gain, and defined its negative effects as “metabolic endotoxemia”. LBP binds LPS, and elevated circulating concentrations of this biomarker are considered to be a marker of metabolic endotoxemia in clinical studies (Gonzalez-Quintela *et al.* 2013). Several reports documented an association between serum LBP and prevalent coronary artery disease, and identified LBP as an independent predictor of coronary atherosclerosis (Lepper *et al.* 2011, Lepper *et al.* 2007, Szeto *et al*. 2008, Serrano *et al*. 2013). Nevertheless, the effects of OSA on serum LBP and the potential links between metabolic endotoxemia and CIMT in such patients remain largely unexplored. Therefore, we investigated the effects of OSA severity on serum LBP, and tested the hypothesis that serum LBP concentrations relate to CIMT in patients with OSA.

Subjects and Methods

Subjects

The study was conducted in the sleep unit at the tertiary referral university hospital. Clinically stable men who had been referred for evaluation of suspected OSA were enrolled to the study. Exclusion criteria were history of known cardiovascular disease (CVD), angina, myocardial infarction, stroke, congestive heart failure, chronic respiratory diseases other than OSA, type 1 or 2 diabetes, hereditary metabolic disorders, hypothyroidism, chronic inflammatory diseases and regular use of sedatives, antidepressant or antipsychotic medication or alcohol. The history was retrieved from complete patients' charts as provided by general practitioners. Anthropometric measurements were obtained in the morning after the polysomnographic examination with the patient standing erect. Body weight, height, neck circumference, waist circumference and hip circumference were measured and recorded. Neck circumference was measured at the level of the cricothyroid membrane; waist circumference at the midpoint between the costal margin and the iliac crest at the end of normal expiration; hip circumference at the level of the greater trochanter. Body mass index (BMI) was defined as weight/height^2 (kg/m^2). The waist-to hip ratio (WHR) was also calculated. The study was in agreement with Helsinki protocol and was approved by the L. Pasteur University Hospital ethics committee. All subjects provided written informed consent before entry to the study.

Polysomnography

All participants underwent attended diagnostic overnight polysomnography (Alice 4; Respironics Inc., Murrysville, Pennsylvania, USA), comprising continuous recording of electroencephalograph, electrooculography, electromyography, electrocardiography, thoracic and abdominal impedance belts for respiratory movements, thermistor for nasal and oral

airflow, pulse oximetry and microphone for snoring. All records were scored manually following the American Academy for Sleep Medicine (AASM) 2012 guidelines (Berry *et al.* 2012). Apnoea was identified as a drop in airflow of ≥ 90% from the baseline excursion for ≥ 10 s; hypopnoea was defined as a reduction in airflow of ≥ 50% of baseline for ≥ 10s accompanied either by a decrease in hemoglobin saturation for ≥ 3%, an EEG-recorded arousal, or both. The apnoea-hypopnoea index (AHI) was defined as the number of apnoea and hypopnoea events per hour of sleep. Oxygen desaturation index (ODI) was defined as the number of oxygen desaturations of hemoglobin of ≥ 3% per hour of sleep. In addition, the length of time with an arterial oxygen saturation measured by pulse oximetry (SpO_2) < 90% was used to assess the degree of nocturnal hypoxia. The classification of OSA severity was based on AASM guidelines: Mild: AHI ≥ 5 and < 15 episodes.h^{-1}; moderate: AHI ≥ 15 and < 30 episodes.h^{-1}, and severe: AHI ≥ 30 episodes.h^{-1} (Berry *et al*. 2012).

Biochemical measurements

Peripheral venous blood samples were collected between 6-7 a.m. following an overnight 12 h fast and polysomnography. Blood sample was taken from the antecubital vein and after immediate centrifugation, aliquots of plasma and serum were stored at -70°C until analysis. Fasting cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, apolipoprotein A-I (ApoA-I), and apolipoprotein B (ApoB) were measured by routine enzymatic methods. Low-density lipoprotein (LDL) cholesterol was derived using the Friedewald equation. Serum insulin was determined with electrochemiluminiscence immunoassay kits (Elecsys) on Roche Elecsys 1010/2010 and modular analytics E170 immunoassay analyzers (Roche Diagnostics GmbH, Mannheim, Germany); plasma glucose was measured by the glucose oxidase method on a Beckman autoanalyzer. Insulin resistance was estimated by the homeostasis model assessment (HOMA-IR) using the following formula: fasting serum insulin (mU.l^{-1}) x fasting

plasma glucose (mmol.l^{-1})/22.5 (Gayoso-Diz *et al*. 2013). LBP levels were assessed using a commercially available kit based on murine monoclonal antibodies specific for human LBP (Human LBP ELISA, Abnova, Taipei City, Taiwan).

Carotid intima-media thickness

Measurement of CIMT was done by high-resolution B-mode ultrasound system (Philips HD11XE) equipped with a linear array 7 MHz transducer. 3-lead electrocardiographic signal was obtained along with ultrasound scanning and CIMT was measured at the end-diastole. Semiautomatic border detection program QLab© was used for offline CIMT measurement on the segment of the common carotid artery (CCA) free of atherosclerotic plaque with clearly defined lumen-intima and media-adventitia interfaces. The measurement was done on the far wall of CCA at a distance of at least 10 mm below its distal end. Values from right and left CIMT were calculated as the mean of three measurements. Average CIMT (mm) was calculated as the mean of the right and left CIMT values (Touboul *et al.* 2012).

