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EFFECTS OF LONG-TERM CPAP TREATMENT AND CPAP WITHDRAWAL IN PATIENTS WITH OBSTRUCTIVE SLEEP APNEA

PhD thesis

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LIST OF ABBREVIATIONS

AASM	American Academy of Sleep Medicine
AC	acceleration index
ACE	angiotensin-converting enzyme
AHI	apnea-hypopnea index
AI	apnea index
AutoCPAP	autotitrating continuous positive airway pressure
BIPAP	bilevel positive airway pressure
BL	baseline
BMI	body mass index
BV	baseline visit
CAD	coronary artery disease
CI	confidence interval
CIH	chronic intermittent hypoxia
COPD	chronic obstructive pulmonary disease
CPAP	continuous positive airway pressure
CRP	C-reactive protein
CVD	cardiovascular diseases
DC	deceleration index
ESS	Epworth sleepiness scale
FEV_1	forced expiratory volume in 1 second
FFA	free fatty acid
FVC	forced vital capacity

GERD	gastroesophageal reflux disease
HbA1c	glycated hemoglobin
HDL-C	high-density lipoprotein cholesterol
HI	hypopnea index
HIF-1	hypoxia inducible factor-1
HR	heart rate
HT	hypertension
IL	interleukin
LDL-C	low-density lipoprotein cholesterol
MMP	matrix metalloproteinase
MS	metabolic syndrome
NO	nitric oxide
NREM	non-rapid eye movement
ODI	oxygen desaturation index
OSA	obstructive sleep apnea
ox-LDL	oxidized low-density lipoprotein
$PaCO_2$	partial arterial carbon dioxide tension
PaO_2	partial arterial oxygen tension
PAP	positive airway pressure
REM	rapid eye movement
ROS	reactive oxygen species
SaO_2	arterial oxygen saturation
SCD-1	stearoyl coenzyme A desaturase-1
SD	standard deviation

SREBP-1	sterol regulatory element binding protein-1
TC	total cholesterol
TG	triglyceride
TIB	time in bed
TIB90%	percentage of time in bed with arterial oxygen saturation less than 90%
TIMP	tissue inhibitors of metalloproteinase
TNF-α	tumor necrosis factor-α
VLDL	very low-density lipoprotein
vWAT	visceral white adipose tissue
WBC	white blood cell count

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1. INTRODUCTION

1.1. Obstructive sleep apnea

The most common form of sleep-related respiratory disorders is obstructive sleep apnea (OSA). OSA is characterized by repetitive overnight hypoxic episodes and subsequent microarousals caused by a complete or partial collapse of the upper airways. OSA has a negative impact on the health-related quality of life the risk of work- and traffic-related accidents worldwide and is a highly underdiagnosed and undertreated disease. Its importance is based on the fact that it increases the risk of cardiovascular and cerebrovascular diseases, hypertension, neuropsychiatric disorders, diabetes mellitus, dyslipidemia, mental health problems, personality changes, and daytime sleepiness, among others.

1.1.1. Prevalence

Prevalence data depend on the definition of OSA, the measurement methodology, and the selected population. In general, when defined as repetitive upper airway obstruction during sleep, OSA is a very common disorder, with recent data from the United States and Europe suggesting that between 14% and 49% of middle-aged men have clinically significant OSA. Nonetheless, as mentioned above, it is an underdiagnosed disease worldwide, i.e. ~85% of all cases are not diagnosed (1).

It is important to note that the prevalence of OSA is approximately three to four times higher in patients with cardiovascular diseases (CVD) and particularly high in patients with hypertension (70-83%), heart failure (> 50%) and stroke. (2).

1.1.2. Pathogenesis

In general, any pathological change or normal variant that narrows the upper airway when awake will predispose the individual to obstructive apnea or hypopnea when asleep. The pharyngeal part of the airways (pharynx) can be considered as a flexible tube whose exceptional mobility is essential for speech and swallowing, but its ability to collapse due to its structure can disturb breathing. The diameter of this part of the airways depends largely on the activity of the dilator muscles in the airway walls that play a key role in maintaining the airways open during sleep (3,4).

It is well known that during the non-rapid eye movement (NREM) phase of sleep, the activity of the pharyngeal muscles decreases while the activity of the intercostal muscles increases. In contrast, in rapid eye movement (REM) sleep, the diaphragm becomes the main inspiratory muscle - so the activity of the muscles of inspiration outweighs the activity of the muscles holding the pharynx wide.

During inspiration, the air pressure in the pharynx is below atmospheric pressure, and the size of the pharyngeal lumen depends on the balance between the narrowing force that results from this suction pressure and the dilating force generated by the small muscles attached to the upper airway, which contract with each inspiration and normally stabilize the floppy walls of the pharynx. At sleep onset, there is a reduction in pharyngeal luminal area and a reduction in upper airway muscle activity, both of which are exaggerated in OSA. Surface mucosal factors may also influence airway patency, especially in subjects with mucosal inflammation from repetitive trauma and resultant loss of sensation (3,4).

All factors that impair the function of the pharyngeal dilatator muscles (fatigue, myopathy, neuropathy, anatomical abnormalities narrowing the airway lumen, increased amount of peripharyingeal adipose tissue, fluid retention [edema] in tissues due to heart failure or end-stage renal disease, changes in respiratory stimulation during sleep triggered by changes in partial arterial oxygen tension [PaO_2] and/or in partial arterial carbon dioxide tension [$PaCO_2$]) act in the direction to narrow the airway lumen. Depending on the degree of airway narrowing the process can generate symptoms from snoring to severe OSA.

Due to the factors mentioned above, patients with OSA breathe with increased mechanical stress in the upper respiratory tract during sleep. In order to maintain the conductivity of the upper respiratory tract, it is necessary to over-activate the neuronal compensatory mechanisms, which in turn however leads to the exhaustion of the neuromuscular reflex function in the long run. In support of this concept, neuropathological studies have described degeneration of pharyngeal mucosal receptors, motor and sensory neuropathy, and neuronal cell death in motor nuclei. In the background of the process, the role of vibration damage caused by snoring and chronic intermittent hypoxia (CIH)-induced airway inflammation can be assumed, which leads to irreversible damage and remodeling over time (3)

1.1.3. Sleep fragmentation

As discussed above, in OSA, partial or complete occlusion of the upper airway between the soft palate and the larynx occurs during sleep, primarily during inhalation. As a result, airflow decreases (hypopnea) or stops (apnea). During an apnea, the patient performs gradually more powerful, but ineffective breathing movements, however, breathing cannot start until an awakening reaction increases or restores the tone of the upper airway muscles. This cycle of apnea, hypopnea and micro-awakenings is repeated several times per hour during the night, so the lack of normal breathing is usually accompanied by oxygen desaturation and a gradual increase in $PaCO_2$ in repeated, relatively long episodes.

As a result of a series of micro-awakenings, the sleep structure is fragmented and the quality of sleep will be significantly deteriorated leading finally to daytime deficit symptoms (5). The main clinical symptoms of OSA include loud snoring, episodes of stopped breathing during sleep, frequent nocturnal awakenings, nycturia, morning weakness and occasionally headache, dry mouth, excessive daytime sleepiness, difficulties in concentration during the day, mood changes, attention and memory deficits, and decreased libido.

1.1.4. Risk factors

OSA is more common in obese individuals with increased neck circumference (males: >42 cm, females: >37cm), even in the absence of other associated risk factors. Recent studies have shown that the size of intraabdominal fat mass, which can be estimated by abdominal circumference, is strongly correlated with the severity of OSA. This may be due to the resistance to leptin, a molecule that is produced by adipocytes and regulates appetite and energy release (6).

In addition to obesity, the main risk factors include age and male gender. There is a significant gender-related effect in OSA since the disease is 2-3 times more common in men than in women. It has been suggested that female sex hormones may play a protective role in the process since the prevalence of OSA after menopause becomes similar to that of men.

OSA can occur at any age, but the incidence of sleep apnea increases with age. Typically, men around the age of 50 are diagnosed with the disease, and then the prevalence is rising to the age of 65, while there is finally a so-called plateau phase. Age-

dependent anatomical changes leading to a greater risk of collapse of the upper airways may explain, at least in part, the increase in the prevalence of OSA in the elderly population (7).

Other risk factors of OSA include upper airway narrowing due to craniofacial and soft tissue abnormalities, genetic predisposition (positive family history), smoking, nasal congestion, certain medical conditions (hypertension [HT], diabetes, Marfan syndrome, acromegaly, hypothyroidism, end-stage renal disease, congestive heart failure, chronic obstructive pulmonary disease [COPD], neurological disorders and pregnancy), medications and substances (including alcohol, benzodiazepines, and narcotics) (6).

1.1.5. Classifications

The American Academy of Sleep Medicine (AASM) classifies OSA according to the apnea-hypopnea index (AHI), a parameter representing the number of apnea and hypopnea events per hour of sleep. The AHI values for adults are categorized as follows: mild OSA is 5<AHI<15 events/hour; moderate OSA is 15<AHI<30 events/hour and severe OSA is when AHI is ≥30 events/hour (8). To set up the diagnosis, the gold standard method is polysomnography that should be performed in an established sleep laboratory.

1.2. OSA and cardiovascular diseases

1.2.1. Changes in hemodynamic parameters during an apnea episode

In OSA, the obstruction of the upper airways results in hypoxia, and as a cardiac response to hypoxia reflex bradycardia (diving reflex) occurs instead of lung inflation at the beginning of the apnea cycle. Inhalation against obstructed airways results in a significant decrease in intrathoracic pressure, an increase in cardiac afterload, and further acute changes both in pulmonary arterial pressure and blood flow. Increased venous reflux that occurs as a result of the decreased intrathoracic pressure, initially causes increased right ventricular diastolic volume and a leftward shift of the intraventricular septum.

Consequently, the left ventricular volume decreases leading again to adverse hemodynamic changes, i.e. lower cardiac output and systemic blood pressure level. However, during an apnea episode, the process moves in the opposite direction, when

(mainly due to the increasing hypoxemia and hypercapnia) there is a pronounced increase in heart rate and blood pressure due to increased sympathetic tone induced by the awakening response (9). Thus, the blood pressure decreases at the beginning of an apnea episode and then increases until the end of apnea (15-80 mmHg). Similarly, synchronized with periods of apnea and hypopnea, a decrease in heart rate is followed by an increase in this parameter due to micro-awakenings forming thereby the so-called bradycardia-tachycardia swing phenomenon (48-150/min fluctuation) (10).

The episodes of apneas, hypopneas, and micro-awakenings repeat over and over during the period of sleep causing thereby a significant hemodynamic load on the whole circulation and generating acute heart failures (i.e. brady- and tachyarrhythmias).

Moreover, it is important to note that the effects of the acute cardiovascular stress factors mentioned above accumulate over time that increase the risk for cardiovascular diseases in patients with OSA. The homeostatic control mechanisms of the cardiovascular system will be overloaded and autonomic dysfunctions will occur when the autonomic nervous system does not work properly.

1.2.2. Effects of OSA on the nervous system

During physiological sleep the autonomic nervous system exerts a number of important regulatory functions in our body: the whole circulatory system takes a rest, the blood pressure and the heart rate decrease by 10-15% when compared to the state of wakefulness, the parasympathetic activity dominates in the NREM phase of the sleep, while the sympathetic activity increases in the REM period. In OSA, sleep fragmentation increases the sympathetic activity resulting in the lack of the aforementioned physiological changes. Moreover, there is evidence that in patients with OSA, there is an increased sensitivity to hypoxemic stimulation in peripheral chemoreceptors (carotid and aortic bodies) (9). As a result, vasoconstriction and hypertension may develop.

Under physiological conditions, pulmonary inhalation and exhalation are controlled by the autonomic nervous system via receptors in the lung and chest wall. However, this sympatholysis is deficient in the apneas and hypopneas periods of OSA, thereby also contributing to increased sympathetic tone and noradrenaline secretion (9). In addition, increased sympathetic activation stimulates renin release, leading to increased circulating levels of pressor angiotensin II and aldosterone.

As a result of all these mechanisms, in patients with OSA, there is an increase in sympathetic tone that is coupled with pathological pulse and blood pressure variability, which are known to be risk factors for CVD. OSA-induced hypertension typically does not show a nocturnal decrease and is often therapy-resistant (this is the so-called non-dipper type of hypertension).

In addition, hypoxemia can also act via the chemoreflex to induce vagal activation to the heart simultaneously with sympathetic activation to most other vascular beds (the diving reflex). Profound vagal activation can take place at the beginning of obstructive apnoea in some patients with OSA and can result in bradyarrhythmias ranging from sinus bradycardia and atrial ventricular block to asystole. Thus, during apnea, similar to sympathetic activation, parasympathetic activity is also increased in patients with OSA (6).

1.2.3. Oxidative stress, inflammation, and endothelial dysfunction

Accumulating evidence suggests that the repetitive sequences of desaturation-reoxygenation in OSA lead to endothelial dysfunction, systemic inflammation, and increased formation of reactive oxygen species (ROS), which provoke and maintain oxidative stress (11).

Oxidative stress is responsible for initiating several inflammatory cascades. It promotes systemic and vascular inflammation, vascular endothelial damage, and vascular remodeling, all these processes play a pivotal role in the development and progression of atherosclerosis and atherothrombosis (12). Increased systemic inflammation in OSA is evident by increased levels of C-reactive protein (CRP), oxidized low-density lipoprotein (ox-LDL), and pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin-6 (IL-6). Furthermore, it has been suggested that ROS produced during CIH can also directly activate matrix metalloproteinases (MMPs). Some investigators found a positive correlation between serum levels of MMP-9 and IL-6 and TNF-α in patients with OSA indicating that inflammation may play a role in the regulation of certain members of the MMP family (6,13).

In OSA, endothelial dysfunction is the result of complex processes. The endothelium plays a key role in vascular homeostasis by regulating vasoconstriction, vasodilation, intravascular coagulation, and inflammation. A major component of this

process is the decreased bioavailability of the vasodilator molecule nitric oxide (NO) coupled with increased endothelial production of vasoconstrictor agent endothelin. NO mediates various anti-inflammatory, antioxidant, and antithrombotic effects that prevent endothelial cell damage, inhibit vascular smooth muscle proliferation and increase platelet activation and aggregation (14).

