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Ocena regulacji biodostępności tlenku azotu w erytrocytach u osób z zespołem bezdechu sennego

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ROZPRAWA DOKTORSKA

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SPIS TREŚCI

1. LISTA PUBLIKACJI WCHODZĄCYCH W SKŁAD CYKLU:

1.1. **Jakub Mochol**, Jakub Gawryś, Damian Gajecki, Ewa Szahidewicz-Krupska, Helena Martynowicz and Adrian Doroszko.

Cardiovascular Disorders Triggered by Obstructive Sleep Apnea—A Focus on Endothelium and Blood Components

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1.2. Jakub Mochol, Jakub Gawryś, Ewa Szahidewicz-Krupska, Jerzy Wiśniewski, Paulina Fortuna, Piotr Rola, Helena Martynowicz and Adrian Doroszko

Effect of Obstructive Sleep Apnea and CPAP Treatment on the Bioavailability of Erythrocyte and Plasma Nitric Oxide

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2. WYKAZ SKRÓTOW I SYMBOLI

ADMA (asymmetric dimethylarginine) - asymetryczna dimetyloarginina

CPAP (continuous positive airway pressure) - stałe dodatnie ciśnienie w drogach oddechowych – metoda leczenia bezdechu protezą powietrzną

DDAH (dimethylarginine dimethylaminohydrolase) – dimetyloaminohydrolaza dimetyloargininy -enzym rozkładający ADMA

ESM-1 (endothelial cell-specific molecule-1, Endocan) -specyficzna cząsteczka 1 śródłonka

HIF-1α (hypoxia-inducible factor 1) czynnik indukowany niedotlenieniem 1 alfa

hsCRP (high-sensitivity CRP) badanie o wysokiej czułości białka C-reaktywnego

LDF (laser doppler flowmetry) laserowa przepływometria dopplerowska

NO (nitric oxide) - tlenek azotu

NOS (nitric oxide synthase) - syntaza tlenku azotu

NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) - czynnik transkrypcyjny, Czynnik jądrowy wzmacniany lekkimi łańcuchami kappa aktywowanych limfocytów B

OBS - obturacyjny bezdech senny

OSA (obstructive sleep apnoea) - obturacyjny bezdech senny

TNFα (tumor necrosis factor α) - czynnik martwicy nowotworów alfa

VEGF (vascular endothelial growth factor) - czynnik wzrostu śródbłonka naczyniowego

VCAM-1 (vascular cell adhesion molecule 1) - cząsteczka adhezyjna-1 komórki naczyniowej

3. OMÓWIENIE PUBLIKACJI

Dane epidemiologiczne z ostatnich lat jednoznacznie wskazują, że występowanie obturacyjnego bezdechu sennego (OBS) w populacji ogólnej wykazuje tendencję wzrostową i rozpoznanie to obecnie dotykać może około miliarda osób na całym świecie. Wśród czynników mogących odpowiadać za powstawanie sercowo-naczyniowych powikłań w przebiegu OBS wymienia się zwiększoną aktywację układu współczulnego, wzrost stresu oksydacyjnego wtórnie do nawracających epizodów hipoksji-reoksygenacji, prowadząc do zwiększenia ekspresji czynników prozapalnych oraz do rozwoju dysfunkcji śródbłonka naczyniowego. Mechanizmy patofizjologiczne są jednak złożone i mogą być również związane ze zmienioną funkcją elementów morfotycznych krwi.

Tlenek azotu (NO) oraz regulacja jego biodostępności odgrywają kluczową rolę w patogenezie chorób układu sercowo-naczyniowego. NO jest jednym z najważniejszych czynników wazodylatacyjnych, a jego zmniejszona biodostępność prowadzi do pojawienia się wazodylatacyjnej dysfunkcji śródbłonka. W wielu badaniach podkreślana jest szczególna rola asymetrycznej dimetyloargininy (ADMA), kompetycyjnego inhibitora syntazy tlenku azotu (NOS), którego zwiększone stężenie w osoczu jest wczesnym i niezależnym wskaźnikiem wyższego ryzyka wystąpienia incydentów sercowo-naczyniowych. Obecność ADMA stwierdzono nie tylko w kompartmencie zewnątrzkomórkowym, ale także wewnątrz komórek śródbłonka naczyniowego. Ponieważ ADMA powstaje wskutek lizy białek bogatych w metylowane reszty argininy, zwłaszcza histonów, karioliza towarzysząca megakario- i erytropoezie może powodować, że erytrocyty i płytki krwi mogą stanowić istotne źródło ADMA. Ponadto, erytrocyty – wskutek dużej objętości tworzonego przez nie kompartmentu w zakresie przestrzeni wewnątrznaczyniowej – mogą stanowić naturalny jej rezerwuar. Dodatkowo, tlenek azotu poprzez modyfikacje potranslacyjne białek szkieletu komórkowego może wpływać na elastyczność erytrocytów i właściwości reologiczne krwi. Jednakże mimo udowodnienia ekspresji syntazy tlenku azotu (NOS) wewnątrz erytrocytów oraz obecności ADMA w cytozolu, ich parakrynna rola w regulacji homeostazy śródbłonka naczyniowego nie została jeszcze dokładnie zbadana. Ocena biodostępności NO poprzez analizę stężeń intermediatów szlaku jego biotransformacji wewnątrz erytrocytów oraz w osoczu w grupie osób z OBS była przedmiotem prowadzonych przeze mnie badań w ramach projektu doktorskiego.

Z uwagi na złożoność mechanizmów mających wpływ na zwiększenie ryzyka sercowo-naczyniowego w bezdechu sennym, w pracy poglądowej pt. „*Cardiovascular Disorders Triggered by Obstructive Sleep Apnea—A Focus on Endothelium and Blood Components*”, dokonano przeglądu i podsumowania aktualnego stanu wiedzy na ten temat, co dało podstawy do nakreślenia

celów pracy doktorskiej i sformułowania hipotez zerowych. Praca ta opisuje szereg zmian toczących się na poziomie komórkowym wywołanych przez następujące po sobie okresy hipoksji i reoksygenacji. Wzrost stresu oksydacyjnego przyczynia się do powstawania reaktywnych form tlenu powodujących rozprzęganie syntazy tlenku azotu i produkcję nadtlenoazotynów, powodujących potranslacyjne modyfikacje białek (nitrowanie, S-nitrozylacja) i w konsekwencji zmianę ich funkcji.

Wzrost ekspresji czynników zapalnych wpływa, łącznie ze zmniejszoną biodostępnością tlenku azotu, na zwiększoną adhezję leukocytów do śródbłonka naczyniowego i przyczynia się do rozwoju miażdżycy. Ekspresja specyficznej dla komórek śródbłonka cząsteczki-1 (ESM-1, Endocan), czynnika wzrostu śródbłonka naczyniowego (VEGF), cząsteczki adhezyjnej komórek naczyniowych 1 (VCAM-1) i czynnika indukowanego niedotlenieniem 1 alfa (HIF-1α) jest zwiększona w komórkach śródbłonka poddanych przerywanej hipoksji. HIF-1α aktywuje czynnik transkrypcyjny NFκB, który z kolei reguluje ekspresję kilku genów prozapalnych, w tym TNFα oraz interleukiny (IL-8 i IL-6). ESM-1 wraz z innymi czynnikami zapalnymi odgrywa rolę w zwiększaniu adhezji między monocytami a komórkami śródbłonka, chemotaksją i promowaną polaryzacją makrofagów w kierunku fenotypu prozapalnego.

