SEMMELWEIS EGYETEM

DOKTORI ISKOLA

Ph.D. értekezések

2753.

MÉSZÁROS MARTINA

Légzőszervi megbetegedések
című program

Programvezető: Dr. Losonczy György, egyetemi tanár

Témavezető: Dr. Bikov András, klinikai szakorvos

The role of systemic inflammation and dyslipidaemia in the pathogenesis of obstructive sleep apnoea

PhD thesis

Martina Mészáros, MD

Doctoral School of Károly Rácz Clinical Medicine

Semmelweis University

Supervisor: Andras Bikov, MD, PhD

Official reviewers: Noemi Eszes, MD, PhD
Istvan Ruzsics, MD, PhD

Head of the Complex Examination Committee:
Gyorgy Purebl, MD, D.Sc
Members of the Complex Examination Committee:
Peter Torzsa, MD, D.Sc
Eva Vizi, MD, PhD

Budapest
2022

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List of Abbreviations

AASM – American Academy of Sleep Medicine

ABC – ATP-binding transport protein

ADMA – asymmetric dimethylarginine

AHI – apnoea-hypopnoea index

AI – arousal index

AIP – atherogenic index of plasma

ANGPTL – angiopoietin-like protein

apo – apolipoprotein

ATGL – adipocyte triglyceride lipase

AUC – area under the curve

BH4 – tetrahydrobiopterin

BMI – body mass index

BP – blood pressure

CALR – calreticulin

CE – cholesteryl ester

CETP – cholesterol ester transport protein

CM – chylomicron

COPD – chronic obstructive pulmonary disease

COX-2 – cyclooxygenase 2

CRP – C-reactive protein

DAG – diacylglycerol

DL – detection limit

DNA – deoxyribonucleic acid

DZ – dizygotic

ECM – extracellular matrix

eNOS – endothelial nitric oxide synthase

ESS – Epworth Sleepiness Scale

FC – free cholesterol

FFA – free fatty acid

HA – hyaluronic acid

HAS – hyaluronic acid synthase

HDL – high-density lipoprotein

HDL-C – high-density lipoprotein cholesterol

HIF-1α – hypoxia inducible factor 1 alpha

HIF-2 – hypoxia inducible factor 2

HL – hepatic lipase

HMW-HA – high molecular weight hyaluronic acid

HSL – hormone sensitive lipase

HYAL-1 – hyaluronidase 1

ICAM-1 – intracellular adhesion molecule 1

IDL – intermediate-density lipoprotein

IH – intermittent hypoxia

IL – interleukin

iNOS – inducible nitric oxide synthase

LCAT – lecithin/cholesterol acyltransferase

LDL – low-density lipoprotein

LDL-C – low-density lipoprotein cholesterol

LDLR – LDL receptor

LMW-HA – low molecular weight hyaluronic acid

LpL – lipoprotein lipase

LRP-1 – LDL receptor-related protein 1

MAD – mandibular advancement device

MAG – monoacylglycerol

MCP-1 – monocyte chemoattractant protein 1

$MinSatO_2$ – minimal oxygen saturation

MMP – matrix metalloproteinase

mRNA – messenger ribonucleic acid

MZ – monozygotic

NADPH – nicotinamide adenine dinucleotide

NF-κB – nuclear factor kappa B

NO – nitric oxide

NOX – NADPH oxidase

ODI – oxygen saturation index

$ONOO^-$ – peroxynitrite

OSA – obstructive sleep apnoea

oxLDL – oxidised-LDL

PG – polygraphy

PL – phospholipid

PLTP – phospholipid transfer protein

PON-1 – paraoxonase 1

PPAR-γ – peroxisome proliferator-activated receptor-gamma

PSG – polysomnography

PSGL-1 – P-selectin glycoprotein ligand 1

RCT – reverse cholesterol transport

REM% – the percentage of rapid eye movement sleep of the total sleep time

RhoE – environmental correlation

RhoG – genetic correlation

ROC – Receiver operating characteristic analysis

ROS – reactive oxygen species

SCAP – SREBP cleavage-activating protein

SCD-1 – stearoyl-coenzyme A desaturase 1

Sleep% – sleep efficiency

sLRP-1 – soluble LDL receptor-related protein 1

SMC – smooth muscle cell

SOD – superoxide dismutase

SPT – sleep period time

SR-BI – scavenger receptor class B type I

SREBP-1 – sterol regulatory element-binding protein 1

TAG – triacylglycerol

TC – total cholesterol

TG – triglyceride

TLR – toll-like receptor

TNF-α – tumour necrosis factor alpha

TST – total sleep time

TST90% – the percentage of total sleep time spent with saturation below 90%

TyG index – triglyceride-glucose index

VCAM-1 – vascular cell adhesion molecule 1

VLDL – very low-density lipoprotein

WAT – white adipose tissue

1. Introduction

Obstructive sleep apnoea (OSA) is the most common sleep-related breathing disorder which is characterised by the recurrent collapses of the upper airways during the sleep leading to chronic intermittent hypoxia (IH) and sleep fragmentation. IH and sleep fragmentation contribute to sympathetic overactivity, oxidative stress and systemic inflammation with consequential metabolic alterations, including dyslipidaemia, atherosclerosis and insulin resistance [1]. Its prevalence ranges between 7.8-77.2% in the general adult population [2] and it has increased in the last two decades [3]. The most common clinical signs/symptoms of OSA are excessive daytime sleepiness, fatigue or insomnia, non-restorative sleep, witnessed snoring and/or breathing interruptions, gasping, waking up with choking [4]. Consequently, OSA leads to impaired work performance and driving abilities, and it most importantly represents an independent risk for cardiovascular morbidity and mortality [5]. Polysomnography is considered as the gold standard diagnostic tool for OSA; however, in selected cases and highly suspected diagnosis, a limited channel cardiorespiratory polygraphy can be used [6]. According to the International Classification of Sleep Disorders (Third Edition) criteria, OSA is defined as more than five obstructive respiratory events per hour of sleep with signs or symptoms of OSA (such as excessive daytime sleepiness, fatigue, snoring, observed apnoea during sleep, gasping or choking) or associated comorbidities (such as hypertension, coronary artery disease, atrial fibrillation, diabetes, cognitive dysfunction) [7]. Alternatively, more than fifteen respiratory events reach the criteria without associated symptoms or comorbidities [7]. The classical severity groups of OSA are defined according to the baseline apnoea-hypopnoea index as mild (5≤ to <15 events/h), moderate (15≤ to <30/h) and severe (30≤ events/h) subgroups.

Continuous positive airway pressure (CPAP) is the primary recommended treatment which improves quality of life, daytime symptoms and decreases blood pressure [8], but did not affect cardiovascular morbidity [9]. Apart from hypertension, vascular inflammation and dyslipidaemia are the two main processes leading to atherosclerosis and cardiovascular disease [10]. Despite numerous articles investigating inflammation and lipid abnormalities in OSA, the link between OSA and these processes is not fully understood. It has an essential clinical importance as it may lead to development of more comprehensive interventions in OSA than CPAP alone.

1.1. Main pathophysiological mechanisms in OSA

1.1.1. Intermittent hypoxia and oxidative stress

Chronic IH is termed as short cycles of oxygen desaturation followed by quick reoxygenation. Thus, IH regulates several redox-sensitive transcriptional factors, such as hypoxia inducible factor 1 alpha (HIF-1α). HIF-1α is a master regulator of over 100 different genes which are involved in adaptive responses under hypoxic conditions [11]. HIF-1α activation was detected in animal models of OSA [12, 13] and patients with OSA too [14, 15]. HIF-1α activates the mitochondrial nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase = NOX) family members (including NOX1, NOX2 etc.) mainly in phagocytes and also in endothelial cells which are the major sources of reactive oxygen species (ROS) [16]. Especially NOX1 and NOX4 seem to be the primary contributors of oxidative stress in case of IH [17]. The levels of NOX4 were significantly higher in patients with OSA and correlated with the disease severity [18]. In contrast to HIF-1α activation, HIF-2 is degraded by IH resulting down-regulation of antioxidant enzymes, for example, superoxide dismutase (SOD) [19]. Patients with OSA had significantly lower levels of SOD suggesting impaired antioxidant defence in OSA [20]. This redox imbalance leads to oxidative stress which was widely investigated in OSA. Several markers of oxidative stress were detected in high concentrations in patients with OSA. For example, increased levels of malondialdehyde (product of lipid peroxidation) or 8-hydroxy-2'-deoxyguanosine (product of deoxyribonucleic acid (DNA) oxidation) were measured in urine [21] and serum samples [18]. Another important prooxidant enzyme, xanthine oxidase is also activated by IH [22] and it contributes to HIF-1α up-regulation [23] and HIF-2 degradation [22] worsening oxidative stress. Not only IH but also sleep fragmentation enhances oxidative stress by promoting NOX2 activation [24]. IH itself [25] and the consequential oxidative stress [26] activate the sympathetic nervous system in OSA [27] which plays a crucial role in the pathomechanism of OSA-related inflammation and cardiovascular consequences [4].

1.1.2. Vascular inflammation and systemic inflammation

Systemic inflammation in response to IH and sympathetic overdrive has emerged as a pivotal factor in the pathomechanism of OSA (*Figure 1*). IH promotes the activation of endothelial cells [28] and several types of immune cells, such as neutrophil leukocytes [29] and monocytes [30, 31] by up-regulating nuclear factor kappa B (NF-κB)

transcription factor in a HIF-1α dependent manner [32]. Activated NF-κB also increases the messenger ribonucleic acid (mRNA) levels of HIF-1α [33] suggesting bidirectional crosstalk between these transcriptional factors in OSA. NF-κB is a master regulator of inflammatory responses by upregulating the expression of several pro-inflammatory genes including genes of cytokines and adhesion molecules.

Thus, the activated cells produce pro-inflammatory cytokines, such as tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), IL-8 [34, 35]. Their circulating levels were found to be elevated in OSA [36-38]. Obesity is the major risk factor for OSA. However, it is also associated with low-grade systemic inflammation with elevated levels of TNF-α and IL-6 [39], their increased levels were independent of the body mass index (BMI) in patients with OSA [40].

Adhesion molecules are mainly expressed by the vascular endothelium and their soluble form can be measured in the circulation [41]. They are responsible for leukocyte adhesion by slowing down their rolling on endothelial cells and facilitating their extravasation. The up-regulation of adhesion molecules is suggested to be the initial step of atherosclerosis [42]. There are several types of adhesion molecules that plays important role in the pathogenesis of OSA: Selectins, such as E-selectin and P-selectin are expressed by activated endothelial cells whereas L-selectin is found to be on the leukocyte surface [43]. Of note, activated platelets can also express P-selectin resulting in platelet adhesion and aggregation and consequential hypercoagulation in OSA [44]. P-selectin glycoprotein ligand 1 (PSGL-1) is the main ligand of selectins and it is expressed primarily by leukocytes [45]. P-selectin levels were found to be increased in OSA in some [46, 47] but not all studies [48, 49]. The presence of obesity [49] and the disease severity [50] may cause these contradictory results. PSGL-1 levels were unchanged in OSA suggesting other regulating mechanisms [50]. L-selectin [51, 52] and E-selectin [51] concentrations were also higher in patients with OSA. Several immunoglobulins, such as intracellular adhesion molecule 1 (ICAM-1) or vascular cell adhesion molecule 1 (VCAM-1) are highly expressed on endothelial cells by inflammatory mediators, such as TNF-α [53]. Elevated concentrations of ICAM-1 [51, 54] and VCAM-1 [51, 54] were reported in OSA in association with the disease severity [51]. However, obesity likely influences these findings [55]. Overall, pro-inflammatory cytokines and adhesion

molecules are good markers of cell activation involved in vascular and systemic inflammation and endothelial dysfunction in OSA.

Not only IH, but also sleep fragmentation affects the endothelial and immune cell activation by enhancing inflammation: levels of pro-inflammatory cytokines, such as IL-6 and TNF-α were increased in subjects with sleep deprivation [56]. Moreover, fragmented sleep leads to sympathetic activation [57] and catecholamines promote endothelial activation through local sympathetic innervation [58, 59].

The mechanisms described above lead to endothelial dysfunction which is an early predictor of atherosclerosis [60] and play an important role in increased cardiovascular risk associated with OSA. Endothelial dysfunction is generally defined as altered endothelial vasodilatation due to lower nitric oxide (NO) availability [61]. NO is one of the main regulators of vascular tone [62]. IH and its pathophysiological responses reduce the NO availability due to three main mechanisms: (1) IH decreases the expression of endothelial nitric oxide synthase (eNOS) directly [63] and indirectly by proinflammatory mediators (such as TNF-α) through NF-kB-activation [64]. Moreover, IH upregulates caveolin 1 expression which is a main regulator of eNOS by blocking its activity [65]. (2) ROS rapidly reacts with NO forming peroxynitrite ($ONOO^-$) that directly decreases the NO bioavailability [66]. ONOO- and other ROS oxidize the eNOS cofactor tetrahydrobiopterin (BH4) [67] resulting in its decreased levels in OSA [68]. Decreased BH4 availability promotes eNOS to produce further superoxide [69]. (3) This alteration is defined as “eNOS uncoupling” which is a critical mechanism in OSA-associated endothelial dysfunction [68]. Decreased levels of L-Arginine (substrate of the NO production) [70] and increased concentration of asymmetric dimethylarginine (ADMA; competitive inhibitor of eNOS)[71] can be also implicated in eNOS uncoupling [72].

Cyclooxygenase 2 (COX-2) and inducible NOS (iNOS) are also upregulated by IH resulting in further increased ROS production [73]. Moreover, elevated levels of NOX2 are associated with endothelial dysfunction in OSA [74]. Impaired endothelial repair capacity can be also impaired by lower endothelial progenitor cell levels due to inflammation in OSA [73]. The consequences of the impaired endothelial function are altered crosstalk between the endothelium and the circulating inflammatory cells, thus enhanced oxidative stress and inflammatory processes.

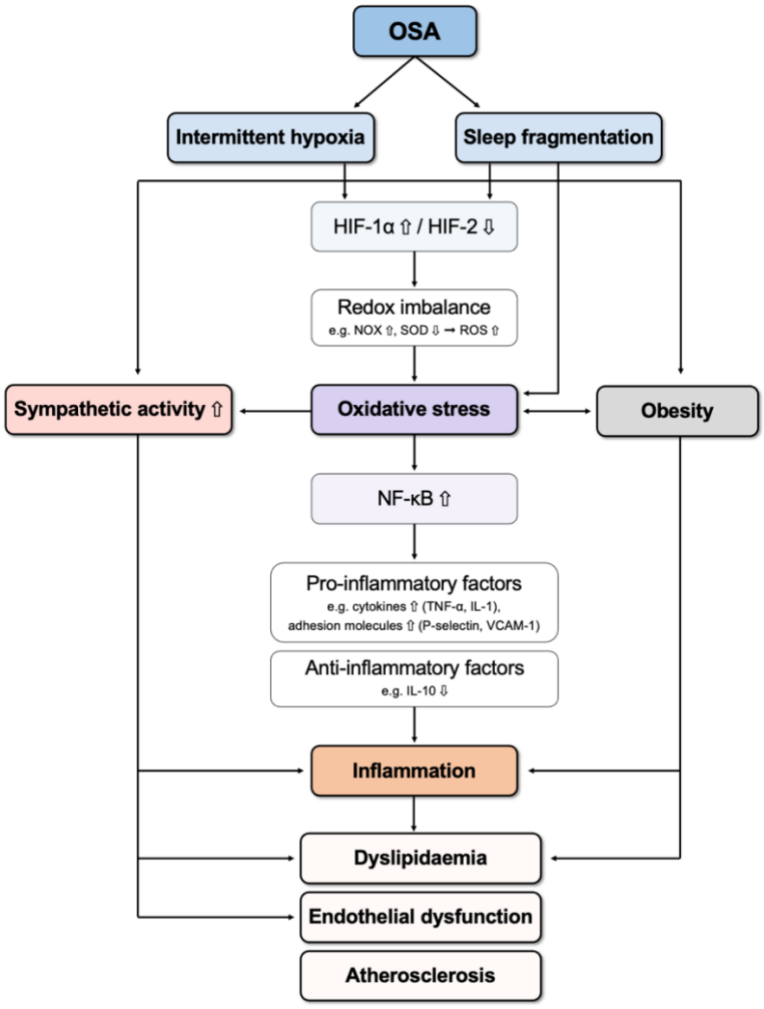

Figure 1. The main mechanisms in the pathogenesis of OSA. Systemic inflammation in response to intermittent hypoxia, sleep fragmentation and sympathetic overdrive has emerged as a pivotal factor in the pathomechanism of OSA. These alterations contribute to consequential metabolic abnormalities, including dyslipidaemia, endothelial dysfunction and atherosclerosis. *Own figure made by the candidate.*
HIF-1α – hypoxia inducible factor 1 alpha; HIF-2 – hypoxia inducible factor 2; IL – interleukin; NF-κB – nuclear factor kappa B; NOX – NADPH oxidase; OSA – obstructive sleep apnoea; ROS – reactive oxygen species; SOD – superoxide dismutase; TNF-α – tumour necrosis factor alpha; VCAM-1 – vascular cell adhesion molecule 1.

Collectively, previous evidence suggests that IH, sleep fragmentation and consequential oxidative stress, increased sympathetic activation and systemic inflammation are the pivotal factors in the pathomechanism of OSA-associated

conditions and comorbidities, such as endothelial dysfunction, dyslipidaemia and consequential atherosclerosis.

1.2. Dyslipidaemia in OSA

Strong evidence suggests that OSA is associated with impaired lipid profile, including elevated total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) levels and decreased high-density lipoprotein cholesterol (HDL-C) levels [75]. These alterations were commonly found in patients with OSA independently of other confounders, such as obesity [76].

1.2.1. Overview of physiological lipid metabolism

Lipoproteins are responsible for the transport of TG and cholesterol-esters (CE) in the circulation surrounded by free cholesterol (FC), phospholipids (PL), and apolipoproteins in different proportions. The main lipoproteins are the chylomicrons (CM), very low-density lipoproteins (VLDL), intermediate-density lipoprotein (IDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL) (*Table 1*).

Table 1. Characteristics of the main circulating lipoproteins (*Modified after Feingold et al.[77]*).