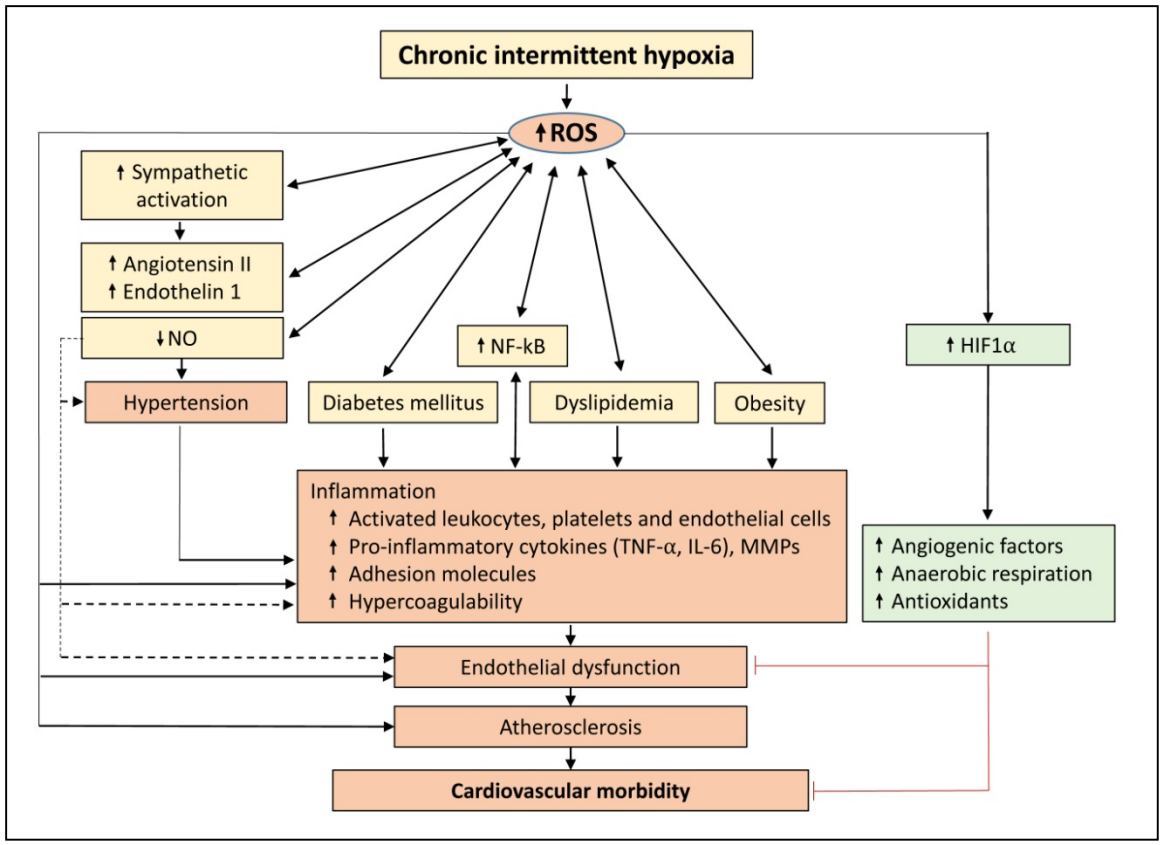

Figure 1. **Relationship between oxidant production, inflammation, endothelial dysfunction and development of cardiovascular complications in OSA**
ROS: reactive oxygen species, NO: nitric oxide, NF-κB: nuclear factor-κB, HIF-1α: hypoxia-inducible factor-1α, IL-6: interleukin-6, MMP: matrix metalloproteinase, TNF-α: tumor necrosis factor-α (adapted from 6)

1.2.4. Atherosclerosis

Atherosclerosis involves several interrelated processes such as oxidative stress, vascular inflammation, and sympathetic activity (Figure 1) (6). OSA can be considered a provocative state of atherosclerosis due to a number of factors: OSA is associated with dyslipidemia, elevated inflammatory markers, insulin resistance, hypertension, and oxidative stress, all of which cause endothelial dysfunction, intimal arterial cell migration,

foam cell formation, and smooth muscle cell migration and proliferation in the endothelium (12).

Accordingly, several studies have reported that OSA is associated with increased carotid intima-media thickness and a higher incidence of cervical plaques (early atherosclerotic lesions), independently of other cardiovascular and metabolic diseases. In line with this view, a correlation has been found between oxygen saturation and the intimal-media layer thickness of the common carotid artery (12). Given that cardiovascular consequences are primarily induced by CIH, the severity of these complications is usually more closely correlated with the oxygen desaturation index (ODI) than with the apnea-hypopnea index (AHI).

Arterial stiffness, an established predictor of late cardiovascular events is independently associated with OSA; and a further increase in this parameter can be observed when OSA is associated with hypertension or metabolic syndrome (MS) (6).

1.2.5. OSA as a cardiovascular risk factor

On one hand, OSA can generate acute cardiac alterations that occur already during one sleep cycle. On the other hand, there is evidence that OSA is associated with a number of adverse health consequences in the long run including for example the development and progression of CVD (15). The acute cardiovascular stress signals that occur during recurrent episodes of apnea, hypopnea, and micro-awakening in patients with OSA include hypercapnia, hypoxemia, fluctuations in intrathoracic pressure, and the awakening response elicited by the central nervous system (arousal). Overall, it is believed these factors cause oscillations in hemodynamic parameters including heart rate, blood pressure, and other cardiac functions, even within one sleep cycle.

Moreover, accumulating evidence suggests that these repetitive sequences of desaturation-reoxygenation lead to sympathetic hyperreactivity, oxidative stress, systemic inflammation, endothelial dysfunction, hypertension, dyslipidemia and insulin resistance, which all may contribute to increased cardiovascular morbidity and mortality in patients with OSA (11,15).

1.2.6. Role of MMPs in OSA-induced atherosclerosis

In recent years a number of new markers have emerged with the potential to predict patients' risk for CVD including members of the family of MMPs (16,17). MMPs are substrate-specific endopeptidases that catalyze the degradation of various structural proteins of the extracellular matrix. In health, MMP activity is closely controlled by their specific antagonists, the tissue inhibitors of metalloproteinases (TIMPs). An imbalance of MMPs and TIMPs has been widely implicated in the development of atherosclerosis and its complications (18-21). Disruption of the balance between MMPs and TIMPs can lead to smooth muscle cell proliferation, inflammatory cell infiltration, collagen deposition, vascular remodeling, and plaque formation.

Several lines of evidence indicate that MMPs are important mediators in the process of accelerated atherosclerosis in the CIH-induced oxidative stress in OSA, as well (22). Accordingly, an early study by Ye et al. reported that intermittent hypoxia/reoxygenation was a predictor of enhanced circulating MMP-9 in OSA patients (23), while Chuang et al. found that MMP-9, but not MMP-1, -2, -3 and TIMP-1 increases during sleep in patients with OSA (24). The contribution of MMP-9 to the development of CVD in OSA has been suggested in other studies as well (25,26).

1.3. OSA and metabolic dysregulation

1.3.1. Dyslipidemia

Dyslipidemia (alone or as part of MS) is a major contributor to the development of cardiovascular diseases in OSA. In general, the condition is characterized by increased total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG), and decreased high-density lipoprotein cholesterol (HDL-C) levels.

Several mechanisms are responsible for the development of dyslipidemia in OSA as depicted in Figure 2 (27,28). In addition, genetic factors may play a role in the process (29). On the one hand, sleep fragmentation *per se* results in increased appetite which directs dietary preferences towards increased fat and carbohydrate intake and reduces satiety.

On the other hand, CIH up-regulates hypoxia-inducible factor-1 (HIF-1) in the liver, which activates sterol regulatory element-binding protein-1 (SREBP-1) and stearoyl

coenzyme A desaturase-1 (SCD-1). As a result, hepatic steatosis and increased lipoprotein secretion occur. Furthermore, there is evidence that CIH increases lipolysis in the adipose tissue increasing thereby free fatty acids (FFA) flux to the liver. Additionally, CIH may also inhibit lipoprotein clearance. Overall, the increase in lipoprotein secretion and the inhibition of lipoprotein clearance lead to dyslipidemia, often with a rise in very-low-density lipoprotein (VLDL) fraction as well (30-32).

Additionally, there is a disruption in the regulation of the normal hormonal changes associated with sleep/wake cycles in the body (e.g., thyroid and growth hormones, etc.) which may also adversely affect lipid metabolism. It appears that these hormonal changes in OSA and their metabolic and cardiometabolic consequences are more or less reversible (32,33).

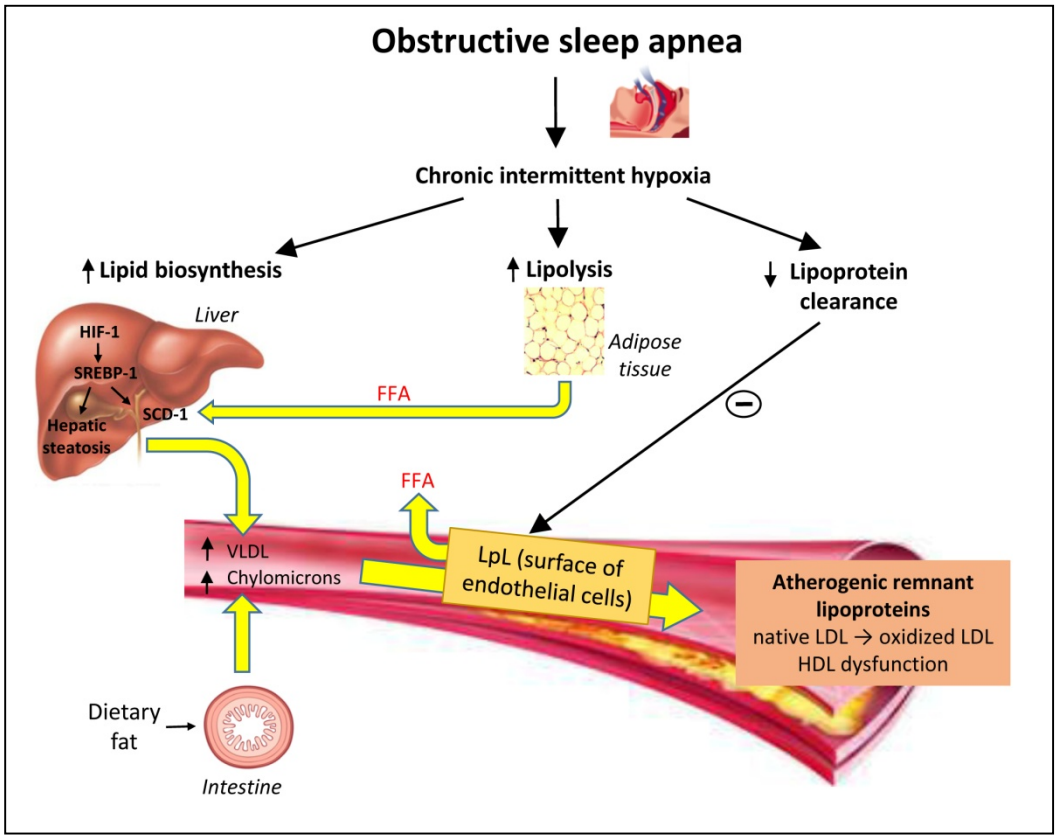

Figure 2. **Dysregulation of lipid metabolism in OSA**

FFA: Free fatty acids, HIF-1: Hypoxia-inducible factor-1, SREBP-1: Sterol regulatory element-binding protein-1, SCD-1: Stearoyl coenzyme A desaturase-1, VLDL: very low-density lipoprotein, LDL: low-density lipoprotein (adapted from 28)

In addition to these regulatory processes, the generation of oxidized and therefore dysfunctional lipids as a consequence of increased oxidative stress may be an important factor in the development of dyslipidemia in OSA. In support of this concept, ox-LDL is produced in patients with OSA due to lipid peroxidation, which is much more atherogenic than its non-oxidized form. On the other hand, HDL dysfunction can also be detected in OSA, which is manifested in a decreased antiatherogenic effect of HDL: i.e. the inhibitory effect of HDL on the oxidative conversation of LDL is reduced. The degree of HDL dysfunction correlates with the severity of OSA and the degree of oxidative stress. This oxidative stress accelerates the pathogenic processes associated with dyslipidemia in OSA and thus leads to the development of progressive atherosclerosis.

Indeed, recent studies in animal models have identified dyslipidemia as a potentially important mediator of accelerated atherosclerosis in OSA patients (34,35). Nonetheless, although dyslipidemia is common in patients with OSA (36), the causal relationship between OSA and dysregulation of the lipid metabolism remains contradictory (30,31).

1.3.2. Impaired glucose tolerance

In OSA, impaired glucose tolerance is mainly attributed to increased sympathetic tone. CIH reduces glucose uptake in muscles, while the oxidative stress-induced decrease in pancreatic B-cell function leads to the development of glucose intolerance, insulin resistance, and type 2 diabetes. Diabetes-induced micro- and macroangiopathy, as well as OSA-induced dyslipidemia and increased ox-LDL production all promote the development and progression of atherosclerosis in patients with OSA (6, 37-39).

1.3.3. Obesity

In OSA sleep fragmentation induces changes in leptin-signaling pathways in the hypothalamus and thus in appetite regulation which in turn moves dietary preferences towards increased fat and carbohydrate intake and a reduced feeling of satiety. As a consequence, the desire for energy-boosting foods, and a constant appetite leads to obesity (29). The effect of OSA-induced processes on visceral white adipose tissue (vWAT) is twofold: while the increased sympathetic activity due to CIH promotes the lipolysis in this tissue, the sleep fragmentation causes proliferation of adipocytes (29).

According to the traditional view of vWAT, it is defined as an energy store. Nonetheless, the role of vWAT is more complex in metabolic regulation than just an endocrine organ with a large number of secretory cells and adipocytes. Equally important, adipocytes secrete several hormones and cytokines (e.g., adipokines) that play an important role in the development of cardiovascular and metabolic complications (i.e. metabolic syndrome) in OSA. In addition, it should be noted that the visceral adipose tissue contributes to the development of chronic, low-grade systemic inflammation independently of OSA as well. (29). Overall, it can be concluded that vWAT is involved in the development of both the OSA and metabolic syndrome (a pathologic condition characterized by the combination of insulin resistance, dyslipidemia, and hypertension).

1.4. Therapy of OSA

1.4.1 Positive airway pressure therapy

The most effective treatment of severe OSA is positive airway pressure (PAP) therapy (40). This treatment aims to provide continuous airway pressure through the upper respiratory tract to the lungs through a special mask (non-invasive respiratory support). The pressure is measured in centimeters of water pressure (cm H_2O). During this type of treatment, air flow is introduced into the airways to maintain a continuous pressure to constantly stent the airways open in people who are breathing spontaneously. The biggest advantage of the treatment is that it acts along the entire length of the pharyngeal section that may collapse, thus in contrast to surgical procedures it is not a prerequisite for its application to know the exact location of the occlusion.

When used, PAP eliminates obstructive sleep events by stabilizing the upper respiratory tract. Improvements in daytime somnolence, neurocognitive function, and quality of life have been shown to be also associated with continuous PAP use through the elimination of sleep fragmentation (41). An important societal consequence is that improvements in neurocognitive function also reduce the risk of work- and traffic-related accidents (6). The fact that PAP can alleviate the adverse cardiovascular consequences of OSA and thus can reduce the cardiovascular risk of treated patients is extremely important because the majority of patients with OSA are obese and generally have a number of other cardiovascular risk factors.

1.4.2. Types of PAP therapy

Several types of PAP therapy can be used such as the continuous positive airway pressure (CPAP), the bilevel positive airway pressure (BIPAP), and the auto-titrating continuous positive airway pressure (AutoCPAP) therapy. During CPAP treatment, which is the most common treatment option for OSA, the device provides a constant pressure during exhalation and inhalation.

BIPAP treatment on the other hand produces higher inspiratory pressure and lower expiratory pressure. The difference between the two modalities is the pressure support. This improves alveolar ventilation, which may be useful in OSA complicated with COPD, but it has not been shown to be more beneficial in the treatment of OSA in general. Higher pressure can be generated with this mode.