W pracy tej usystematyzowano również wpływ bezdechu sennego na wystąpienie innych manifestacji chorób układu sercowo-naczyniowego, jak nadciśnienie tętnicze, cukrzyca i w konsekwencji miażdżyca, niewydolność serca czy migotanie przedsionków. Ponadto podjęto próbę opisania zmian wywołanych terapią CPAP i podsumowano doniesienia naukowe na temat wspomagającego wpływu jej stosowania w aspekcie ich leczenia. W wielu pracach nie wykazano bezpośredniego korzystnego efektu terapią CPAP na funkcję śródbłonka naczyniowego. Potwierdzono jednak, że regularne stosowanie może przynieść inne korzyści, w tym zmniejszenie ryzyka nawrotu migotania przedsionków (stabilizacja rytmu zatokowego u pacjentów z napadową postacią tej tachyarytmii), zmniejszenie końcoworozkurczowej objętości lewej komory czy zmniejszenie insulinooporności.

W ostatnich rozdziałach omawianej pracy opisano także zmiany funkcji płytek krwi i właściwości erytrocytów w kontekście obturacyjnego bezdechu sennego. OBS jest związany ze zwiększoną adhezją erytrocytów mierzoną za pomocą protokołów optycznych.

Entuzjazm dotyczący potencjalnej przydatności klinicznej terapii CPAP jest osłabiony przez brak mocnych badań z randomizacją potwierdzających jej skuteczność w redukcji ryzyka sercowo-naczyniowego. Wprawdzie regularne i długotrwałe stosowanie terapii CPAP (≥4 h na dobę) wydaje się skutkować nieznacznym zmniejszeniem częstości występowania incydentów sercowo-naczyniowych, co wykazano w kilku badaniach, jednakże rozbieżności w wynikach badań klinicznych oraz pewne trudności translacyjne w implementacji wyników badań

podstawowych na grunt kliniczny mogą wynikać z równoczesnej roli śródbłonka i składników krwi w patofizjologii powikłań zakrzepowo-zatorowych związanych z OBS oraz wskazują na ich wspólną rolę jako celu terapeutycznego stosując CPAP. Dokonując przeglądu literatury dostrzeżono brak badań z zakresu medycyny translacyjnej wiążących patofizjologię OBS oraz oceniających wpływ terapii CPAP na poziomie molekularnym na wybrane aspekty ryzyka sercowo-naczyniowego. Z tego względu postanowiłem przeprowadzić badanie, którego celem było podjęcie próby falsyfikacji następujących hipotez zero dotyczących braku różnic w zakresie metabolizmu tlenku azotu w erytrocytach oraz osoczu u osób z OBS i bez takiego rozpoznania, następnie braku różnic w zakresie funkcji wazodylatacyjnej śródbłonka ocenionej metodą Laser Doppler w grupie osób z OBS i bez takiego rozpoznania, a także braku wpływu terapii CPAP na oceniane parametry biochemiczne szlaku biodostępności tlenku azotu i funkcji wazodylatacyjnej śródbłonka. Efektem tego jest powstanie pracy oryginalnej z niniejszego cyklu publikacji.

Druga praca wchodząca w skład niniejszej rozprawy doktorskiej nosi tytuł *„Effect of Obstructive Sleep Apnea and CPAP Treatment on the Bioavailability of Erythrocyte and Plasma Nitric Oxide”* i jest to pierwsze oryginalne doniesienie naukowe opisujące ekspresję osi biotransformacji tlenku azotu w erytrocytach osób z bezdechem sennym. Do badania kwalifikowano osoby z podejrzeniem OBS w przedziale 30-70 lat. Chcąc zbadać wpływ OBS jako możliwie izolowaną patologię, z badania dyskwalifikowano osoby z cukrzycą, klinicznie zaawansowaną miażdżycą i opornym nadciśnieniem tętniczym. W związku z tymi kryteriami włączenia do badania z pierwotnie sporej liczby pacjentów branych pod uwagę ostatecznie do grupy badanej włączono ostatecznie zaledwie 46 osób, pozostawiając te z otyłością i nadciśnieniem tętniczym I stopnia wg ESC/ESH/PTNT, z którymi obturacyjny bezdech senny często jest nierozerwalnie związany. Po przeprowadzeniu polisomnografii, pacjentów podzielono na podgrupy w zależności od ciężkości bezdechu. Od osób zakwalifikowanych do udziału w projekcie, pobrano około 40 ml krwi żylnej celem analizy podstawowych parametrów biochemicznych służących do oceny ryzyka sercowo-naczyniowego. Cześć materiału zabezpieczono do dalszych badań biochemicznych oceniających osoczowe i wewnątrz-erytrocytarne elementy osi biotransformacji NO. U każdego pacjenta wykonywano nieinwazyjną ocenę wazodylatacyjnej funkcji śródbłonka naczyniowego za pomocą przepływomierza laserowego (*Laser-Doppler*). U osób ze średnim i ciężkim bezdechem, u których wdrożono terapię CPAP przeprowadzono ponowną prospektywną ewaluację funkcji śródbłonka - *follow-up* w 1-rocznej obserwacji i oznaczono metabolity szlaku tlenku azotu po około roku od rozpoczęcia regularnego stosowania terapii. Do ponownej oceny brani byli jedynie pacjenci, który stosowali terapię regularnie.

W badaniu wykazano brak istotnych różnic pomiędzy stężeniem metabolitów tlenku azotu w kompartmencie wewnątrz-erytrocytarnym w zależności od ciężkości OBS. Erytrocyty miały średnio prawie dwukrotnie niższe stężenie ADMA niż osocze, mogąc stanowić wysokoobjętościowy bufor dla tego inhibitora syntazy tlenku azotu. Wśród parametrów osoczowych wykazano obniżone stężenie L-Cytruliny w grupie osób z ciężkim OBS. L-Cytrulina, która jest łatwo konwertowana do L-Argininy, głównego substratu dla syntazy tlenku azotu, może stanowić pierwszy osoczowy marker zmniejszonej biodostępności tlenku azotu.