Name	Density (g/mL)	Size (nm)	Major lipids	Major apolipoproteins	Function
CM	<0.93	75-1200	TG	ApoB-48, A-I, A-II, A-IV, C, E	Transport of dietary lipids to the peripheral tissues and liver
CM remnant	0.93-1.01	30-80	TG, CE	ApoB-48, E	
VLDL	0.93-1.01	30-80	TG	ApoB-100, E, C	Transport of endogenous TG (and CE) to the peripheral tissues
IDL	1.01-1.02	25-35	TG, CE	ApoB-100, E, C	
LDL	1.02-1.06	18-25	CE	ApoB-100	Transport of CE to the peripheral tissues and liver
HDL	1.06-1.21	5-12	CE, PL	Apo A-I, A-II, C, E, F, J, M	Reverse CE transport

This table shows the main characteristics of the circulating lipoproteins. CM has the biggest size and the smallest density. HDL has the smallest size and the biggest density. Apo – apolipoprotein; CE – cholesteryl ester; CM – chylomicron; HDL – high-density lipoprotein; IDL – intermediate-density lipoprotein; LDL – low-density lipoprotein; PL – phospholipid; TG – triglyceride; VLDL – very low-density lipoprotein.

CMs are large TG-rich lipoproteins that contain apolipoprotein B-48 (apoB-48). CM delivers dietary lipids from the small intestine to the liver and other peripheral tissues (*"exogenous lipid transport")* [78]. Dietary TGs and CEs are hydrolysed by lipases such as pancreatic and gastric lipases resulting in free fatty acid (FFA), monoacylglycerol (MAG; from TGs) and FC (from CEs). These molecules are absorbed by the enterocytes by passive diffusion (for MAG) or transporters (cluster determinant 36, fatty acid–transport protein 4 for FFAs; Niemann-Pick C1-like protein, ATP-binding cassette protein G5 (ABCG5) and G8 (ABCG8) or scavenger receptor class B type I (SR-BI) for FCs). In the enterocytes triacylglycerol (TAG; from FFA+MAG) [79-82] and CEs (from FC+FFA) are resynthesized via several steps [83]. ApoB-48 is the only specific marker of CM and each CM particle has only one apoB-48 molecule [83]. ApoB-48 is truncated from the hepatic-derived apoB-100 representing 48% of its initial length. ApoB-48, apoA-I and apoA-IV are incorporated in the CMs in the enterocyte [83]. After leaving the enterocyte through its basolateral membrane, nascent CM gain further lipoproteins resulting in its mature form. In the circulation CM is metabolised by lipoprotein lipase (LpL) to produce CM remnants. CM remnants can be recognised via their apoE and taken up by the liver via LDL-receptor (LDLR) or LDL receptor-related protein 1 (LRP-1) [84].

VLDL transports hepatic lipids to the peripheral tissues (*"endogenous lipid transport"*). VLDL is produced by the hepatocytes from FFAs which can be originated from adipocytes and CM remnants [85]. The first step of VLDL synthesis is the lipidation of apoB-100 (under inadequate TG availability apoB-100 is degraded in the endoplasmic reticulum) [86]. Thus, conformational changes of apoB-100 allow VLDL particles to be expanded by further lipoproteins and VLDL particles leave the liver [87]. Several tissues, such as adipose tissue, muscle and heart tissue express VLDL-receptor which recognises apoB-100 and apoE [88]. VLDL is metabolised by LpL in the circulation resulting IDL. IDL is able to transfer apoE to HDL and their TG content is further hydrolysed by hepatic lipase (HL) resulting CE-rich LDL particles. The hepatic clearance of ILDs and LDLs is mediated by their apoE via mainly LDLR [89].

LpL catalyses the hydrolysis of VLDL- and CM-associated TAGs to FFAs and MAGs and promotes their uptake by the tissues. LpL is produced by different cell types, such as adipocytes, skeletal or cardiac muscle cells and macrophages and transported to the luminal surface of endothelial cells in extrahepatic tissues [90]. ApoC-II is the most

important activator of LpL [91]. On the contrary, ApoC-I and apoC-III [92], angiopoietin-like proteins, including ANGPTL3, ANGPTL4 and ANGPTL8 inhibit LpL [93, 94]. Hormones, such as insulin, glucocorticoids and adrenalin also regulate LpL activity [95].

LDL particles are derived from VLDL and IDL particles [77]. LDL is the main cholesterol transporter in the circulation. The unique apolipoprotein of LDL is a single molecule apoB-100 [96]. Several modified LDL particles were identified, such as oxidised-LDL (oxLDL), small dense-LDL and desialylated-LDL with strong atherogenic potential [97-99]. LDLR removes circulating LDL particles in the liver (70%) and in peripheral tissues (30%) [100] after recognition of apoB-100 and apoE [95].

HDL particles are responsible for reverse cholesterol transport (RCT) in which cholesterol is delivered from the peripheral cells back to the liver for further metabolism [101]. The two main forms of HDL are the small lipid-poor *discoid HDL* and the larger lipid-rich *spherical HDL* [102, 103] which is takes the majority of the circulating HDL particles [103]. The main apolipoprotein associated with HDL is apoA-I [104] which is produced mainly by the liver [105] (and partly by the intestine [106]). Lipid-poor apoA-I interacts with ABCA1 on peripheral cells (such as on hepatocyte, macrophage [107]) transporting FC and PL from the cells to apoA-I [108] and gains plus one apoA-I [103]. This *discoidal HDL* reacts quickly with lecithin/cholesterol acyltransferase (LCAT). LCAT promotes the esterification of FC to CE and incorporation of more apoA-I resulting *spherical HDL* [101, 103] which is dynamically modified in the RCT. Phospholipid transfer protein (PLTP) transfers more PL and FC from VLDL to HDL increasing HDL size [109]. HDL particles can be catabolised by two ways: (1) SR-BI expressed on hepatocytes and steroidogenic cells has a great affinity for CE and apoA-I of HDL particles [110, 111]. (2) In the *indirect pathway* HDL particles are modified by cholesterol ester transport protein (CETP) which circulates bound with HDL [112]. CETP mediates the transport of CE from HDL to apoB-containing lipoproteins (to LDL, as well to VLDL and CM) and exchanges TG in the opposite direction. It is important to highlight other functions of HDL. By mediating cholesterol efflux from the peripheral tissues (via ABCA1, ABCG1 and SR-BI) HDL plays a protective role against foam cell formation [113-115]. HDL inhibits the oxidation of LDL particles and inactivates the products of lipid peroxidation [116, 117]. HDL decreases the expression of inflammatory molecules (cytokines, adhesion molecules) and inhibits inflammatory cell activation [118, 119].

Moreover, the HDL-associated apolipoproteins (including apoA-I, A-II, E, J, M) [102] and enzymes (such as paraoxonase 1 (PON-1)) have also anti-inflammatory properties [120]. In conclusion, HDL is an important anti-inflammatory factor and antioxidant and protects against consequential atherosclerotic processes.

1.2.2. The three main explanations of dyslipidaemia in OSA

There are three classic pathophysiological mechanisms mediated by IH, inflammation and sleep fragmentation which can lead to OSA-associated lipid alterations (*Figure 2*): (1) increased peripheral lipolysis, (2) decreased lipoprotein clearance because of the impaired function of LpL and (3) increased lipid production of the liver. (4) Other important mechanisms have been also investigated including the role of genetic factors, adipose tissue dysfunction and HDL dysfunction.

1.2.2.1. Increased peripheral lipolysis

White adipose tissue (WAT) is the main storage of fatty acids (mainly in the form of TAG) [121]. TAGs can be mobilised through the steps of lipolysis: *(1)* The hydrolysis of TAG is catalysed by adipocyte triglyceride lipase (ATGL) resulting FFA and diacylglycerol (DAG) [122] *(2)* which is further hydrolysed by hormone-sensitive lipase (HSL) to MAG and FFA [123]. *(3)* Finally, MAG lipase produces the end-products FFA and glycerol [124].

Increased peripheral lipolysis has been reported in OSA. Catecholamines are major activators of lipolysis [125]. The presence of sympathetic overactivity is well-known in OSA [126] providing background of OSA-associated altered lipolysis. IH resulted in increased lipolysis by 264% in mice [127] and by 211% in human adipocytes too [128] resulting in decreased adipocyte size by 8% (mice) [127] and lower intracellular lipid stores by 37% (human) [128]. Human subjects exposed to two weeks of IH showed an increase in lipolysis through increased HSL expression caused by sympathetic hyperactivity [129]. Not only IH, but sleep fragmentation itself also enhances lipolysis by increasing adrenocorticotropin and cortisol secretion [130] which are known lipolysis activators [131]. Oxidative stress is an activator of HSL [132] and ATGL [133]. Furthermore, cytokines associated with OSA, such as TNF-α [36] or IL-6 [37], stimulate lipolysis [134, 135].

Lipolysis can be suppressed by insulin. In insulin resistance, which is often present in OSA [136], insulin loses its ability to inhibit lipolysis [137]. Furthermore, its

anti-lipolytic effect is influenced by the oxygen tension of adipose tissue [138], in hypoxia it is inhibited [139].

Obesity is characterised by increased basal levels of lipolysis [140] through altered production of adipokines. Leptin is a lipolysis activator [141], but adiponectin exerts inhibitory properties [142]. In line with this, OSA-associated increased leptin [143] and decreased adiponectin levels [144] may also partly explain the increased lipolysis in OSA.

1.2.2.2. Decreased lipoprotein clearance because of altered LpL function

Drager et al. reported impaired clearance of CEs and TGs in mice because of >5-fold decrease in LpL activity under IH [145]. This delayed clearance showed a direct correlation with markers of nocturnal hypoxaemia and disease severity [146]. Patients with OSA had lower serum LpL concentrations compared to controls and LpL concentrations decreased with disease severity [147].

IH decreases LpL activity directly [145] and the depth of hypoxia correlates with the delayed TG clearance [148, 149]. IH also impairs LpL function indirectly by activating its inhibitors: in mice models of OSA IH caused a 80% increase in mRNA and protein levels of ANGPLT4 in adipose tissue [145] in a HIF-1α-dependent manner and ANGPTL4 mRNA levels correlated with the severity of nocturnal hypoxia [150]. Increased circulating levels of ANGPTL4 and ANGPTL8 were detected in patients with OSA compared to controls [151].

Inflammatory cytokines, such as TNF-α and IL-1 can limit LpL activity [152-154]. In line with this, an inverse correlation was found between circulating LpL and CRP levels suggesting the negative impact of inflammation on LpL function [147].

Insulin resistance leads to decreased activity of LpL [155]. Catecholamines have also an inhibitory effect on LpL activity directly [156, 157] and indirectly through the activation of ANGPTL4 [158]. Leptin decreases [159, 160], whereas adiponectin increases the activity of LpL [161]. In conclusion, impairment in LpL function leads to decreased peripheral lipid uptake resulting in increased circulating lipid levels.

1.2.2.3. Increased lipid production of the liver

The hepatic lipid production is influenced mainly by the following mechanisms: (1) de novo lipogenesis of the hepatocytes (genetic factors, effects of IH), (2) increased

FFA delivery and uptake from the periphery (a) because of LpL dysfunction (b) increased lipolysis and (c) high dietary intake of lipids and carbohydrates.

Sterol regulatory element-binding protein 1 (SREBP-1) is a transcriptional factor which is the main regulator of the hepatic lipid biosynthesis in OSA through HIF-1α activation [162, 163]. SREBP-1 upregulates several genes which produce the substrates for VLDL synthesis, including stearoyl-coenzyme A desaturase 1 (SCD-1). SCD-1 is the regulator of the synthesis of monosaturated fatty acids [164] which increase TG and CE synthesis for VLDL production [165]. Partial HIF-1α-deficiency in a mice model led to lower hepatic levels of mRNA and protein levels of SREBP cleavage-activating protein (SCAP) and SCD-1 and protein levels of SREBP-1 compared to the wild-type mice [162]. Moreover, lower hepatic fat accumulation was detected in the case of partial HIF-1α-deficiency [162]. In SCAP knockout mice, 5 days of IH did not have effects on levels of serum and hepatic lipids and the expression of SREBP-1 and SCD-1 were not altered [166]. The duration and the severity of IH have also an impact on SREBP-1 expression: only severe IH (defined as oxygen nadir of 5%) seems to increase the SCD-1 levels in the liver [75]. In genetically obese rats 5 days of IH not [163], but 12 weeks of IH led to elevated lipid levels and SREBP-1 and SCD-1 transcription [167]. These findings suggested the main role of the HIF-1α/SREBP-1/SCD-1 pathway in OSA-associated dyslipidaemia. IH-induced increased sympathetic tone also supports lipid alterations in OSA, because the sympathetic nervous system stimulates the VLDL-production [168]. Not only IH, but also oxidative stress itself leads to lipid overproduction in the liver by enhancing HIF-1α [169] and inducing hepatic lipid peroxidation enhancing the vicious circle [75]. For note, the pre-existence of dyslipidaemia due to high food intake was suggested to be required for atherogenic consequences of IH [170].

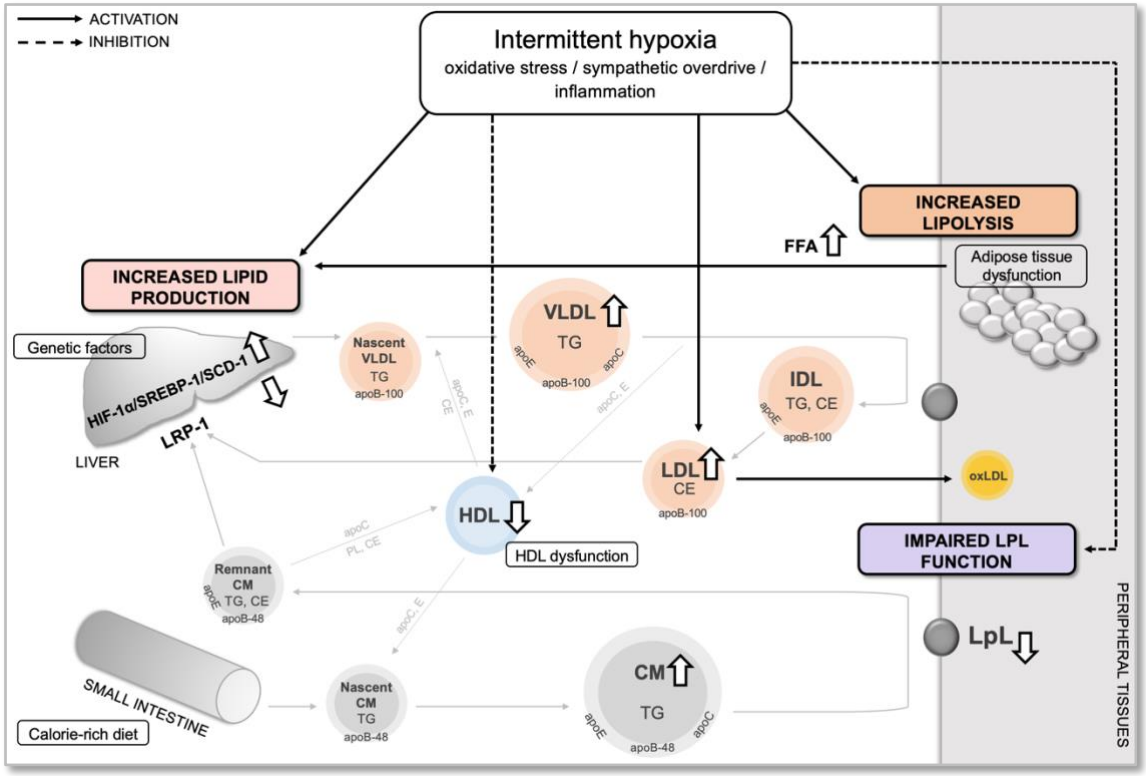

Figure 2. The main mechanisms of OSA-associated dyslipidaemia. The light grey lines show the physiological mechanisms. The continuous black lines show the enhanced pathways in OSA. The dashed black lines show the impaired pathways in OSA. *Own figure made by the candidate.*

Apo – apolipoprotein; CE – cholesteryl ester; CM – chylomicron; FFA – free fatty acid; HDL – high-density lipoprotein; HIF-1α – hypoxia inducible factor 1 alpha; LpL – lipoprotein lipase; LRP-1 – LDL receptor-related protein 1; oxLDL – oxidised-LDL; OSA – obstructive sleep apnoea; SCD-1 – stearoyl-coenzyme A desaturase 1; SREBP-1 – sterol regulatory element-binding protein 1; TG – triglyceride; VLDL – very low-density lipoprotein.

1.2.3. Other important mechanisms

1.2.3.1. Genetic factors

Genetic factors may also contribute to dyslipidaemia in OSA. Genetic susceptibility was reported for dyslipidaemia [171] and OSA too [172]. Furthermore, common genetic loci are shared by dyslipidaemia and OSA, such as peroxisome proliferator-activated receptor-gamma (PPAR-γ) [173, 174] or APOE polymorphism [175] suggesting a common pathophysiological pathway.

1.2.3.2. Adipose tissue dysfunction

Obesity is the main risk factor for developing OSA: at least 30% of obese patients have OSA and 60% of the patients with OSA suffer from obesity [176, 177]. Dysfunctional WAT plays an important role in the metabolic alterations of OSA [178]. The adaptive response of WAT to the high levels of circulating lipids is the storage of lipids by increasing the number (hyperplasia) and the size (hypertrophy) of the adipocytes [179]. In contrast to hyperplasia, hypertrophy leads to pathological changes including oxidative stress and inflammation [180]. Moreover, IH itself is associated with specific changes in WAT independently of obesity [181]. However, shrunken adipocytes in the WAT and decreased reduced epididymal fat mass were also detected in mice model under IH [182, 183, 127], as results of the increased lipolysis.

The hypertrophic adipose tissue has lower oxygen tension leading to hypoxic damage. Thus, the production of pro-inflammatory cytokines and adipokines is further enhanced by IH [178]. Hypertrophic WAT shows a pro-inflammatory cell profile infiltrated by CD8+ cytotoxic T-cells and Th1 cells with increased production of pro-inflammatory cytokines (TNF-α, IL-6) and M1 type macrophages [181]. M1-type macrophages produce further cytokines, such as monocyte chemoattractant protein 1 (MCP-1) which further regulates inflammatory functions [184] and increase the hepatic expression of SREBP-1 [184]. The levels of MCP-1 were found to be increased in patients with OSA [185].

Several adipokines associated with adipose tissue dysfunction can affect metabolic dysfunction in OSA. Leptin is a master regulator of food intake and body energy balance. Its levels found to be elevated in patients with OSA [186-191]. IH was postulated to elevate leptin levels in OSA via activating the sympathetic nervous system, renin-angiotensin-system, hypothalamic-pituitary-adrenal axis [192, 181] and adipose tissue dysfunction [193]. Leptin may contribute to lipid alterations in OSA. Leptin activates peripheral lipolysis [141] and hepatic lipid production [168] through the activation of the sympathetic nervous system and inhibits LpL [159]. Adiponectin is another important adipokine which has anti-inflammatory and antioxidant effects. Its levels are decreased by IH [194] and were reported to be lower in OSA by most [195, 196], but not all studies [197, 198]. Adiponectin plays a protective role in lipid

metabolism by enhancing HDL assembly [199, 200] and TG-rich lipoprotein catabolism through the activation of LpL [201].