Regarding to AutoCPAP, it automatically adjusts pressure values during sleep according to the severity of each obstructive breathing event. Using this treatment option pressure can be better tolerated by most patients, which can improve the compliance to the treatment.

1.4.3. Current recommendations with CPAP therapy in Hungary

Currently, CPAP therapy is recommended for patients with moderate (AHI>15)/severe (AHI>30) OSA, and for patients with mild OSA when it is associated with certain other conditions (84). BIPAP is recommended for severe OSA, if obstructive symptoms cannot be eliminated with CPAP or deterioration in symptoms occurs during proper treatment (40).

Several lines of evidence indicate that the effect of fixed pressure CPAP and AutoCPAP devices on cardiac and/or metabolic parameters is different. It has been suggested that AutoCPAP devices may have limitations in their ability to adjust the pressure to the actual needs of the patients (42). This can lead to extra sympathetic stimuli, which can cause adverse cardiovascular and/or metabolic effects. In fact, for the above reasons, AutoCPAP is not recommended in patients with complicated OSA.

OSA in younger and middle-aged individuals has a different phenotype than in the elderly patients, i.e. it is more closely associated with metabolic syndrome in younger patients. In elderly patients, due to advanced age airway anatomy and collapsibility play a relatively greater role in the pathogenesis of OSA. Taken together, these findings suggest that cardiometabolic diseases in the elderly occur independently of OSA, which

is worth considering in terms of differential diagnosis, prognosis, and treatment options in terms of the expected therapeutic effect of CPAP.

1.4.4. Effect of CPAP on sleep parameters and lung function

CPAP treatment improves respiratory mechanics and prevents the repetitive overnight hypoxic episodes and subsequent microarousals caused by complete or partial collapse of the upper airway. The effectiveness of the therapy is related to a significant reduction in AHI and better SaO_2 (8). When using CPAP, day and night symptoms of OSA will improve, i.e. sleep efficiency will be better, excessive daytime sleepiness will decrease, daytime performance and cognitive dysfunction will improve. Many of the beneficial effects of CPAP will occur already after a few days of treatment (6).

The application of CPAP eliminates vibration-related injuries caused by snoring and improves the decreased neuromuscular compensatory mechanisms (4). Additionally, improvements in lung mechanics result in increased vital capacity, functional residual capacity, oxygenation (43), and elimination of the extreme fluctuations in the intrathoracic pressure.

Although CPAP therapy effectively eliminates the collapse of the upper airways and improves the symptoms, it does not always have a demonstrable beneficial effect on cardiovascular and metabolic parameters (29).

1.4.5. Effect of CPAP on the cardiovascular effects of OSA

1.4.5.1. Hypertension

Several studies including meta-analyses on cardiovascular outcomes have shown that CPAP lowers blood pressure and that this improvement is more pronounced in the so-called OSA-induced non dipper hypertensive patients (29,44). In general, the decrease in blood pressure in normotensive patients is modest (~2.5 mmHg per day during treatment) while patients already taking hypertensive antihypertensive drugs or those with more severe OSA show a more pronounced decrease (>5 mmHg) (14).

1.4.5.2. Cardiac arrhythmias

Some studies suggest that CPAP is able to reduce the number of fatal and non-fatal cardiovascular events, including arrhythmias, and myocardial infarctions, which are predominantly associated with repetitive sympathicotonia (40,44).

In other studies, the effect of CPAP on hypertension and cardiovascular events was not significant, but in a subgroup of patients who used the device for more than 4 hours per night, a close association could be demonstrated. Another recent study analyzing the long-term effects of CPAP showed a beneficial effect of CPAP treatment on blood pressure in those subjects in whom it was combined with weight loss. Indeed, several studies failed to show a positive effect of CPAP therapy alone (6). However, sympathetic activation and increased blood pressure which are consequences of intermittent hypoxia can be improved with CPAP therapy.

1.4.5.3. Heart failure

Patients with heart failure also benefit from improved hemodynamic status due to increased ejection fraction, reduced afterload, and improved diastolic function (45). The function of the right and left ventricles may also improve.

1.4.6. Effect of CPAP on the metabolic effects of OSA

Regarding metabolic outcomes, several previous studies suggest that OSA may independently contribute to the development of metabolic syndrome (MS). Components of MS include systemic arterial hypertension, insulin resistance, dyslipidemia, and abdominal obesity. Of note that all of these four diseases are independently associated also with increased cardiovascular and cerebrovascular risk and therefore it may be a critical point to reduce cardiometabolic risk successfully.

The effect of CPAP therapy on MS has been studied by several research groups. In a randomized, placebo-controlled study of patients with MS and OSA, after 3 months of CPAP therapy, 20% of patients do no longer meet the criteria for MS. In contrast, another randomized controlled trial with shorter CPAP therapy (only up to 6 weeks) showed no effect on this outcome (38).

In terms of the components of MS, CPAP treatment appears to have the greatest effect on arterial blood pressure. Several randomized, placebo-controlled studies showed a significant reduction in arterial blood pressure with CPAP therapy in OSA. In another study, when CPAP therapy was withdrawn from previously CPAP-treated patients, arterial pressure increased significantly.

The other main component of MS is insulin resistance. The percentage of glycated hemoglobin (HbA1c), a marker of long-term glycemic control in diabetic individuals,

was positively correlated with the severity of OSA in type 2 diabetes. Several studies have shown an improvement in HbA1c level after 3 months of CPAP treatment in patients who have used CPAP for more than 4 hours per day. Thus, CPAP treatment may improve glucose metabolism in type 2 diabetes, but a good adherence to CPAP treatment is essential to achieve this beneficial result.

With regard to abdominal or visceral obesity, in a randomized, controlled trial in which nearly half of participants had both OSA and type 2 diabetes, a significant decrease in body mass index (BMI) was observed after 3 months of CPAP therapy along with a decrease in visceral and subcutaneous fat. However, in non-diabetic OSA patients, there was no evidence of an effect of CPAP therapy on adipose tissue distribution.

Overall, the adverse effects of OSA on metabolic parameters in adult individuals may be masked by the concomitant presence of obesity, which also has a negative impact on metabolic health. Long-standing obesity may also inhibit the ability of CPAP treatment to reverse OSA-mediated pathology on the basis that obesity can cause similar organ dysfunction (46).

1.4.7. Effect of CPAP on dyslipidemia

Although regular CPAP therapy relieves patients of the most common symptoms of OSA, its effect on lipid profile is inconclusive. For example, a recent meta-analysis concluded that CPAP improves dyslipidemia (47), and there is also evidence for a reduction in postprandial triglyceride and total cholesterol levels as a result of CPAP treatment (48), Campos-Rodriguez et al. reported that 3 months long CPAP therapy had no additive beneficial effect on the lipid profile of women with moderate-to-severe OSA compared with conservative treatment (49). Likewise, Keenan et al. found no differences in fasting lipid levels between CPAP adherent and non-adherent OSA patients (50). Based on meta-analyses of randomized controlled trials, Lin et al. concluded that CPAP lowers TC, TG, and HDL-C levels (51), while Xu et al. argued that only TC, but not TG, HDL-C, and LDL-C fractions are significantly modified by CPAP in patients with OSA (52).

It is important to emphasize that the duration of the trials investigating the effect of pressure therapy on lipid profiles mentioned above was generally short, i.e. less than 12 months. However, OSA is a lifelong condition with several late cardiovascular and other complications. The development of dyslipidemia in OSA is also a long process that

is affected by numerous factors including the severity of the disease, nocturnal hypoxia, obesity, sympathetic activity, diet, and exercise (31,53,54). Thus, it is clear that findings obtained in short-term studies cannot be simply extrapolated to subjects treated with CPAP for longer periods of time.

1.4.8. Effect of CPAP on MMPs

As mentioned above, CPAP treatment results in nearly complete remission of symptoms of OSA. However, the effects of CPAP on OSA comorbidities including cardiovascular outcomes are much less unambiguous. A number of studies have been published on the short-term effects of CPAP on established CVD risk factors, for example on oxidative stress (55). Regarding MMPs, it was found that 1-month CPAP treatment significantly decreases serum levels of MMP-9 but does not affect TIMP-1 levels in a population of patients with mixed severity of OSA (13).

Nonetheless, the development of OSA-induced CVDs, and in particular atherosclerosis, is a long and progressive process that is modulated by numerous OSA independent factors such as systemic inflammation, sympathetic activity, obesity, diet, and exercise (56). Thus, it would be a mistake to extrapolate findings on the short-term effects of CPAP treatment on CVD risk factors and assume that they will be sustained over the long term. Indeed, the utility of CPAP in preventing CVDs in OSA has been questioned by a recent meta-analysis (57) that generated interesting pro and con arguments in this field (58,59).

1.4.9. Other therapeutic recommendations

In addition to CPAP therapy, general recommendations for patients with OSA include weight control, healthy eating, avoiding a sedentary lifestyle, and regular exercise. For example, it is believed that a 10-15% weight loss can reduce the AHI by 20-30%. However, on their own, these factors are usually effective only in mild forms of OSA and their effectiveness needs to be confirmed by a subsequent sleep study. Avoidance of drugs and stimulants that increase the symptoms of OSA can also be considered.

1.4.10. CPAP compliance in clinical practice

It is well known that after discontinuation of CPAP therapy, many symptoms of OSA usually return rapidly, thus continuous treatment is necessary from night to night to

prevent the adverse consequences of OSA. However, adaptation/compliance to therapy is often limited. The minimum expected CPAP usage time per day is 4 hours. According to studies investigating compliance to CPAP, patients give up airway therapy in 10-20% for various reasons, and many use the instrument less than 4 hours per day. Even patients with good therapeutic adherence occasionally discontinue CPAP therapy, such as on weekends or holidays, and perhaps during episodes of nasal congestion (60).

1.4.11. Consequences of CPAP withdrawal in OSA

As described earlier the pathophysiological mechanisms underlying hypertension and heart rate changes in OSA have been associated with intermittent hypoxia, increased sympathetic activity, increased oxidative stress, endothelial dysfunction, and changes in intrathoracic pressure that exert increased transmural pressure on the heart. These processes can generate sudden critical conditions and may result in the long-term structural transformation of the cardiovascular system, i.e. vascular remodeling. Increased sympathetic activity and autonomic disorders such as OSA-associated chemoreflex and baroreflex disorders may show reversibility with CPAP therapy, but the structural changes caused by OSA are irreversible.

Withdrawal of CPAP treatment provides information on acute functional effects related to existing structural changes, as the physiological consequences are reactivated by CPAP withdrawal. The mechanisms underlying the association between OSA and cardiovascular disease are manifold (e.g., the role of obesity-induced low-density background inflammation in maintaining OSA symptoms) and are not yet fully understood. The short-term CPAP treatment withdrawal allows patients to serve as their own "control group", bypassing most of the confounding factors typically associated with a multimorbid condition (60,61).

The CPAP withdrawal model is suitable for the study of the short-term physiological effects of OSA, apnea-related repetitive sympathicotonia, changes in blood pressure, and arrhythmias induced by changes in microcirculation.

In several studies, the immediate recurrence of disease-specific abnormal respiratory events following CPAP withdrawal was associated with more moderate oxygen desaturation compared to baseline, ie. before the initiation of CPAP therapy. Possible mechanisms of this effect include benign changes in upper airway anatomy,

ventilation (e.g., reduction of upper airway edema), and upper airway control mechanisms attributable to CPAP therapy (41). In a study that included 42 patients with severe OSA, two nights after CPAP withdrawal, the apnoe index (AI), AHI was 4% lower and was accompanied with a moderate oxygen desaturation compared to baseline. In contrast, patients with mild to moderate OSA did not exhibit such an improvement. A possible explanation is that in patients with mild disease, the tendency of the upper airways to collapse is less pronounced at baseline, and CPAP therapy has less room to improve on that (41).

Schwarz et. al have investigated the role of OSA as a risk factor for secondary systemic hypertension (60). The starting point of their study was based on previous meta-analyzes showing that CPAP therapy has a modest antihypertensive effect, a decrease of 2-3 mmHg. Withdrawal of CPAP for 2 weeks resulted in a statistically significant and clinically important increase in systolic and diastolic blood pressure values of 9 and 8 mmHg, respectively. The observed effect of CPAP was much greater than in previous studies. Changes in blood pressure after withdrawal were more pronounced in those individuals whose baseline blood pressure was lower, suggesting that OSA is a mediator of secondary hypertension in these patients.

Based on 1 and 2 week CPAP withdrawal studies, Phillips et al. found a link between increased blood pressure and heart rate and increased urinary catecholamine excretion, and progressively impaired endothelial function. However, this was not associated with changes in the levels of vascular inflammatory markers (CRP, IL-6, TNF-α), suggesting that acutely increased sympathetic activity and endothelial dysfunction may underlie the observed increases in blood pressure and heart rate (62).

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2. OBJECTIVES

Although OSA is a lifelong condition, most previous studies investigating the effect of CPAP therapy on various complications of OSA had covered often a limited time frame. Therefore, to explore the long-term effects of CPAP on dyslipidemia and cardiovascular risk factors, we had initiated a longitudinal study with a 5-year follow-up period in a cohort of patients with severe OSA. Additionally, we intended to test the effect of CPAP withdrawal in the same cohort.

Thus, the specific aims of my PhD thesis were:

1. To investigate the short- and long-term effect of CPAP therapy on disturbed lipid metabolism in a cohort of patients with newly diagnosed severe OSA by measuring levels of fasting lipids such as TC, TG, LDL-C, and HDL-C at various time-points between diagnosis and 5 years of CPAP treatment (Study 1).

2. To investigate the short- and long-term effect of CPAP therapy on established cardiovascular risk factors in a cohort of patients with newly diagnosed severe OSA by measuring the expression of MMPs and TIMPs at various time-points between diagnosis and 5 years of CPAP treatment (Study 2).

3. To investigate the effect of a 1-week CPAP withdrawal on sleep and cardiac parameters in a cohort of patients receiving 5-year CPAP therapy due to severe OSA (Study 3).

3. METHODS

3.1. Study 1 and 2

All methods were described in detail in the published articles attached to the dissertation.

3.2. Study 3

3.2.1. Study patients and design

Patients from the previous lipid and MMP related studies were approached to participate in the CPAP withdrawal study. Inclusion and exclusion criteria are detailed in Figure 3. In general, those who refused to take part in this study or had severe symptoms of CVDs, or were very high risk for these conditions were not selected. The research protocol was approved by the National Ethics Committee (OGYÉI/29037). All subjects gave written informed consent to participate in the study.

Diagnosis of OSA was established by overnight polygraphy at baseline (SOMNOscreen RC, SOMNOmedics GmbH, Electro Oxygen Ltd., Hungary) as described in detail earlier (63). During the 5-year control visit and after the 1-week CPAP withdrawal visit, patients were re-evaluated by overnight polygraphy for sleep and cardiac parameters such as AHI, ODI, percentage of time in bed (TIB) with <90% oxygen saturation (TIB90%), and the mean and the lowest SaO_2. The following morning participants were assessed for blood gases, lung function, BMI, smoking habit, comorbidities, and Epworth sleepiness scale (ESS).