Porównując grupę średniego i ciężkiego OBS z grupą z lekkim OBS i osobami zdrowymi wykazano różnice w zakresie parametrów biochemicznych - wyższe stężenie kwasu moczowego, hsCRP oraz insuliny na czczo, wskazując tym samym na profil wyższego ryzyka kardio-metabolicznego. Molekularne mechanizmy zwiększonego stresu oksydacyjnego i wzrost aktywności zapalnej w bezdechu sennym dają teoretyczne przesłanki do wytłumaczenia większość z tych zmian. Nie wykazano istotnych różnic w zakresie metabolitów szlaku tlenku azotu w erytrocytach i osoczu u pacjentów przed i po stosowaniu leczenia terapią CPAP. Stwierdzono, jednakże tendencję do wyższego stężenia L-Argininy w erytrocytach, przy zachowanym stosunku substrat/inhibitor kompetycyjny (L-Arginina/ADMA). Częściowo może być to związane z niższą aktywnością arginazy, która jest aktywowana w warunkach wzmożonego stresu oksydacyjnego, głównie w warunkach silnych wahań potencjału redox. Arginaza I, która konkuruje z syntazą tlenku azotu o Argininę, może być punktem uchwytu dla działania leków, które poprzez hamowanie jej aktywności mogłyby się przyczynić do poprawy funkcji śródbłonka naczyniowego. Terapia OBS metodą CPAP nie wykazała istotnej statystycznie poprawy wszystkich parametrów funkcji śródbłonka, jakkolwiek wiązała się z tendencją do zwiększonej odpowiedzi wazodylatacyjnej na bodziec termiczny w badaniu *Laser Doppler Flowmetry* (LDF), a także spadkiem stężenia *hs*CRP w surowicy.

Podsumowując, w toku realizacji projektu będącego podstawą niniejszej pracy doktorskiej wykazano, że u osób ze średnim i ciężkim OBS jeszcze bez rozwiniętych powikłań naczyniowych różnice w zakresie metabolitów tlenku azotu wewnątrz erytrocytów nie były istotne statystycznie w porównaniu do grupy osób z lekkim OBS i osobami zdrowymi. Kardiometaboliczne wykładniki ryzyka sercowo-naczyniowego, jak podwyższone stężenie kwasu moczowego, insuliny na czczo i hsCRP w surowicy lepiej korelowały ze stopniem ciężkości OBS niż zmiany dotyczące metabolitów biodostępności tlenku azotu. Układy regulujące szlak wydzielania tlenku azotu u osób bez zaawansowanych zmian naczyniowych ulegać mogą zatem w dużej mierze kompensacji. Ponadto, na stężenie ADMA ma wpływ ekspresja i aktywność dimetyloaminohydrolazy dimetyloargininy (DDAH), której obecność również została potwierdzona wewnątrz erytrocytów i stanowi główny mechanizm degradacji ADMA. Na

pewnym etapie mechanizmy zmiatające wolne rodniki, kompensujące nadmierny stres oksydacyjny mogą również mieć istotny wpływ przeciwdziałający rozprzęganiu syntazy tlenku azotu zmniejszając nasilenie stresu nitrozacyjnego. Przy złożoności mechanizmów regulujących biodostępność tlenku azotu konieczne są dalsze szczegółowe badania, w tym określenie wszystkich składników biorących udział w regulacji aktywności NOS. Brak korelacji pomiędzy osoczowym i wewnątrz-erytrocytarnym stężeniem ADMA przemawia za częściową niezależnością tych dwóch kompartmentów, a także znacznej roli transportu przezbłonowego w regulacji stężeń aminokwasów i innych molekuł pomiędzy komórkami śródbłonka, osoczem i erytrocytami.

4. PRACA NR 1:

Cardiovascular Disorders Triggered by Obstructive Sleep Apnea—A Focus on Endothelium and Blood Components

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Molecular Sciences

MDPI

Review

Cardiovascular Disorders Triggered by Obstructive Sleep Apnea—A Focus on Endothelium and Blood Components

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Abstract: Obstructive sleep apnea (OSA) is known to be an independent cardiovascular risk factor. Among arousal from sleep, increased thoracic pressure and enhanced sympathetic activation, intermittent hypoxia is now considered as one of the most important pathophysiological mechanisms contributing to the development of endothelial dysfunction. Nevertheless, not much is known about blood components, which justifies the current review. This review focuses on molecular mechanisms triggered by sleep apnea. The recurrent periods of hypoxemia followed by reoxygenation promote reactive oxygen species (ROS) overproduction and increase inflammatory response. In this review paper we also intend to summarize the effect of treatment with continuous positive airway pressure (CPAP) on changes in the profile of the endothelial function and its subsequent potential clinical advantage in lowering cardiovascular risk in other comorbidities such as diabetes, atherosclerosis, hypertension, atrial fibrillation. Moreover, this paper is aimed at explaining how the presence of OSA may affect platelet function and exert effects on rheological activity of erythrocytes, which could also be the key to explaining an increased risk of stroke.

Keywords: obstructive sleep apnea (OSA); endothelial dysfunction (ED); oxidative stress; nitric oxide (NO); asymmetric dimethylarginine (ADMA)

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1. Introduction

Obstructive sleep apnea (OSA) is characterized by recurrent obstruction of the upper airway during sleep causing intermittent hypoxia (IH). The prevalence is increased by advanced age, male sex, higher body mass index and ranges, according to some estimations, from 9% to 38% in general population [1]. OSA is known to be an independent cardiovascular risk factor. The incidence during three years observation of cardiovascular mortality, myocardial infarction, stroke, and unplanned revascularization in patients undergoing percutaneous coronary intervention was higher in the OSA group (18.9% versus 14.0% in the non-OSA group) [2]. Numerous possible mechanisms contributing to the progression of cardiovascular disorders remain in the focus of interest (Figure 1).

Among increased thoracic pressure and activation of the sympathetic system, intermittent hypoxia (IH) is now being recognized as a potential major factor contributing to the pathogenesis of OSA-related comorbidities. IH is characterized by cycles of hypoxemia followed by reoxygenation that contribute to the development of the ischemia-reperfusion injury [3]. The biochemical consequences of hypoxia comprise the inhibition of the Krebs cycle and promotion of lactate synthesis. The impairment of mitochondrial oxidative phosphorylation in the course of hypoxia, followed by subsequent reoxygenation, induces the production of reactive oxygen species (ROS). ROS generation involves the mitochondrial respiratory chain and numerous enzyme complexes, including the NADPH oxidase, nitric oxide (NO) synthase and the xanthine oxidase [4].

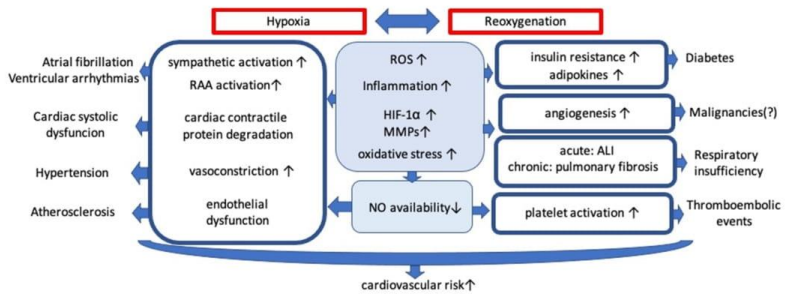

Figure 1. Mechanisms contributing to the progression of cardiovascular disorders. Abbreviations: ROS: reactive oxygen species; HIF-1α: hypoxia inducible factor 1α; MMPs: matrix metalloproteinases; ALI: acute lung injury; RAA: renin-angiotensin-aldosterone; NO: nitric oxide; ↑: increased; ↓: decreased.