1.2.3.3. HDL dysfunction

Pathophysiological mechanisms, such as IH, oxidative stress and inflammation damage the structure of HDL resulting its dysfunctional form [202]. Several molecules of the RCT are down-regulated by IH in a NF-κB dependent manner [203], such as ABCA1 [204] and SR-BI [163]. Oxidative stress enzymes associated with OSA [205], such as myeloperoxidase plays role in the oxidation of HDL components, such as apoA-I [206, 207] resulting its destroyed structure and impaired functionality [208]. Modified apoA-I is also not able to activate LCAT impairing the RCT [209]. Impaired activity of PON-1 leads to HDL dysfunction [210] and its levels were lower in patients with OSA compared to the control [211-214]. Moreover, oxidised-HDL particles increase the expression of pro-inflammatory molecules in a NF-κB-dependent manner [215].

Circulating HDL-C levels have been widely evaluated in OSA finding decreased HDL-C levels in the most [216, 217] but not all studies [218].

Tan et al. evaluated the antioxidant properties of HDL by measuring plasma oxLDL levels [219]. Plasma oxLDL concentrations were higher in patients with OSA independent of cardiovascular comorbidities compared to the controls. The authors suggested that dysfunctional HDL particles may explain their findings in OSA [219].

Increased levels of pro-atherogenic small HDL (HDL 8-10) subfractions and decreased levels of anti-atherogenic large HDL (HDL 1-3) subfractions were measured recently in OSA in correlation with disease severity [218]. In addition, sleep fragmentation was also inversely related to HDL-C and large HDL subfractions [218].

Atherogenic Index of Plasma (AIP) is a marker of atherosclerosis and coronary heart disease. AIP is calculated according as log(TG/HDL-C) [220]. AIP is hypothesised to show a dysregulation between anti- and pro-atherogenic lipoproteins, thus may predict the HDL dysfunction. Significantly higher AIP values were found in patients with OSA compared to the controls [221-223].

1.3. Potential biomarkers in OSA-associated inflammation and dyslipidaemia

1.3.1. The role of hyaluronic acid in OSA

Endothelial cells assert most of their regulator functions through their glycocalyx. The glycocalyx is a polysaccharide-rich coat on the luminal endothelial surface that

contains proteoglycans, glycosaminoglycans, glycoproteins and glycolipids binding several other proteins and enzymes, such as eNOS or LpL [224, 225]. Hyaluronic acid (HA) is an essential glycosaminoglycan of the endothelial glycocalyx. Its biological functions depend on the molecular size: under physiological circumstances HA is mainly synthetised in a high molecular weight form (HMW-HA; >1000 kDa) which plays a role in scaffolding functions of extracellular matrix (ECM), tissue regeneration and morphogenesis by interacting with a wide range of HA-binding proteins. HMW-HA displays anti-inflammatory, antioxidative, immunosuppressive and anti-angiogenic properties mainly through its CD44 receptor [226, 227]. Under pathological conditions, such as inflammation or oxidative stress, HMW-HA undergoes enzymatic degradation mainly by hyaluronidase 1 (HYAL-1) and HYAL-2. The non-enzymatic degradation happens due to ROS in a lesser extent [228]. The degradation results in low molecular weight fragments (LMW-HA; 150–350 kDa) that exert mainly pro-inflammatory effects by stimulating immune cells and leading to cytokine production [227].

1.3.1.1. The role of HA in inflammation

HA has a dual role in inflammation according to its molecular size. In a mice model HMW-HA significantly decreased the pro-inflammatory cytokine production of immune cells, such as TNF-α in T-cells [229] and increased anti-inflammatory cytokine production, such as IL-10 in regulatory T-cells or macrophages [230, 231]. Elevated levels of TNF-α [40] and decreased levels of IL-10 [232] were detected in patients with OSA. Moreover, HMW-HA represses the classically activated M1 macrophage polarisation [233, 231]. The anti-inflammatory properties of HMW-HA are mainly mediated by the suppression of the NF-κB pathway [233, 234]. Furthermore, HMW-HA is known to bind and neutralise ROS resulting in mitigated oxidative stress [235, 236] (*Figure 3*).

Under inflammation HA promotes pro-inflammatory mechanisms indirectly by HMW-HA degradation and directly by LMW-HA formation. Degraded HMW-HA loses its anti-inflammatory properties discussed above. It is important to note that HMW-HA has a pivotal role to preserve glycocalyx thickness and its selective permeability. Under inflammation, oxidative stress (ROS) or hypoxia HYAL-1 leads to glycocalyx damage by degrading HMW-HA [237], which is an early step in endothelial dysfunction. Various other pro-inflammatory factors promote glycocalyx shedding, including matrix

metalloproteinases (MMPs), ROS and TNF-α [238]. TNF-α, which levels are increased in OSA [36], directly activates MMP-9 [239] and enhances the ROS and protease production of leukocytes [240, 241]. ROS are able to activate further MMPs and depolymerise HMW-HA [236, 240]. Increased levels of MMP-9 were detected in patients with OSA [242]. Furthermore, HYAL-mediated HMW-HA degradation inhibits eNOS with consequential reduced endothelial mechanosensitivity [243, 244]. The damaged endothelial surface provides surface for adhesion of leukocytes and platelets resulting vascular inflammation. These findings suggest that decreased HMW-HA content may potentially lead to enhanced inflammation and endothelial dysfunction.

It is important to note that some studies detected increased endothelial HMW-HA synthesis mediated by IL-1 or TNF-α [245]. But it is hypothesised, that parallel increased HYAL activity diminishes this effect.

HMW-HA degradation results in smaller HA fragments: LMW-HA leads to pro-inflammatory signalling by activating not only CD44 [246], but also toll-like receptors (TLRs) [227] on the immune cells. LMW-HA fragments facilitate monocyte and macrophage activation and enhance their cytokine and protease production, including TNF-α and IL-6, IL-8 [247, 248]. Furthermore, LMW-HA products also increase MMP-9 levels [249] enhancing the inflammatory vicious circle.

1.3.1.2. The role of HA in dyslipidaemia and atherosclerosis

Hyaluronic acid can affect lipid metabolism mainly by impairing the integrity of the endothelium in association with inflammation. LpL is located on the endothelial lumen bounded by the glycocalyx [250]. Thus, the shedding of endothelial glycocalyx (by HYAL, oxLDL or cytokines) [227] leads to the displacement of LpL resulting in its dysfunction with consequential impaired lipoprotein clearance [251] (*Figure 3*).

Thinner glycocalyx because of HMW-HA degradation is associated with increased permeability. The consequential intimal LDL accumulation is one of the initial steps in atherosclerotic plaque formation [252]. Moreover, oxLDL particles also increase the expression of HA synthase 2 (HAS2) in vascular smooth muscle cells (SMC) [253]. It is hypothesised that HMW-HA promotes LDL retention indirectly: it interacts with versican which can bind LDL particles [254]. HA further activates immune cells binding CD44 and TLR receptors. Thus, interstitial HMW-HA facilitates the migration and differentiation of SMC (in media and neointima) in the atherosclerotic plaque [255]. The

increased production of LMW-HA probably enhances these mechanisms. In summary, the roles of interstitial HMW-HA seem to be opposed by its protective effects in the endothelial glycocalyx [255]. (Noteworthy, that several previous studies did not differentiate HA according to the molecular weight, thus it is hard to interpret the exact mechanisms in this field.)

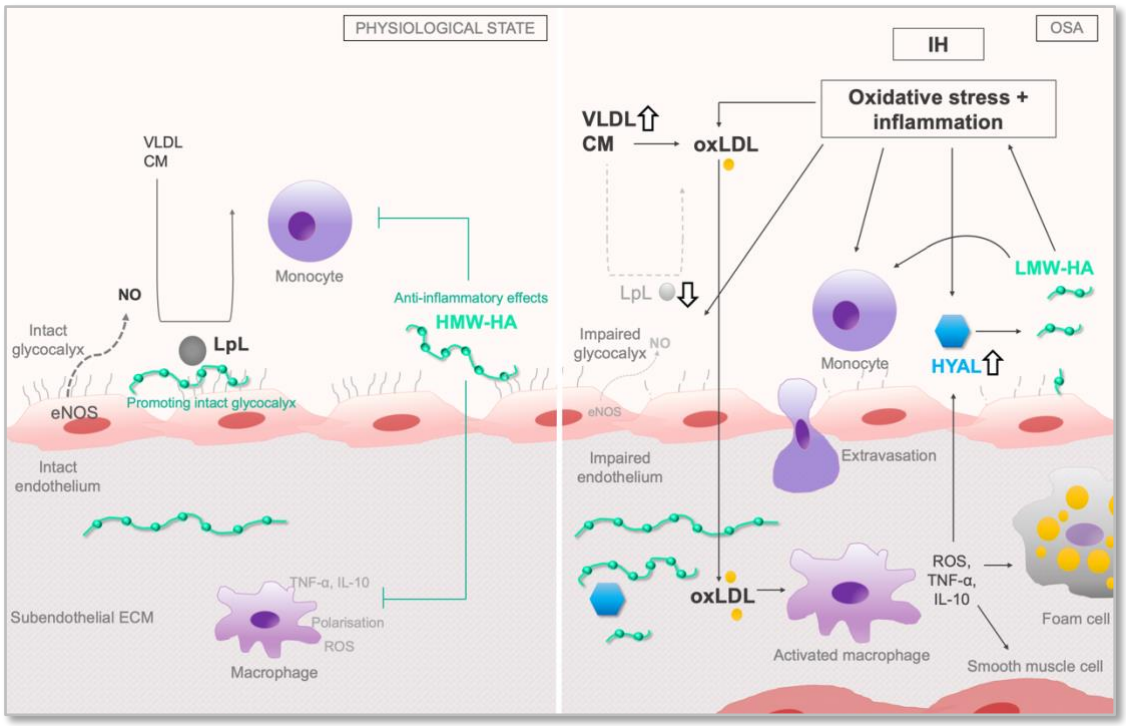

Figure 3. The main roles of the hyaluronan metabolism in the physiological state and in OSA. In physiological conditions, HMW-HA has anti-inflammatory effects, and it protects the integrity of the glycocalyx. In OSA, HMW-HA is degraded by the hyaluronidases and inflammatory cytokines. This shedding leads to the displacement of LpL resulting in its dysfunction with consequential impaired lipoprotein clearance. Moreover, HMW-HA degradation is associated with increased permeability of the glycocalyx. The consequential intimal oxLDL accumulation activates macrophages resulting in enhanced inflammation and foam cell formation. *Own figure made by the candidate.*

CM – chylomicron; ECM – extracellular matrix; eNOS – endothelial nitric oxide synthase; HMW-HA – high molecular weight hyaluronic acid; HYAL-1 – hyaluronidase 1; IH – intermittent hypoxia; IL – interleukin; LMW-HA – low molecular weight hyaluronic acid; LpL – lipoprotein lipase; NO – nitric oxide; OSA – obstructive sleep

apnoea; oxLDL – oxidised-LDL; ROS – reactive oxygen species; TNF-α – tumour necrosis factor alpha; VLDL – very low-density lipoprotein.

1.3.2. The roles of LDL receptor protein 1 in the pathomechanism of OSA

LRP-1 (also known as CD91) is a multifunctional receptor expressed in several tissues (hepatocytes, adipocytes, muscle cells, macrophages) [256]. LRP-1 consists of an extracellular α-chain (515-kDa) and a transmembrane β-chain (85-kDa) with a cytoplasmatic domain [257]. The expression of LRP-1 is tightly regulated by metabolic and inflammatory processes [258].

1.3.2.1. The roles of LRP-1 in inflammation

LRP-1 has been shown to be an anti-inflammatory molecule (*Figure 4*): In response to inflammatory stimuli the intracellular domain of LRP-1 limits the expression of pro-inflammatory genes including TNF-α and IL-6 [259]. In line with this, the inhibition of LRP-1 increases the inflammatory cytokine expression (TNF-α, IL-1, IL-6) via the NF-κB pathway [260] which is activated in OSA too [32]. LRP-1 promotes the conversion of macrophages from the inflammatory M1 to the anti-inflammatory M2-phenotype [261].

In pathophysiological circumstances LRP-1 can be shed by several factors including MMPs [262], atherogenic lipoproteins [263] and proinflammatory cytokines [264] resulting in its soluble form (sLRP-1). sLRP-1 consists of the α-chain and part of the β-chain of transmembrane LRP-1 [258]. The inflammatory role of sLRP-1 is unclear. Under inflammatory stimuli sLRP-1 promoted further the pro-inflammatory cytokine expression, such as TNF-α [258]. It has been also suggested that sLRP-1 may be a good marker of atherosclerosis. Despite these findings, several studies found sLRP-1 to be an anti-inflammatory biomarker: sLRP1 inhibited αMβ2 integrin-mediated adhesion properties by mediating its internalisation, thus hypothesising the anti-inflammatory function of sLRP-1 [265]. sLRP-1 was found to decrease TNF-α and IL-1 expression [266].

Calreticulin (CALR) is a ligand of LRP-1 which acts as a pro-inflammatory molecule: by binding LRP-1 CALR mediates the phagocytosis of apoptotic cells promoting pro-inflammatory responses in macrophages [267].

1.3.2.2. The roles of LRP-1 in dyslipidaemia and atherosclerosis

The primary function of LRP-1 is the clearance of apoE-containing lipoproteins (VLDL and CM remnants) mainly in the absence of LDLR [268] (*Figure 4*): Inactivation of hepatic LRP-1 altered the remnant lipoprotein clearance resulting in their increased plasma concentration [269]. LRP-1 regulates the HDL metabolism by enhancing the recycled apoE accumulation in early endosomes for HDL production [270]. Besides the lipid metabolism, LRP-1 internalises more than 100 ligands including coagulation factors, growth factors and matrix proteins and is able to regulate cellular signalling in response to a wide range of extracellular stimuli (such as hypoxia, inflammation) [257]. In case of dyslipidaemia, LRP-1 seems to be atheroprotective by regulating intracellular lipid levels and inflammatory responses in several cell types (*Figure 4*): The deletion of macrophage LRP-1 was associated with elevated plasma CE and TG levels resulting accumulation of circulating TG-rich lipoproteins [271]. Macrophage LRP-1 has been recently shown to regulate intracellular lipid levels through the expression of the major cholesterol exporter ABCA1 [272]. Moreover, macrophages with LRP-1 deletion showed increased apoptotic cell accumulation in atherosclerotic lesions enhancing monocyte recruitment [273]. On the contrary, other studies demonstrated atherogenic effects of LRP-1: macrophage LRP-1 can also mediate the accumulation of cholesterol in macrophages [271] and the cell-mediated LDL oxidation [274]. Similarly, inactivated LRP-1 in SMC resulted in impaired vascular integrity and SMC proliferation suggesting its preventing function in atherosclerosis [275]. Under physiological circumstances endothelial cells express LRP-1 in a low concentration [276]. On the contrary, under pathologic conditions, LRP-1 expression is higher to mediate endothelial cell migration, differentiation and survival resulting in improved endothelial function [277, 257]. On the other hand, LRP-1 avoids ECM degradation by promoting the clearance of several proteases: LRP-1 interacts with MMP-9 leading to its endocytosis and lysosomal degradation [278]. Plasminogen activators lead to plasmin-mediated ECM degradation [279]. LRP-1 ameliorates this degradation by internalising plasminogen activators and mediating their degradation [280, 281]. In conclusion, LRP-1 seems to be an important anti-inflammatory molecule.

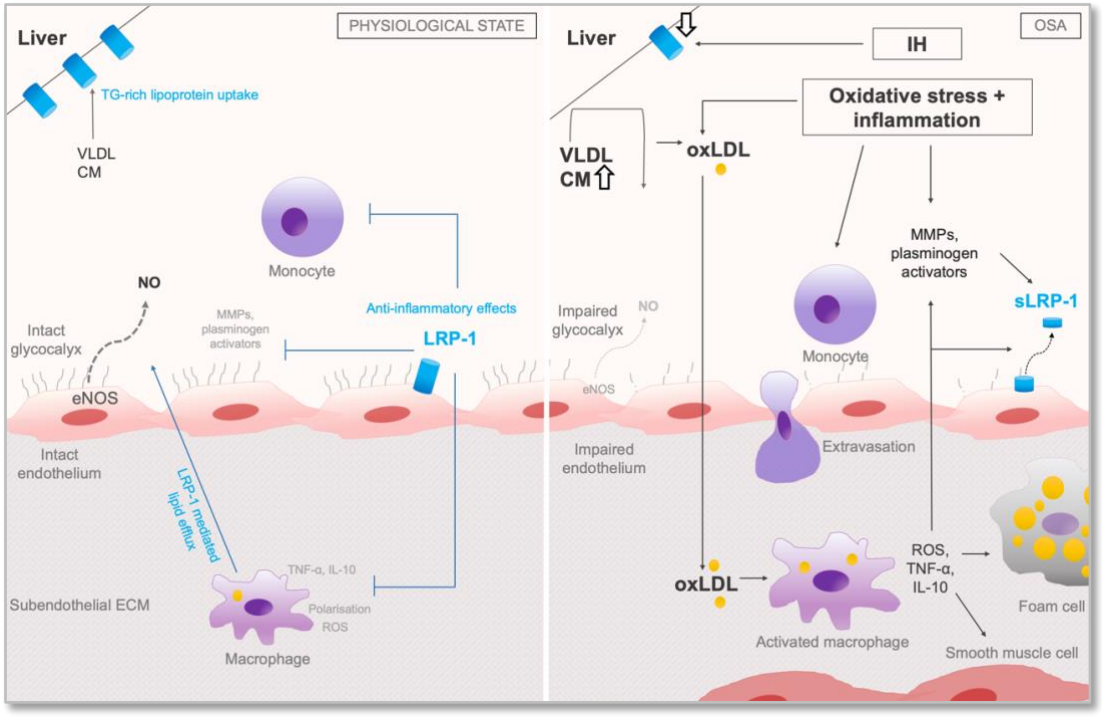

Figure 4. The main roles of the LRP-1/sLRP-1 in the physiological state and in OSA. In physiological conditions, the primary function of LRP-1 is the clearance of apoE-containing lipoproteins in the liver. Moreover, it has anti-inflammatory and atheroprotective effects too. In OSA, IH decreases the expression of the hepatic LRP-1 resulting in altered lipoprotein clearance. The consequential intimal oxLDL accumulation activates macrophages resulting in enhanced inflammation and foam cell formation. Moreover, pro-inflammatory cytokines and MMPs mediate the shedding of LRP-1 resulting in its soluble form (sLRP-1). *Own figure made by the candidate.*

CM – chylomicron; ECM – extracellular matrix; eNOS – endothelial nitric oxide synthase; HYAL-1 – hyaluronidase 1; IL – interleukin; LpL – lipoprotein lipase; LRP-1 – LDL receptor-related protein 1; MMP – matrix metalloproteinase; NO – nitric oxide; OSA – obstructive sleep apnoea; oxLDL – oxidised-LDL; ROS – reactive oxygen species; sLRP-1 – LRP-1 – soluble LDL receptor-related protein 1; TNF-α – tumour necrosis factor alpha; VLDL – very low-density lipoprotein.