Statistical analysis

Analyses were conducted using SPSS software for Windows (version 14.0; IBM, Chicago, Illinois, USA); two-tailed $p < 0.05$ was considered significant. The data of continuous variables are presented as mean ± SD; differences between the groups were analyzed using analysis of variance (ANOVA). Kolmogorov-Smirnov test was used to check for normality of the values distribution within each variable. Chi-square test was used to compare the proportion of categoric variables between groups. Correlation analyses were performed using the Pearson product moment correlation method. Multiple linear regression models were used with LBP as a dependent variable and age, WHR, smoking, hypertension, ODI, serum fasting glucose, triglycerides and HDL cholesterol levels as independent variables. Furthermore, multiple linear regression models were used to assess independent predictors of CIMT.

Results

Characteristics of the subjects

One hundred and seventeen subjects participated in the study; 10 had no OSA, 50 suffered from mild to moderate, and 57 from severe OSA. Basic demographic characteristics and polysomnographic findings in the study groups are summarized in Table 1. Higher age, BMI, neck circumference, waist-to hip ratio, systolic and diastolic blood pressure (BP) were all associated with greater severity of OSA. In the entire cohort, 35.0% of patients were hypertensive, and were using the following anti-hypertensive drugs: angiotensin-converting enzyme inhibitors (n = 25), sartans (n = 6), diuretics (n = 6), calcium-antagonists (n = 14) and beta-blockers (n = 17).

Serum lipids and insulin sensitivity

Serum LDL/HDL ratio, atherogenic index, ApoB levels, and ApoB/ApoA ratio significantly increased from patients without OSA to those with mild-moderate and severe OSA ($p = 0.019$, $p = 0.005$, $p = 0.030$, $p = 0.046$, respectively) (Table 2). In addition, fasting glucose, insulin and HOMA-IR all increased with greater severity of OSA; patients with severe OSA had significantly higher HOMA-IR compared to participants with no OSA ($p < 0.05$).

Circulating LBP

Circulating LBP concentrations increased from patients without OSA to those with mild-moderate and severe OSA (from 32.1 ± 10.3 to 32.3 ± 10.9 to 38.1 ± 10.3 $\mu g.ml^{-1}$, $p = 0.015$) (Figure 1). Patients with severe OSA had significantly higher serum LBP compared to patients with mild-moderate OSA ($p < 0.05$). After adjustments for age and BMI, serum LBP levels were significantly related to indices of central obesity (neck circumference and WHR), and to ODI (Table 3). ODI ($p = 0.023$) and WHR ($p = 0.039$) predicted serum LBP levels

independently of age, smoking, hypertension, serum fasting glucose, triglycerides and HDL cholesterol levels in the multivariate regression analysis (*P* of the model = 0.002, R^2 = 0.154). We also analyzed an independent effect of an interaction term (WHR*ODI) on serum LBP levels, nevertheless, this interaction term did not reach statistical significance in the multivariate analysis.

Carotid intima-media thickness

Both the right and left common CIMT increased from participants with no OSA to patients with mild-moderate and severe OSA (p = 0.006; p = 0.002, respectively) (Table 4). Figure 2 illustrates the average CIMT in subjects with no OSA, patients with mild-moderate and those with severe OSA (0.52 ± 0.09 mm versus 0.58 ± 0.06 mm versus 0.62 ± 0.10 mm, p = 0.004). Patients with severe OSA had higher average CIMT compared to subjects with no OSA (p < 0.05).

Figure 3 illustrates direct relationship between average CIMT and serum LBP levels (r = 0.287, p = 0.002). In multivariate analysis with average CIMT as the dependent variable and age, WHR, ODI, arterial hypertension, serum cholesterol, smoking and serum LBP levels as independent variables, the following variables independently predicted CIMT: age (p<0.001), cholesterol (p=0.041), smoking (p=0.039) and serum LBP (p=0.012) (*P* of the model < 0.001 R^2 = 0.321). Thus serum LBP was retained in the model as a significant predictor of -CIMT independently of WHR.

Discussion

The present study provides a novel observation on the relationship between OSA severity, endotoxemia and subclinical atherosclerosis in patients with OSA. Our data demonstrate increases in serum LBP concentrations in patients with severe OSA, in association with increases in CIMT. OSA severity as reflected by ODI was related to metabolic endotoxemia independently of obesity. Furthermore, serum LBP levels predicted CIMT independently of other known risk factors of atherosclerosis including obesity. Recently, low-grade endotoxemia was observed in children with OSA (Kheirandish-Gozal *et al.* 2014), and relationships between snoring (but no OSA) and higher LBP were demonstrated in adults (Sun *et al*. 2011). Nevertheless, these studies did not evaluate an independent effect of OSA on LBP levels, and in addition no direct assessment of clinical or subclinical atherosclerosis was performed. Therefore, by concomitant assessment of OSA severity, serum LBP levels and CIMT, our findings are the first to suggest that OSA severity as reflected by ODI relates to metabolic endotoxemia, and that endotoxemia is linked to increases in CIMT in adult patients with OSA. Importantly, the observed relationships were independent of the confounding effects of obesity.