Oxidative stress results from an imbalance between the pro-oxidants formation and neutralization. Reactive oxygen species (ROS) are toxic highly reactive and unstable compounds. Superoxide reacts with NO creating peroxynitrite ($ONOO^-$) which is a very potent entity, being ~1000× stronger as an oxidizing agent than is H_2O_2. The $ONOO^-$ can influence posttranslational protein modifications altering their structure, activity and function, leading in turn to impairment of signaling pathways. Markers of $ONOO^-$ formation (such as nitrotyrosines or isoprostanes) can be found in many disease states including brain injury [5], heart injury [6], preeclampsia [7], and inflammation [8].

Reactive oxygen species (ROS) can limit NO bioavailability by reacting with cofactors of NO synthase (NOS). ROS cause depletion of tetrahydrobiopterin and alter the ratio of oxidized to reduced glutathione inducing NOS S-glutathionylation [9]. Oxidative stress is strongly attributed to endothelial NOS dysfunction (eNOS uncoupling). Converted to a superoxide-producing enzyme, uncoupled eNOS not only leads to reduction of the NO generation but also potentiates the preexisting oxidative stress. An understanding of the biology of NO, $O2^-$ and $ONOO^-$ as well as the importance of the NOS uncoupling in physiological and pathological setting are crucial for understanding the nitrosative stress-related controversies. There is a critical balance between cellular concentrations of NO, $O2^-$, and superoxide dismutase, which physiologically favors NO production but in pathological conditions such as ischemia/reperfusion(I/R) results in $ONOO^-$ generation.

The NO bioavailability can be also diminished in other possible mechanism involving asymmetric dimethylarginine (ADMA) and monomethylated arginine (L-NMMA) which are endogenous competitive inhibitors of the NOS. ADMA plays a role in the development of endothelial dysfunction and is considered as a marker of oxidative stress. Most importantly, OSA is associated with a decrease in the NO bioavailability and changes in the platelet function and erythrocyte rheological disturbances [10].

2. Molecular Consequences of Hypoxia/Reoxygenation (H/R) on Endothelial Function in OSA before CPAP Treatment

There are many mechanisms triggered by the hypoxia/reoxygenation injury. Most of them are associated with ROS overproduction and cellular damage. The impact of hypoxemia causing endothelial dysfunction was examined in numerous animal and human in vitro and in vivo models. Intermittent hypoxia in rats impaired endothelial function by attenuating the integrity of endothelium and lowering the number of endothelial progenitor cells (EPCs) in the blood [11]. Endothelial progenitor cells (EPCs) are circulating bone marrow-derived precursors which are capable of excreting microvesicles (MVs) containing gene messages (mRNAs and miRNAs). MicroRNAs (miRNAs) are small non-coding RNAs which play a role in several cellular processes. Not only progenitor cells, but also activated endothelial cells and white blood cells are capable of producing the micropar-

ticles. MVs may have either beneficial or detrimental effects on endothelial cells. In the medium containing tumor necrosis factor α (TNFα) they activate the caspase 3 which leads to apoptosis. MVs transduce information associated with ROS production, inducing angiogenesis and activation of the PI3K/eNOS/NO pathway [12]. MVs released during hypoxia/reoxygenation injury are pro-apoptotic and pro-oxidative [13]. Another study confirms that circulating MVs cause increased permeability and disruption of tight junctions along with increased adhesion molecule expression, reduce eNOS expression and promote increased monocyte adherence. Comparing the influence of microvesicles isolated before and after PAP therapy, the disturbances in endothelial cells function such as increased permeability and disruption of tight junctions were attenuated with treatment [14].

Priou et al. found higher levels of microvesicles derived from granulocytes and activated leukocytes (CD62L+) in patients with the oxyhemoglobin desaturation index (ODI) ≥ 10. MVs have increased expression of endothelial adhesion molecules (E-selectin, ICAM-1, Integrin alpha-5) and cyclooxygenase 2.

MVs from desaturating patients injected into mice impaired the endothelium-dependent relaxation of vascular smooth muscle cells (VSMCs) in aorta and the flow-mediated dilation (FMD) in small mesenteric arteries resulting from decreased NO production. The endothelial NO synthesis negatively correlated with the number of active leukocytes and the sleep apnea severity. In vitro, MVs from desaturating patients reduced endothelial NO production by enhancing phosphorylation of eNOS at the site of inhibition and increasing expression of caveolin-1. Caveolin-1 is a membrane protein which regulates endothelial nitric oxide synthase (eNOS) activity and takes part in cellular insulin-signaling. In the study by Sharma et al. chronic 3-day intermittent hypoxia (IH) exposure on human coronary artery endothelial cells increased caveolin-1 and endothelin-1 expression resulting in decreased NO bioavailability [15,16].

Skin biopsies obtained from OSA patients with severe nocturnal hypoxemia demonstrate a significant upregulation of eNOS, TNFα-induced protein 3, hypoxia-inducible factor 1 alpha (HIF-1α), vascular endothelial growth factor (VEGF) and vascular cell adhesion molecule 1 (VCAM-1) [17]. The expression of endothelial-cell-specific molecule-1 (ESM-1, Endocan), VEGF and HIF-1α was also significantly increased in the human umbilical vein endothelial cells (HUVEC) subjected to IH and in patients with OSA. ESM-1 is upregulated by the HIF-1α/VEGF pathway under IH in endothelial cells, playing a critical role in enhancing adhesion between monocytes and endothelial cells [18]. IH increased advanced glycation end products formation and activated NF-κB signaling in monocytes, resulting in enhanced monocyte adhesion, chemotaxis, and promoted macrophage polarization toward a pro-inflammatory phenotype [19]. Enhancing adhesion and infiltration activity monocyte chemoattractant protein-1 (MCP-1) was also increased in monocytes under IH [20]. Moreover increased macrophage population with pro-inflammatory expression of CD36 and Ly6c was confirmed in the aortic wall in a murine model of IH [21]. In mice nocturnal intermittent hypoxia increased mRNA levels of 5-lipoxygenase and CysLT1 receptor, which was strongly associated with atherosclerosis lesion size. That confirms cysteinyl-leukotrienes (CysLT) pathway activation as a potential mechanism responsible for developing atherosclerosis connected with IH [22]. Another study trying to explain OSA-dependent atherogenesis found an increased expression of toll-like receptors (TLRs) and receptor for advanced glycation end-products (RAGE) in atherosclerotic plaques from patients with severe OSA [23].

An in vitro model of OSA shows that endothelial cells originating from distinct vascular beds respond differently to intermittent hypoxia. In human dermal microvascular endothelial cells IH decreased the expression of eNOS and HIF-1α, while in coronary artery endothelial cells HIF-1α expression was increased [17]. The HIF-1α activates the NFκB, a transcription factor which regulates several pro-inflammatory genes, including TNFα, interleukin (IL)-8, and IL-6 [24]. IL-6, the epidermal growth factor family ligands, and tyrosine kinase receptors induced by IH may be involved in the proliferation of vascular smooth muscle cells [25]. Another study showed that a further vascular and cardiac dys-

function in mice under IH can be triggered by trombospondin-1 though cardiac fibroblast activation and increasing angiotensin II activity [26].

In conclusion, OSA may result in endothelial dysfunction by limiting the NO availability, promoting oxidative stress, up-regulating the expression of pro-inflammatory cytokines. Microparticles and signaling factors lead to lower permeability of endothelial cells, enhanced adhesion and increased apoptosis (Figure 2).