2. Objectives

The aim of the PhD work was to investigate unexplored processes linking OSA with inflammation and dyslipidaemia. These were the following:

2.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

- Our main aim was to investigate the plasma levels of HMW-HA and HYAL-1 in patients with OSA to understand the role of hyaluronan metabolism in the pathogenesis of OSA.
- Our exploratory aim was to investigate the relationship between biomarker levels and clinical variables.
- Our further exploratory aim was to investigate the effect of obesity on the biomarker levels.

2.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

- Our main aim was to investigate the plasma levels of sLRP-1 and CALR in patients with OSA to understand their role in the pathogenesis of OSA.
- Our exploratory aim was to investigate the relationship between biomarker levels and clinical variables.

2.3. The role of heritable factors in the pathomechanism of dyslipidaemia in OSA

- Our main aim was to investigate the heritability of the relationship between OSA and serum lipid levels.
- Our further exploratory aims were to investigate the heritability of the relationship between OSA and blood pressure as well as the relationship between OSA and serum glucose levels.

2.4. Atherogenic Index of Plasma in OSA

- Our main aim was to investigate the AIP values in patients with OSA in relation to disease severity.
- Our exploratory aim was to compare the clinical value of AIP with other lipid parameters to predict OSA.
- Our exploratory aim was to investigate the effect of statin therapy on the relationship between AIP and OSA.

3. Methods

3.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

3.1.1. Study design and subjects

The study had a case-control design in which 108 participants were referred to our sleep laboratory due to suspected OSA (i.e. symptoms of snoring, witnessed apnoea, daytime sleepiness, obesity or cardiometabolic comorbidities). None of them was diagnosed with OSA before and they have not been treated with CPAP or mandibular advancement device (MAD). We excluded patients with malignancy within 10 years, infection within 2 months, autoimmune disorders or uncontrolled chronic disease (for example acute heart failure, uncontrolled diabetes). Following informed consent, detailed medical history was taken including comorbidities and smoking history. We defined the comorbidities based on the patients' report, available medical history and current medications. Cardiovascular diseases included current or previous stable and unstable angina, stroke, transient ischaemic attack and significant atherosclerosis. Smoking status was defined as self-reported current smoking or previous history of smoking. The participants filled out the Epworth Sleepiness Scale (ESS) [282]. In the morning following the full night sleep study, blood pressure (BP) was measured and fasting venous blood was collected for HA, HYAL-1, glucose, TC, TG, LDL-C, HDL-C, lipoprotein(a) and C-reactive protein (CRP) levels.

All procedures performed in the study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. The local Ethics Committee approved the study (Semmelweis University, TUKEB 30/2014, RKEB 172/2018). Each volunteer provided written informed consent.

3.1.2. Biomarker measurements

Blood samples were taken into EDTA-tubes. They were processed within 30 min after collection and centrifuged at 4℃ for 10 min at 1500 rpm. Immediately following the centrifugation, plasma samples were separated and stored at -80℃ until further analysis. Biomarker levels were measured using commercially available ELISA kits (Human Hyaluronic Acid (HA) ELISA kit from Corgenix Inc (Catalogue number: 029-001), Colorado, USA; Human Hyaluronidase-1 (HYAL-1) ELISA Kit from Cusabio Technology Llc (Catalogue number: CSB-EL010918HU), Houston, USA) according to

the manufacturers` instructions in duplicates. We reported the mean concentrations as inputs for analysis. The detection limit (DL) values were 11 ng/mL for HMW-HA and 0.31 ng/mL for HYAL-1. Concentrations under the DL were presented as DL in the analysis. The intra-assay variability was 4.0±2.9% for HA and 8.6±7.9% for HYAL-1, respectively.

3.1.3. Sleep studies

Full-night cardiorespiratory polygraphy (PG; Somnoscreen RC device, Somnomedics GmbH, Germany) and polysomnography (PSG; Somnoscreen Plus Tele PSG, Somnomedics GmbH, Germany) were performed according to the American Academy of Sleep Medicine (AASM) recommendations [283]. Sleep stages, movements and cardiopulmonary events were scored manually according to AASM guidelines [283]. Apnoea was defined as ≥90% decrease in the nasal flow lasting for ≥10 s. Hypopnoea was defined as ≥30% decrease in the nasal flow lasting for ≥10 s which is associated with either ≥3% drop in the oxygen saturation (for both PG and PSG) or arousal (for PSG). We recorded total sleep time (TST), sleep period time (SPT) and minimal oxygen saturation ($MinSatO_2$). Apnoea-hypopnoea index (AHI), oxygen desaturation index (ODI), arousal index (AI) and the percentage of total sleep time spent with oxygen saturation below 90% (TST90%) were calculated. OSA was defined with an AHI>5/h according to the International Classification of Sleep Disorders (Third Edition) criteria [7]. The OSA group was divided into mild (AHI 5–14.9/h), moderate (AHI 15–29.9/h) and severe (AHI ≥ 30/h) subgroups.

3.1.4. Statistical analysis

JASP 0.11.1 (University of Amsterdam, Amsterdam, Netherlands) and Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA, USA) were used for statistical analysis. The normality of the data was assessed with Shapiro-Wilk test which showed non-parametric distribution for HMW-HA and HYAL-1 levels. Demographics and clinical characteristics were compared between the OSA and control groups using t-test, Mann-Whitney test and Chi-square test. We used non-parametric ANCOVA after adjustment on age, gender, BMI and smoking to evaluate the differences in HMW-HA, HYAL-1 levels and HMW-HA/HYAL-1 ratio between OSA and control groups as well as among the severity groups. Further comparison analyses were performed after adjustment for statin and steroid use. We performed comparisons between the OSA and control groups

also after excluding outlier data (defined as 75th percentile+1.5×interquartile range) as well as patients with chronic airway disease (asthma and chronic obstructive pulmonary disease) and chronic heart failure. Spearman test was used to compare HA and HYAL-1 levels with clinical parameters and markers of sleep architecture. The HMW-HA, HYAL-1 and HMW-HA/HYAL-1 results were presented as median with interquartile range. A p-value <0.05 was considered significant.

The sample size was calculated to detect significant differences in HA and HYAL-1 levels between the OSA and control groups with an effect size of 0.60, statistical power of 0.80 and alpha error probability of 0.05.

3.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

3.2.1. Study design and subjects

The study had a case-control design in which 76 participants were referred to our sleep laboratory due to suspected OSA (i.e. symptoms of snoring, witnessed apnoea, daytime sleepiness, obesity or cardiometabolic comorbidities). None of them was diagnosed with OSA before and they have not been treated with CPAP or MAD. We excluded patients with malignancy within 10 years, infection within 2 months, autoimmune disorders or uncontrolled chronic disease (for example acute heart failure, uncontrolled diabetes) or those who were on statin therapy. Following informed consent, detailed medical history was taken including comorbidities and smoking history. We defined the comorbidities based on the patients' report, available medical history and current medications. Cardiovascular diseases included current or previous stable and unstable angina, stroke, transient ischaemic attack and significant atherosclerosis. Smoking status was defined as self-reported current smoking or previous history of smoking. The participants filled out the ESS [282]. In the morning following the full night sleep study, BP was measured and fasting venous blood was collected for sLRP-1 and CALR, glucose, TC, TG, LDL-C, HDL-C, apoA-I, apoB and CRP levels. Triglyceride-glucose (TyG) index was calculated as ln(TG (mg/dL)*glucose (mg/dL)/2) and AIP was calculated as log(TG (mmol/L)/HDL-C (mmol/L)).

All procedures performed in the study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. The local Ethics Committee approved the study (Semmelweis University, TUKEB 30/2014, RKEB 172/2018). Each volunteer provided written informed consent.

3.2.2. Biomarker measurements

Blood samples were taken into EDTA-tubes in the morning. They were processed within 30 min after collection and centrifuged at 4℃ for 10 min at 1500 rpm. Immediately following the centrifugation, plasma samples were separated and stored at -80℃ until further analysis. Biomarker levels were measured using commercially available ELISA kits (Human Low Density Lipoprotein Receptor Related Protein 1 ELISA Kit from Bioassay Technology Laboratory, Shanghai Korain Biotech Co. Ltd. Inc, Shanghai, China; Human Calreticulin ELISA Kit from Cusabio Technology Llc., Houston, USA) according to the manufacturers` instructions in duplicates. We reported the mean concentrations as inputs for analysis. The DL values were 0.027 mg/L for sLRP-1 and 0.039 ng/mL for CALR. All concentrations were above the detection limit. The intra-assay variability was 9.3±9.9% for sLRP-1 and 14.4 ± 12.2% for CALR, respectively.

3.2.3. Sleep studies

Full-night cardiorespiratory PG (Somnoscreen RC device, Somnomedics GmbH, Germany) and PSG (Somnoscreen Plus Tele PSG, Somnomedics GmbH, Germany) were performed according to the AASM recommendations [283]. Sleep stages, movements and cardiopulmonary events were scored manually according to AASM guidelines [283]. Apnoea was defined as ≥90% decrease in the nasal flow lasting for ≥10 s. Hypopnoea was defined as ≥30% decrease in the nasal flow lasting for ≥10 s which is associated with either ≥3% drop in the oxygen saturation (for both PG and PSG) or arousal (for PSG). We recorded TST, SPT and $MinSatO_2$. AHI, ODI and TST90% were calculated. OSA was defined with an AHI>5/h according to the International Classification of Sleep Disorders (Third Edition) criteria [7]. The OSA group was divided into mild (AHI 5–14.9/h), moderate (AHI 15–29.9/h) and severe (AHI ≥ 30/h) subgroups.

3.2.4. Statistical analysis

JASP 0.11.1 (University of Amsterdam, Amsterdam, Netherlands) and Graph Pad Prism 5.0 (GraphPad Software, San Diego, CA, USA) were used for statistical analysis. The normality of the data was assessed with Shapiro-Wilk test which showed non-parametric distribution for sLRP-1 and CALR levels. Demographics and clinical characteristics were compared between the OSA and control groups using t-test, Mann-Whitney test and Chi-square test. We used non-parametric ANCOVA after adjustment on age, gender, BMI and the lipid profile (TC, HDL-C, LDL-C, TG, lipoprotein (a)) to

evaluate the differences in sLRP-1 and CALR levels between OSA and control groups as well as among the severity groups. Spearman test was used to compare sLRP-1 and CALR levels with clinical parameters and markers of sleep architecture. Multivariate logistic regression analysis was performed to investigate further potential relationship between sLRP-1 and CALR levels and clinical variables: the biomarker levels were grouped into low- and high sLRP-1 and CALR groups as dependent variables (according to the median 1.84 mg/L for sLRP-1 and 0.23 ng/mL for CALR). The sLRP-1 and CALR results were presented as median with interquartile range. A p-value <0.05 was considered significant.

The sample size was calculated to detect significant differences in sLRP-1 and CALR levels between the OSA and control groups with an effect size of 0.50, statistical power of 0.80 and alpha error probability of 0.05.

3.3. Heritable factors in the pathomechanism of dyslipidaemia in OSA

3.3.1. Study design and subjects

We included 69 Caucasian twin pairs from the Hungarian Twin Registry [284]. None of them was diagnosed with OSA before and they have not been treated with CPAP or MAD. Following informed consent, detailed medical history was taken including comorbidities and smoking history. We defined the comorbidities based on the patients' report, available medical history and current medications. Cardiovascular diseases included current or previous stable and unstable angina, stroke, transient ischaemic attack and significant atherosclerosis. Smoking status was defined as self-reported current smoking or previous history of smoking. Data on alcohol consumption and diet were also collected. The participants filled out the ESS [282]. In the morning following the full night sleep study, BP was measured and fasting venous blood was collected for glucose, TC, TG, LDL-C, HDL-C and lipoprotein(a).

All procedures performed in the study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. The local Ethics Committee approved the study (Semmelweis University, TUKEB 30/2014). Each volunteer provided written informed consent.

3.3.2. Sleep studies

Full-night PSG (Somnoscreen Plus Tele PSG, Somnomedics GmbH, Germany) were performed according to the AASM recommendations [283] on the same night for

both twins. Sleep stages, movements and cardiopulmonary events were scored manually according to AASM guidelines [283]. Apnoea was defined as ≥90% decrease in the nasal flow lasting for ≥10 s. Hypopnoea was defined as ≥30% decrease in the nasal flow lasting for ≥10 s which is associated with either ≥3% drop in the oxygen saturation or arousal. AHI, ODI and TST90% were calculated. OSA was defined with an AHI>5/h according to the International Classification of Sleep Disorders (Third Edition) criteria [7].

3.3.3. Statistical analysis

SOLAR Eclipse version 8.1.1 (http://www.solar-eclipse-genetics.org/) was used for statistical analysis. The normality of the data was assessed with the Kolmogorov-Smirnov test. Demographics and clinical characteristics and monozygotic (MZ) and dizygotic (DZ) twins were compared between the OSA and control groups using t-test, Mann-Whitney test and Chi-square test. Indices of interest, including AHI, ODI and TST90%, as well as ESS, were log-transformed and linear regression analysis was carried out with serum glucose, serum lipids and lipoproteins and morning BP. In subsequent steps, the heritability was estimated only for the associations with a $p<0.1$.

Bivariate co-twin correlation (Pearson's test) was used to calculate a descriptive estimate of the genetic influence in MZ (rMZ) and DZ (rDZ) pairs. If the similarity for a phenotype within the pairs is greater in MZ than DZ pairs, it means that the trait is genetically influenced. On the contrary, higher rDZ than rMZ values provide evidence for an environmental influence. A bivariate Cholesky decomposition was calculated to derive the magnitude of covariation between the investigated phenotypes. With this test we estimated what proportion of this correlation is attributable to shared genetic and environmental factors. For genetic correlation (RhoG), two separate hypotheses were tested: (a) RhoG = 0 (the test for overlapping genetic correlations) or (b) RhoG = 1 or −1 (the test for non-overlapping positive or negative genetic correlations). If a p-value for RhoG = 0 is significant ($p<0.05$), the two traits share common genetic loci.

If a p-value for RhoG = 1 or −1 (without RhoG = 0 being significant) is significant, the two traits have high heritability, but they are determined by different genes (they did not share common genetic loci). The environmental correlation (RhoE) and RhoG values were computed and reported along with their estimated standard errors. The -testrhoE option tested the significance of the rhoE difference from zero. The -testrhoG option tested the significance of the rhoG parameter from zero and also from either 1 or −1

(depending on whether rhoG is negative or positive, and not exactly 1 or −1 already). We also performed pleiotropy tests with adjustment for age, gender, BMI and smoking status (ever vs. never smokers). We performed the analyses also after excluding patients treated with medications on glucose and lipid levels.

3.4. Atherogenic Index of Plasma in OSA

3.4.1. Study design and subjects

This dual-centre study had case-control design in which 560 participants were referred to the sleep laboratories (Semmelweis University, Budapest, Hungary and University of Medicine and Pharmacy Timisoara, Timisoara, Romania) due to suspected OSA (i.e. symptoms of snoring, witnessed apnoea, daytime sleepiness, obesity or cardiometabolic comorbidities). None of them was diagnosed with OSA before and they have not been treated with CPAP or MAD. We excluded patients with acute respiratory, heart or renal failure. Following informed consent, detailed medical history was taken including comorbidities and smoking history. We defined the comorbidities based on the patients' report, available medical history and current medications. Smoking status was defined as self-reported current smoking or previous history of smoking. The participants filled out the ESS [282]. In the morning following the full night sleep study, fasting venous blood was collected for TC, TG, LDL-C and HDL-C levels. AIP was calculated as log(TG (mmol/L)/HDL-C (mmol/L).

All procedures performed in the study involving human participants were in accordance with the ethical standards of the 1964 Helsinki declaration and its later amendments. The local Ethics Committee approved the study (Semmelweis University, TUKEB 30/2014, RKEB 172/2018; University of Medicine and Pharmacy Timisoara 22/2014/24.07.2019). Each volunteer provided written informed consent.

3.4.2. Sleep studies

Full-night cardiorespiratory PG and PSG were performed according to the AASM recommendations [283]. Sleep stages, movements and cardiopulmonary events were scored manually according to AASM guidelines [283]. Apnoea was defined as ≥90% decrease in the nasal flow lasting for ≥10 s. Hypopnoea was defined as ≥30% decrease in the nasal flow lasting for ≥10 s which is associated with either ≥3% drop in the oxygen saturation (for both PG and PSG) or arousal (for PSG). We recorded TST, SPT, percentage of total sleep time spent in rapid eye movement stage, $MinSatO_2$. AHI, ODI,

TST90% and sleep efficiency (TST/SPT) were calculated. OSA was defined with an AHI>5/h according to the International Classification of Sleep Disorders (Third Edition) criteria [7]. The OSA group was divided into mild (AHI 5–14.9/h), moderate (AHI 15–29.9/h) and severe (AHI ≥ 30/h) subgroups.

3.4.3. Statistical analysis

JASP 0.14 (JASP Team, University of Amsterdam, Amsterdam, The Netherlands) and MedCalc 19.5.3 (MedCalc Software Ltd., Ostend, Belgium) were used for statistical analysis. The normality of the data was assessed with Shapiro-Wilk test. Demographics and clinical characteristics were compared between the OSA and control groups using Mann-Whitney test and Chi-square test. We used non-parametric ANCOVA after adjustment on age, gender and BMI to evaluate the relationship between lipids and OSA severity. Further comparison analyses were performed in people not taking any statin and in those who had PSG as a diagnostic test. We used multivariate logistic regression adjusted to age, gender and BMI to evaluate the relationship between lipid levels and AIP as well as OSA. Spearman test was used to compare lipid levels with clinical parameters and markers of sleep architecture. The predictive value of AIP for OSA, cardiovascular disease, hypertension and diabetes was evaluated with the Receiver operating characteristic (ROC) analysis and compared to lipid levels with the DeLong test. Lipid levels were presented as median with interquartile range. A p-value <0.05 was considered significant.