LBP is an endogenously produced biomarker produced by hepatocytes, intestinal epithelial cells and adipocytes in response to intestinal microbial translocation, *i.e.*, in response to the movement of gut microbial species or their products across intestinal mucosal barrier without overt bacteremia (Cani *et al.* 2008, Teixeira *et al.* 2012, Patel *et al.* 2015). LBP binds LPS in various pathologic states including obesity and insulin resistance which are recognized as leading clinical conditions associated with endotoxemia (Kim *et al.* 2016, Boutagy *et al*. 2016). In agreement with previous reports our data demonstrate relationships between serum LBP and parameters of central obesity and insulin sensitivity (Gonzalez-

Quintela *et al.* 2013, Kheirandish-Gozal *et al*. 2014, Sun *et al.* 2011, Zhu *et al.* 2016), and extend these findings further by suggesting an independent effect of OSA on serum LBP levels. The presence of intermittent episodes of hypoxia during sleep due to the repetitive apnoea-hypopnoeas is a hallmark of OSA that links this disorder to its comorbidities (Dewan *et al.* 2015). Indeed, in our recent study we have documented that ODI provides a solid reflection of the degree of intermittent hypoxaemia during sleep that predicts arterial hypertension in OSA patients (Tkacova *et al.* 2014). The role of hypoxia is further supported by the present results that suggest that ODI is related to serum LBP levels independently of the confounding effects of central obesity and other confounders. Which mechanisms might link chronic hypoxia to increases in endotoxemia markers? Several pathogenetic pathways are likely involved such as hypoxia-induced deterioration in the intestinal barrier function (Chun *et al.* 2016, Shah *et al.* 2016), and activation of different cellular adaptive mechanisms in hepatocytes (Liu *et al.* 2014, Savransky *et al.* 2007, Savransky *et al.* 2007). Hypoxia-inducible factor (HIF) transcription factors represent master regulators of the cellular responses to the hypoxia that might be key elements also in the control of immune cell metabolism and functionality (Palazon *et al.* 2014). Nevertheless, assessment of these mechanisms was beyond the scope of the present investigation, and thus further studies are needed to explore the pathogenetic links between hypoxia and metabolic endotoxemia.

Recent studies identified serum LBP as a significant and independent predictor of cardiovascular morbidity and mortality which significantly increased the research and clinical interests in assessing circulating LBP levels. Serrano *et al.* (2013) documented a consistent association between serum LBP and the CIMT within the FLORINASH project. Moreover, Lepper *et al.* (2007) observed significantly increased LBP in patients with angiographically documented coronary disease compared with angiographically negative patients, and in

another study demonstrated that circulating LBP was a significant and independent predictor of total and cardiovascular mortality (Lepper *et al.* 2011). Moreover, consistent associations were observed between serum LBP and CIMT in other disorders such as patients in chronic peritoneal dialysis (Szeto *et al.* 2008). Our present report extends these previous observations by demonstrating increases in serum LBP in association with increased CIMT in patients with severe OSA, independently of other risk factors of atherosclerosis. To the best of our knowledge this is the first study that reports a relationship between endotoxemia and subclinical atherosclerosis in patients with sleep disordered breathing, a condition associated with increased atherosclerotic morbidity and mortality (Marin *et al.* 2005, Ayas *et al.* 2016).

Multiple studies had linked OSA to the both traditional and novel risk factors of atherosclerosis such as arterial hypertension (Tkacova *et al*. 2014), the metabolic syndrome (Quian *et al*. 2016), systemic inflammation and oxidative stress (DeMartino *et al*. 2016). Our present findings coupled with reports of others (Lepper *et al*. 2011, Serrano *et al.* 2013) raise the possibility that LBP might, indeed, represent an additional risk factor of atherosclerosis. A question arises about the pathogenetic role of LBP in the development of the atherosclerotic plaque. LBP is the first protein to encounter LPS and to deliver it to its cellular targets, and thus it seems to be the first step in activating proinflammatory cascade of innate immune responses which plays an important role in the pathophysiology of atherosclerosis (Ding *et al.* 2014, Schumann *et al*. 2011). Nevertheless, further studies are needed to thoroughly investigate the role of LBP as a cardiovascular risk factor.

Studying a well-defined cohort of adult men with OSA who all underwent full attended overnight polysomnography represents one of the main strengths of this study. Importantly, for the assessment of CIMT we have used an automatically based method that is both precise and highly reproducible (Bauer *et al.* 2012). There are several limitations to this

study that need to be acknowledged. First, only a limited number of subjects were studied. However, compared to participants with no OSA, patients with severe OSA had mean CIMT values increased by 19% which was associated with increases in serum LBP by 12%. Therefore, although our results are robust to gain some understanding on the role of LBP in OSA-related increases in CIMT, they should be considered preliminary and hypothesis generating. In addition, cross-sectional nature of the present study does not allow for the determination of time-course relationship between LBP and CIMT.

In conclusion, our study highlights associations between OSA severity, endotoxemia and CIMT in patients with OSA that are independent of other known risk factors of atherosclerosis including obesity. Further studies are needed to address the pathological mechanisms underlying the observed relationships in more details.