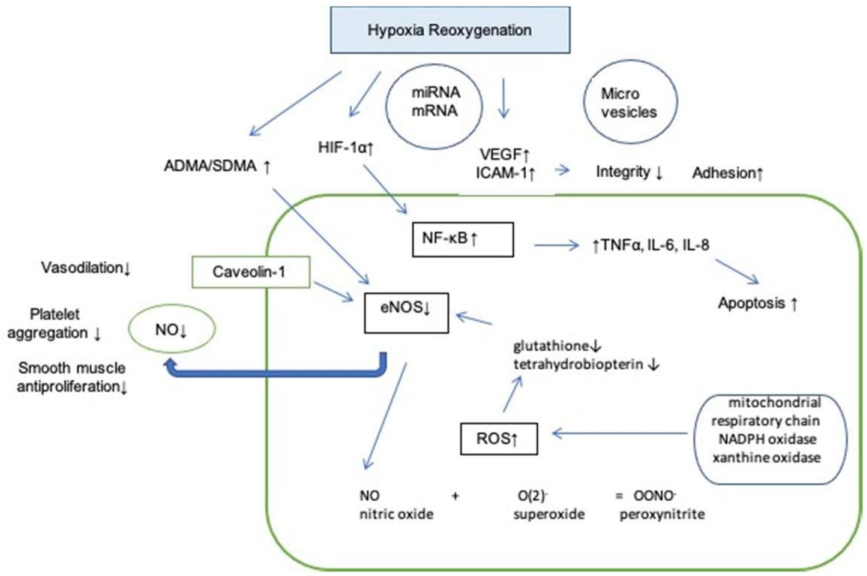

Figure 2. Influence of hypoxia reoxygenation on endothelium. Abbreviations: HIF-1α: hypoxia inducible factor 1 alpha; VEGF: vascular endothelial growth factor; ICAM-1: intercellular adhesion molecule 1; NF- κB: nuclear factor kappa-light-chain-enhancer of activated B cells; TNFα: tumor necrosis factor alpha; IL-6: interleukin 6; IL-8: interleukin 8; SDMA: symmetric dimethylarginine; ADMA: asymmetric dimethylarginine; ROS: reactive oxygen species; eNOS: endothelial nitric oxide synthase; NO: nitric oxide; ↑: increased; ↓: decreased.

3. Endothelial Function after Treatment with CPAP

An appropriate CPAP therapy in randomized control trials conducted on OSA patients improved flow mediated dilation (FMD), suggesting its potentially beneficial role in cardiovascular risk reduction [27,28]. A meta-analysis confirms that CPAP increases the absolute FMD value by a mean of 3.87% [29]. Flow mediated dilation (FMD) measures the change of the brachial artery diameter after a brief period of forearm ischemia. It helps to assess endothelial function and its ability to dilate the vessel by producing NO and prostacyclin. There are many other physical methods to assess endothelial function including venous occlusion plethysmography, peripheral arterial tonometry (PAT) and optical techniques using laser doppler flowmetry (LDF) that can be coupled with provocation tests (post-ischemic hyperemia, local heating). However, more studies are needed in order to validate their usefulness in assessing endothelial dysfunction.

As far as the literature is concerned, the effect of CPAP on inflammatory reaction is not proven yet. Inflammatory markers such as IL-8, hs-CRP, and TNF-α did not significantly change from baseline after 1 year CPAP therapy [30]. However, the use of CPAP for at least 5 h per night decreased TNF-α levels in women suffering from OSA [31]. Interestingly, CPAP tends to lower TNF-α, which is initially increased in OSA patients [32]. These diverse effects of CPAP treatment could be explained by a low adherence to the therapy and the

fact that better effects are observed with the therapy duration of ≥3 months and more adequate compliance (≥4 h/night).

Another positive effect after 12 weeks of CPAP therapy was a decreased expression of angiotensin receptors type-1 (AT-1R) measured in the gluteal subcutaneous tissue [33]. The AT-1R mediates the major cardiovascular effects of angiotensin II, including cardiac hypertrophy, augmentation of peripheral noradrenergic activity and vascular smooth muscle cells proliferation.

Three months of CPAP treatment significantly increased the level of sirtuin 1 (SIRT1) and serum levels of NO derivative in the blood [34]. SIRT1 is a histone/protein deacetylase which regulates the eNOS, restores the NO availability and is involved in different aspects of aging, metabolism, stress resistance and cardiovascular disease.

Patients with OSA showed a higher adventitial vasa vasorum density, correlating with AHI [35]. This could be explained by increased VEGF activity induced by IH. One meta-analysis confirmed that CPAP therapy improved endothelial function associated with VEGF lowering [36]. On the contrary, short return of OSA using sham CPAP for 2 weeks was not associated with changes in endocan, ET-1, resistin and VEGF. However, a significant decrease in vasodilatory peptide adrenomedullin was found [37,38]. Adrenomedullin is a protective endothelial product stimulated by IH, which could partially explain why the CPAP therapy may deteriorate endothelial function or exert a neutral effect in the short-term observational studies.

In the moderate to severe OSA, the 2-month CPAP treatment vs. sham did not reduce the plasma concentrations oxidative stress-related markers [39]. In a study by Borges comparing the 8-week CPAP therapy with aerobic training, no significant changes regarding oxidative stress markers and cell-free DNA levels were detected [40]. Interesting outcomes were shown in a mice model study, where the animals treated with a high-fat diet revealed a positive effect of the low-frequency hypoxia. The serum levels of the oxidative stress markers were increased in the mice treated with a high-frequency intermittent hypoxia (60 hypoxic events/h) and decreased by treating with a low frequency hypoxia (10 events/h) [41]. That could be partially explained by the activation of protective mechanisms during IH.

Although there is substantial evidence that CPAP improves endothelial function (Figure 3), the antioxidant capacity is not changed significantly. The median plasma nitrite level and total antioxidant status did not show any significant difference between the OSA and the control groups. Nevertheless, the oxidant-antioxidant balance was shifted toward the oxidant side in OSA cases [42].

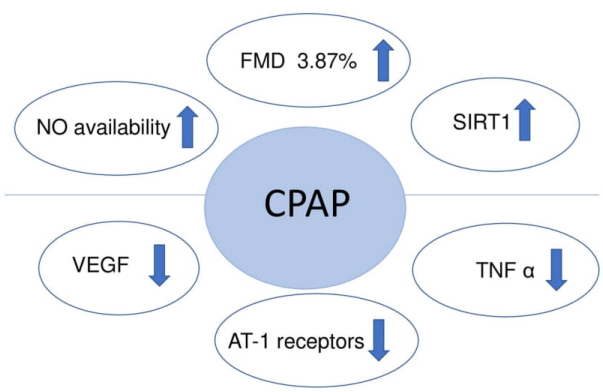

Figure 3. Endothelial function after treatment with CPAP. Abbreviations: VEGF: vascular endothelial growth factor; NO: nitric oxide; FMD: flow mediated dilation; SIRT1: sirtuin 1; TNFα: tumor necrosis factor alpha; AT-1 receptors: expression of angiotensin receptors type-1; ↑: increased; ↓: decreased.