3.3.2. Statistical analysis

Data normality was tested by the Kolmogorov-Smirnov test. Clinical and polygraphic variables were analyzed either by the Friedman test followed by the Dunns test (non-parametric data) or repeated-measures analysis of variance with post hoc test (Newman-Keuls test) for multiple comparisons (parametric data). Correlation coefficients were calculated by Spearman's method. Calculations were performed by GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA). The threshold of significance was set at $p<0.05$. Data are presented as mean±SEM or median with interquartile ranges when appropriate.

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4. RESULTS

4.1. Study 1

4.1.1. Enrollment, demographics, and clinical characteristics

From patients referred to our sleep laboratory at the National Koranyi Institute of Pulmonology for suspicion of OSA during the period of recruitment, 62 fulfilled inclusion criteria and agreed to participate in Study 1 (Figure 3). During follow-up, 29 patients had to be withdrawn during the follow-up period. Demographic and clinical data of the 33 patients who completed Study 1 are presented in Table 1.

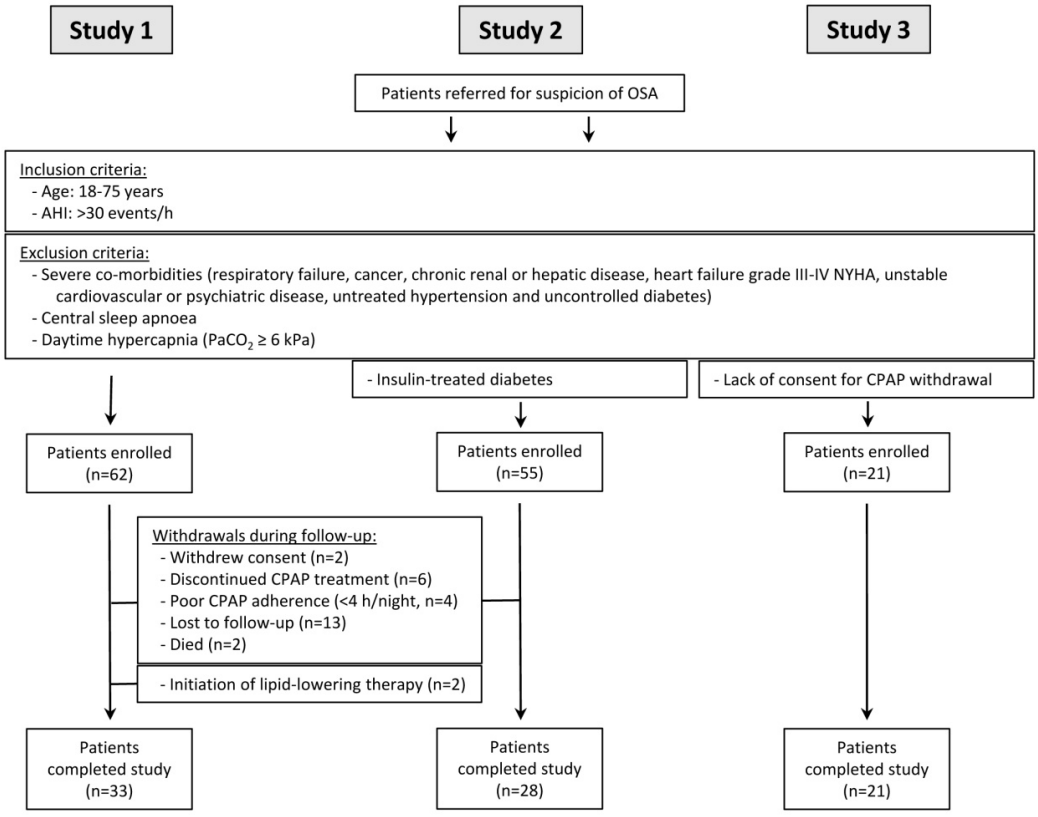

Figure 3. **Flow chart showing the profile of studies 1, 2 and 3**

OSA: obstructive sleep apnea, CPAP: continuous positive airway pressure, AHI: apnoea-hypopnoea index, $PaCO_2$: partial arterial carbon dioxide tension (adapted from 63, 83 + unpublished data)

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Table 1.

Demographic and clinical characteristics of patients who completed the study

	Study 1	**Study 2**	**Study 3**
	Measure		
Demographics			
Subjects (n)	33	28	21
Age (years)	54.2 ± 10.3	54.0 ± 9.1	55.7 ± 1.7
Sex (male/female, n, %)	27 (82) / 6 (18)	22 (79) / 6 (21)	16 (76) / 5 (24)
Smoking history (n, %)			
Smokers	5 (15.2)	5 (17.9)	1 (4.8)
Ex-smokers	11 (33.3)	9 (32.1)	7 (33.3)
Non-smokers	17 (51.5)	14 (50.0)	13 (61.9)
Medical history (n, %)[#]			
Hypertension	20 (60.6)	16 (57.1)	13 (61.9)
GERD	5 (15.2)	3 (10.7)	4 (19.0)
CAD	4 (12.1)	4 (14.3)	-
Asthma/COPD	4 (12.1)	4 (14,3)	-
Diabetes	3 (9.1)	2 (7.1)	2 (9.5)
Allergic rhinitis	3 (9.1)	-	-
Major medication (n, %)			
Antihypertensives	12 (36.4)	12 (42.9)	8 (38.1)
Statins	5 (15.2)	3 (10.7)	3 (14.3)
Inhaled bronchodilators / cortico-steroids	4 (12.1)	4 (14.3)	1 (4.8)
Antidiabetics	3 (9.1)	2 (7.1)	1 (4.8)
Pulmonary function			
FVC (% predicted)	99.5 ± 13.8	100.7 ± 14.5	100.3 ± 14.3
FEV_1 (% predicted)	92.9 ± 18.5	94.6 ± 20.4	93.2 ± 17.3
FEV_1/FVC (%)	74.1 ± 8.32	74.7 ± 9.0	73.9 ± 8.5
Blood gases			
$PaCO_2$ (kPa)	5.11 ± 0.36	5.03 ± 0.4	5.13 ± 0.38
PaO_2 (kPa)	9.21 ± 1.27	9.40 ± 1.4	8.90 ± 0.74

Data are presented as mean±SD unless stated otherwise. CAD: coronary artery disease, GERD: gastroesophageal reflux disease, COPD: chronic obstructive pulmonary disease, FVC: forced vital capacity, FEV_1: forced expiratory volume in 1 second, $PaCO_2$: partial arterial carbon dioxide tension, PaO_2: partial arterial oxygen tension. [#]Co-morbidities affecting <3% of study subjects were not indicated, (adapted from 63, 83 + unpublished data)

4.1.2. Effect of CPAP therapy on clinical and polygraphic variables

As expected, initiation of CPAP therapy was associated with an improvement of several polygraphic variables recorded at baseline ($p<0.0001$ for each, Table 2). Likewise, subjective sleepiness, as assessed by the ESS score, normalized along with treatment ($p<0.0001$). In contrast, BMI, fasting glucose and CRP levels did not change in CPAP-treated subjects ($p>0.05$).

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With regard to the CPAP adherence of our patients, the mean nightly duration of CPAP usage was >4 hours in each subject at the 2- and 6-month and the 5-year visits; these data (mean ±SD) for the whole cohort are indicated in Table 2. Moreover, the percentage of days when patients used the device >4 hours/day was at least 70% for each subject, both at the beginning and at the end of the study. At 2 months and at the final (5-year) visit this index was 92.4±9.2 and 89.4±9.1%, respectively.

Table 2.

Effect of CPAP therapy on clinical and polygraphic variables during follow-up in Study 1

	Baseline visit	CPAP		
		2 months	6 months	5 years
Polygraphic data				
AHI (events/h)	58.0 (50.1-72.5)	1.7 (0.5-3.0)**	0.5 (0-2.1)**	2.3 (1.4-4.1)**
ODI (events/h)	61.1 (47.1-67.5)	3.0 (1.8-5.4)**	2.0 (1.0-4.2)**	2.3 (1.1-4.5)**
Mean SaO_2 (%)	91 (89-92)	94 (92-95)**	93 (92-94)*	94 (93-95)**
Minimal SaO_2 (%)	71 (64-77)	87 (85-89)**	85 (83-88)*	89 (86-91)**
TIB90% (%)	28.0 (14.8-42.5)	0.1 (0-3.4)*	0 (0-6.4)*	0 (0-0.3)*
ESS score	11.0 (7.5-14.5)	4.0 (4.0-5.0)*	3.0 (3.0-4.0)*	4.0 (2.0-7.0)**
Laboratory data				
WBC ($\times 10^9$/L)	7.1 ± 1.5	6.4 ± 1.3	7.1 ± 3.0	6.9 ± 1.2
CRP (mg/L)	8.0 ± 6.4	6.6 ± 7.1	8.7 ± 7.0	6.2 ± 4.3
Glucose (mmol/L)	6.6 ± 2.3	6.6 ± 2.4	6.9 ± 2.3	6.0 ± 1.8
BMI (kg/m^2)	35.2 ± 5.5	35.6 ± 5.8	35.1 ± 5.0	35.1 ± 5.8
CPAP adherence (h/night)#	–	6.21 ± 0.96	6.23 ± 1.14	6.43 ± 1.17

Data are presented as mean±SD or median (interquartile ranges). CPAP: continuous positive airway pressure, AHI: apnea-hypopnea index, ODI: oxygen desaturation index, SaO_2: arterial oxygen saturation, TIB90%: percentage of time in bed with arterial oxygen saturation less than 90%, ESS: Epworth sleepiness scale, WBC: white blood cell count, CRP: C-reactive protein, BMI: body mass index. #CPAP adherence data for the 2-month, 6-month and 5-year visits represent CPAP use in the first two months, months 3-6, and the last 6 months before the end of the study, respectively. *$p<0.01$ and **$p<0.0001$ vs. baseline visit, (63)

4.1.3. Effect of CPAP therapy on lipid profile

Following 2-month CPAP treatment, both TC and LDL-C levels were significantly decreased compared to baseline (TC: 5.62±1.25 vs. 5.18±1.08 mmol/L, $p<0.05$; LDL-C: 3.52±1.02 vs. 3.19±1.04 mmol/L, $p<0.05$; Figure 4). Similarly, 6 months post-treatment levels of these lipid fractions were reduced (TC: 4.83±1.2 mmol/L, $p<0.01$; LDL-C: 2.89±1.01 mmol/L, $p<0.01$). In contrast, serum TG and HDL-C levels at 2 and 6 months did not change significantly compared to baseline (TG: 2.1±0.7 and 1.97±0.95 vs. 2.13±1.05 mmol/L, $p>0.05$; HDL-C: 1.07±0.27 and 1.1±0.26 vs. 1.13±0.26 mmol/L, $p>0.05$).

Five year long CPAP treatment was associated with reduced levels of TC and LDL-C levels (TC: 5.1±1.02 mmol/L and LDL-C: 2.86±0.91 mmol/L, $p<0.01$ for each when compared to baseline). Additionally, there was some tendency towards increased HDL-C levels in these subjects (1.23±0.26 mmol/L, $p>0.05$). TG levels, on the other hand, still did not change (2.22±1.43 mmol/L, $p>0.05$). Mean changes in lipid fractions were similar following the different CPAP treatment periods (data not shown).

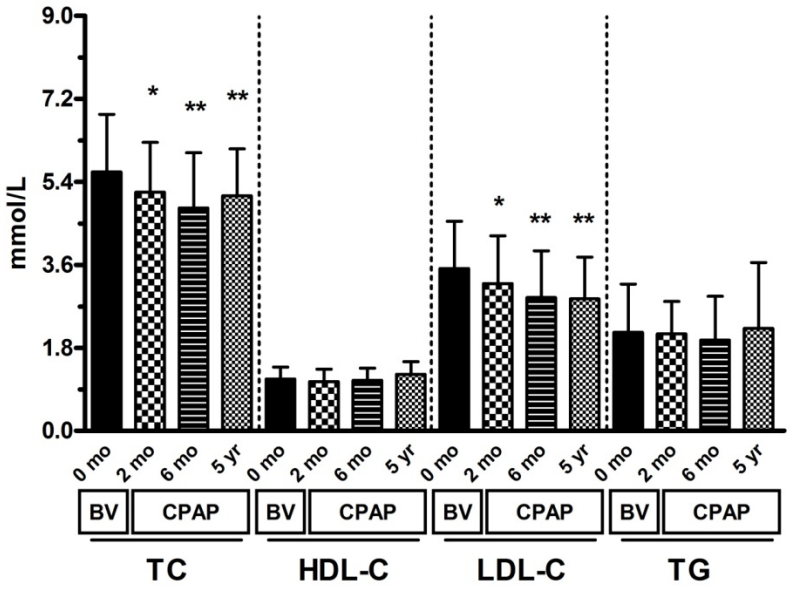

Figure 4. **Effect of CPAP therapy on serum lipid profile of patients with OSA during follow-up**

OSA: obstructive sleep apnea, BV: baseline visit, CPAP: continuous positive airway pressure, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TC: total cholesterol, TG: triglyceride. Error bars represent SD. $^{*}p < 0.05$ and $^{**}p < 0.01$ versus baseline visit, (63)

4.1.4. Subgroup analysis

First, we investigated whether the outcome of the study is affected when the analysis is restricted to patients not using lipid-lowering (i.e. statins) medications. Such restriction had no effect on the outcome, this subgroup of patients (n=28) was not different from the whole cohort (i.e. both short- and long-term CPAP therapy lowered their serum TC and LDL-C, but not TG and HDL-C levels) (Table 3).

Table 3.

Effect of CPAP therapy on lipid levels in patients (n=28) not using lipid-lowering medications

Lipids (mmol/L)	Baseline visit	CPAP		
		2 months	6 months	5 years
TC	5.82 ± 1.17	5.22 ± 1.17**	5.06 ± 1.16*	5.17 ± 1.05**
HDL-C	1.14 ± 0.26	1.09 ± 0.27	1.13 ± 0.25	1.25 ± 0.27
LDL-C	3.7 ± 0.95	3.25 ± 1.1*	3.11 ± 0.94*	2.96 ± 0.86**
TG	2.14 ± 1.09	2.04 ± 0.73	1.93 ± 0.85	2.11 ± 1.14

Data are presented as mean±SD. CPAP: continuous positive airway pressure, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride. *$p<0.05$ and **$p<0.01$ vs. baseline visit, (63)

To further explore the effect of CPAP treatment, changes in lipid levels were analyzed in patients stratified by median age and BMI (Table 4). We found that 5 years past treatment initiation, the mean difference in change in TC and LDL-C levels was greater in younger patients (n=17) and those with higher BMI (n=17) ($p<0.05$ for each). In contrast at 2 or 6 months of CPAP treatment no such differences between the subgroups could be detected (data not shown).

Table 4.