4. Results

4.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

4.1.1. Patient characteristics

Sixty-eight patients were diagnosed with OSA (53 years /47–64/, 49 men). They were older and had significantly higher BMI, systolic and diastolic BP and ESS values, higher levels of CRP, glucose, TG and lipoprotein(a) and lower levels of HDL-C (all $p<0.05$). The prevalence of hypertension was higher in OSA ($p<0.01$). The OSA group had higher AHI, ODI, SPT, TST, and TST90% and lower $MinSatO_2$ values (all $p<0.05$; *Table 2*).

Table 2. Subjects' characteristics.

	Control (n=40)	**OSA (n=68)**	**Total (n=108)**	**p**
Age (years)	51 /39-60/	53 /47-64/	53 /44-62/	0.08
Males (%)	28	72	56	**< 0.01**
BMI (kg/m^2)	24.75 ± 4.48	31.98 ± 5.97	29.30 ± 6.48	**< 0.01**
Hypertension (%)	38	71	58.3	**< 0.01**
Diabetes (%)	13	21	17.6	0.29
Dyslipidaemia (%)	30	37	34.3	0.47
Cardiovascular disease (%)	10	15	13	0.48
Chronic cardiac failure (%)	5	12	9	0.24
Cardiac arrythmia (%)	8	21	15.7	0.07
Asthma (%)	10	15	13	0.48
COPD (%)	5	9	7	0.46
Smokers (%)	5	46	30.6	**< 0.01**
Cigarette pack years	0 /0-0/	0 /0-10/	0 /0-5/	**< 0.01**
Systolic BP (mmHg)	120 /110-130/	132 /124-138/	130 /120-136/	**< 0.01**
Diastolic BP (mmHg)	70 /70-80/	80 /74-89/	78 /70-84/	**< 0.01**
CRP (mg/L)	1.47 /0.83-2.83/	3.51 /1.76-8.27/	2.175 /1.15-4.85/	**< 0.01**
Glucose (mmol/L)	4.6 /4.2-5.2/	5.3 /4.9-6.7/	5.1 /4.7-6.1/	**< 0.01**
Cholesterol (mmol/L)	5.64 ± 1.1	5.45 ± 1.26	5.52 ± 1.20	0.43
HDL-C (mmol/L)	1.61 /1.39-1.95/	1.21 /0.98-1.33/	1.33 /1.14-1.63/	**< 0.01**
LDL-C (mmol/L)	3.39 ± 0.98	3.46 ± 1.05	3.44 ± 1.02	0.73
Triglyceride (mmol/L)	1.13 /0.88-1.39/	1.67 /1.25-2.17/	1.35 /1.07-1.96/	**< 0.01**
Lipoprotein (a) (mmol/L)	0.25 /0.07-0.56/	0.74 /0.37-0.99/	0.32 /0.12-0.67/	**< 0.01**

AHI (1/h)	1.90 /0.78-2.70/	18.1 /10.3-41.5/	9.25 /2.38-28.23/	**< 0.01**
ODI (1/h)	0.75 /0.20-1.53/	15.9 /8.5-33.7/	6.95 /1.08-22.38/	**< 0.01**
AI (1/h)	45.11 ± 18.85	48.06 ± 14.57	46.09 ± 17.36	0.65
SPT (min)	422.5 /398.3-439.3/	451.0 /404-485/	435.0 /403.6-479.1/	**0.04**
TST (min)	381.26 ± 45.93	415.32 ± 62.62	402.27 ± 58.81	**0.03**
TST90% (%)	0.0 /0.0-0.8/	3.45 /0.53-12.35/	0.7 /0.0-6.9/	**< 0.01**
$MinSatO_2$ (%)	91 /89-93/	83 /78-87/	87 /81-91/	**< 0.01**
ESS	5.5 /2.0-6.8/	6 /4-9/	6 /4-8/	**0.02**

This table shows the patients' characteristics of the patients with OSA, the controls and the overall study population. Data are presented as mean ± standard deviation or median /25-75% percentile/. The significant differences were shown in bold.

AHI – apnoea-hypopnoea index; AI – arousal index; BMI – body mass index; BP – blood pressure; COPD – chronic obstructive pulmonary disease; CRP – C-reactive protein; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; MinSatO2 – minimal oxygen saturation; ODI – oxygen saturation index; OSA – obstructive sleep apnoea; SPT – sleep period time; TST – total sleep time; TST90% – the percentage of total sleep time spent with saturation below 90%.

4.1.2. Circulating HA and HYAL-1 levels

Plasma HYAL-1 concentrations were significantly higher (0.59 /0.31– 0.88/ ng/mL vs. 0.31 /0.31–0.58/ ng/mL; p=0.005, *Figure 5a*) and HMW-HA levels were lower (31.63 /18.11–59.25/ ng/mL vs. 46.83 /25.41–89.95/ ng/mL; p=0.068, *Figure 5b*) in the OSA group compared to the control group after adjustment for age, gender, BMI and smoking. HMW-HA/HYAL-1 ratio differed also significantly between the OSA and control groups (61.8 /25.74–107.54/ vs. 118.12 /44.23–260.39/; p=0.005). After adjustment for hypertension and cardiovascular diseases HYAL-1 levels remained significantly higher (p=0.005), HMW-HA concentrations were lower (p=0.068) in the OSA group. The difference in HMW-HA/HYAL-1 ratio was also significant between the two groups after the adjustments (p=0.005).

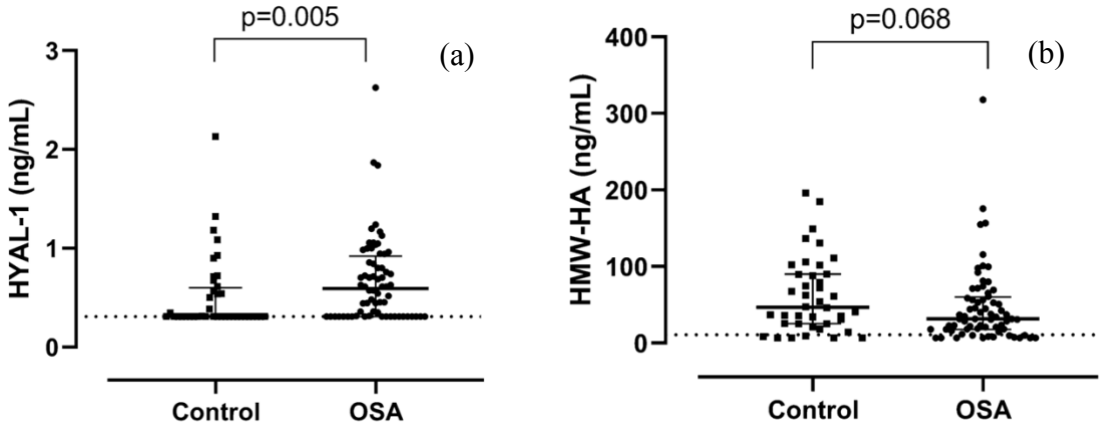

Figure 5. Circulating HYAL-1 (a) and HMW-HA (b) levels between OSA and control groups. Data are presented as median with interquartile range. Dashed line means the limit of detection.

OSA – obstructive sleep apnoea; HMW-HA – high molecular weight hyaluronic acid; HYAL-1 – hyaluronidase 1.

Ten outliers were detected (2 controls and 4 patients for HMW-HA; 1 control and 3 patients for HYAL-1) who were not different in their general characteristics or comorbidities from the others. After excluding them, the HYAL-1 levels and HMW-HA/HYAL-1 ratio remained significantly different (p=0.013; p=0.002). The difference in HMW-HA concentrations between the two groups became significant after the exclusion (p=0.027).

We detected significantly lower HMW-HA (28.62 /16.50–44.86/ ng/mL vs. 47.45 /25.38–89.90/ ng/mL; p=0.021) and significantly higher HYAL-1 (0.61/0.31–0.94/ ng/mL vs. 0.31/0.31–0.54/ ng/mL; p=0.002) levels in OSA after excluding patients with chronic airway disease and chronic heart failure (33 controls vs. 50 patients). The difference in HMW-HA/HYAL-1 ratio remained also significant (p=0.001).

In 19% (n=13) of the OSA group and in 15% (n=6) of the control group we measured concentrations below the detection limit for HMW-HA (p=0.59) and in 28% (n=19) of patients with OSA and in 58% (n=23) of control patients for of HYAL-1 (p=0.002).

4.1.3. Relationship between the biomarker levels and clinical variables

Plasma HMW-HA levels significantly and inversely correlated with AHI (ρ=−0.195, p=0.043, *Figure 6a*). However, we did not find any correlations between

HMW-HA and the other sleep parameters (all p>0.05). A significant correlation was found between plasma between HYAL-1 levels and AHI (ρ=0.30, p<0.01, *Figure 6b*) and ODI (ρ=0.26, p<0.01). Furthermore, we found a tendency for correlation between HYAL-1 levels and TST90% (ρ=0.186, p=0.057) and MinSatO_2 (ρ=− 0.184, p=0.059), however they did not reach the level of significance.

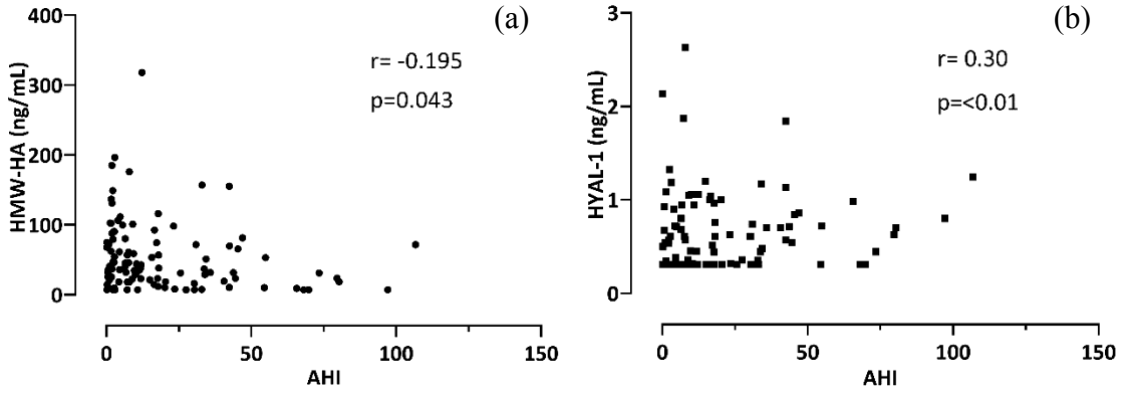

Figure 6. (a) Correlation between HMW-HA levels and AHI. (b) Correlation between HYAL-1 levels and AHI.
AHI – apnoea-hypopnoea index; HMW-HA – high molecular weight hyaluronic acid; HYAL-1 – hyaluronidase 1.

Comparing biomarker levels between the OSA severity groups, we observed a significant difference in HYAL-1 concentration (p=0.03) as well as HMW-HA/HYAL-1 ratio (p=0.005, *Figure 7*), but not in HMW-HA levels (p=0.12).

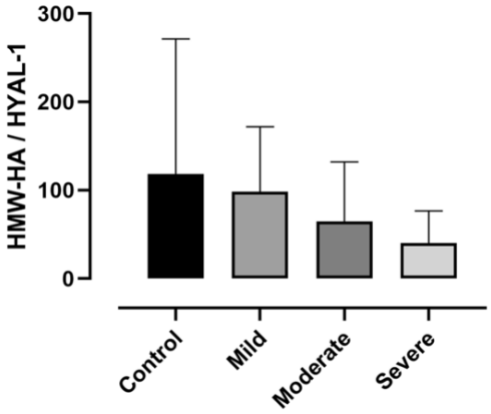

Figure 7. HMW-HA/HYAL-1 ratio between the OSA severity groups. Data are presented as median with interquartile range.
HMW-HA – high molecular weight hyaluronic acid; HYAL-1 – hyaluronidase 1.

There was a direct correlation between HMW-HA levels and age (ρ=0.41, $p<0.01$; *Table 3*). We measured higher HYAL-1 concentrations in men than women (0.56 /0.31–0.85/ng/mL vs. 0.31 /0.31–0.72/ ng/mL, p=0.01) and HYAL-1 levels were directly related to glucose (ρ=0.32; p=0.002), CRP (ρ=0.30; p=0.005) and triglyceride levels (ρ=0.24; p=0.014) and indirectly to HDL-C concentrations (ρ=−0.21; p=0.036). No correlation was detected between the other clinical variables as well as HMW-HA or HYAL-1 (all $p>0.05$). There was no correlation between HMW-HA and HYAL-1 levels (ρ=−0.12; p= 0.20), neither in the control (ρ=−0.25; p=0.12), nor in the OSA group (ρ=0.06; p=0.63).

Table 3. Summary of the correlations between the biomarkers and the clinical variables.

	HMW-HA	**HYAL-1**
Age (age)	**Significant (positive)**	Non-significant
BMI (kg/m^2)	Non-significant	Non-significant
Cigarette pack years	Non-significant	Non-significant
Systolic BP (mmHg)	Non-significant	Non-significant
Diastolic BP (mmHg)	Non-significant	Non-significant
CRP (mg/L)	Non-significant	**Significant (positive)**
Glucose (mmol/L)	Non-significant	**Significant (positive)**
Cholesterol (mmol/L)	Non-significant	Non-significant
HDL-C (mmol/L)	Non-significant	**Significant (negative)**
LDL-C (mmol/L)	Non-significant	Non-significant
Triglyceride (mmol/L)	Non-significant	**Significant (positive)**
Lipoprotein (a) (mmol/L)	Non-significant	Non-significant
AHI (1/h)	**Significant (negative)**	**Significant (positive)**
ODI (1/h)	Non-significant	**Significant (positive)**
AI (1/h)	Non-significant	Non-significant
SPT (min)	Non-significant	Non-significant
TST (min)	Non-significant	Non-significant
TST90% (%)	Non-significant	Non-significant*****
MinSatO_2 (%)	Non-significant	Non-significant*****
ESS	Non-significant	Non-significant
		******* *Tendency for correlation*

This table shows the correlation between HMW-HA, HYAL-1 and the clinical variables. AHI – apnoea-hypopnoea index; AI – arousal index; BMI – body mass index; BP – blood pressure; CRP – C-reactive protein; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; HMW-HA – high molecular weight hyaluronic acid; HYAL-1 – hyaluronidase 1; LDL-C – low-density lipoprotein cholesterol; MinSatO_2 – minimal oxygen saturation; ODI – oxygen saturation index; SPT – sleep period time; TST – total sleep time; TST90% – the percentage of total sleep time spent with saturation below 90%.

4.1.4. The effect of obesity on biomarker levels

We observed a significant correlation between HYAL-1 and BMI in the total cohort of subjects (ρ=0.25; p=0.01). However, this correlation was insignificant in controls (ρ=0.25; p=0.12) or patients with OSA (ρ=0.06; p=0.65) separately. We did not observed correlation between HMW-HA and BMI either in all subjects, or in the control

or OSA subgroups separately (all p>0.05). After adjustment for age, gender, smoking and AHI we compared HMW-HA and HYAL-1 levels between non-obese and obese (BMI≥30 kg/m^2) controls and patients with OSA. HYAL-1 levels did not differ significantly between the non-obese (n=25; 0.61 /0.36–0.86/ ng/mL) and obese (n=43; 0.54 /0.31–0.89/ ng/mL) patients with OSA (p=0.68). Interestingly, we detected higher HYAL-1 levels in obese controls (n=6; 0.72 /0.59–0.99/ ng/mL) compared to the non-obese controls (n=34; 0.31 /0.31–0.47/ ng/mL; p=0.01). There was no difference in HMW-HA levels between non-obese and obese participants either in controls (46.83 /25.48–90.04/ ng/mL vs. 56.53 /23.98–77.24/ ng/mL; p=0.87), or in OSA group (34.42 /22.22– 50.90/ ng/mL vs. 30.95 /12.34–67.49/ ng/mL; p=0.63).

4.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

4.2.1. Patient characteristics

Forty-six patients were diagnosed with OSA (54 years /46-62/, 31 men). They were older and had significantly higher systolic and diastolic BP, higher levels of CRP, glucose and TG levels, higher AIP and TyG index values and lower levels of HDL-C (all p<0.01). The prevalence of males, hypertension and smokers was higher in OSA (p<0.01). The OSA group had higher AHI, ODI and TST90% and lower MinSatO_2 values (all p<0.01, *Table 4*).

Table 4. Subjects' characteristics.

	Control (n=30)	**OSA (n=46)**	**p**
Age (years)	43 /30-51/	54 /46-62/	**< 0.01**
Males (%)	23	67	**< 0.01**
BMI (kg/m^2)	23.89 /20.96-26.99/	29.89 /25.11-37.56/	**< 0.01**
Hypertension (%)	30	67	**< 0.01**
Diabetes (%)	10	15	0.51
Dyslipidaemia (%)	27	30	0.72
Cardiovascular disease (%)	7	9	0.75
Cardiac arrythmia (%)	13	26	0.18
Smokers (%)	3	30	**< 0.01**
Systolic BP (mmHg)	120.0 /110.0-128.8/	135.5 /122.0-148.0/	**< 0.01**
Diastolic BP (mmHg)	70.0 /66.3-80.0/	84.0 /78.5-90.0/	**< 0.01**
CRP (mg/L)	1.75 /0.84-2.50/	3.20 /1.79-4.79/	**< 0.01**
Glucose (mmol/L)	4.70 /4.30-5.20/	5.10 /4.90-6.20/	**< 0.01**
Cholesterol (mmol/L)	5.63 ± 1.03	5.59 ± 1.03	0.877
HDL-C (mmol/L)	1.59 /1.38-1.97/	1.25 /1.06-1.47/	**< 0.01**
LDL-C (mmol/L)	3.39 ± 0.83	3.58 ± 0.88	0.33
Triglyceride (mmol/L)	1.29 /0.93-1.56/	1.64 /1.26-2.15/	**< 0.01**
Lipoprotein (a) (mmol/L)	0.22 /0.08-0.58/	0.35 /0.02-0.54/	0.94
ApoA1 (g/L)	1.66 /1.50-1.73/	1.46 /1.29-1.64/	0.09
ApoB (g/L)	1.17 /1.09-1.38/	1.19 /0.99-1.39/	0.79
TyG index	8.37 ± 0.32	8.92 ± 0.50	**< 0.01**
AIP	-0.13 ± 0.25	0.11 ± 0.25	**< 0.01**
AHI (1/h)	2.15 /1.13-3.08/	26.05 /12.50-35.63/	**< 0.01**
ODI (1/h)	0.90 /0.23-1.65/	22.0 /9.33-33.40/	**< 0.01**
SPT (min)	431.56 ± 47.41	447.59 ± 41.43	0.27
TST (min)	401.31 ± 41.52	415.04 ± 31.75	0.25
TST90% (%)	0 /0-0/	4.50 /0.70-16.40/	**< 0.01**
MinSatO_2 (%)	91 /89-92/	83 /75-87/	**< 0.01**
ESS	6 /4-8/	7 /5-10/	0.50

This table shows the patients' characteristics of the patients with OSA and the controls. Data are presented as mean ± standard deviation or median /25-75% percentile/. The significant differences were shown in bold.