Acknowledgments:

This work was supported by the Slovak Research and Development Agency of the Ministry of Education, Slovakia (APVV-0134-11), and grants VEGA 1/0863/15, VEGA 1/0208/16.

The authors wish to express their gratitude to Mrs. Anna Schejbalova and Mrs. Zuzana Lazarova, the technicians in the Sleep Laboratory, Department of Respiratory Medicine, Safarik University, Medical Faculty and L. Pasteur University Hospital, Kosice, Slovakia.

Conflict of interest:

There is no conflict of interest.

References

AYAS NT, TAYLOR CM, LAHER I: Cardiovascular consequences of obstructive sleep apnea. *Curr Opin Cardiol* **31**: 599-605, 2016.

BAUER M, CAVIEZEL S, TEYNOR A, ERBEL R, MAHABADI AA, SCHMIDT-TRUCKSÄSS A: Carotid intima-media thickness as a biomarker of subclinical atherosclerosis. *Swiss Med Wkly* **142**: w13705, 2012.

BERRY RB, BUDHIRAJA R, GOTTLIEB DJ, GOZAL D, IBER C, KAPUR VK, MARCUS CL, MEHRA R, PARTHASARATHY S, QUAN SF, REDLINE S, STROHL KP, DAVIDSON WARD SL, TANGREDI MM; AMERICAN ACADEMY OF SLEEP MEDICINE: Rules for scoring respiratory events in sleep: update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine. *J Clin Sleep Med* **8**: 597-619, 2012.

BOUTAGY NE, MCMILLAN RP, FRISARD MI, HULVER MW. Metabolic endotoxemia with obesity: Is it real and is it relevant? *Biochimie* **124**: 11-20, 2016.

CANI PD, BIBILONI R, KNAUF C, WAGET A, NEYRINCK AM, DELZENNE NM, BURCELIN R: Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* **57**: 1470-1481, 2008.

CHUN C, ZHENG L, COLGAN SP: Tissue metabolism and host-microbial interactions in the intestinal mucosa. *Free Radic Biol Med* **S0891-5849**: 30441-30445, 2016.

DAMIANI MF, ZITO A, CARRATÙ P, FALCONE VA, BEGA E, SCICCHITANO P, CICCONE MM, RESTA O: Obstructive Sleep Apnea, Hypertension, and Their Additive Effects on Atherosclerosis. *Biochem Res Int* **2015**: 84193, 2015.

DEMARTINO T, GHOUL RE, WANG L, BENA J, HAZEN SL, TRACY R, PATEL SR, AUCKLEY D, MEHRA R: Oxidative Stress and Inflammation Differentially Elevated in Objective Versus Habitual Subjective Reduced Sleep Duration in Obstructive Sleep Apnea. *Sleep* **39**: 1361-1369, 2016.

DEWAN NA, NIETO FJ, SOMERS VK: Intermittent hypoxemia and OSA: implications for comorbidities. *Chest* **147**: 266-274, 2015.

DING PH, JIN LJ. The role of lipopolysaccharide-binding protein in innate immunity: a revisit and its relevance to oral/periodontal health. *J Periodontal Res* **49**: 1-9, 2014.

DRAGER LF, BORTOLOTTO LA, LORENZI MC, FIGUEIREDO AC, KRIEGER EM, LORENZI-FILHO G: Early signs of atherosclerosis in obstructive sleep apnea. *Am J Respir Crit Care Med* **172**: 613-618, 2005.

FOX N, AYAS N, PARK JE, FLEETHAM J, FRANK RYAN C, LEAR SA, MULGREW A, CHAN S, HILL J, JOHN MANCINI GB, WONG GC: Carotid intima media thickness in patients with obstructive sleep apnea: comparison with a community-based cohort. *Lung* **192**: 297-303, 2014.

GAYOSO-DIZ P, OTERO-GONZÁLEZ A, RODRIGUEZ-ALVAREZ MX, GUDE F, GARCÍA F, DE FRANCISCO A, QUINTELA AG: Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: effect of gender and age: EPIRCE cross-sectional study. *BMC Endocr Disord* **13**: 47, 2013.

GONZALEZ-QUINTELA A, ALONSO M, CAMPOS J, VIZCAINO L, LOIDI L, GUDE F: Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. *PLoS One* **8**: e54600, 2013.

KHEIRANDISH-GOZAL L, PERIS E, WANG Y, TAMAE KAKAZU M, KHALYFA A, CARRERAS A, GOZAL D: Lipopolysaccharide-binding protein plasma levels in children: effects of obstructive sleep apnea and obesity. *J Clin Endocrinol Metab* **99**: 656-663, 2014.

KIM KE, CHO YS, BAEK KS, LI L, BAEK KH, KIM JH, KIM HS, SHEEN YH: Lipopolysaccharide-binding protein plasma levels as a biomarker of obesity-related insulin resistance in adolescents. *Korean J Pediatr* **59**: 231-238, 2016.

LEPPER PM, KLEBER ME, GRAMMER TB, HOFFMANN K, DIETZ S, WINKELMANN BR, BOEHM BO, MÄRZ W: Lipopolysaccharide-binding protein (LBP) is associated with total and cardiovascular mortality in individuals with or without stable coronary artery disease--results from the Ludwigshafen Risk and Cardiovascular Health Study (LURIC). *Atherosclerosis* **219**: 291-297, 2011.