Mean changes (compared to baseline) in lipid fractions in patients stratified by the median age and BMI after 5 years of CPAP treatment

Clinical variables	Lipids			
	TC	HDL-C	LDL-C	TG
	mmol/L			
Age				
<56 years old (n=17)	-0.94 (-1.55, -0.32)	0.07 (-0.04, 0.17)	-1.0 (-1.48, -0.52)	0.01 (-0.79, 0.8)
>56 years old (n=16)	-0.08 (-0.45, 0.29)*	0.14 (0.01, 0.27)	-0.29 (-0.65, 0.07)*	0.17 (-0.72, 1.1)
BMI				
<35 kg/m2 (n=16)	-0.14 (-0.51, 0.24)	0.12 (-0.01, 0.26)	-0.33 (-0.7, 0.04)	0.16 (-0.78, 1.1)
>35 kg/m2 (n=17)	-0.88 (-1.5, -0.24)#	0.08 (-0.03, 0.19)	-0.97 (-1.5, -0.48)#	0.02 (-0.74, 0.77)

Data are presented as mean (95% confidence intervals [CI]). CPAP: continuous positive airway pressure, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, TG: triglyceride, BMI: body mass index. *$p<0.05$ vs. patients <56 years old, #$p<0.05$ vs. patients with BMI <35 kg/m^2, (63)

4.1.5. Correlations

At baseline, significant positive correlations were found between BMI and AHI ($r=0.436$, $p<0.05$), ODI ($r=0.458$, $p<0.01$) or TIB90% ($r=0.345$, $p<0.05$). In addition, BMI showed a weak negative association with HDL-C (BMI: $r=-0.263$, $p<0.05$), but not with other lipid fractions. No correlations were found between polygraphic variables and lipid levels or other clinical variables (data not shown). As expected, the above correlations disappeared upon CPAP treatment, and during the follow-up visits no clinically important associations emerged (data not shown).

4.2. Study 2

4.2.1. Enrollment, demographics, and clinical characteristics

From patients referred to our sleep laboratory for suspicion of OSA during the period of recruitment, 55 fulfilled inclusion criteria and agreed to participate in Study 2 (Figure 3). During follow-up, 27 patients had to be withdrawn for various reasons during the follow-up period. Demographic and clinical data of the remaining 28 patients who completed Study 2 are presented in Table 1.

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Table 5.

Effect of CPAP therapy on clinical and polygraphic variables during follow-up in Study 2

	Baseline visit	CPAP		
		2 months	6 months	5 years
Polygraphic data				
AHI (events/h)	57.9 (51.3-72.5)	1.6 (0.5-2.95)**	0.6 (0-2.1)**	2.3 (1-4.0)**
ODI (events/h)	61.1 (50-67.5)	3.0 (2-5.35)**	1.8 (0.9-4.5)**	2.3 (1.1-4.5)**
Mean SaO_2 (%)	90 (88-94)	93 (92-95)**	94 (91-95)*	93 (92-95)**
Minimal SaO_2 (%)	73 (65-77)	86 (82-88)**	85 (82-89)*	87 (85-92)**
TIB90% (%)	27.0 (14.8-45.0)	0.2 (0-4.2)*	0.1 (0-6.4)*	0 (0-0.25)*
ESS score	12.0 (7.2-14.1)	3.7 (3.5-5.8)*	2.9 (2.9-4.2)*	3.8 (1.8-7.1)**
Laboratory data				
WBC ($\times 10_9$/L)	7.3 ± 0.3	6.5 ± 0.3	7.3 ± 0.7	7.0 ± 0.2
CRP (mg/L)	8.6 ± 1.3	6.4 ± 1.6	9.4 ± 1.6	6.4 ± 0.9
Glucose (mmol/L)	6.6 ± 0.4	6.8 ± 0.5	6.5 ± 0.4	5.8 ± 0.3
BMI (kg/m^2)	35.7 ± 1.1	35.8 ± 1.2	35.1 ± 1.8	35.7 ± 1.2
CPAP adherence (h/night)#	–	6.07 ± 0.18	6.09 ± 0.22	6.47 ± 0.24

Data are presented as mean±SD or median (interquartile ranges). CPAP: continuous positive airway pressure, AHI: apnea-hypopnea index, ODI: oxygen desaturation index, SaO_2: arterial oxygen saturation, TIB90%: percentage of time in bed with arterial oxygen saturation less than 90%, ESS: Epworth sleepiness scale, WBC: white blood cell count, CRP: C-reactive protein, BMI: body mass index. #CPAP adherence data for the 2-month, 6-month, and 5-year visits represent CPAP use in the first two months, months 3-6, and the last 6 months before the end of the study, respectively. *$p<0.01$ and **$p<0.0001$ vs. baseline visit, (83)

4.2.2. Effect of CPAP therapy on sleep and clinical variables

Compared to baseline, initiation of CPAP therapy resulted in marked improvements in sleep parameters such as AHI, ODI, SaO_2, TIB90% ($p<0.01$ or better for each, Table 5). According to the ESS score, CPAP therapy normalized subjective sleepiness as well ($p<0.0001$). BMI and CRP levels on the other hand did not change significantly during the 5-year follow-up period ($p>0.05$).

The mean nightly duration of CPAP usage was >4 hours in each subject at 2 and 6 months and the 5-year visit (Table 5). Each subject used the CPAP device >4 hours/day

in at least 70% of days throughout the study with 89.6±9.2%.in the last 6 months preceding the final visit.

4.2.3. Early effects of CPAP therapy on serum MMP profile

Using undiluted serum, all analytes but MMP-13 fell within the detection range of the protein array. Serum levels of MMP-8 and MMP-9 markedly decreased at the 2-month control visit as compared to those at the time of OSA diagnosis (146 (79-237 [95% CI 85-217]) vs. 287 (170-560 [95% CI 226-403]) pg/mL at baseline, p=0.028 and 10.1 (7.1-14.1 [95% CI 7.8-13.2]) vs. 12.7 (10.4-15.6 [95% CI 10.8-15.0]) ng/mL at baseline, p=0.029, respectively; Figure 5), while the level of the remaining analytes did not markedly change during this early period of CPAP treatment. At 6 months only MMP-8 was significantly below the level observed at OSA diagnosis, but a similar tendency for a decrease could be observed for MMP-9 as well (146 (54-276 [95% CI 54-276]) vs. 287 (170-560 [95% CI 226-403]) pg/mL at baseline, p=0.018 and 8.1 (4.7-13.6 [95% CI 4.7-13.6]) vs. 12.7 (10.4-15.6 [95% CI 10.8-15.0]) ng/mL at baseline, p=0.083, respectively).

4.2.4. Serum MMP profile after 5 years of CPAP therapy

Despite uninterrupted CPAP therapy, at the 5-year control visit increased levels of MMP-8, MMP-9 and TIMP-4 were detected compared to those at the time of OSA diagnosis (578 (255-1167 [95% CI 295-1070]) vs. 287 (170-560 [95% CI 226-403]) pg/mL at baseline, p=0.017; 15.0 (12.4-24.2 [95% CI 12.9-22.7]) vs. 12.7 (10.4-15.6 (95% CI 10.8-15.0]) ng/mL at baseline, p=0.014 and 893 (496-1542 [95% CI 586-1428]) vs. 828 (387-1211 [95% CI 482-1047]) pg/mL at baseline, p=0.023, respectively; Figure 5). The 5-year change in the level of other MMPs did not reach statistical significance compared to baseline but some, such as MMP-2 and MMP-10 when compared to early time-points following initiation of CPAP treatment also significantly increased by the end of the study (Figure 5).

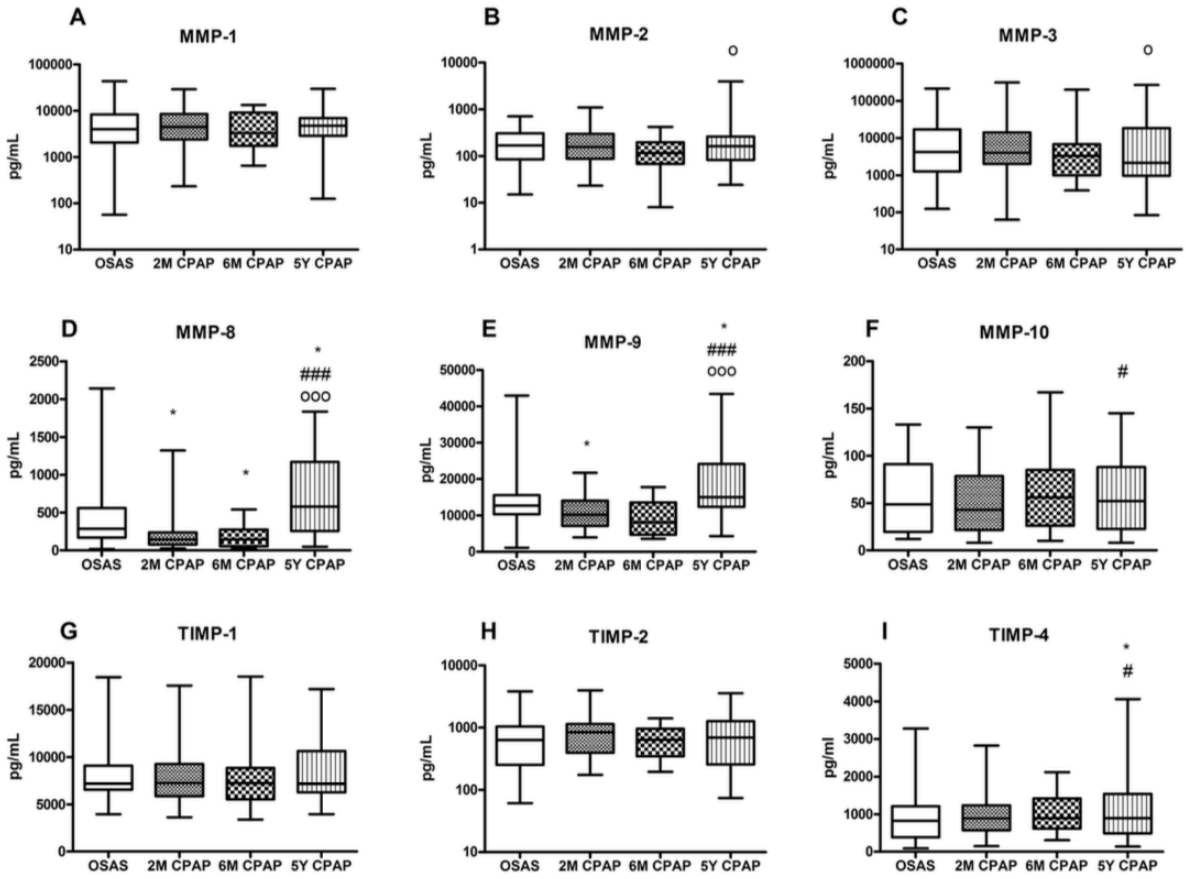

Figure 5. **Serum MMP (Panel A–F) and TIMP (Panel G–I) levels of study subjects during the 5-year follow-up**

Marker levels were assessed at the time of diagnosis (OSA), at 2 and 6 months (2M and 6M), and 5 years of CPAP therapy (5Y). OSAS: obstructive sleep apnea syndrome (baseline visit), CPAP: continuous positive airway pressure, MMP: matrix metalloproteinase, TIMP: tissue inhibitor of matrix metalloproteinase. Standard box plots with median (25th and 75th percentiles) and whiskers (at the minimum and maximum values) are shown. $^{*}p < 0.05$ vs. baseline visit; $^{\#}p < 0.05$ and $^{\#\#\#}p < 0.001$ vs. 2-month visit; $^{\circ}p < 0.05$ and $^{\circ\circ\circ}p < 0.001$ vs. 6-month visit, (Simon et al., Sci Rep, 10: 8609), (83)

4.2.5. Subgroup analysis

To explore the possibility that short- and long-term changes in serum MMP-8, MMP-9, and TIMP-4 levels were affected by patient characteristics, data were reanalyzed after stratifying patients by median age, BMI, and whether or not they have been taking medication for hypertension at the time of OSA diagnosis. However, both at 2 months and 5 years past CPAP treatment initiation the mean difference in change between these subgroups was similar (Table 6).

Table 6.

Mean changes in MMP-8 and MMP-9 and TIMP-4 levels after 2 months and 5 years of CPAP treatment in patients stratified by the median age and body mass index and whether or not they have been taking medication for hypertension at the time of OSA diagnosis.

	MMP-8		MMP-9		TIMP-4	
	Δ2M-BL pg/mL	Δ5Y-BL pg/mL	Δ2M-BL pg/mL	Δ5Y-BL pg/mL	Δ2M-BL pg/mL	Δ5Y-BL pg/mL
Age						
<56 years old (n=15)	-137 (-366-91)	473 (225-722)	-3057 (-6914-800)	5085 (1019-9152)	-34 (-206-139)	311 (-132-755)
≥56 years old (n=13)	-257 (-674-160)	2 (-429-433)	-4041 (-10652-2570)	2935 (-1639-7510)	28 (-97-153)	239 (-93-571)
BMI						
<35 kg/m^2 (n=14)	-304 (-687-79)	195 (-218-609)	-5317 (-11788-1155)	5393 (797-9989)	34 (-98-167)	379 (-157-914)
>35 kg/m^2 (n=14)	-85 (-311-142)	313 (5-622)	-1804 (-5003-1395)	2782 (-1151-6714)	-44 (-220-131)	177 (4-351)
HT						
no HT (n=12)	-29 (-332-274)	252 (-177-681)	-1256 (-4444-1932)	3240 (-223-6703)	26 (-151-202	191 (-151-533)
treated for HT (n=16)	-297 (-591-(-)4)	256 (-66-577)	-4979 (-10300-342)	4722 (92-9353)	-28 (-173-117)	343 (-77-763)

Data are presented as mean (95% confidence intervals [CI]). OSA: obstructive sleep apnea, CPAP: continuous positive airway pressure, MMP: matrix metalloproteinase, TIMP: tissue inhibitor of matrix metalloproteinase, 2M: 2 months of CPAP treatment, 5Y: 5 years of CPAP treatment, BL: baseline, HT: hypertension, BMI: body mass index, (83 suppl. inf.)

4.2.6. Correlations between MMPs and sleep parameters

No correlations were found between MMPs, TIMPs, and main polygraphic variables such as AHI, ODI, and TIB90% at the time of OSA diagnosis (Figure 7).

4.2.7. Correlations between MMPs and clinical variables

At baseline, a strong positive association was found between MMP-8 and white blood cell count (WBC) ($r=0.62$, $p<0.001$; for neutrophils: $r=0.76$, $p<0.001$) and a weaker one for CRP ($r=0.47$, $p=0.019$). Notably, both of these correlations disappeared after 2 months of CPAP treatment ($r=-0.02$, $p=0.933$ for WBC; $r=-0.04$, $p=0.855$ for CRP). MMP-3 and MMP-9 showed a weak positive correlation with WBC only at OSA diagnosis ($r=0.40$, $p=0.033$ and $r=0.43$, $p=0.021$; respectively). Another potentially important, albeit weak negative correlation was observed between MMP-1 and BMI ($r=-0.37$, $p=0.049$). No other clinically important correlations were detected (Figure 8).