AHI – apnoea-hypopnoea index; AIP – atherogenic index of plasma; apo – apolipoprotein; BMI – body mass index; BP – blood pressure; CRP – C-reactive protein; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; MinSatO_2 – minimal oxygen saturation; ODI – oxygen saturation index; OSA – obstructive sleep apnoea; SPT – sleep period time; TST – total sleep time; TST90% – the percentage of total sleep time spent with saturation below 90%; TyG index – triglyceride-glucose index.

4.2.2. Circulating sLRP-1 and CALR levels

Plasma sLRP-1 concentrations were significantly lower (1.67 /0.90-2.11/ mg/L vs. 1.99 /1.53-3.51/ mg/L; p=0.04; *Figure 8a*) in the OSA group compared to the controls after adjustment for age, gender, BMI and lipid profile. CALR did not differ significantly between the patients with OSA and the controls (0.23 /0.17-0.34/ ng/mL vs. 0.24 /0.20-0.36/ ng/mL p=0.76; *Figure 8b*) after the same adjustments.

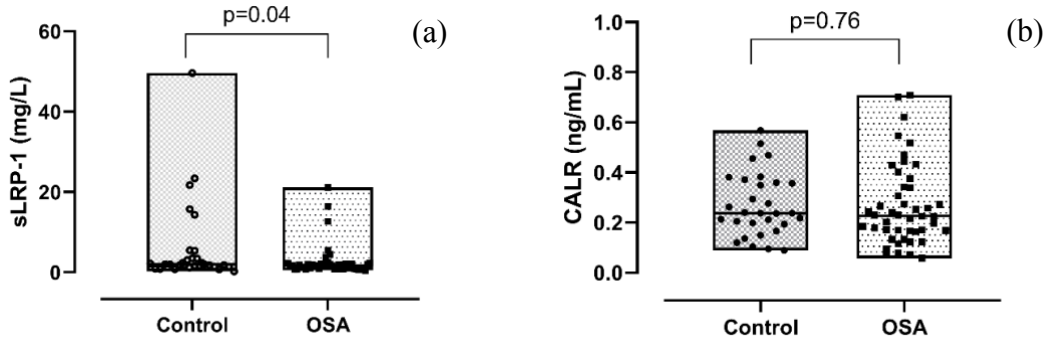

Figure 8. Circulating sLRP-1 (a) and CALR (b) levels between OSA and control groups. Data are presented as median, minimum and maximum values.
CALR – calreticulin; OSA – obstructive sleep apnoea; sLRP-1 – soluble LDL receptor-related protein 1.

There was no difference between the severity groups neither in sLRP-1 (p=0.15; *Figure 9a*), nor in CALR levels (p=0.44; *Figure 9b*).

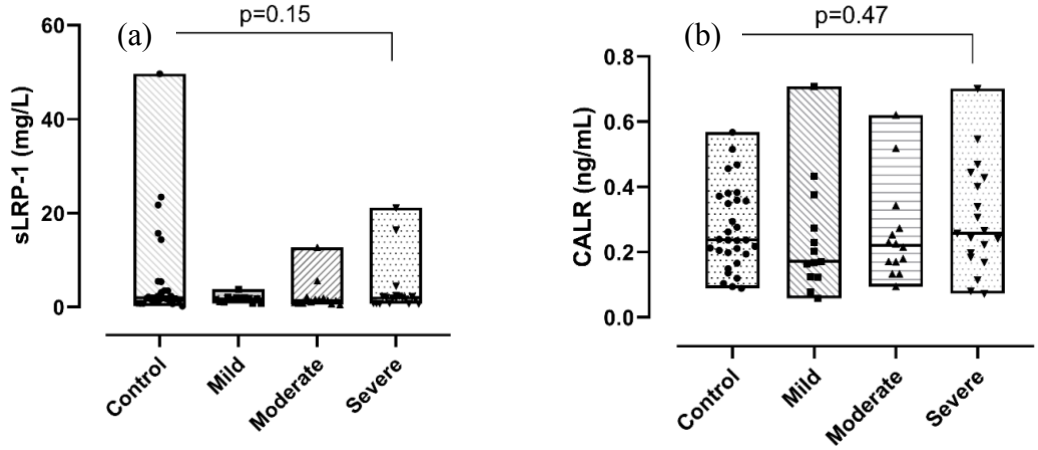

Figure 9. Circulating sLRP-1 (a) and CALR (b) levels between the OSA severity groups. Data are presented as median, minimum and maximum values.
CALR – calreticulin; sLRP-1 – soluble LDL receptor-related protein 1.

4.2.3. The relationship between the biomarker levels and clinical variables

Plasma sLRP-1 levels significantly and inversely correlated with AHI (ρ=-0.29, p=0.01) and ODI (ρ=-0.23, p=0.04; *Table 5*). However, we did not find any correlations between sLRP-1 levels and the other sleep parameters (all p>0.05). CALR levels did not correlate with any sleep parameters (all p>0.05). A significant inverse correlation was found between plasma sLRP-1 levels and BMI (ρ=-0.35, p<0.01), cigarette pack years (ρ=-0.31, p<0.01), LDL-C (ρ=-0.23, p=0.04), TG (ρ=-0.27, p=0.02), TyG index (ρ=-0.37, p=<0.01) and AIP (ρ=-0.27, p=0.02). Plasma CALR correlated only with age (ρ=0.35, p<0.01) and BMI (ρ=0.31, p<0.01), but none of the other variables all (p>0.05). Using multivariate regression analysis, BMI (β=-0.16, p=0.01) and ODI (β=-0.04, p=0.04) were independently associated with lower sLRP-1 levels and none of the clinical variables were associated with altered CALR levels (all p>0.05). We did not find correlation between sLRP-1 and CALR levels (ρ=-0.17, p=0.15).

Table 5. Summary of the correlations between the biomarkers and the clinical variables.

	sLRP-1	**CALR**
Age (age)	Non-significant	**Significant (positive)**
BMI (kg/m^2)	**Significant (negative)**	**Significant (positive)**
Cigarette pack years	**Significant (negative)**	Non-significant
Systolic BP (mmHg)	Non-significant	Non-significant
Diastolic BP (mmHg)	Non-significant	Non-significant
CRP (mg/L)	Non-significant	Non-significant
Glucose (mmol/L)	Non-significant	Non-significant
Cholesterol (mmol/L)	Non-significant	Non-significant
HDL-C (mmol/L)	Non-significant	Non-significant
LDL-C (mmol/L)	**Significant (negative)**	Non-significant
Triglyceride (mmol/L)	**Significant (negative)**	Non-significant
Lipoprotein (a) (mmol/L)	Non-significant	Non-significant
ApoA1 (g/L)	Non-significant	Non-significant
ApoB (g/L)	Non-significant	Non-significant
TyG index	**Significant (negative)**	Non-significant
AIP	**Significant (negative)**	Non-significant
AHI (1/h)	**Significant (negative)**	Non-significant
ODI (1/h)	**Significant (negative)**	Non-significant
SPT (min)	Non-significant	Non-significant
TST (min)	Non-significant	Non-significant
TST90% (%)	Non-significant	Non-significant
MinSatO_2 (%)	Non-significant	Non-significant
ESS	Non-significant	Non-significant

This table shows the correlation between sLRP-1, CALR and the clinical variables.

AHI – apnoea-hypopnoea index; AIP – atherogenic index of plasma; apo – apolipoprotein; BMI – body mass index; BP – blood pressure; CALR – calreticulin; CRP – C-reactive protein; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; $MinSatO_2$ – minimal oxygen saturation; ODI – oxygen saturation index; sLRP-1 – soluble LDL receptor-related protein 1; SPT – sleep period time; TST – total sleep time; TST90% – the percentage of total sleep time spent with saturation below 90%; TyG index – triglyceride-glucose index.

4.3. Heritable factors in the pathomechanism of dyslipidaemia in OSA

4.3.1. Patient characteristics

Ninety-four MZ (51 years /37-62/, 34 men) and forty-four DZ twins (61 years /53-69/, 6 men) were included. The MZ twins were significantly younger and had significantly lower systolic and diastolic BP, higher BMI and lipoprotein(a) values (all $p<0.01$, except for diastolic BP $p=0.02$). The prevalence of males was higher in the MZ group ($p=0.01$). ESS was higher ($p=0.03$) and TST90% was lower (<0.01) among the MZ twins. The other sleep parameters did not differ significantly between the two groups (all $p>0.05$). Fifty-eight subjects were diagnosed with OSA in the whole study population and the prevalence of OSA was not different between the groups ($p=0.46$; *Table 6*).

Table 6. Subjects' characteristics.

	Total (n=138)	**MZ (n=94)**	**DZ (n=44)**	**p**
Age (years)	54 /39-63/	51 /37-62/	61 /53-69/	**<0.01**
Male (%)	29	36	14	**0.01**
BMI (kg/m^2)	25.4 /22.0-28.7/	24.6 /21.1-27.8/	27.3 /23.1-31.6/	**<0.01**
Hypertension (%)	30	29	32	0.96
Diabetes (%)	7	3	14	0.05
Dyslipidaemia (%)	13	12	16	0.68
Smokers (%)	11%	14%	5%	0.67
Cigarette pack years	0 /0-0/	0 /0-0/	0 /0-0/	0.29
Systolic BP (mmHg)	120 /120-140/	120 /110-140/	130/120-140/	**<0.01**
Diastolic BP (mmHg)	80 /70-80/	75 /70-80/	80 /70-90/	**0.02**
Glucose (mmol/L)	4.7 /4.3-5.3/	4.7 /4.2-5.3/	4.9 /4.6-5.4/	0.94
Cholesterol (mmol/L)	5.40 /4.70-6.30/	5.40 /4.68-6.33/	5.60 /4.80-6.20/	0.91
HDL-C (mmol/L)	1.58 /1.26-1.96/	1.59 /1.21-1.98/	1.57/1.34-1.82/	0.75
LDL-C (mmol/L)	3.26 ± 1.14	3.26 ± 1.15	3.27 ± 1.15	0.94
Triglyceride (mmol/L)	1.26 /0.91-1.75/	1.26 /0.91-1.76/	1.24 /0.89-1.74/	0.88
Lipoprotein(a) (mmol/L)	0.20 /0.05-0.50/	0.27 /0.09-0.57/	0.12 /0.01-0.26/	**<0.01**
AHI (1/h)	4.00 /1.68-9.43/	4.15 /1.57-9.55/	3.75 /1.93-9.18/	0.74
ODI (1/h)	2.25 /0.70-7.20/	1.90 /0.70-7.60/	2.90 /0.75-7.07	0.40
TST90% (%)	0.05 /0.0-1.7/	0.0 /0.0-1.35/	0.10 /0.0-2.4/	**<0.01**
ESS	6 /3-8/	6 /4-10/	4 /2-7/	**0.03**
OSA (%)	42	45	36	0.46
Mild OSA (%)	30	33	25	0.88
Moderate OSA (%)	9	9	9	
Severe OSA (%)	3	3	2	

This table shows the patients' characteristics of the monozygotic- and dizygotic twins and the overall study population. Data are presented as mean ± standard deviation or median /25-75% percentile/. The significant differences were shown in bold.

AHI – apnoea-hypopnoea index; BMI – body mass index; BP – blood pressure; DZ – dizygotic; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; MZ – monozygotic; ODI – oxygen saturation index; OSA – obstructive sleep apnoea; TST90% – the percentage of total sleep time spent with saturation below 90%.

4.3.2. Bivariate co-twin correlation

A higher bivariate co-twin correlation of BP, glucose, lipid- and sleep parameters were found in the MZ group than in DZ group. All correlations were statistically significant ($p<0.01$) among MZ twins and nominally higher than in the DZ group. We detected significant correlations in systolic BP ($p<0.01$), LDL-C ($p=0.02$) and TG ($p=0.01$) levels among the DZ twins (*Table 7*).

Table 7. Bivariate co-twin correlation.

	MZ		DZ	
	R	**P**	**R**	**P**
Systolic BP (mmHg)	0.57	< **0.01**	0.68	**<0.01**
Diastolic BP (mmHg)	0.61	< **0.01**	0.14	0.59
Glucose (mmol/L)	0.77	< **0.01**	0.21	0.47
Cholesterol (mmol/L)	0.84	< **0.01**	0.37	0.10
HDL-C (mmol/L)	0.85	< **0.01**	0.43	0.07
LDL-C (mmol/L)	0.62	< **0.01**	0.53	**0.02**
Triglyceride (mmol/L)	0.64	< **0.01**	0.58	**0.01**
Lipoprotein (a) (mmol/L)	0.92	< **0.01**	0.07	0.75
AHI (1/h)	0.71	< **0.01**	0.19	0.39
ODI (1/h)	0.74	< **0.01**	0.20	0.36
TST90% (%)	0.53	< **0.01**	-0.07	0.75
ESS	0.67	< **0.01**	0.44	0.09

This table shows the results of the bivariate co-twin correlation among the monozygotic- and dizygotic twins. The significant values were shown in bold.

AHI – apnoea-hypopnoea index; BP – blood pressure; DZ – dizygotic; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; MZ – monozygotic; ODI – oxygen saturation index; TST90% – the percentage of total sleep time spent with saturation below 90%.

4.3.3. Linear regression between sleep parameters, blood pressure, glucose and lipids

AHI significantly correlated with systolic BP ($r=0.22$; $p=0.02$), diastolic BP ($r=0.22$, $p=0.02$), TG ($r=0.36$, $p<0.01$), lipoprotein(a) ($r=0.20$, $p=0.02$), and glucose ($r=0.20$, $p=0.05$). We found a significant association between ODI and systolic BP ($r=0.30$, $p<0.01$), diastolic BP ($r=0.29$, $p<0.01$), TG ($r=0.45$, $p<0.01$), lipoprotein (a) ($r=0.26$, $p<0.01$), glucose ($r=0.26$, $p<0.01$), HDL-C ($r=-0.20$, $p=0.03$), TC ($r=0.18$, $p=0.03$) and LDL-C ($r=0.18$, $p=0.05$). Significant association was detected between

TST90% and systolic BP (r=0.20, p=0.03), TG (r=0.39, p<0.01, r=0.39), TC (r=0.24, p<0.01), LDL-C (r=0.22, p=0.02), lipoprotein (a) (r=0.22, p=0.02). None of the parameters were related to ESS (p>0.05).

4.3.4. The heritability of associations between indices of OSA severity, blood pressure, glucose and lipids

The significant associations of the bivariate analysis in all subjects were adjusted for age, gender and BMI. Genetic correlations for the associations between AHI and TG, ODI and TG, TST90% and TG were significant (both p-values for RhoG were <0.05) (*Table 8*). Significant RhoG=1 or -1 value and non-significant RhoG=0 value suggests that both traits were significantly heritable but they did not share common genetic loci. The environment did not influence the relationships (p>0.05). However, only the genetic correlation between TST90% and TG remained significant after FDR correction. This correlation remained significant after the adjustment for smoking (both p-values for RhoG were <0.05).

The heritable relationships between AHI and TG, ODI and TG and TST90% and TG remained significant after taking into consideration the effects of antidiabetic and lipid-lowering medications. The relationships between TST90% and TG (p=0.04), TST90% and LDL-C (p=0.01) were significantly determined by the environment (*Table 9*). After FDR correction, the heritable relationships between ODI and TG levels and TST90% and TG levels remained significant (both p-values for RhoG were <0.05). However, the genetic relationship between AHI, TG and the environmental associations became insignificant (p>0.05). After adjustment for smoking, associations for non-overlapping genetic correlations became insignificant between serum TG and ODI (p=0.09 for RhoG different from 1 or -1) as well as TST90% (p=0.08). P-values for RhoG different from 0 did not change (both p=0.02) suggesting shared genetic loci between markers of overnight hypoxaemia and TG levels in our study population.

Table 8. Results of the bivariate Cholesky decomposition adjusted for age, gender and BMI.

Variable 1	Variable 2	Genetic correlation ($r_G \pm SE$)	Environmental correlation ($r_E \pm SE$)	p-values for hypotheses			FDR-corrected p-values		
				RhoG different from zero	RhoG different from 1 or -1.0	RhoE different from zero	RhoG different from zero	RhoG different from 1 or -1.0	RhoE different from zero
AHI	Triglyceride	0.37 ± 0.16	- 0.02 ± 0.14	**0.03**	**<0.01**	0.91	0.19	**<0.01**	0.94
	Systolic BP	- 0.13 ± 0.19	0.23 ± 0.15	0.48	**<0.01**	0.14	0.32	**<0.01**	0.32
	Diastolic BP	- 0.07 ± 0.18	0.23 ± 0.15	0.70	**<0.01**	0.16	0.32	**<0.01**	0.32
	Glucose	0.05 ± 0.21	0.16 ± 0.16	0.82	**<0.01**	0.34	0.93	**<0.01**	0.60
ODI	Triglyceride	0.46 ± 0.17	0.05 ± 0.15	**0.01**	**<0.01**	0.73	0.13	**<0.01**	0.86
	Systolic BP	- 0.15 ± 0.19	0.09 ± 0.16	0.45	**<0.01**	0.59	0.32	**<0.01**	0.32
	Diastolic BP	- 0.17 ± 0.18	0.27 ± 0.15	0.38	**<0.01**	0.09	0.32	**<0.01**	0.32
	Glucose	0.09 ± 0.22	0.11 ± 0.18	0.70	**<0.01**	0.55	0.86	**<0.01**	0.81
TST90%	Triglyceride	0.63 ± 0.15	- 0.12 ± 0.17	**<0.01**	**<0.01**	0.46	**0.01**	**0.01**	0.77
	Cholesterol	0.11 ± 0.16	0.18 ± 0.17	0.92	**<0.01**	0.31	0.80	**<0.01**	0.60
	LDL-C	0 ± 0.17	0.29 ± 0.16	0.51	**<0.01**	0.09	1.00	**<0.01**	0.32
	Lipoprotein (a)	0.12 ± 0.13	0.31 ± 0.17	0.99	**<0.01**	0.09	0.78	**<0.01**	0.32
	Systolic BP	- 0.25 ± 0.18	0.31 ± 0.15	0.37	**<0.01**	0.05	0.13	**<0.01**	0.32

This table shows the results of the bivariate Cholesky decomposition after adjustment for age, gender and BMI. If a p-value for RhoG = 0 is significant, the two traits share common genetic loci. If a p-value for RhoG = 1 or −1 (without RhoG = 0 being significant) is significant, the two traits have high heritability, but they did not share common genetic loci. The significant values were shown in bold.