4. Endothelial Function after Treatment with CPAP in Specific Subgroups

The molecular background of the tissue damage in the course of hypoxia and the hypoxia followed by reoxygenation has already been studied at molecular and functional level in numerous basic science-based studies. Since the presence of obstructive sleep apnea might be easily mimicked by the episodes of recurrent tissue hypoxia in the long-term observation, its treatment with the CPAP, leading to the improvement of the oxygen supply does not represent in fact easily a reoxygenation injury. Once the CPAP treatment begins, the hypoxia episodes do not appear or are much less profound, which is accompanied by an optimal oxygen supply preventing from reoxygenation injury. Hence, the clinically observed OSA followed the onset of its treatment mimics hypoxia-induced injury accompanied by initial reoxygenation injury, but its treatment with CPAP reflects rather its prevention and "wound healing" in the long-term outcome. Therefore, the use of CPAP cannot be simply attributed to the reversal of all molecular changes observed in the studies on H-R injury, and its effects may not inhibit or reverse simply all the changes observed in the chronically reversible H-R conditions. The following sections comment on the clinical studies on subjects with the OSA treatment aiming at the explanation of the molecular background of the putative therapeutic effect of CPAP on the overall cardiovascular risk (Table 1).

4.1. Atherosclerosis

Patients with the apnea-hypopnea index ≥20 showed an increased risk for arterial stiffness correlated with the arousal index and with mean O2 saturation compared to other poststroke patients with similar age, sex, body mass index, hypertension and diabetes mellitus status. However, no significant differences were seen in endothelial function measured by Endo-PAT 2000 in patients suffering from sleep apnea [43]. Another study including elderly subjects did not detect significant differences of pulse wave velocity depending OSA severity [44].

In a metanalysis, the pulse wave velocity was more associated with age, systolic blood pressure and diabetes than with the apnea parameters [45].

The coronary artery plaque burden was significantly associated with AHI and was independent of other traditional cardiovascular risk factors. AHI index correlates better with more advanced coronary atherosclerosis than the severity of arterial desaturation [46]. In another study moderate/severe OSA was associated with 10% lower hyperemia index measured using the Endo-PAT device and 35% higher coronary artery calcium (CAC) quantified by electron beam computed tomography, which did not reach the statistical significance ($p = 0.08$ for both comparisons) [47]. Participants with moderate-to-severe OSA were 1.6x more prone to have an ascending thoracic aorta calcification than those without OSA, and the calcification was greater in patients with higher epicardial fat volume [48]. Additionally, the non-dipper profile of nocturnal hypertension makes the OSA patients more prone to a high-risk atherosclerosis [49].

There were different studies results regarding carotid intima media complex. Carotid IMT was not increased in adults with moderate to severe OSA versus controls and does not change following 4 months of PAP treatment in one study [50]. On the contrary, in the study of Catala, the carotid IMT decreased markedly in the CPAP group [51]. In a metanalysis CPAP had no impact on carotid IMT in OSA patients, carotid IMT was significantly decreased after CPAP treatment in more severe OSA patients and patients with long CPAP usage [52].

4.2. Myocardial Infarction

Hypoxia-reoxygenation (H/R) injury is observed during an early phase of myocardial infarction and at the beginning of reperfusion therapy as well as in the severe OSA desaturations, which mimics asphyxia (hypoxia). The increased $ONOO^-$ formation in the heart during H/R, may change several proteins which compose the contractile machinery of the heart leading to systolic dysfunction and cardiac injury [53]. Peroxynitrites formed during the injury may lead to the nitration of cardiac contractile proteins (including the

myosin light chain 1 and 2, MLC1 and MLC2) leading to their increased susceptibility to subsequent proteolysis by the matrix metalloproteinases (i.e., MMP-2) [54,55] Similar changes were observed in an ex vivo model of myocardial infarction [56], pointing thus at the same cardiotoxic pathophysiological mechanisms of the ischemia/reperfusion and hypoxia/reoxygenation cardiac injury. In that studies, compensatory increase in the tissue inhibitor of matrix metalloproteinases-4 (TIMP-4) expression was observed during ischemia, but not reperfusion, which reflects its role in the ischemic preconditioning.

In clinical setting, the prevalence of moderate/severe OSA in patients with coronary artery disease was associated with diminished plasma level of C1q/TNF-related protein-9 (CTRP9) [57]. CTRP9 is an adipokine that protects the heart against ischemic injury and ameliorates cardiac remodeling, which in turn could explain the role of OSA in exacerbating the coronary artery disease.

The role of OSA in increased incidence of myocardial infarction can be also considered together with the progression of atherosclerosis and altered platelet function. The role of CPAP effectiveness in reducing the myocardial infarction incidence is not proven yet. The ISAACC study showed that among non-sleepy patients with acute coronary syndrome, the presence of OSA was not associated with an increased prevalence of cardiovascular events and treatment with CPAP did not significantly reduce this prevalence [58]. On the contrary, RICCADSA trial confirmed that CPAP treatment may reduce this risk, if the device is used at least 4 h/day [59].

4.3. Heart Function

Advanced and untreated sleep apnea, where the recurring prolonged periods of hypoxia followed by reoxygenation mimic to some extend the pathophysiological cascade observed in the course of asphyxia may deteriorate heart function.

Among men 40 to 70 years old, those with AHI > or = 30 were 68% more prone to develop coronary artery disease than those with AHI < 5 [60]. Bakker et al. study showed that the 3 months of CPAP therapy resulted in lowering the left ventricle (LV) end-diastolic volume, as assessed by the magnetic resonance imaging (MRI) [61]. Conversely, in another study no significant changes were noted in ventricular dimensions, systolic and diastolic function, valvular function and coronary vasodilation to nitroglycerin after 3 months of CPAP. The OSA patients display right ventricle dilatation and an increased wall thickening (eccentric hypertrophy) [62].

The coronary flow reserve (CFR), which is decreased in patients with moderate to severe OSA, improved after 3 months of CPAP [63]. CPAP treatment in subjects with congestive heart failure may achieve symptomatic and functional improvements, but exercise alone improved quality of life more than CPAP.

Interestingly, another type of positive pressure therapy (adaptive servo-ventilation therapy) is contraindicated in patients with ejection fraction $\leq$ 45%. Although the SERVE-HF control trial showed increased mortality of any cause, there is no clear mechanism of adverse effects of the therapy. There is a hypothesis that an increased ventilation could cause alkalosis which may interfere with the ion transmembrane transport resulting in proarrhythmic action.

4.4. Diabetes

The common synergism for glucose intolerance, insulin resistance, developing diabetes and OSA can multiply the negative effect of endothelial dysfunction and may increase a cardiovascular risk significantly. Oxidative stress, protein glycation, impairment of the NO bioavailability can be a link multiplying endothelial dysfunction in comorbidity of OSA and diabetes. Molecular changes are partially reversible by the CPAP (Continuous Positive Airway Pressure) therapy, lowering the BMI and lifestyle changes.

Diabetes can exacerbate endothelial dysfunction through ADMA generation stimulated by glyceraldehyde-derived advanced glycation end products (glycer-AGEs). Dys-

lipidemia works through oxidized LDL, which stimulates endothelial cell inflammation, oxidative stress, and apoptosis.