LEPPER PM, SCHUMANN C, TRIANTAFILOU K, RASCHE FM, SCHUSTER T, FRANK H, SCHNEIDER EM, TRIANTAFILOU M, VON EYNATTEN M: Association of lipopolysaccharide-binding protein and coronary artery disease in men. *J Am Coll Cardiol* **50**: 25-31, 2007.

LIBBY P: Inflammation and atherosclerosis. *Nature* **420**: 868-874, 2002.

LIU Y, MA Z, ZHAO C, WANG Y, WU G, XIAO J, MCCLAIN CJ, LI X, FENG W: HIF-1α and HIF-2α are critically involved in hypoxia-induced lipid accumulation in hepatocytes through reducing PGC-1α-mediated fatty acid β-oxidation. *Toxicol Lett* **226**: 117-123, 2014.

MARIN JM, CARRIZO SJ, VICENTE E, AGUSTI AG: Long-term cardiovascular outcomes in men with obstructive sleep apnea-hypopnea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* **265**: 1046-1053, 2005.

MARIN JM, CARRIZO SJ, VICENTE E, AGUSTI AG: Long-term cardiovascular outcomes in men with obstructive sleep apnea-hypopnea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* **365**: 1046-1053, 2005.

MONNERET D, PEPIN JL, GODIN-RIBUOT D, DUCROS V, BAGUET JP, LEVY P, FAURE P: Association of urinary 15-F2t-isoprostane level with oxygen desaturation and

carotid intima-media thickness in nonobese sleep apnea patients. *Free Radic Biol Med* **48**: 619-625, 2010.

PALAZON A, GOLDRATH AW, NIZET V, JOHNSON RS: HIF transcription factors, inflammation, and immunity. *Immunity* **41**: 518-528, 2014.

PATEL PN, SHAH RY, FERGUSON JF, REILLY MP: Human experimental endotoxemia in modeling the pathophysiology, genomics, and therapeutics of innate immunity in complex cardiometabolic diseases. *Arterioscler Thromb Vasc Biol* **35**: 525-534, 2015.

PUNJABI NM, CAFFO BS, GOODWIN JL, GOTTLIEB DJ, NEWMAN AB, O'CONNOR GT, RAPOPORT DM, REDLINE S, RESNICK HE, ROBBINS JA, SHAHAR E, UNRUH ML, SAMET JM: Sleep-disordered breathing and mortality: a prospective cohort study. *PLoS Med* **6**: e1000132, 2009.

QIAN Y, XU H, WANG Y, YI H, GUAN J, YIN S: Obstructive sleep apnea predicts risk of metabolic syndrome independently of obesity: a meta-analysis. *Arch Med Sci* **12**: 1077-1087, 2016.

SAVRANSKY V, BEVANS S, NANAYAKKARA A, LI J, SMITH PL, TORBENSON MS, POLOTSKY VY: Chronic intermittent hypoxia causes hepatitis in a mouse model of diet-induced fatty liver. *Am J Physiol Gastrointest Liver Physiol* **293**: G871-877, 2007.

SAVRANSKY V, NANAYAKKARA A, VIVERO A, LI J, BEVANS S, SMITH PL, TORBENSON MS, POLOTSKY VY: Chronic intermittent hypoxia predisposes to liver injury. *Hepatology* **45**: 1007-1013, 2007.

SCHUMANN RR: Old and new findings on lipopolysaccharide-binding protein: a soluble pattern-recognition molecule. *Biochem Soc Trans* **39**: 989-993, 2011.

SERRANO M, MORENO-NAVARRETE JM, PUIG J, MORENO M, GUERRA E, ORTEGA F, XIFRA G, RICART W, FERNÁNDEZ-REAL JM: Serum lipopolysaccharide-binding protein as a marker of atherosclerosis. *Atherosclerosis* **230**: 223-227, 2013.

SHAH NA, YAGGI HK, CONCATO J, MOHSENIN V: Obstructive sleep apnea as a risk factor for coronary events or cardiovascular death. *Sleep Breath* **14**:131-136, 2010.

SHAH YM: The role of hypoxia in intestinal inflammation. *Mol Cell Pediatr* **1**: 1, 2016.

SUN L, PAN A, YU Z, LI H, SHI A, YU D, ZHANG G, ZONG G, LIU Y, LIN X: Snoring, inflammatory markers, adipokines and metabolic syndrome in apparently healthy Chinese. *PLoS One* **6**: e27515, 2011.

SZETO CC, KWAN BC, CHOW KM, LAI KB, CHUNG KY, LEUNG CB, LI PK: Endotoxemia is related to systemic inflammation and atherosclerosis in peritoneal dialysis patients. *Clin J Am Soc Nephrol* **3**: 431-436, 2008.

TEIXEIRA TF, COLLADO MC, FERREIRA CL, BRESSAN J, PELUZIO MDO C: Potential mechanisms for the emerging link between obesity and increased intestinal permeability. *Nutr Res* **32**: 637-647, 2012.