AHI – apnoea-hypopnoea index; BP – blood pressure; LDL-C – low-density lipoprotein cholesterol; ODI – oxygen desaturation index; r_E – environmental correlation coefficient; r_G – genetic correlation coefficient; RhoE – environmental correlation; RhoG – genetic correlation; SE – standard error; FDR-False Discovery Rate; TST90% – total sleep time spent with oxygen saturation below 90%.

Table 9. Results of the bivariate Cholesky after exclusion of participants taking antidiabetic and lipid lowering drugs.

Variable 1	Variable 2	Genetic correlation ($r_G \pm SE$)	Environmental correlation ($r_E \pm SE$)	p-values for hypotheses			FDR corrected p-values		
				RhoG different from zero	RhoG different from 1 or -1.0	RhoE different from zero	RhoG different from zero	RhoG different from 1 or -1.0	RhoE different from zero
AHI	Triglyceride	0.45 ± 0.18	-0.09 ± 0.15	**0.02**	**<0.01**	0.54	0.09	**<0.01**	0.81
	Glucose	0.15 ± 0.22	0.19 ± 0.19	0.51	**<0.01**	0.33	0.74	**<0.01**	0.55
ODI	Triglyceride	0.63 ± 0.19	-0.09 ± 0.18	**<0.01**	**0.04**	0.64	**0.03**	**0.05**	0.81
	Glucose	0.18 ± 0.23	0.11 ± 0.25	0.45	**<0.01**	0.67	0.69	**<0.01**	0.81
TST90%	Triglyceride	0.58 ± 0.18	-0.36 ± 0.16	**<0.01**	**0.03**	**0.05**	**0.03**	**0.03**	0.35
	Cholesterol	-0.05 ± 0.17	0.22 ± 0.18	0.78	**<0.01**	0.24	0.85	**<0.01**	0.55
	LDL-C	-0.22 ± 0.14	0.45 ± .015	0.21	**<0.01**	**0.01**	0.41	**<0.01**	0.35
	Lipoprotein (a)	0.21 ± 0.14	0.25 ± 0.19	0.14	**<0.01**	0.21	0.31	**<0.01**	0.55

This table shows the results of the bivariate Cholesky decomposition after exclusion of participants taking antidiabetic and lipid lowering drugs. If a p-value for RhoG = 0 is significant, the two traits share common genetic loci. If a p-value for RhoG = 1 or −1 (without RhoG = 0 being significant) is significant, the two traits have high heritability, but they did not share common genetic loci. The significant values were shown in bold.

AHI – apnoea-hypopnoea index; BP – blood pressure; LDL-C – low-density lipoprotein cholesterol; ODI – oxygen desaturation index; rE – environmental correlation coefficient; rG – genetic correlation coefficient; RhoE – environmental correlation; RhoG – genetic correlation; SE – standard error; FDR-False Discovery Rate; TST90% – total sleep time spent with oxygen saturation below 90%.

4.4. Atherogenic Index of Plasma in OSA

4.4.1. Patient characteristics

Four hundred sixty-one patients were diagnosed with OSA (54 years /46-62/, 300 men). They were older and had significantly higher BMI values and TG and lower HDL-C levels (all $p<0.01$). The prevalence of males, smokers, statin usage and cardiovascular comorbidities (including hypertension, cardiovascular or cerebrovascular disease and cardiac arrhythmia) was higher in OSA ($p<0.01$). The OSA group had higher ESS, AHI, ODI, and TST90%, longer TST and SPT and lower $MinSatO_2$ values and poorer sleep efficiency (all $p<0.01$). AIP index was significantly higher in patients with OSA compared to the controls ($p<0.01$; *Table 10*).

Table 10. Subjects' characteristics.

	OSA (n=461)	**Control (n=99)**	**p-value**
Age (years)	54 /46-62/	46 /34.5-59.5/	**<0.01**
Gender (% male)	65	31	**<0.01**
BMI (kg/m^2)	31.8 /28.3-35.8/	24.8 /21.7-28.1/	**<0.01**
Smokers (ever%)	42	14	**<0.01**
Cigarette pack years	0 /0-15/	0 /0-0/	**<0.01**
Hypertension (%)	68	38	**<0.01**
Cardiovascular or cerebrovascular disease (%)	29	5	**<0.01**
Cardiac arrhythmia (%)	26	12	**<0.01**
Diabetes mellitus (%)	20	13	0.09
COPD (%)	12	6	0.08
Statin users (%)	24	6	**<0.01**
Cholesterol (mmol/L)	5.1 /4.3-5.9/	5.2 /4.7-6.1/	0.06
LDL-C (mmol/L)	2.9 /2.4-3.8/	3.1 /2.4-3.8/	0.87
HDL-C (mmol/L)	1.1 /1.0-1.3/	1.6 /1.3-2.0/	**<0.01**
Triglyceride (mmol/L)	1.7 /1.3-2.1/	1.2 /0.9-1.6/	**<0.01**
AIP	0.18 /0.03-0.31/	-0.15 /-0.34-0.06/	**<0.01**
AHI (1/h)	28.6 /14.9-47.7/	2.3 /1.2-3.5/	**<0.01**
ODI (1/h)	26.0 /13.5-47.9/	1.1 /0.4-2.2/	**<0.01**
SPT (min)	470 /431-503/	423 /397-444/	**<0.01**
TST (min)	423 /375-463/	400 /358-425/	**<0.01**
Sleep%	91 /82-97/	95 /88-99/	**<0.01**
REM%	15.1 /11.6-20.2/	16.1 /12.2-20.8/	0.95
TST90% (%)	7.3 /1.3-25.4/	0 /0-0.1/	**<0.01**
$MinSatO_2$ (%)	82 /75-87/	91 /88-93/	**<0.01**
ESS	8 /5-11/	6 /4-9/	**<0.01**

This table shows the patients' characteristics of the patients with OSA and the controls. Data are presented as mean ± standard deviation or median /25-75% percentile/. The significant differences were shown in bold.

AHI – apnoea-hypopnoea index; AIP – atherogenic index of plasma; BMI – body mass index; COPD – chronic obstructive pulmonary disease; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; $MinSatO_2$ – minimal oxygen saturation; ODI – oxygen saturation index; OSA – obstructive sleep apnoea; REM% – the percentage of rapid eye movement sleep of the total sleep time; Sleep% – sleep efficiency; SPT – sleep period time; TST – total sleep time; TST90% – the percentage of total sleep time spent with saturation below 90%.

4.4.2. Relationship between lipids and OSA

Higher AIP, lower HDL-C and surprisingly lower TC levels were associated with OSA after adjusting for age, gender and BMI. The other lipids were not associated with OSA (*Table 11*).

Table 11. The relationship between lipidic fractions and OSA.

	β	**p-value**
Triglyceride (mmol/L)	0.33	0.07
Cholesterol (mmol/L)	-0.27	**0.02**
LDL-C (mmol/L)	-0.07	0.24
HDL-C (mmol/L)	-1.02	**<0.01**
AIP	2.02	**<0.01**

This table shows the relationship between lipidic fractions and OSA. The significant values were shown in bold.

AIP – atherogenic index of plasma; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

HDL-C levels significantly correlated with ESS (ρ=-0.26), SPT (ρ=-0.13), AHI (ρ=-0.40), ODI (ρ=-0.43) TST90% (ρ=0.32) and $MinSatO_2$ (ρ=0.27; all $p<0.05$; *Table 12*). TG levels significantly correlated with ESS (ρ=0.15), AHI (ρ=0.32), ODI (ρ=0.43), TST90% (ρ=0.31) and $MinSatO_2$ (ρ=-0.25; all $p<0.05$). AIP values significantly positively correlated with ESS (ρ=0.19), AHI (ρ=0.40), ODI (ρ=0.43), TST90% (ρ=0.36) and negatively correlated with $MinSatO_2$ (ρ=-0.28, all $p<0.05$). The other lipid parameters were correlated none of the sleep parameters (all $p>0.05$).

Table 12. Summary of the correlations between lipids, AIP and sleep parameters.

	Triglyceride	**Cholesterol**	**LDL-C**	**HDL-C**	**AIP**
AHI (1/h)	**Significant (positive)**	Non-significant	Non-significant	**Significant (negative)**	**Significant (positive)**
ODI (1/h)	**Significant (positive)**	Non-significant	Non-significant	**Significant (negative)**	**Significant (positive)**
SPT (min)	Non-significant	Non-significant	Non-significant	**Significant (negative)**	Non-significant
TST (min)	Non-significant	Non-significant	Non-significant	Non-significant	Non-significant
Sleep%	Non-significant	Non-significant	Non-significant	Non-significant	Non-significant
REM%	Non-significant	Non-significant	Non-significant	Non-significant	Non-significant
TST90% (%)	**Significant (positive)**	Non-significant	Non-significant	Non-significant	**Significant (positive)**
$MinSatO_2$ (%)	**Significant (negative)**	Non-significant	Non-significant	**Significant (positive)**	**Significant (negative)**
ESS	**Significant (positive)**	Non-significant	Non-significant	**Significant (negative)**	**Significant (positive)**

This table shows the correlation between lipids, AIP and the investigated sleep parameters.

AHI – apnoea-hypopnoea index; AIP – atherogenic index of plasma; ESS – Epworth Sleepiness Scale; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; $MinSatO_2$ – minimal oxygen saturation; ODI – oxygen saturation index; REM% – the percentage of rapid eye movement sleep of the total sleep time; Sleep% – sleep efficiency; SPT – sleep period time; TST – total sleep time; TST90% – the percentage of total sleep time spent with saturation below 90%.

Comparing lipid levels between the OSA severity groups, we observed progressively increasing TG ($p<0.01$) and AIP ($p<0.01$) and progressively decreasing HDL-C ($p<0.01$) levels among the severity groups (*Figure 10*).

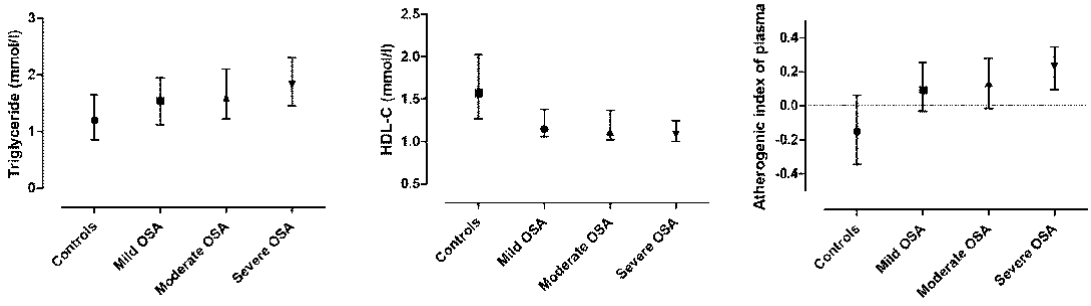

Figure 10. The relationship between lipid fractions and increasing disease severity. Data are presented as median /25-75% percentile/.
HDL-C – high-density lipoprotein cholesterol; OSA – obstructive sleep apnoea.

According to the ROC analysis, HDL-C, TG and AIP were found to be significant predictors (all $p<0.05$). The areas under the curves (AUC) with their 95% confidence intervals were 0.816 /0.769-0.863/ for HDL-C, 0.707 /0.649-0.765/ for TG and 0.778 /0.723-0.833/ for AIP. Comparing the AUC values, HDL-C was associated with significantly higher AUC than AIP ($p=0.03$) and TG ($p<0.01$). AIP was associated significantly higher AUC than TG ($p<0.01$) (*Figure 11*).

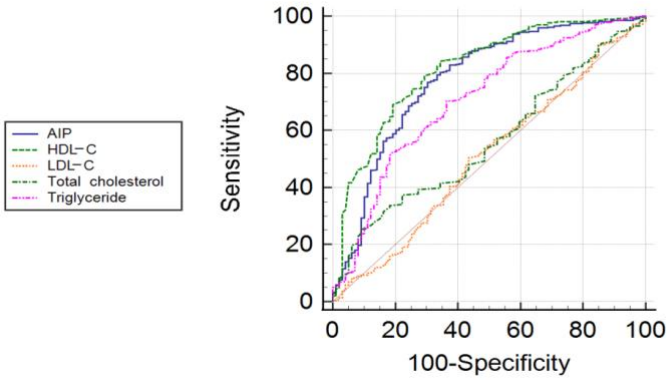

Figure 11. Receiver operating characteristic curve for specific lipid fractions to detect OSA.
AIP – atherogenic index of plasma; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

4.4.3. The relationship between lipids and OSA in subjects not taking statins

Four hundred forty-one subjects did not take statins. Among them, higher AIP and lower HDL-C levels were associated with OSA after adjusting for age, gender and BMI (*Table 13*).

Table 13. The relationship between lipidic fractions and OSA in subjects not taking statins.

	β	**p-value**
Triglyceride (mmol/L)	0.30	0.12
Cholesterol (mmol/L)	-0.23	0.05
LDL-C (mmol/L)	-0.07	0.21
HDL-C (mmol/L)	-0.84	**0.02**
AIP	1.81	**<0.01**

This table shows the relationship between lipidic fractions and OSA in subjects not taking statins. The significant values were shown in bold.
AIP – atherogenic index of plasma; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol.

In this subpopulation we observed progressively increasing TG ($p<0.01$) and AIP ($p<0.01$) and progressively decreasing HDL-C ($p<0.01$) levels among the severity groups. According to the ROC analysis, HDL-C, TG and AIP were found to be significant predictors (all $p<0.05$). The AUC values were 0.815 /0.776-0.850/ for HDL-C, 0.704 /0.659-0.747/ for TG and 0.771 /0.729-0.810/ for AIP. Comparing the AUC values, HDL-C was associated with significantly higher AUC than AIP ($p=0.01$). HDL-C and AIP were associated with significantly higher AUC than TG (both $p<0.01$).

4.4.4. The relationship between lipids and OSA in subjects having PSG

Four hundred fifty-eight subjects had PSG. Among them higher AIP and lower HDL-C and TG levels were associated with OSA after adjusting for age, gender and BMI (*Table 14*).

Table 14. The relationship between lipidic fractions and OSA in subjects undergoing diagnostic PSG.

	β	**p-value**
Triglyceride (mmol/L)	0.12	0.49
Cholesterol (mmol/L)	-0.34	**0.02**
LDL-C (mmol/L)	-0.05	0.45
HDL-C (mmol/L)	-0.98	**<0.01**
AIP	1.79	**<0.01**

This table shows the relationship between lipidic fractions and OSA in subjects undergoing diagnostic PSG. The significant values were shown in bold.

AIP – atherogenic index of plasma; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; PSG – polysomnography.

In this subpopulation we observed progressively increasing TG ($p<0.01$) and AIP ($p<0.01$) and progressively decreasing HDL-C ($p<0.01$) levels among the severity groups. According to the ROC analysis, HDL-C, TG and AIP were found to be significant predictors (all $p<0.05$). The AUC values were 0.824 /0.786-0.858/ for HDL-C, 0.696 /0.651-0.738/ for TG and 0.763 /0.721-0.801 for AIP. Comparing the AUC values, HDL-C was associated with significantly higher AUC than AIP and TG (both $p=0.01$). AIP were associated with significantly higher AUC than TG ($p<0.01$).

5. Discussion

5.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

In our study higher levels of HYAL-1 and lower levels of HMW-HA were detected in OSA. OSA-associated IH and systemic inflammation may explain these findings. In the study of Gao et al., HYAL was upregulated by IH [285]. Our results support these findings, because we detected a significant association between plasma HYAL-1 levels and the markers of overnight hypoxia. However, IH upregulates HMW-HA which may be immediately degraded due to increased HYAL activity [285]. Moreover, hypoxia may lead to higher HA uptake from the circulation by increasing the expression of CD44 [286]. Oxidative stress associated with OSA can also explain our results. ROS directly oxidate HMW-HA polymers [287] and also induce the expression of HYAL [288] resulting in further HMW-HA degradation. The loss of the anti-oxidant SOD3, which levels were low in OSA [289], was associated with increased HYAL expression and activity [290]. Pro-inflammatory cytokines, including IL-1β and TNF-α, elevate HA levels by upregulating HA-synthase enzymes [245] and increase HYAL levels in plasma too [291]. Thus, it is possible that increased HMW-HA can be quickly eliminated due to high HYAL-1 activity.

The high activity of HYAL enzymes promotes the production of pro-inflammatory LMW-HA levels [243]. Furthermore, HYAL itself leads to endothelial glycocalyx shedding resulting in endothelial dysfunction and vascular inflammation [243]. The degradation of HMW-HA due to inflammation reduces extracellular viscosity and increases tissue permeability through glycocalyx destabilization [292] leading to macrophage extravasation to the arterial wall. Moreover, the damaged endothelium promotes immune cell adhesion via adhesion molecules, such as VCAM-1, which levels were detected to be increased in OSA [293, 52]. Elevated HYAL levels are involved in intima aging [294] and its activity was shown to be higher in patients with coronary artery disease [295]. These results suggest that HYAL plays an important role in the pathogenesis of atherosclerosis. In addition, glycocalyx shedding by HYAL results in altered endothelial mechanosensitive response leading to dysregulated vascular tone [296, 244]. Supporting this, arterial stiffness was significantly associated with increased plasma HYAL concentrations indicating its potential role in the pathophysiology of

hypertension [297]. The association between OSA and increased cardiovascular morbidity and mortality is well-known [298]. We mentioned above that the destruction of endothelial glycocalyx leads to the impaired function of LpL leading to altered lipoprotein clearance and consequential high circulating lipid levels [251]. As a "sheddase" of HMW-HA in the glycocalyx, HYAL-1 may also promote this process. Supporting this fact, we detected a significant correlation between HYAL-1 and circulating TG levels in our study population. In summary, our results support the theory that altered hyaluronan metabolism may lead to inflammation, endothelial dysfunction and dyslipidaemia in OSA.

Obesity is a common comorbidity associated with OSA. Our study found a significant relationship between HYAL-1 levels and BMI, and HYAL-1 concentrations were higher in the obese participants compared to the non-obese controls. We hypothesise that obesity could partially contribute to our findings. Most importantly, HMW-HA and HYAL-1 levels were not different in obese vs. non-obese patients with OSA after adjustment for age, gender, smoking and BMI. This suggests that OSA-related changes in the investigated biomarkers were not exclusively due to differences in BMI between the OSA and control groups.