Lifestyle intervention and successful weight reduction significantly improved AHI, BMI, serum triglycerides and insulin resistance in mild OSA patients [64]. The CPAP effect on improving endothelial function measured by the FMD is even greater in OSA patients with coexisting diabetes [61]. The treatment efficiency in coexisting diabetes and OSA could be explained by common mechanisms leading to endothelial dysfunction, which comprises ROS overproduction. In diabetes, ROS are generated as an effect of increased expression of NADPH oxidase, cyclooxygenase, lipoxygenase and impaired antioxidant defense. Similar to OSA, the pathophysiological role of microparticles and increased inflammatory markers are observed. The benefit of CPAP therapy on endothelial function may be explained by similar mechanism in both diseases.

The treatment with CPAP after 12 or 24 weeks showed no effectiveness in changing glycated hemoglobin ($HbA1_c$) levels [65]. CPAP treatment significantly improved the HOMA index, but no significant changes in fasting glucose were observed [66]. CPAP has a favorable effect on insulin resistance, but it is not associated with any significant changes in the total adiponectin levels [67]. Prospective studies showed that regular CPAP use was associated with reduction of diabetes incidence from 3.41 to 1.61 per 100 person-years [68].

4.5. Hypertension

Sympathetic hyper-activation and alteration in the renin-angiotensin-aldosterone axis may play a pathophysiological role in patients with obstructive sleep apnea. Increased renin generation is induced by efferent renal sympathetic nerve activation and leads to activation of the renin-angiotensin-aldosterone system (RAAS). OSA causes systemic inflammation and oxidative stress, which results in increased endothelin-1 generation and decreased NO production in endothelial cells.

Endothelin receptor antagonist, bosentan, suppressed the increase in SBP during a 5 min hypoxic challenge (143 $\pm$ 5 mmHg vs. 127 $\pm$ 3 mmHg), which confirms the role of endothelin in response to acute hypoxia in patients with severely untreated OSA [69].

OSA is related to an increased risk of resistant hypertension [70]. In patients with resistant hypertension and OSA catheter-based renal sympathetic denervation after 6 months reduced the mean ambulatory blood pressure by 8.3/6.2 mmHg, with no significant changes in the sleep apnea severity [71].

CPAP treatment may improve the hypertension and cardiovascular outcomes by reducing aldosterone excess in resistant hypertensive individuals with OSA. There was a borderline significant reduction in 24 h urine collection for aldosterone after 6 months of follow-up [72].

Although it is well known that the treatment of sleep apnea lowers blood pressure, the effectiveness of the therapy is not very high, but its effect translated into CV events and mortality reduction over the long term, is not completely negligible. After 6 months, the CPAP caused greater reduction of night-time systolic blood pressure at 4.7 mm Hg. The CPAP was associated with significant reductions of the 24 h ambulatory systolic blood pressure of 2.32 mm Hg and diastolic blood pressure of 1.98 mm [73,74]. A better effect of CPAP was observed in the resistant hypertension subjects -5.40 mmHg and -3.86 mmHg respectively [75]. In the subjects with severe oxygen desaturations (SpO2 < 77%) with good CPAP adherence the reduction of systolic blood pressure was observed [76]. Severe desaturations but not AHI were associated with better hypotensive response of CPAP. That could be explained by reducing the ROS production during hypoxia/reoxygenation injury. Indication for the therapy should comprise not only not only the frequency but also the depth and duration of sleep-related upper airway obstructions. Another aspect is that deeper hypoxia leads to stronger stimulation of the peripheral chemoreceptors, which increase sympathetic system. Other study showed that a 2 week withdrawal of CPAP leads to a relevant increase in morning blood pressure of nearly 10 mm Hg in the moderate to severe sleep apnea patients [77]. Interestingly supplemental oxygen, which reduced

intermittent hypoxia (IH) and had a minimal effect on AHI abolished the rise in morning blood pressure during CPAP withdrawal [78]. Therefore, IH, and not recurrent arousals, appears to be the dominant cause of daytime increases in blood pressure in OSA. There is a need to better understand the effects of CPAP on BP since a better characteristic of patients who benefit most might help to tailor therapy according to the expected benefit to reduce BP and in general improve the patient's cardiovascular risk profile. Interestingly, a systematic review and meta-analysis of randomized controlled trials (RCTs) compared the effect of CPAP on BP in particular subgroups of patients and aimed at defining the group with the best response to treatment. The meta-analysis has defined younger age, uncontrolled blood pressure and severe OSA-related oxygen desaturations as positive predictors of a favorable blood pressure response to OSA treatment. Desaturation of less than 77% were associated with a greater BP drop at follow-up in treated patients further supporting the role of intermittent hypoxia in the pathogenesis of OSA- related hypertension [76]. This observation points at oxidative stress as on the important therapeutic target.

4.6. Pulmonary Hypertension

The prevalence of pulmonary hypertension in OSA ranges from 17 to 53% [79]. Small pulmonary arteries constrict in the presence of alveolar hypoxia. It helps to redirect blood flow from poorly-ventilated lung regions to those which are well-ventilated. It is also known as hypoxic pulmonary vasoconstriction (HPV) or the van Euler–Liljestrand mechanism. During global alveolar hypoxia, HPV leads to pulmonary hypertension. ROS originating during hypoxia/reoxygenation in OSA patients can interact with protein kinases, phospholipases, and other ion channels modulating response of the pulmonary arterial smooth muscle cells [80]. The patients suffering from OSA had increased serum levels of C-reactive protein and 8-isoprostane, TNFα, interleukin (IL)-1β and IL-6 in the pulmonary tissue [81]. Another study revealed that limited NO bioavailability caused by IH could be compensated by increased pulmonary vascular smooth muscles sensitivity to NO and cGMP [82].

In one study the CPAP therapy was associated with a significant decrease in pulmonary artery pressure in patients with isolated OSA and pulmonary hypertension [83].

4.7. Atrial Fibrillation and Other Arrhythmias

Chronic OSA induces sympathetic activation followed by a structural and electrical remodeling of the atria contributing to the AF maintenance and recurrence of AF paroxysms [84]. Higher AHI is a known factor associated with persistent or permanent AF [85].

Reactive oxygen species and chronic inflammation decrease $Na^{+/}K^{+}$ ATPase currents, alter the $Ca2^{+}$ homeostasis and down-regulate some proteins, such as connexin-43 [86]. Changed ion currents contribute to increased proarrhythmic activity.

The effects of a 3-month CPAP therapy were observed in patients with arrhythmias such as supraventricular and ventricular extrasystoles, atrial fibrillation, non-sustained ventricular tachycardia (nsVT), and sinus pauses [87]. There was a significant decrease in atrial and ventricular ectopy count in patients with AF [88]. Another study confirms that CPAP therapy has an impact on reversing the atrial remodeling in patients with OSA. Following the treatment, the atrial pressure, volume overload and serum BNP levels were significantly reduced. These observations may suggest that the substrate predisposing to AF may be reversible and measured by total atrial conduction time assessed by tissue doppler imaging (PA-TDI interval) and BNP [89].