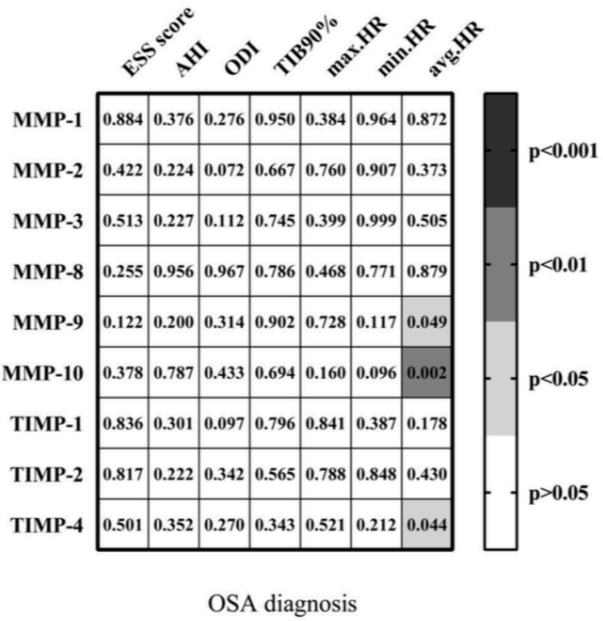

Figure 7. **Correlations between MMPs/TIMPs and main polygraphic variables and heart rate at OSA diagnosis**

P-values of Spearman correlations are indicated in the cell for each pair and gray scale coded according to the label at right. OSA: obstructive sleep apnea, MMP: matrix metalloproteinase, TIMP: tissue inhibitor of matrix metalloproteinase, ESS: Epworth sleepiness scale, AHI: apnea-hypopnea index, ODI: oxygen desaturation index, TIB90%: percentage of time in bed with arterial oxygen saturation less than 90%, HR: heart rate, avg: average, (83 suppl. inf.)

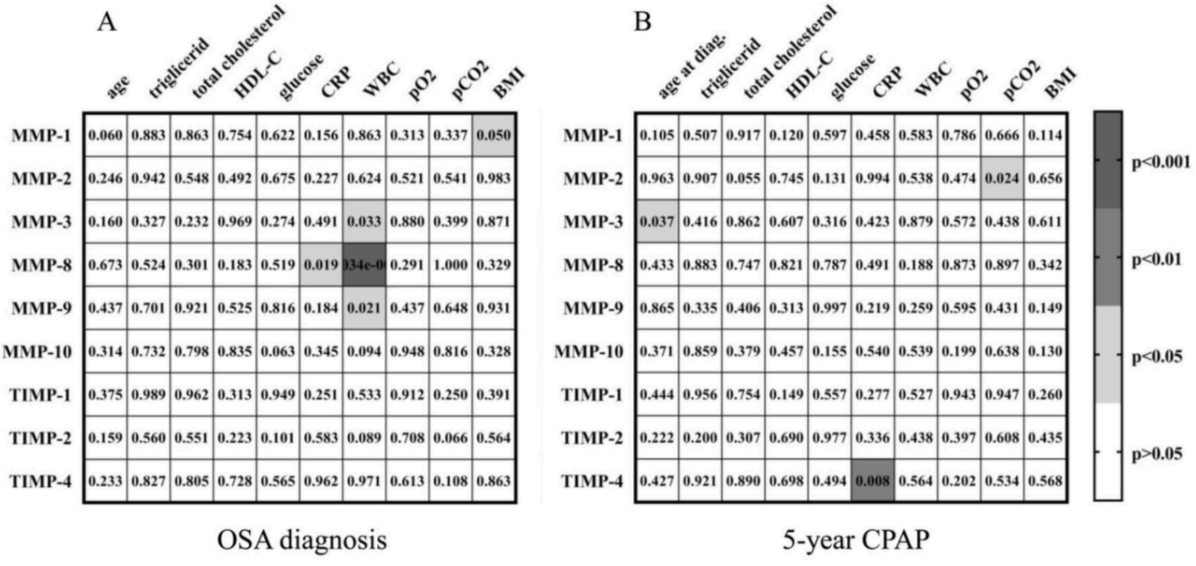

Figure 8. **Correlations between MMPs/TIMPs and clinical variables at OSA diagnosis (Panel A) and after 5 years of CPAP treatment (Panel B)**

P-values of Spearman correlations are indicated in the cell for each pair and gray scale coded according to the label at right. OSA: obstructive sleep apnea, CPAP: continuous positive airway pressure, MMP: matrix metalloproteinase, TIMP: tissue inhibitor of matrix metalloproteinase,

HDL- C: high-density lipoprotein cholesterol, FVC: forced vital capacity, FEV_1: forced expiratory volume in 1 second, CRP: C-reactive protein, WBC: white blood cell count, $PaCO_2$: partial arterial carbon dioxide tension, PaO_2: partial arterial oxygen tension, BMI: body mass index, (83 suppl. inf.)

4.3. Study 3

4.3.1. Sleep physiological parameters

At the time of diagnosis (baseline), the sleep diagnostic parameters of participating patients were in the pathological range (AHI: 57.6±3.9 events/h, ODI: 57.9±4.0 events/h, min SaO_2: 72.2±2.1%, mean SaO_2: 90.9±0.6%, TIB90%: 26.7±4.5%). Following the initiation of CPAP therapy, all the above parameters quickly improved, reaching normal values already in the short-term (2-6 months) that remained so in the long-term (5 years) (Figure 9).

However, at 1 week after CPAP withdrawal, all sleep parameters except AI and ODI (41.2±4.4 vs. 32.4±4.4 and 57.0±4.0 vs. 44.3±4.6 events/h, at baseline and after 1-week CPAP withdrawal, respectively; $p<0.01$) deteriorated close to the value measured at the time of OSA diagnosis ($p>0.05$). Interestingly, 1-week CPAP withdrawal had no effect on the ESS score.

4.3.2. Cardiovascular parameters

Long-term CPAP treatment also improved several cardiac parameters such as maximum and mean heart rate (HR), acceleration (AC), deceleration (DC) and arrhythmia indices compared with those at the time of OSA diagnosis (all with $p <0.001$) (Figure 10). After 1 week of CPAP withdrawal, mean HR values and the DC capacity significantly deteriorated compared to the 5-year CPAP control visit (63.0±1.8 vs. 67.4±1.8; $p<0.01$ and 6.7±3.0 vs. 12.7±3.4 $p<0.05$, respectively), while the arrhythmia index and the AC capacity (5.9±2.5 vs. 10.0±3.7 and 6.8±3.0 vs. 12.8±3.4; $p>0.05$, respectively) hardly changed.

Compared to the 5-year control values, 1 week CPAP withdrawal resulted in mild but significant increase of morning systolic blood pressure values (127.6±3.6 vs. 135.0±4.5 mmHg; $p=0.02$), while it only led to a trend level deterioration of morning diastolic blood pressure (79.5±2.0 vs. 83.9±2.4 mmHg; $p=0.064$) and blood gas values (9.7±0.15 vs. 9.3±0.26 kPa: $p=0.072$ for PaO_2 and 5.2±0.09 vs. 5.5±0.23 kPa for $PaCO_2$: $p=0.058$).

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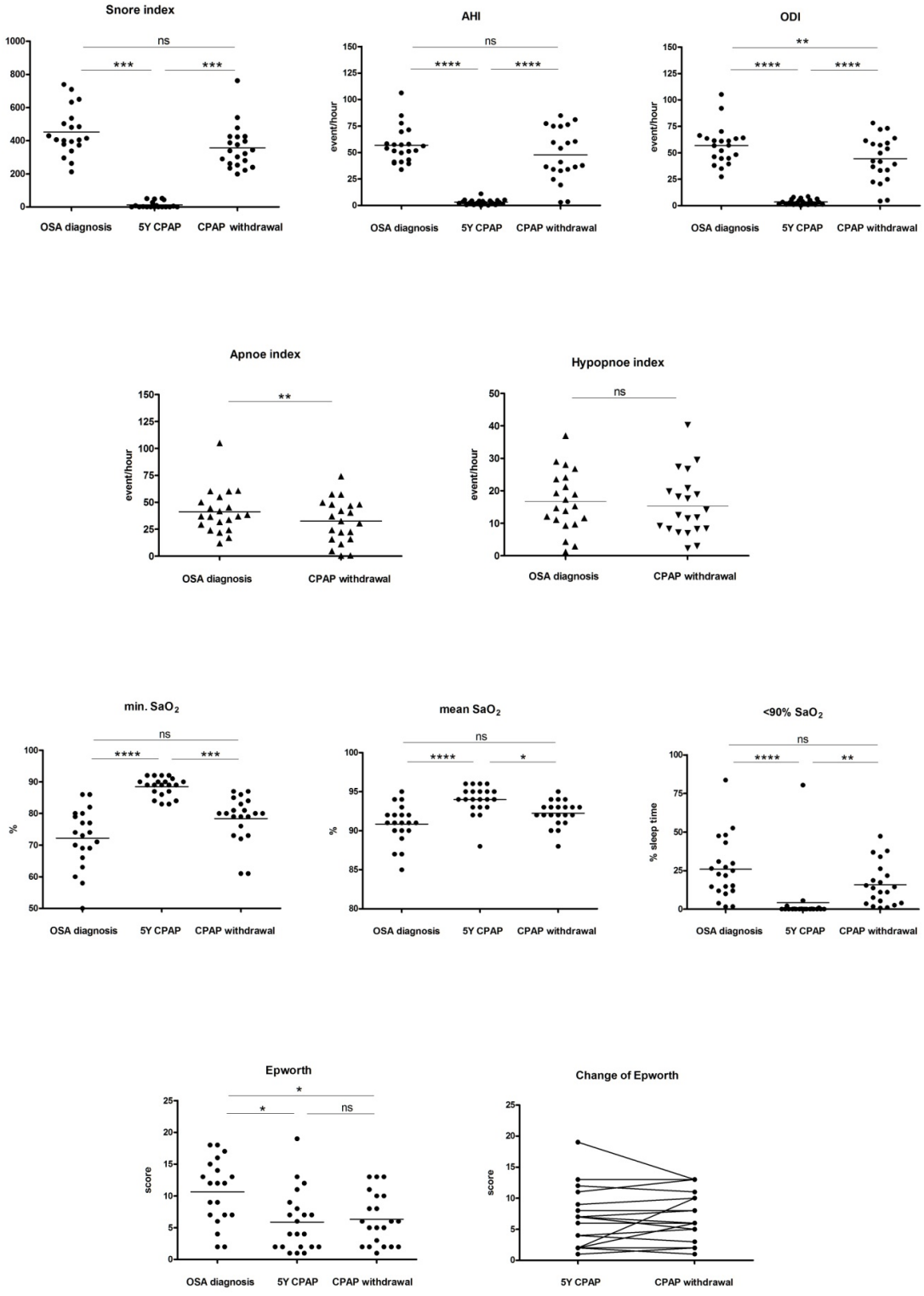

Figure 9. **Changes of sleep parameters and ESS scores after 1 week of CPAP withdrawal**

Horizontal lines indicate mean. OSA: obstructive sleep apnea, AHI: apnea-hypopnea index, ODI: oxygen desaturation index, SaO_2: oxygen saturation, CPAP: continuous positive airway pressure, ESS: Epworth sleepiness scale, ns: non-significant. $^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p<0.001$, $^{****}p<0.0001$, (unpublished figure)

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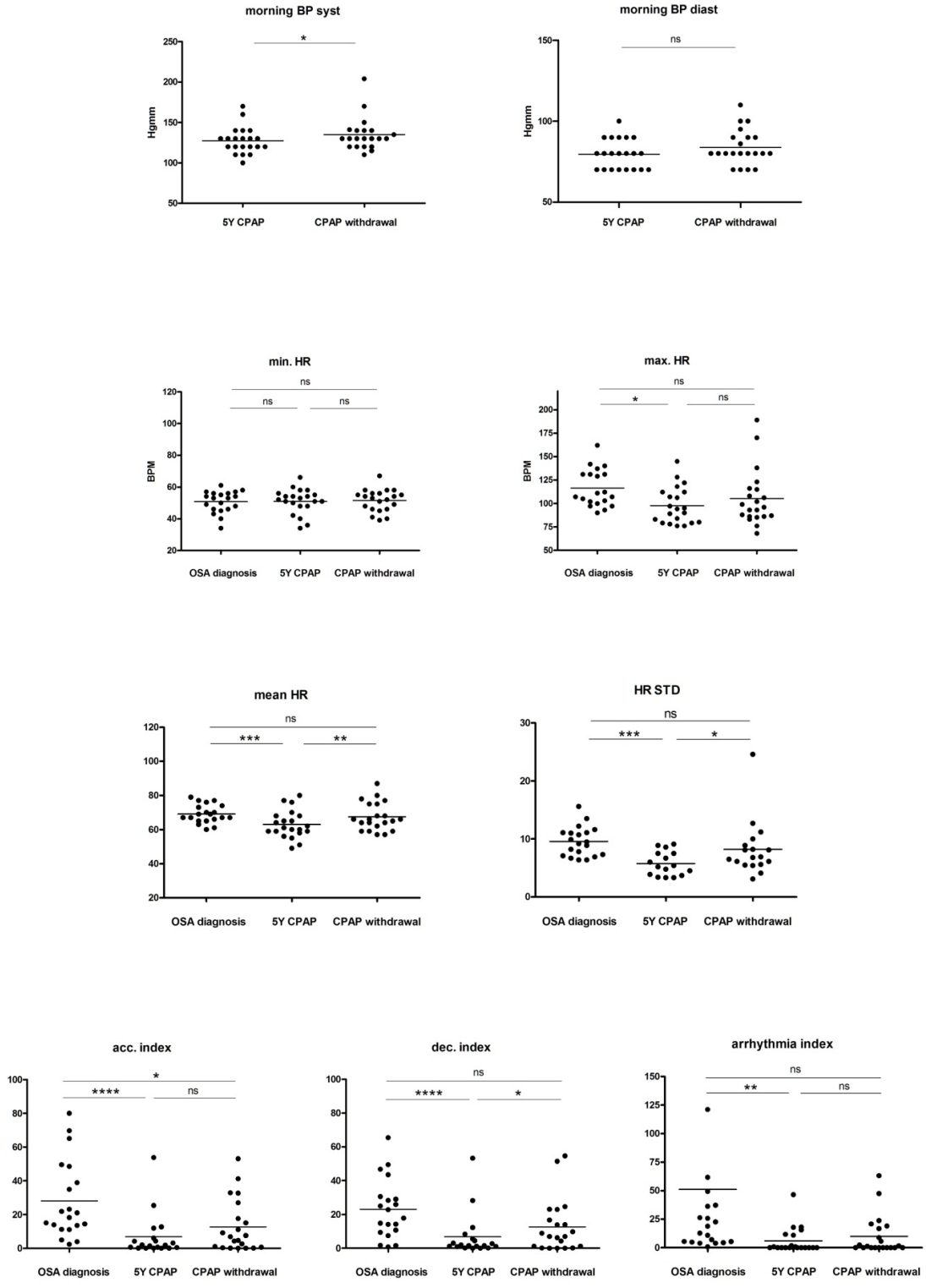

Figure 10. **Changes of cardiovascular parameters after 1 week of CPAP withdrawal**