TKACOVA R, MCNICHOLAS WT, JAVORSKY M, FIETZE I, SLIWINSKI P, PARATI G, GROTE L, HEDNER J; EUROPEAN SLEEP APNOEA DATABASE STUDY COLLABORATORS: Nocturnal intermittent hypoxia predicts prevalent hypertension in the European Sleep Apnoea Database cohort study. *Eur Respir J* **44**: 931-941, 2014.

TOUBOUL PJ, HENNERICI MG, MEAIRS S, ADAMS H, AMARENCO P, BORNSTEIN N, CSIBA L, DESVARIEUX M, EBRAHIM S, HERNANDEZ HERNANDEZ R, JAFF M, KOWNATOR S, NAQVI T, PRATI P, RUNDEK T, SITZER M, SCHMINKE U, TARDIF JC, TAYLOR A, VICAUT E, WOO KS: Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011. *Cerebrovasc Dis* **34**: 290-296, 2012.

ZHU Q, ZHOU H, ZHANG A, GAO R, YANG S, ZHAO C, WANG Y, HU J, GOSWAMI R, GONG L, LI Q: Serum LBP Is Associated with Insulin Resistance in Women with PCOS. *PLoS One* **11**: e0145337, 2016.

Table 1. Basic demographic characteristics and polysomnographic findings in subjects grouped by OSA severity.

	Entire cohort (n = 117)	No OSA (n = 10)	Mild – Moderate OSA (n = 50)	Severe OSA (n = 57)	P (ANOVA)
Age, years	46.5 ± 9.5	36.3 ± 10.1	45.3 ± 9.2	49.4 ± 8.3*	< 0.001
BMI, $kg.m^{-2}$	30.3 ± 4.2	26.9 ± 2.3	29.4 ± 3.6	31.7 ± 4.4*†	< 0.001
Neck circumference, cm	43 ± 3	41 ± 2	42 ± 3	44 ± 3*†	0.006
Waist-to hip ratio	0.98 ± 0.05	0.95 ± 0.07	0.98 ± 0.05	1.00 ± 0.05*	0.019
Current smoker, n (%)	23	1 (10.0)	11 (22.0)	11 (19.3)	0.681
Arterial hypertension, n (%)	41	1 (10.0)	18 (36.0)	22 (38.6)	0.213
BP systolic, mmHg	125.7 ± 13.7	110.5 ± 8.0	123.9 ± 12.3	130.0 ± 13.7*†	< 0.001
BP diastolic, mmHg	82.0 ± 9.1	76.0 ± 9.4	80.1 ± 8.8	84.7 ± 8.4*†	0.001
Polysomnography NREM, min	359 ± 45	349 ± 59	354 ± 48	367 ± 38	0.308
S1 NREM, min	55 ± 34	33 ± 20	49 ± 28	65 ± 38*†	0.002
S2 NREM, min	242 ± 55	237 ± 56	236 ± 52	249 ± 57	0.410
SWS, min	65 ± 37	79 ± 20	71 ± 39	57 ± 36	0.063
REM, min	69 ± 29	66 ± 21	74 ± 30	65 ± 29	0.233
AHI, $events.hour^{-1}$	32.0 ± 23.2	2.8 ± 1.1	16.3 ± 6.8*	50.1 ± 18.9*†	< 0.001
ODI, $events.hour^{-1}$	27.2 ± 24.4	2.0 ± 1.4	11.6 ± 8.4	45.6 ± 22.4*†	< 0.001
Arousal index, $events.hour^{-1}$	38.3 ± 22.0	17.7 ± 8.9	25.3 ± 11.7	53.5 ± 20.3*†	< 0.001
SpO_2 < 90%, min	21.6 ± 48.2	0.0 ± 0.1	2.9 ± 7.1	46.0 ± 64.8*†	< 0.001
Lowest SpO_2, %	80.5 ± 12.1	92.2 ± 1.9	86.6 ± 5.4*	73.0 ± 12.8*†	< 0.001

Values are given as the mean ± SD, if not indicated otherwise.

* $p < 0.05$ compared to no OSA

† $p < 0.05$ compared to mild - moderate OSA

Abbreviations: OSA, obstructive sleep apnoea; BMI, body mass index; BP, blood pressure; NREM , non-rapid eye movement; S1, stage 1; S2, stage 2; SWS, slow wave sleep; REM, rapid eye movement; AHI, apnea/hypopnea index; ODI, oxygen desaturation index; SpO_2, arterial oxygen saturation measured by pulse oximetry.

Table 2. Serum lipid and glucose metabolism markers in subjects grouped by OSA severity.