The main limitation of our study was that the levels of HYAL-1 and HMW-HA were below the detection limit in 39% and 18% of all cases. We replaced the values with the DL and this may lead to skewing our data. However, after their exclusion we also made a sub-analysis.

In conclusion, our study shows that plasma HYAL-1 levels are elevated in OSA and may decrease the circulating HMW-HA concentrations. These derangements may potentially enhance OSA-associated systemic inflammation and could be a potential link between OSA and its comorbidities.

5.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

In our study lower levels of plasma sLRP-1 were detected in OSA which were associated with the severity of nocturnal hypoxia. We found no difference in CALR levels between the OSA and control groups.

Several OSA-related mechanisms can explain our results. SREBP-1, the master regulator of OSA-associated lipid metabolism, probably down-regulates LRP-1 expression [299]. In line with this, silenced SREBP-1 expression resulted in an enhanced

LRP-1 expression in human vascular smooth cells and in macrophages [300, 299]. Furthermore, vitamin D [301] and klotho protein [302] can induce LRP-1 expression and these factors were found to be decreased in OSA [303, 304]. On the contrary, hypoxia seems to up-regulate LRP-1 expression in a HIF-1α-dependent manner [305, 306] and HIF-1α may mediate OSA-associated metabolic abnormalities [307]. We suggest that the former mechanisms mediated by SREBP-1 are more dominant leading to decreased LRP-1 levels than the latter one. Age can negatively affect the LRP-1 expression in the liver leading to impaired CM remnant clearance in a mice model [308]. In line with this, we detected a significant negative relationship between sLRP-1 levels and age. Although, LRP-1 is extensively shed by several factors associated with inflammation and dyslipidaemia [262-264] (please see the Introduction) leading to elevated sLRP-1 levels in the circulation. It is contradictory to our results as there was an inverse correlation between sLRP-1 levels and markers of dyslipidaemia. This suggests that the suppressive effects of SREBP-1 are stronger than the shedding effect of atherogenic lipids on sLRP-1 levels. A previous study showed a direct association between circulating sLRP-1 and TC and LDL-C concentrations in patients with severe hypercholesterinaemia (LDL-C>190 mg/dL) [263]. These findings suggest that the severity of dyslipidaemia also affects sLRP-1 levels and our study population these parameters were in the normal range. Lower LRP-1 expression in OSA could contribute to metabolic dysfunction by losing its protective functions discussed above. In line with this, we detected a significant negative relationship between sLRP-1 levels and lipid profile.

It has been reported that LRP-1 as well sLRP-1 were associated with decreased levels of pro-inflammatory molecules including TNF-α [266, 309], IL-1β [266], IL-6 [309] and MMPs [310]. Moreover, LRP-1 was found to promote the anti-inflammatory M2-phenotype of macrophages [261]. According to these results, altered sLRP-1 levels in our study may enhance vascular inflammation in OSA.

It is noteworthy that LRP-1 also induces the expression of insulin receptors and the translocation of glucose transporter 2 [311]. Supporting this, we found an inverse correlation between sLRP-1 levels and TyG index which is a marker of insulin resistance [312]. Decreased LRP-1 concentrations may play role in OSA-associated insulin resistance.

Unaltered CALR levels can be explained by the following: in obesity, which is often associated with OSA, the expression of CALR is down-regulated [313]. In our study the patients with OSA were overweight (BMI 29.9 (25.1-37.6) kg/m^2) suggesting a counterbalancing mechanism leading to unaltered CALR levels.

The main limitation of this study was that during the patient recruitment we did not limit the BMI values and we included patients with various degrees of obesity. Obesity is commonly associated with OSA [314] it leads to systemic inflammation and reduced antioxidant capacity [315]. To reduce this bias, we adjusted our analyses for BMI.

In conclusion, we reported lower sLRP-1 levels in patients with OSA independently of the gender, age, BMI or lipid profile. We hypothesised that the altered LRP-1 pathway could contribute to the metabolic and inflammatory changes associated with OSA.

5.3. Genetic factors in the pathomechanism of dyslipidaemia in OSA

In this study, we firstly estimated the contribution of heritable and environmental factors to the associations between OSA and its common comorbidities. We found that the relationship between the variables of nocturnal hypoxaemia and serum TG levels is genetically determined.

Previous literature suggests that several disorders including hypertension [171], diabetes [316] and dyslipidaemia [171] are partly genetically determined. The estimated heritability was 46% for hypertension [317], 50-92% for type 2 diabetes mellitus [318, 316] and 25-60% for dyslipidaemia [171]. Genetic susceptibility was suggested to contribute to the development of OSA too [172, 319]. Our previous twin study reported approximately 70% heritability of OSA parameters in a Hungarian twin cohort [320]. Supporting these, the relationship among MZ twins for the investigated parameters was stronger than in DZ twins in our study.

The OSA severity measures correlated with BP, glucose and TG values but only ODI correlated significantly with TC levels. A previous study may explain this result: ODI-based severity grouping showed a stronger association with lipid abnormalities than the AHI-based grouping [321]. In line with this, only overlapping genetic correlations between markers of IH and serum TG levels were significant after adjustment for multiple comparisons (using FDR-correction).

The postulated mechanisms leading to OSA-associated dyslipidaemia are well-known (please see the Introduction). Our results suggest that common genetic loci may also influence the relationship between OSA and dyslipidaemia. Several polymorphisms have been identified playing role in the pathogenesis of OSA and related dyslipidaemia. Transcriptional factor PPAR-γ is an important regulator of glucose and lipid metabolism [322]. In humans, polymorphism Pro12Ala of PPAR-γ gene was found to be associated with susceptibility to OSA [174] and increased risk of hyperlipidaemia in OSA [323]. Polymorphism of APOE E2 and E4 alleles, which are associated with higher LDL-C and lower HDL-C levels [324], may be associated with OSA as well [175, 325]. Furthermore, these alleles were suggested to predispose to comorbidities associated with OSA [325]. Moreover, different APOE genotypes were associated with different lipid profiles irrespective of nocturnal hypoxia in OSA [321]. The HIF-1α/SREBP-1/SCD-1 pathway has been suggested to be the main mechanism leading to dyslipidaemia in OSA (please see the Introduction). Thus, the genetic variability of these genes may also promote dyslipidaemia in OSA. These previous findings suggest that genetic susceptibility may determine whether patients with OSA have dyslipidaemia too.

The main limitation of this study was the relatively low sample size. Thus, the lack of associations needs to be interpreted carefully. Moreover, the low ratio of DZ twins may lead to underestimated environmental effects.

In conclusion, our twin study suggests that OSA and hypertriglyceridemia share a common genetic background. Thus, it may be suggested to screen for OSA in patients suffering from dyslipidaemia and screen for dyslipidaemia in patients with OSA.

5.4. Atherogenic Index of Plasma in OSA

In our study, we evaluated the AIP index in OSA which values were higher in patients with OSA compared to the control group and correlated with the disease severity.

The TG/HDL-C ratio or its logarithm (AIP) has been studied in OSA before. Two studies included exclusively men participants and found elevated TG/HDL-C ratio/AIP in patients with OSA [221, 223]. However, gender may influence AIP [326]. Three further studies investigated TG/HDL-C or AIP in OSA: increased TG/HDL-C ratio and AIP were detected in OSA compared to the controls [327, 222] and they were related to AHI values [327, 328]. However, Wysocki et al. did not include control participants [328]. In the study of Silva et al., there was no correlation between TG/HDL-C ratio and

sleepiness or any parameters of sleep quality [222]. Supporting the previous findings, we also found significantly elevated AIP values in participants with OSA. Nor our study, neither the previous studies [327, 223] found a stronger association with OSA between HDL-C and AIP.

OSA is commonly associated with dyslipidaemia caused by IH and the disruption of the sleep architecture. As discussed in the Introduction, IH is suggested to be the most important mechanism in OSA-associated dyslipidaemia. In line with this, we found a significant correlation between AIP and the markers of nocturnal hypoxaemia (ODI, $MinSatO_2$). However, sleep fragmentation can also promote dyslipidaemia through increased lipolysis or hyperphagia [1]. In our study, we failed to detect any relationship between the parameters of sleep quality and AIP.

Statins are the most widely used medications to treat dyslipidaemia. It is known that statins may cause an elevation in HDL-C levels [329]. This fact may lead to potential bias in our study. After the exclusion of the statin-taking patients, the results were similar to the whole study population. The only exception was that reduced TC levels were no longer significantly associated with OSA. Our conclusion is that statins did not change the clinical value of AIP in OSA. We performed another sub-analysis on patients with PSG to make our study more comparable with the previous studies [222, 223, 328].

Obesity is a potential covariate influencing the relationship between AIP and OSA [221, 223]. Although, OSA is associated with elevated TG and decreased HDL-C levels independently of obesity [76]. In line with this, adjustment for BMI in this study did not change our main results.

In summary, we reported elevated AIP values in OSA which were related to the disease severity, but not the quality or quantity of sleep. In this study, we could not prove the superiority of AIP over other lipid parameters in evaluating OSA-associated dyslipidaemia.

6. Conclusions

6.1. The role of hyaluronic acid and hyaluronidase 1 in the pathomechanism of OSA

- Plasma HYAL-1 levels were higher and plasma HMW-HA levels were lower in the OSA group compared to the controls.
- There was a direct relationship between HYAL-1 levels and disease severity and markers of nocturnal hypoxaemia.
- There was an inverse relationship between HMW-HA levels and the disease severity.
- There was a direct relationship between HYAL-1 levels and BMI values and HYAL-1 levels were higher in the obese compared to the non-obese controls.

6.2. The role of sLRP-1 and calreticulin in the pathomechanism of OSA

- Plasma sLRP-1 levels were lower in the OSA group compared to controls.
- There was an inverse relationship between sLRP-1 levels and lipid parameters as well as markers of nocturnal hypoxaemia.
- Plasma CALR did not differ between the two groups.

6.3. Heritable factors in the pathomechanism of dyslipidaemia in OSA

- The relationship of OSA and serum TG levels was heritable suggesting a common genetic background.
- The relationships between OSA and glucose levels and blood pressure were not significantly determined by heritable factors.

6.4. Atherogenic Index of Plasma in OSA

- AIP were higher in the OSA group compared to the controls and related to the disease severity.
- AIP did not have a stronger predictive utility than the other lipid parameters in assessing dyslipidaemia in OSA.
- Statin therapy did not influence the AIP results.

7. Summary

A wide range of clinical studies has provided evidence that OSA is frequently associated with abnormal lipid profiles. Intermittent hypoxia, sleep fragmentation, oxidative stress and consequential systemic inflammation have been considered as the main factors mediating metabolic dysfunction in this disorder. However, the exact mechanisms of the bidirectional relationship between OSA-associated inflammation and dyslipidaemia in humans have not been clearly elucidated. Thus, the main objective of this PhD work was to study the role of inflammation- and dyslipidaemia-associated factors which may lead to lipid alterations in OSA.

We were the first to investigate the role of hyaluronan metabolism in OSA. We have found higher levels of circulating hyaluronidase 1 and lower levels of high molecular weight hyaluronic acid in OSA. Our findings suggest that intermittent hypoxia may cause these alterations enhancing further inflammation and leading to consequential comorbidities in OSA.

We were also the first to show that the soluble levels of LDL receptor protein 1 were decreased and the levels of its ligand calreticulin were unchanged in OSA. The impaired availability of this receptor may potentially enhance OSA-associated systemic inflammation and could be a potential link between OSA and dyslipidaemia.

We were the first to report that the relationship between OSA and high triglyceride levels is genetically determined.

We demonstrated that the atherogenic index of plasma, which is a marker of the dysregulation between anti- and pro-atherogenic lipoproteins, was elevated in OSA and related to the disease severity. Thus, its value to predict cardiovascular disease may be affected by the presence of OSA.

In conclusion, this PhD thesis expanded the previous understanding that metabolic implications are strongly influenced by inflammatory processes in OSA.

8. Összefoglalás

Klinikai vizsgálatok széles köre bizonyította, hogy az obstruktív alvási apnoe gyakran abnormális lipid profillal társul. Az OSA-ban megfigyelt metabolikus diszfunkció kórélettanában az intermittáló hypoxia, az alvás fragmentáció, az oxidatív stressz és a következményes szisztémás gyulladás kiemelt szerepet játszik. Az OSA-val összefüggő gyulladásos folyamatok és a dyslipidaemia közötti kétirányú kapcsolat pontos mechanizmusait azonban még nem tisztázták egyértelműen. A jelen PhD munka legfőbb törekvése az volt, hogy tanulmányozza a gyulladással és dyslipidaemiával összefüggő faktorok szerepét OSA-ban.

Elsőként vizsgáltuk hialuronsav szerepét OSA-ban. Azt találtuk, hogy a hyaluronidáz 1 enzim szintje magasabb, míg a nagy molekulasúlyú hialuronsav szintje alacsonyabb volt OSA-ban szenvedő betegekben. Eredményeink feltételezik, hogy az intermittáló hypoxia okozhatja ezeket a változásokat, melyek fokozhatják a szisztémás gyulladást és következményes társbetegségek kialakulásához vezethetnek OSA-ban.

Szintén elsőként mutattuk ki, a szolubilis LDL receptor protein 1 szintje csökkent, míg a kalretikulin szintje nem változott OSA-ban. Ennek a receptornak a csökkent biológiai elérhetősége potenciálisan fokozhatja az OSA-val összefüggő szisztémás gyulladást és kapcsolatot jelenthet az OSA és a dyslipidaemia között.

Továbbá vizsgáltuk az OSA és társbetegségei genetikai hátterét. Ikervizsgálatunk kimutatta, hogy az OSA és a triglicerid szint közötti kapcsolat genetikailag meghatározott.

Végül kimutattuk, hogy a plazma aterogén indexe, mely az anti- és pro-aterogén lipoproteinek közötti diszfunkció markere, magasabb volt OSA-ban és összefüggött a betegség súlyosságával. Ezáltal feltételezhető, hogy az index szív- és érrendszeri betegségekre vonatkozó prediktív értékét befolyásolhatja az OSA jelenléte.

Összefoglalva elmondható, hogy jelen doktori értekezés új eredményeivel kibővítette a korábbi tapasztalatokat, miszerint a gyulladásos folyamatok hozzájárulnak az OSA-ban megfigyelt metabolikus diszfunkció kialakulásához.

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10. The bibliography of the candidate's publications

10.1. Publications related to the PhD thesis

1. Meszaros M, A. Kis, L. Kunos, A.D. Tarnoki, D.L. Tarnoki, Z. Lazar, and A. Bikov. (2020) *The role of hyaluronic acid and hyaluronidase-1 in obstructive sleep apnoea.* Sci Rep, **10**: 19484.
   Impact factor: 4.380
   Quartile: D1

2. Meszaros M, L. Kunos, A.D. Tarnoki, D.L. Tarnoki, Z. Lazar, and A. Bikov. (2021) *The role of soluble low-density lipoprotein receptor-related protein-1 in obstructive sleep apnoea.* J Clin Med, **10**: 1494.
   Impact factor: 4.964
   Quartile: Q1

3. Meszaros M*, A.D. Tarnoki*, D.L. Tarnoki*, D.T. Kovacs, B. Forgo, J. Lee, J. Sung, J. Vestbo, V. Müller, L. Kunos, and A. Bikov. (2020) *Obstructive sleep apnea and hypertriglyceridaemia share common genetic background: Results of a twin study.* J Sleep Res, **29**: e12979.
   ** Shared first authorship.*
   Impact factor: 3.981
   Quartile: Q1

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   Impact factor: 4.964
   Quartile: Q1

   Σ Impact factor: 18.289

10.2. Other publications

1. Meszaros, M., P. Horvath, A. Kis, L. Kunos, A.D. Tarnoki, D.L. Tarnoki, Z. Lazar, and A. Bikov. (2021) *Circulating levels of clusterin and complement factor H in patients with obstructive sleep apnea.* Biomark Med, **15**: 323-330.

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3. Meszaros, M., A.G. Mathioudakis, M. Xanthoudaki, V. Sircu, E. Nena, J. Vestbo, A. Corlateanu, P. Steiropoulos, and A. Bikov. (2021) *The association between beta-blocker therapy and daytime sleepiness in obstructive sleep apnoea.* Sleep Biol Rhythms, **19**: 399-408.
4. Tarnoki, A.D., D.L. Tarnoki, C. Oláh, M. Szily, D.T. Kovacs, A. Dienes, M. Piroska, B. Forgo, M. Pinheiro, P. Ferreira, L. Kostyál, M. Meszaros, J. Pako, L. Kunos, and A. Bikov. (2021) *Lumbar spine abnormalities in patients with obstructive sleep apnoea.* Sci Rep, 11: 16233.
5. Bikov, A., S.M. Frent, M. Meszaros, L. Kunos, A.G. Mathioudakis, A.G. Negru, L. Gaita, and S. Mihaicuta. (2021) *Triglyceride-glucose index in non-diabetic, non-obese patients with obstructive sleep apnoea.* J Clin Med, **10**: 1932.
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7. Bikov, A., M. Mészáros, and L. Kunos. (2020) *[Characteristics of Hungarian patients with obstructive sleep apnoea].* Orvosi Hetilap, **161**: 2117-2123.
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11. Horváth, P., Z. Lázár, G. Gálffy, R. Puskás, L. Kunos, G. Losonczy, M. Mészáros, Á.D. Tárnoki, D.L. Tárnoki, and A. Bikov. (2020) *Circulating P-selectin glycoprotein ligand 1 and P-selectin levels in obstructive sleep apnea patients.* Lung, **198**: 173-179.
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11. Acknowledgements

Firstly, I would like to thank my supervisor András Bikov, who always motivated me to participate in his research projects. I am very grateful for his trust in me and in my talent. I could not have completed this work without his professional scientific guidance and assistance.

I would like to thank my colleagues at the Department of Pulmonology, Semmelweis University (Zsófia Lázár and László Kunos) and at the Medical Imaging Centre, Semmelweis University (Ádám Domonkos Tárnoki and Dávid László Tárnoki) for providing valuable scientific advice during my studies.

I would like to thank Professor Veronika Müller from Department of Pulmonology, Semmelweis University for providing facilities for my analyses.

I would like to thank Semmelweis University for supporting my participation at international conferences during my PhD studies.

The studies contributing to this PhD thesis were supported by the Grant of Hungarian Respiratory Society to Martina Mészáros (2019 "A CPAP terápia hatásának vizsgálata az inzulinrezisztenciára obstruktív alvási apnoéban" 1.5 million HUF).

I would like to thank my friend, Veronika Párizs for always helping me with her Microsoft Office knowledge. Finally, I would like to thank my family for providing moral support and endless and enormous patience during my PhD studies.