Horizontal lines indicate mean. OSA: obstructive sleep apnea, BP: blood pressure, HR: heart rate, HR STD: heart rate standard deviation, acc: hearth rate acceleration, dec: hearth rate deceleration, CPAP: continuous positive airway pressure, ns: non-significant. $^{*}p< 0.05$, $^{**}p< 0.01$, $^{***}p<0.001$, $^{****}p<0.0001$, (unpublished figure)

DOI:10.14753/SE.2022.2666

		acceleration index	deceleration index	arrhythmia index	max. HR	min. HR	mean HR	HR STD
OSA diagnosis	Epworth score	0.008	0.083	0.951	0.607	0.002	0.058	0.066
	AHI	0.095	0.016	0.867	0.271	0.123	0.464	0.340
	ODI	0.356	0.063	0.319	0.274	0.117	0.210	0.865
	min. SaO_2 (%)	0.728	0.712	0.024	0.075	0.508	0.948	0.996
	baseline SaO_2 (%)	0.758	0.326	0.959	0.245	0.482	0.110	0.767
	mean SaO_2 (%)	0.708	0.723	0.111	0.795	0.476	0.566	0.911
	<90% SaO_2 (%)	0.587	0.715	0.122	0.995	0.401	0.574	0.985
	snore index	0.980	0.985	0.035	0.545	0.608	0.152	0.560
	snoring episodes	0.552	0.618	0.055	0.432	0.840	0.228	0.326
5 year CPAP control visit	Epworth score	0.215	0.116	0.856	0.982	0.510	0.502	0.288
	AHI	0.858	0.600	0.081	0.796	0.156	0.101	0.970
	ODI	0.878	0.661	0.466	0.323	0.782	0.768	0.313
	min. SaO_2 (%)	0.609	0.876	0.774	0.528	0.530	0.413	0.226
	baseline SaO_2 (%)	0.623	0.908	0.532	0.640	0.658	0.951	0.901
	mean SaO_2 (%)	0.637	0.931	0.675	0.514	0.676	0.967	0.671
	<90% SaO_2 (%)	0.155	0.261	0.885	0.378	0.931	0.925	0.919
	snore index	0.917	0.848	0.584	0.784	0.445	0.822	0.815
	snoring episodes	0.890	0.749	0.228	0.762	0.524	0.8567	0.524
1 week CPAP withdrawal	Epworth score	0.521	0.768	0.948	0.971	0.149	0.701	0.307
	AHI	0.161	0.169	0.007	0.083	0.146	0.949	0.057
	ODI	0.130	0.119	0.003	0.078	0.233	0.846	0.064
	min. SaO_2 (%)	0.618	0.601	0.325	0.763	0.383	0.453	0.469
	baseline SaO_2 (%)	0.957	0.851	0.535	0.721	0.704	0.688	0.781
	mean SaO_2 (%)	0.081	0.144	0.426	0.324	0.306	0.460	0.136
	<90% SaO_2 (%)	0.054	0.070	0.105	0.141	0.553	0.377	0.088
	snore index	0.088	0.035	0.278	0.132	0.621	0.100	0.077
	snoring episodes	0.114	0.058	0.314	0.172	0.661	0.123	0.097

p<0.05
p<0.01

Figure 11. **P-values of correlations between sleep and cardiovascular parameters at the time of OSA diagnosis, after 5 years of CPAP use and after 1 week of CPAP withdrawal**

OSA: obstructive sleep apnea, AHI: apnea-hypopnea index, ODI: oxygen desaturation index, SaO_2: oxygen saturation, CPAP: continuous positive airway pressure, BP: blood pressure, HR: heart rate, HR STD: heart rate standard deviation, (unpublished figure)

4.3.3. Correlations

Initiation of CPAP therapy led to the disruption of all correlations between sleep and cardiac parameters that existed at the time of OSA diagnosis. The changes induced by 1 week CPAP withdrawal resulted in the appearance of correlations between AHI, ODI, and the arrhythmia index, correlations that did not exist at baseline. All correlations are shown in Figure 11

5. DISCUSSION

5.1. Study 1

In this study, we demonstrated that the application of CPAP therapy for as short as 2 months reduced fasting TC and LDL-C levels in a cohort of patients with severe OSA. Importantly, the beneficial effect of CPAP was not transient, and sustained even after 5 years of therapy, indicating that CPAP improves the lipid profile of patients with OSA over the long-term, potentially ameliorating the risk of cardiovascular disease associated with OSA.

Although the effect of CPAP treatment on dyslipidemia has been extensively investigated in recent years, previous studies had covered a limited time frame. To the best of our knowledge, our study exploring the effect of CPAP on fasting lipids over 5 years has to date the longest duration. Randomized controlled trials that followed changes in lipid profile as a result of CPAP treatment were limited to a maximum of 24 months (51,52), while the longest observational study had a 2-year follow-up period (50). In the latter study, no effect of CPAP use on lipid profile was observed. Similarly, in a small, but relatively long, observational study (mean duration of treatment: 13.9 months) a negative outcome was reported as well (64). In contrast, in the PREDICT trial, as one of the longest randomized trials, CPAP reduced the levels of TC and LDL-C at 3 months, but the effect was not sustained at 12 months (65).

Differences between the findings of these studies and the one presented here may, at least in part, be explained by differences in study populations: our study was restricted to patients with severe OSA (AHI>30), while in all aforementioned trials (50,64,65) patients with moderate disease (15<AHI<30) were also included. CPAP therapy has been reported to be more effective in OSA patients with severe disease (66,67). Animal studies suggest that the more severe the hypoxic stimulus is, the higher the degree of dyslipidemia that it triggers, and also, the effect of pressure therapy will be more likely beneficial in severe OSA (68). A further difference between the study populations is the age. For example, the PREDICT trial (65) was conducted in elderly patients (>65 years), where CPAP may be less effective. Indeed, as noted already, early favorable effects of CPAP on lipids were not sustained until the end of that study.

Contradictory data on the effects of CPAP therapy on the lipid profile may also be due to the consequence of using different CPAP modalities (auto-adjusting vs. fixed-pressure) in the clinical trials as these may have different effects on cardiac/metabolic parameters AutoCPAP devices may have limitations in their ability to properly adjust the pressure to actual requirements which may lead to extra sympathetic stimulations causing disadvantageous cardiac and/or metabolic effects (42). However, our results were not confounded by this factor since all our patients received fixed-pressure CPAP therapy.

Large cohort studies using for example data from the European Sleep Apnea Database indicate that OSA severity is independently associated with both TC and TG concentrations in patients with OSA (69). However, lipid levels in OSA are often confounded by the effects of comorbid obesity. In line with this theory, BMI but not AHI showed an inverse relationship with HDL-C in our study, in agreement with findings of others (70). Similarly, correlations between AHI and serum lipid levels recently observed both in the REM and non-REM sleep phases were lost after adjustment for BMI (71).

Changes in BMI during therapy may also influence the lipid profile of subjects as there is evidence that weight loss intervention alone can lead to a reduction in serum lipid levels (72). Earlier studies have reported disparate results in terms of BMI: in some cases, BMI increased (50,73), in another, it decreased (74) or in several cases, it remained unchanged (48,64,65) in subjects receiving CPAP therapy. In our study not only the cohort-wide average BMI did not change along with CPAP treatment, but the change of BMI over the 5 years was very small for individual subjects as well (the mean of the absolute values of change of BMI was 0.9 kg/m^2 while the SD of the change was only 1.55, with over 80% of patients having less than 5% change in BMI). This suggests that in our study cohort the observed changes in lipid profile cannot be attributed to changes in body weight.

The role of CPAP in disturbed glucose metabolism in OSA is again controversial. Some studies documented an improvement (75), while others saw no effect of pressure treatment on the glycaemic profile of patients with OSA (49,76). In our study we did not observe changes in fasting glucose, however, other possible measures (HbA1c, insulin resistance, etc.) had not been investigated.

Fifteen percent of our patients (n=5) used lipid-lowering agents (i.e. statins) at baseline (use of such medication was not an exclusion criterion). This policy likely had

some impact on our lipid data; however, in participants not using statins a similar beneficial effect of CPAP on the lipid status was demonstrated. Importantly, subjects in whom lipid-lowering medication was initiated during the follow-up were excluded from our study; thus, the effect of these agents could not be responsible for the improvements in lipids.

Our findings in subgroup analysis corroborated the results of a recent meta-analysis indicating that the effect of CPAP on lipids is more pronounced in patients who are younger and more obese (52). However, this relationship was detectable only after 5 years of treatment.

It is important to note that patients with poor CPAP adherence and those who discontinued CPAP therapy were excluded from our study. As a consequence, the nightly average duration of CPAP use (>6.4 h/night at 5 years) was much higher in our cohort than in many clinical trials published to date, where this parameter was found to be a critical determinant of the study outcome. We hypothesize that the excellent CPAP adherence might have significantly contributed to our favorable long-term findings.

The mechanism by which CPAP improves lipid metabolism is unclear. It can be speculated that a decrease in oxidative stress or elimination of intermittent hypoxia, sympathetic hyperreactivity, and sleep fragmentation are indirectly involved in the process (11,51).

Theoretically, CPAP independent factors could also explain the observed improvements in lipid profile. Although increasing daytime physical activity and switching to a healthier diet is routinely recommended to our patients during ambulatory visits, a limitation of our study is that such lifestyle factors were not directly controlled during follow-up. In retrospect, however, it is not likely that these factors played a significant role in our study, otherwise BMI, which can be considered as a surrogate marker of lifestyle (51), would be expected to improve as well. Since the BMI of most study participants did not change during the 5 years of the follow-up, no fundamental alterations in patients' diet and/or physical activity could be suspected.

Nonetheless, to gain at least some information on the current lifestyle of our patients, we made a short, post-trial survey among our patients using the so-called Simple Lifestyle Indicator Questionnaire (SLIQ) (provided in the Supplementary Appendix). It is a validated health-measurement scale suitable for the assessment of various individual

lifestyle components such as (i) diet, (ii) exercise, (iii) alcohol consumption, (iv) smoking, and (v) life stress (77,78). Since each component is equally weighted and can have a category score of 0, 1, or 2, the overall SLIQ scores, which are based on the five category scores, can range from 0 to 10. In general, a higher score represents a healthier lifestyle. During the survey, we were able to reach 30 study participants by phone. Their mean SLIQ score was 5.167±1.262, indicative of an average, moderately healthy lifestyle. Thus, it appears that our patients do not pay extra attention to lifestyle factors.

Some antihypertensives, including angiotensin-converting enzyme (ACE)-inhibitors and beta-blockers, are not lipid neutral; however, during the study, these drugs were initiated only for 18% (n=6) of the patients. When we repeated the TC and LDL-C analysis using data from the remaining 27 subjects (i.e. excluding data for the above-mentioned 6 patients), we found that the significance of the changes was not lost for either TC or LDL-C. Therefore, we can reasonably assume that the effect of newly prescribed antihypertensives, if any, did not play a significant role in the study outcome.

Polygraphy was a suitable method for the diagnosis of severe OSA in our cohort as the diagnosis was established as part of a comprehensive sleep study in patients with a high pretest likelihood of the disease. Polysomnography may yield a more accurate AHI and could more accurately distinguish between mild, moderate, and severe OSA cases. However, we can exclude the possibility that our patients were misclassified since at the time of diagnosis their median AHI was significantly higher (58 events/h) than the cut-off value used for the inclusion (30 events/h).

5.2. Study 2

Our study has demonstrated that CPAP therapy in patients with severe OSA results in a marked reduction in the serum levels of MMP-8 and MMP-9 in the short-term, while at the same time it has lesser or no effect on the concentration of several other members of the MMPs and TIMPs. The most striking finding of our study was that the beneficial effect of CPAP on key MMPs implicated in the progression of atherosclerosis was not sustained over the long-term as by the end of the 5 year follow-up period, the levels of MMP-8, MMP-9, and TIMP-4 increased beyond even those detected at the time of OSA diagnosis.

OSA is considered an independent risk factor for CVD. In support of this concept, a very recent study demonstrated marked coronary plaque formation in patients with OSA using coronary computed tomography angiography (79). As discussed earlier, MMPs have been associated with both oxidative stress and cardiovascular diseases, and hypoxic conditions were shown to influence MMP expression, secretion, and activity making these markers potentially important mediators in the process of accelerated atherosclerosis in OSA (23-26,80).

Notably, it was found that initiation of CPAP treatment of OSA significantly decreases serum levels of MMP-9, but does not affect TIMP-1 levels within 1 month. Our data regarding the short-term effect of CPAP treatment on MMP-9 and TIMP-1 not only reiterates these observations but also adds new information about the behavior of several other MMPs and TIMPs. Taken together, this is consistent with a scenario where the effects of OSA-induced CIH on CVDs are mediated by only a subset of MMPs, including MMP-8 and MMP-9, and that CPAP therapy alleviates this aspect of CVD risk in the short-term by eliminating the burden of CIH.

Little is known about the long-term effect of OSA on CVD, particularly the impact of CPAP treatment on MMP levels. In our study by the end of the follow-up period, increased levels of MMP-8, MMP-9, and TIMP-4 were measured compared to the time of OSA diagnosis. Our original study design had also called for the inclusion of control subjects, i.e. severe OSA patients who for any reason discontinued CPAP therapy during the follow-up period. However, despite our best efforts, for the final visit, we were unable to recruit such patients in sufficient numbers required for statistical analysis. Nevertheless, the reappearance of CIH can be excluded as a reason behind the elevated MMP-8, MMP-9, and TIMP-4 levels, since the initial strong association of MMP-8 and MMP-9 with markers of systemic inflammation had not reappeared at 5 years, consistent with the high adherence of our patients to CPAP.

Our subgroup analysis was carried out to see if independent CVD risk factors such as age, BMI, and a history of hypertension exerted significant influence on the extent of change in the levels of MMP-8, MMP-9, and TIMP-4 during the 5-year follow-up period. Stratification of the subjects based on the above parameters did not uncover differences between the subgroups suggesting that at least in this cohort of patients, age, BMI and hypertension did not play a major role in the short and long-term changes of these

analytes. It must be noted, however, that every single member in our cohort of patients was obese at the time of OSA diagnosis, and initiation of CPAP therapy did not prompt a change in their lifestyle. Moreover, by the end of the 5-year follow-up period, the cohort-wide mean change of BMI was 0.9 kg/m^2, while the SD of the change was only 2.14, with over 80% of the patients having less than 5% change in their BMI. Obesity as a constant comorbid condition could explain why the initial beneficial effect of CPAP could not be sustained in this cohort.

It was shown that obesity and an associated low-grade systemic inflammation modulate MMP-9 levels in children with OSA, independently from OSA severity (56). The authors found that BMI and CRP levels correlate with MMP-9 levels and speculated that a more severe CIH may be responsible for the higher prevalence of systemic inflammation in adults with OSA. On the other hand, there is evidence that exercise and diet lower MMP-9 levels within 2 weeks as was shown in a cohort of overweight children (81). The expression and activity of MMPs are regulated by various hormones and growth factors, including insulin, leptin, and adiponectin, factors involved in adipose tissue expansion (82). It is therefore conceivable that the lack of exercise and diet that led to invariable BMI in our cohort of severe OSA patients may be responsible for the increased levels of a number of MMPs by perpetuating a low-grade systemic inflammation, independent of OSA.