	Entire cohort (n = 117)	No OSA (n = 10)	Mild – Moderate OSA (n = 50)	Severe OSA (n = 57)	P (ANOVA)
Cholesterol, mmol.l^{-1}	5.30 ± 1.01	4.61 ± 0.71	5.30 ± 0.96	5.42 ± 1.05	0.060
Triglycerides, mmol.l^{-1}	1.79 ± 1.11	1.31 ± 0.50	1.52 ± 0.64	2.12 ± 1.39	0.093
HDL cholesterol, mmol.l^{-1}	1.18 ± 0.27	1.14 ± 0.20	1.26 ± 0.32	1.11 ± 0.22	0.094
LDL cholesterol, mmol.l^{-1}	3.44 ± 0.84	3.00 ± 0.78	3.41 ± 0.81	3.54 ± 0.87	0.174
LDL/HDL ratio	3.02 ± 0.79	2.72 ± 0.81	2.83 ± 0.82	3.23 ± 0.72†	0.019
Atherogenic index$^{\#}$	3.68 ± 1.11	3.15 ± 0.81	3.39 ± 1.05	4.01 ± 1.11*†	0.005
ApoA-1, g.l^{-1}	1.56 ± 0.27	1.48 ± 0.22	1.61 ± 0.33	1.54 ± 0.23	0.246
ApoB, g.l^{-1}	1.02 ± 0.21	0.89 ± 0.19	1.00 ± 0.20	1.07 ± 0.21*	0.030
ApoB/ApoA-1	0.67 ± 0.16	0.61 ± 0.12	0.64 ± 0.16	0.70 ± 0.15	0.046
Lp(a), g.l^{-1}	21.09 ± 28.70	12.43 ± 15.80	23.88 ± 33.59	20.27 ± 25.67	0.503
Fasting glucose, mmol.l^{-1}	5.13 ± 0.68	4.77 ± 0.57	5.00 ± 0.55	5.31 ± 0.76*	0.024
Fasting insulin, mmol.l^{-1}	12.18 ± 8.50	7.35 ± 2.26	11.37 ± 6.72	13.68 ± 10.07*	0.026
HOMA-IR	2.85 ± 2.31	1.56 ± 0.52	2.56 ± 1.58	3.31 ± 2.85*	0.008

Values are given as the mean ± SD

$^{\#}$Atherogenic index: (total cholesterol – HDL cholesterol).HDL cholesterol^{-1}

$^{*}p < 0.05$ compared to no OSA

$^{\dagger}p < 0.05$ compared to mild - moderate OSA

Abbreviations: OSA, obstructive sleep apnoea; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Apo, apolipoprotein; Lp, lipoprotein; HOMA-IR, homeostasis model assessment.

Table 3. Linear relationships between serum concentrations of LBP and atherosclerosis risk factors

Variable	Pearson correlation coefficient*	*P* Value*	Beta coefficient†	*P* Value†
Age	0.167	0.073		
BMI	0.274	0.003		
Neck circumference	0.323	<0.001	0.260	0.039
Waist-to hip ratio	0.343	<0.001	0.250	0.028
Total Cholesterol	0.086	0.362	0.053	0.570
HDL cholesterol	-0.127	0.190	-0.044	0.653
Triglycerides	0.122	0.203	0.076	0.416
LDL/HDL cholesterol	0.194	0.043	0.095	0.339
Atherogenic index	0.180	0.061	0.076	0.448
ApoB	0.118	0.223	0.074	0.438
ApoB/ApoA1	0.119	0.221	0.031	0.755
Fasting glucose	0.171	0.068	0.046	0.647
HOMA-IR	0.252	0.009	0.169	0.082
Arterial hypertension	0.280#	0.002	0.195	0.055
AHI	0.302	<0.001	0.184	0.101
ODI	0.333	<0.001	0.232	0.045
Neutrophil count	0.126	0.181	0.047	0.619

* Unadjusted

† Adjusted for age and BMI

Spearman Rho correlation coefficient

Abbreviations: LBP, lipopolysaccharide-binding protein; BMI, body mass index; LDL, low-density lipoprotein; HDL, high-density lipoprotein; Apo, apolipoprotein; HOMA-IR, homeostasis model assessment; AHI, apnoea/hypopnoea index; ODI, oxygen desaturation index

Table 4. Carotid intima-media thickness in subjects grouped by OSA severity.

	Entire cohort (n = 117)	**No OSA (n = 10)**	**Mild – Moderate OSA (n = 50)**	**Severe OSA (n = 57)**	**P** (ANOVA)
CIMT right, mm	0.58 ± 0.09	0.53 ± 0.09	0.57 ± 0.07	0.61 ± 0.10*†	0.006
CIMT left, mm	0.61 ± 0.11	0.52 ± 0.09	0.60 ± 0.08	0.64 ± 0.12*†	0.002
CIMT average, mm	0.60 ± 0.09	0.52 ± 0.09	0.58 ± 0.06	0.62 ± 0.10*	0.004

Values are given as the mean ± SD.

* $p < 0.05$ compared to no OSA

† $p < 0.05$ compared to mild – moderate OSA

Abbreviations: OSA, obstructive sleep apnoea; CIMT, carotid intima-media thickness.

Figure legend

Figure 1. Comparison of circulating lipopolysaccharide-binding protein (LBP) levels in patients without obstructive sleep apnoea (OSA), patients with mild-moderate OSA and patients with severe OSA (ANOVA, p = 0.015).

* p < 0.05 compared to mild – moderate OSA

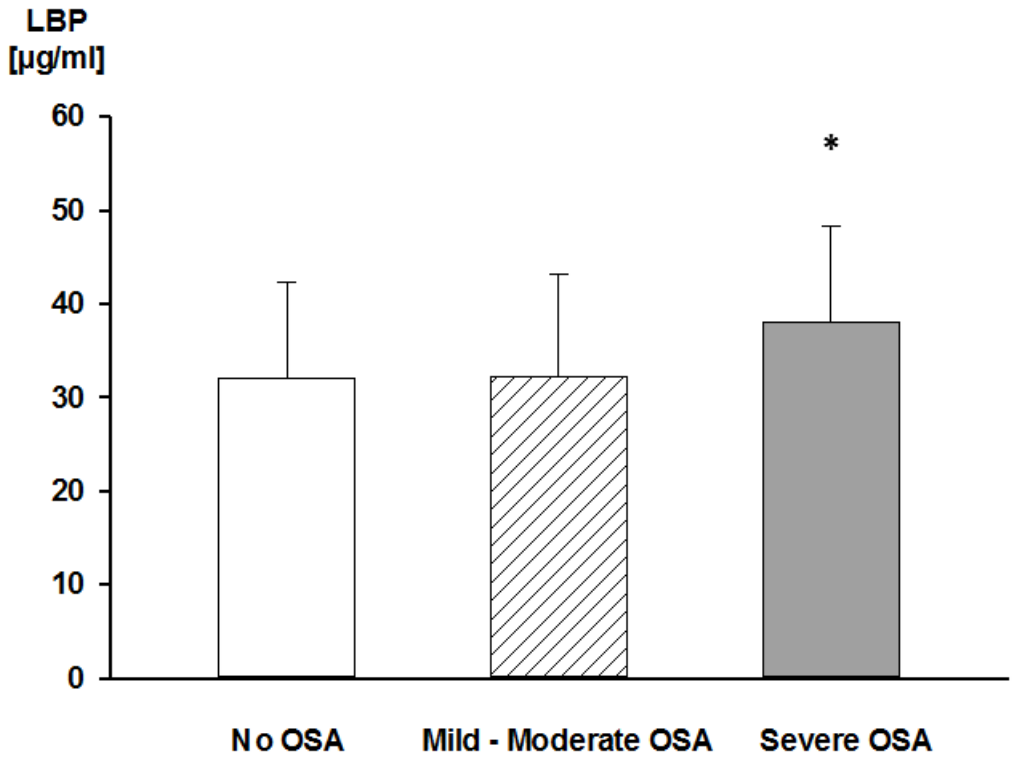

Figure 2. Comparison of average carotid intima-media thickness (CIMT) in patients without obstructive sleep apnoea (OSA), patients with mild-moderate OSA and patients with severe OSA (ANOVA, $p = 0.004$).

* $p < 0.05$ compared to no OSA

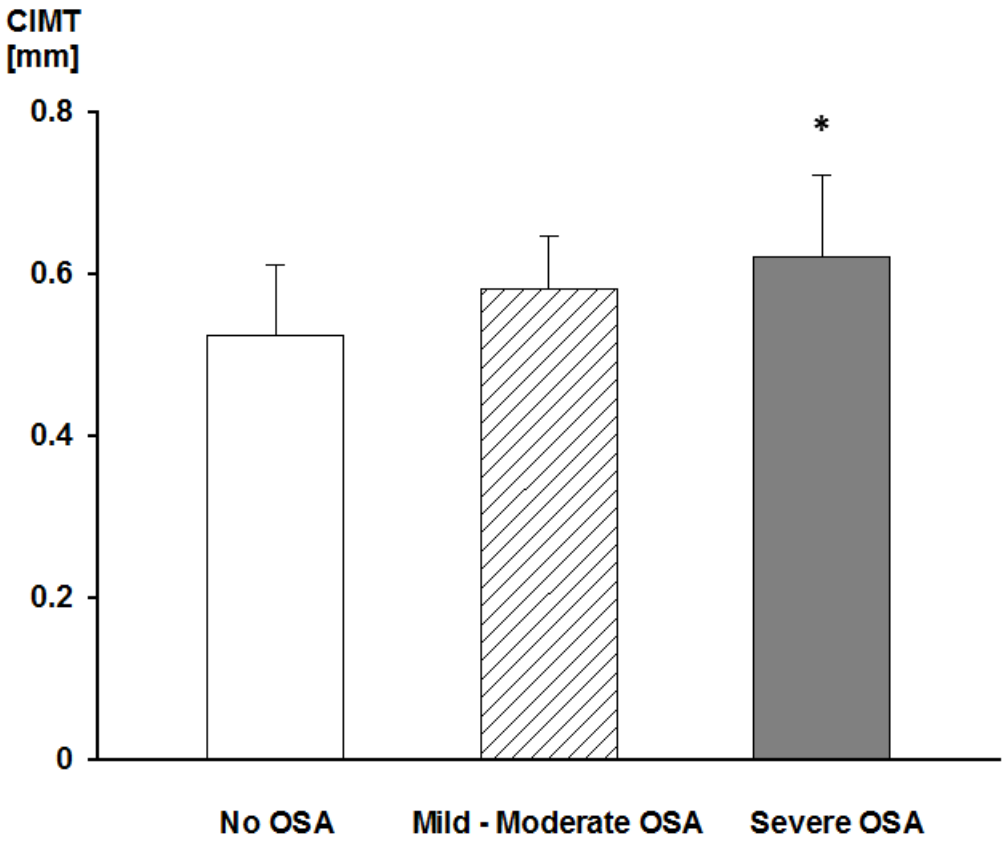

Figure 3. Linear relationship between average carotid intima-media thickness (CIMT) and serum lipopolysaccharide-binding protein (LBP) levels (R = 0.287, p = 0.002).