One of the strengths of our study is the simultaneous measurement of 7 MMPs and 3 TIMPs in a single serum sample by means of a multiplexed antibody array. This has effectively minimized the possibility of errors due to variations between samples and/or conditions, and thus greatly facilitated comparisons between analytes.

We detected positive correlations of MMP-8 with neutrophils and CRP and between MMP-9 and neutrophils at the time of OSA diagnosis. Associations between MMPs and markers of systemic inflammation are not unprecedented; increased serum levels of CRP and MMP-9 in OSA had been reported before (24). The fact that all these correlations disappeared after only 2 months of CPAP treatment is another strong indication that MMP-8 and MMP-9 are central in mediating the systemic effects of CIH.

5.3. Study 3

We demonstrated that in patients diagnosed with severe OSA who were characterized with high adherence to CPAP usage, a well-adjusted CPAP therapy quickly leads to normalized sleep parameters that remain in the normal range provided CPAP use is continuous. However, despite prolonged CPAP treatment, even a short, 1-week long CPAP withdrawal in a deterioration of most sleep parameters close to the abnormal values observed at diagnosis.

The AI and ODI levels were the exceptions that, although increasing after withdrawal, were still significantly lower compared to the values measured at the time of diagnosis. Consistent with the literature, our results suggest that the reduction in the number of apnea events associated with complete airway obstruction during withdrawal may be due in part to the upper airway stabilizing effect of CPAP treatment. Prolonged CPAP use allows for the regenerative process of reversible damage to pharyngeal tissues. CPAP treatment also eliminates the vibrational trauma caused by snoring and may contribute to the reduction of inflammatory and cardiac edema that in turn improves the parapharyngeal tissue tone, muscle tone, and reflex muscle activity. This reduced tendency to collapse leads to fewer and less severe respiratory events like apnea and remains so even upon withdrawal of CPAP. A decrease in the number of apnea events may partly explain the improvement in ODI as apnea is associated with more pronounced oxygen desaturation (41).

Another possible explanation for the improved AI and ODI upon CPAP withdrawal compared to baseline values could be a change in sleep structure. In a 2-night withdrawal study in severe OSA patients, Young et al. registered fewer apnea, more hypopnea/respiratory effort related arousal (RERA) events paralleled by more moderate O_2 desaturation. Changes in sleep architecture were found to be significant in terms of SDB severity, EEG analysis showing a lower REM (15.6% vs. 12.9%, p=0.009) at CPAP withdrawal compared to baseline. As the REM phase is associated with more severe and more frequent abnormal respiratory episodes, it is plausible that less REM phase leads to less apnea and more favorable O_2 desaturation values (41). In the absence of electroencephalography measurements in our cohort, we could not draw any conclusions in this regard.

In a few patients, AHI remained in the normal range even after CPAP withdrawal but that does not mean that these patients were cured of OSA. According to current guidelines, CPAP therapy can only be permanently suspended if a control sleep study after three months of CPAP withdrawal yields favorable results. This is because although the AHI value may be normal, the exacerbation of mechanical damage and airway inflammation caused by vibration trauma due to snoring may result in a recurrence of OSA. Patients in our study insisted on using the CPAP device and did not take advantage of the proposed 3-month therapy suspension.

One week after CPAP withdrawal, morning systolic blood pressure showed a modest but significant, clinically relevant increase, and the morning diastolic blood pressure showed a non-significant increase compared to 5-year CPAP control values. Morning blood pressure is a function of nocturnal hypoxia/hypercapnia associated with OSA, changes in intrathoracic pressure, increased sympathetic nervous system activity induced by micro-awakenings, and acute peripheral vasoconstriction (activation of the renin-angiotensin system). Dominantly, the increase in sympathetic baseline induced by repetitive apnea is transmitted to daytime wakefulness resulting in abnormal levels of pulse and blood pressure variability. Morning systolic blood pressure values showed a significant average increase of 7 mmHg ($p=0.02$) after 1 week of CPAP withdrawal compared to the 5-year CPAP control values, while the average increase in diastolic blood pressure was 4 mmHg ($p=0.06$). The evening blood pressure value is largely a function of daytime activity and no change was observed upon CPAP withdrawal (data not shown). Our results suggest that OSA is an important mediator of secondary hypertension.

CPAP withdrawal also leads to a rapid deterioration of most cardiac parameters, although the AC that measures the activity of the autonomic nervous system remains significantly lower after withdrawal compared to the value at OSA diagnosis.

The autonomic nervous system modulates the physiological activity of the heart through sympathetic and parasympathetic activity. During vasoconstriction, the sympathetic nervous system modulates several cardiovascular parameters that result in increased heart rate, increased cardiac contractility, decreased venous capacity, and increased peripheral vascular resistance. The cardiovascular effects of the parasympathetic nervous system (vagus nerve) include reduction of heart rate by inhibition of the sympathetic nervous system and direct hyperpolarization of the sinus

node. The real-time activity of the parasympathetic and sympathetic nervous systems on the heart is reflected in the electrophysiology of the heart. Heart rate variability, a physiological phenomenon of changes in the interval between heartbeats over time and frequency, are considered promising markers to characterize autonomic nervous system activity. The heart rate decelerates upon parasympathetic activity and accelerates upon sympathetic activity. Pulse acceleration is characterized by the Acceleration index and deceleration by the Deceleration index.

OSA causes the heart rate to move toward bradycardia through the activation of vagotonia and diving reflex at the beginning of apnea events. Sympathetic activation induced by micro-awakenings, intermittent hypoxia, oxidative stress, and increased intrathoracic pressure fluctuations together result in tachycardia and induce electrical instability in the heart muscle (10).

Our observation regarding the acceleration index suggests that our patients had a more pronounced sympathetic effect due to repetitive sympathicotonia at diagnosis compared to the 1-week CPAP withdrawal after 5 years of continuous CPAP use.

Despite the deterioration of objective sleep and cardiac parameters, the subjective ESS scores did not change after CPAP withdrawal suggesting that the physiological effects of short-term withdrawal do not translate to changes in the patient’s sense of condition.

Our analysis of possible correlations between sleep and cardiac parameters demonstrated that the correlations existing at the time of OSA diagnosis are eliminated by CPAP treatment, and upon CPAP withdrawal a different pattern appears. To determine if these correlations are scientifically and clinically relevant could be the subject of further research and in-depth analysis.

6. CONCLUSIONS

6.1. Study 1

In this study, we investigated the effect of long-term CPAP treatment on the lipid profile of patients with severe OSA. Our data suggest that the beneficial effects of CPAP treatment on fasting TC and LDL-C levels, already achieved with short-term treatment periods, could be sustained in the long term. An improved lipid profile may contribute to reducing cardiovascular risk in patients with severe OSA.

6.2. Study 2

In this study, we investigated the effect of CPAP therapy, both short- and long-term, on the MMP profile of patients with severe OSA. We observed that CPAP therapy in the short term lowers the serum concentration of MMPs formerly implicated as CVD risk factors, but these potentially beneficial effects were not sustained over the long term. CPAP treatment may only eliminate some but not all MMP associated cardiovascular risk in obese patients with severe OSA.

6.3. Study 3

Our results indicate that the symptoms of OSA return in a less severe form following a 1-week CPAP withdrawal in patients with severe OSA who have been using the CPAP device for 5 years with high adherence, corroborating data published earlier. The process is dominated by the re-emergence of apnea-related repetitive sympathicotonia. We observed that hypertension associated with OSA is reduced by CPAP; however, withdrawal of CPAP significantly increases the systolic blood pressure. CPAP withdrawal also results in a rapid deterioration of cardiac parameters, suggesting increased sympathicotonia. In conclusion, even a 1-week CPAP withdrawal involves serious health risks.

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7. SUMMARY

Obstructive sleep apnea (OSA), is the most common form of sleep-related breathing disorder and is characterized by recurrent episodes of complete or partial obstruction of the upper airways during sleep. As a result of untreated OSA, severe cardiovascular and metabolic complications, mental dysfunction and depression, may develop.

Continuous positive airway pressure (CPAP) therapy provides a well-documented symptomatic relief for most patients with OSA; however, its effect on dyslipidemia, an important mediator of accelerated atherosclerosis in OSA, remains contradictory. Therefore, in the first part of our work, we investigated the effects of CPAP treatment on the lipid profile of patients with severe OSA. We found that the beneficial effects of this therapy on fasting total cholesterol and low-density lipoprotein cholesterol levels, already achieved with short-term treatment periods, could be sustained even up to 5 years. The reduction in lipid levels was more pronounced in younger patients and in those who had a higher body mass index.

Besides dyslipidemia, several other factors including members of the family of matrix metalloproteinases (MMPs) and their specific antagonists, the tissue inhibitors of matrix metalloproteinases (TIMPs) may play an important role in the development of OSA-induced atherosclerosis. In the second part of our work, the effects of CPAP therapy on these cardiovascular risk factors were investigated in the same cohort of patients with OSA. We found that initiation of CPAP leads to a decrease in the level of key MMPs in the short-term; however, this effect was not sustained over the long-term, and levels of MMP-8, MMP-9, and TIMPs significantly increased at 5 years.

Finally, in the third part of our experiments, we analyzed the effect of CPAP withdrawal on sleep and cardiac parameters in the same patients. Our data demonstrate that in patients using the CPAP device already for a long time with high adherence, sleep parameters deteriorate significantly even after 1 week of CPAP withdrawal. Withdrawal also results in rapid deterioration of cardiac parameters suggesting increased sympathetic activity.

In summary, while the lipid-lowering effect of CPAP treatment was long-lasting, the beneficial effect on members of the MMP/TIMP protein family could only be demonstrated in the short term what may play a role in the development of late cardiovascular complications of the disease. Discontinuation of CPAP therapy, even for a short period, may increase the cardiovascular risk in patients with OSA.

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9. SUPPLEMENTARY APPENDIX

ÉTREND: A kérdések megválaszolásához gondoljon az étkezési szokásaira az elmúlt évben. Jelölje meg, hogy milyen gyakran eszi a következő ételeket. Minden étkezést vegyen figyelembe.

E1: Saláta vagy zöld saláta, más zöldségekkel vagy anélkül

☐ kevesebb, mint heti 1-szer	☐ heti 1-szer	☐ heti 2-3-szor	☐ heti 4-6-szor	☐ naponta 1-szer	☐ naponta 2-szer vagy többször
0	1	2	3	4	5

E2: Gyümölcs (beleértve a friss, konzerv vagy fagyasztott gyümölcsöt), a gyümölcslevek kivételével

☐ kevesebb, mint heti 1-szer	☐ heti 1-szer	☐ heti 2-3-szor	☐ heti 4-6-szor	☐ naponta 1-szer	☐ naponta 2-szer vagy többször
0	1	2	3	4	5

E3: Barna kenyér, teljes kiőrlésű kenyér, müzli.-magas rosttartalmú gabonapehely.

☐ kevesebb, mint heti 1-szer	☐ heti 1-szer	☐ heti 2-3-szor	☐ heti 4-6-szor	☐ naponta 1-szer	☐ naponta 2-szer vagy többször
0	1	2	3	4	5

Étrend nyers pontszám (E1+E2+E3):	_____	Étrend kategória pontszám:	_____	0: 0-5 nyers pontszám között 1: 6-10 nyers pontszám között 2: 11-15 nyers pontszám között

E4: Az alábbiak közül melyik húsféleségből eszi a legtöbbet? (1 aláhútandó) Hal / csirke / marha / sertés

E5: Tart-e diétát? (1 aláhútandó) Nem, Speciális (húsmentes,paleo, kaloriaszegény), orvosi (diabeteses, gluténmentes)

TESTMOZGÁS:

T1:nyugdijas/aktivan dolgozó-ülőmunka, könnyű fizikai munka,nehéz fizikai munka
Ülőmunka estén:
1h,2h,3h,4h,5h,6h,több,mint6H

T2: Naponta hány órát tölt ülőtevékenységgel (tévézéssel, számítógép előtt)? (aláhúzandó)

Soha, 1h, 2h, 3h, 4h, 5h, 6h, több,mint 6h

Az alábbi kérdések megválaszolásához kérjük, jelezze, hogy hetente hányszor vesz részt a következő tevékenységekben legalább 30 percig:

T3: Könnyű testmozgás, mint például az alábbiak:

- könnyű kertészkedés és könnyű házimunka (pl. portörlés, söprés, porszívózás)
- kényelmes sétálás (pl. kutyasétáltatás)
- bowling, horgászás, asztaloskodás, hangszeres zene

☐ 0-szor hetente	☐ hetente 1-3-szor	☐ hetente 4-7-szer	☐ hetente 8-szor vagy többször
0	2	3	4

T4: Mérsékelt testmozgás, mint például az alábbiak:

- tempós séta
- kerékpározás, korcsolyázás, úszás, curling
- kertészkedés (pl. gereblyézés, gyomlálás, ásás)
- tánc, Tai Chi, vagy mérsékelt testgyakorlat

☐ 0-szor hetente	☐ hetente 1-3-szor	☐ hetente 4-7-szer	☐ hetente 8-szor vagy többször
0	4	6	8

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T5: Erőteljes testmozgás, mint például az alábbiak:

- futás, kerékpározás, sífutás, úszás, aerobik
- nehéz kerti munka
- súlyzós edzés
- labdarúgás, kosárlabda vagy egyéb csapatsport

☐ 0-szor hetente	☐ hetente 1-3-szor	☐ hetente 4-7-szer	☐ hetente 8-szor vagy többször
0	6	9	12

Testmozgás nyers pontszám (T3+T4+T5):		Testmozgás kategória pontszám:	0: csak könnyű testmozgás esetén 1: bármely mérsékelt testmozgás esetén 2: bármely erőteljes testmozgás esetén
	_____	_____	

ALKOHOLFOGYASZTÁS: Kérjük, adja meg, hogy az alábbi alkoholos italfajták közül hányat fogyaszt egy átlagos héten.

A1: Bor (1dl-1,5dl-es adag):
A2: Sör (3dl-es adag vagy pohár):
A3: Tömény (3cl-5cl-es adag):

Alkohol nyers pontszám (A1+A2+A3):
Alkohol kategória pontszám:
0: 14 és a fölötti nyers pontszám esetén
1: 8-13 nyers pontszám között
2: 0-7 nyers pontszám között

DOHÁNYZÁS: Kérjük, adja meg a dohányzási szokásait.

D1: Dohányzik Ön jelenleg?
0, Igen
D2: Ha nem, akkor dohányzott Ön valaha?
1, Igen
2, Nem

Dohányzás nyers pontszám (D1+D2):
Dohányzás kategória pontszám: ugyanaz, mint a nyers pontszám:

STRESSZ: A kérdés megválaszolásához karikázza be a mindennapi életben lévő stressz szintjéhez leginkább megfelelő számot.

6, egyáltalán nem stresszes
...
1, nagyon stresszes

Stress nyers pontszám (S1): a bejelölt érték
Stressz kategória pontszám:
0: 1-2 nyers pontszám között
1: 3-4 nyers pontszám között
2: 5-6 nyers pontszám között

SLIQ pontszám: az összes kategória pontszám összege.

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