CPAP therapy resulted in a higher AF-free survival rate and an AF-free survival off antiarrhythmic drugs or repeat ablation. AF recurrence rate of CPAP-treated patients was similar to a group of patients without OSA and was significantly higher in CPAP nonuser patients [90]. Patients with OSA are less likely to remain in sinus rhythm after catheter ablation of AF. Concomitant OSA increased (HR 2.61) and usage of CPAP therapy decreased (HR 0.41) the probability of AF recurrences in prospective study [91]. On the contrary,

randomized controlled trial assessing the impact of treatment of OSA on recurrence of AF after direct current cardioversion (DCCV) did not detect a difference between those treated with PAP versus usual care [92].

Another study found that apnea/hypopnea duration is the main factor for heart conduction disorders [93]. Cardiac activity pauses were correlated with the longest apnea, as well as the AHI and oxygen desaturation index [87].

4.8. Pediatric Population—Effect of Tonsillectomy

Interesting studies showed that OSA in children can induce endothelial dysfunction. The lowest FMD values were found in children with higher AHI [94]. The improvement in FMD following adenotonsillectomy was found together with a decrease in oxidative stress [95]. Hypermethylation of the core promoter region of eNOS gene in the OSA children was related to decreased eNOS expression. Additionally, children with OSA had increased expression of genes encoding pro-oxidant enzymes and decreased expression of genes encoding anti-oxidant enzymes [96]. OSA in adolescents appears to increase independently the risk of dyslipidemia, insulin resistance, and hypertension [97].

Table 1. Endothelial function after treatment in specific subgroups.

OSA Subpopulation	Demonstrated Molecular Pathomechanism	Effect of OSA Treatment
Atherosclerosis	endothelial dysfunction	No effect on endothelium, did not reduced PWV [44] (2019, clinical trial, 101 patients)
Myocardial Infarction	Increased peroxynitrite formation [53], nitration of cardiac contractile proteins (MLC1 and MLC2) and their subsequent degradation (by MMP-2) protective role of TIMP-4 in ischemic preconditioning [54–56]	The ISAACC—among non-sleepy patients with acute coronary syndrome, treatment with CPAP did not significantly reduce the prevalence of acute coronary syndromes [58]. (2020, randomized controlled trial, 1264 subjects) On the contrary, RICCADSA trial confirmed that CPAP treatment may reduce this risk, if the device is used at least 4 h/day [59] (2016, randomized controlled trial, 244 subjects)
Heart Failure	Increased peroxynitrite formation [53]	No effect on endothelium, lowering the left ventricle end-diastolic volume [61] (2020, randomized controlled trial, 141 patients)
Diabetes	impairment of the NO bioavailability, ROS	improved HOMA index, no effect on adipokine level [67] (2015, meta-analysis)
Hypertension	activation of RAAS	SBP—2.32 mm Hg [74] (2015, meta-analysis, 794 patients)
Pulmonary Hypertension	Increased inflammatory cytokines [81]	decrease in pulmonary artery pressure [83] (2010, metanalysis, 222patients)
Atrial Fibrillation	down-regulation connexin-43 [86]	(HR 0.41) the probability of AF recurrences [91] (2012, prospective study, 153 patients)
Children	decreased eNOS expression [96]	FMD improvement after tonsillectomy [95] (2015, clinical trial, 144 patients)

5. Platelet Function

OSA may change the platelet function and contribute to the pro-coagulative state. Platelets play a great role in atherothrombosis and their reactivity appeared to be higher in OSA patients (Table 2).

There were several studies observing that mean platelet volume (MPV) was independently correlated with AHI. MPV was considered as a new marker associated with atherothrombosis. An increased MPV previously was explained by new generated platelets, which have higher volume and density. The new data suggest that there are different platelets subpopulations originating from different megakaryocytes. The high-volume platelets correlate with higher expression of adhesion molecules, increased aggregation, enhanced release of thromboxane TXA2, whereas small and low-density platelets have an enhanced intracellular $Ca2^{+}$ response to thrombin. MPV and platelet distribution width (PDW) are higher in severe OSA compared to control group, but no significant differences between controls and patients with mild and moderate OSA were observed. Mean platelet

volume and red blood cell distribution width changed significantly after 3-month CPAP treatment [98,99].

Arachidonic acid- and adenosine diphosphate (ADP)-induced aggregation in the OSA group was significantly higher than in the non-OSA group [100]. In experimental human and animal models induced hypoxia/reoxygenation contributed to enhanced TXA2 formation and increased activation of matrix metalloproteinase 2 (MMP-2) leading to platelet activation and subsequent aggregation [101,102].

Moreover, patients with OSA were characterized by significantly lower inhibitory rate of the ADP-dependent aggregation. OSA patients were more likely to have high residual platelet reactivity after acetylsalicylic acid or clopidogrel therapy [98]. OSA-related intermittent hypoxia and reoxygenation frequency contributed to platelet hyperaggregability for ADP more than total hypoxic time during the sleep. Patients with one or more vascular risk factors such as diabetes, hypertension, smoking, hyperlipidemia were prone to platelet hyperaggregability for both ADP and collagen. After three months CPAP treatment partially normalized the OSA-related ADP- and collagen-induced platelet hyperaggregability [103]. The possible mechanisms underlying this phenomenon include normalized levels of proinflammatory mediators and decreased activity of sympathetic nervous system after treatment.

Another study showed that desaturating patients had lower GPIb fluorescence in circulating platelets. The platelet surface P-selectin, platelet surface-activated GPIIb/IIIa, platelet-monocyte aggregation, platelet-neutrophil aggregation, CD62P and were not significantly correlated with markers of OSA in one study [104]. Conversely, another study found a progressive increase in the concentrations of soluble E-selectin, P-selectin and L-selectin in OSA patients [105]. Even though the surface selectins remain unchanged, the increased soluble forms may be considered as markers of inflammation of vascular wall with prothrombic activity. Another study confirms that serum content of platelet P-selectin and P-selectin glycoprotein ligand 1 are increased proportionally with OSA severity [106]. CPAP significantly decreased serum levels of sCD40L and sP-selectin in patients with moderate to severe OSA [107,108].

Hypercoagulative State in OSA

Patients with moderate to severe OSA have elevated PT and INR compared with healthy individuals [99]. This could be connected with increased activity of coagulation factor VII.

OSA patients are characterized by elevated plasma fibrinogen levels which is induced by chronic inflammation, exaggerated platelet activity, and reduced fibrinolytic capacity. In hypertensive patients with OSA fibrin clot was characterized by more compact fibrin structure, impaired fibrinolysis and faster clot formation, which was normalized after 3 months of CPAP treatment [109].

Table 2. Cardiovascular thromboembolic disorders and CPAP effectiveness.

Demonstrated Platelet-Derived Molecular Pathomechanism	Thromboembolic Disorders	Clinical Effect of OSA Treatment-Based on Clinical Studies
-platelet hyperaggregability for both ADP and collagen	Stroke	Reduction in risk of stroke in elderly patients [110] (2021, retrospective cohort study, 5757 patients)
-lower inhibitory rate of the ADP-dependent aggregation -high residual platelet reactivity after acetylsalicylic acid or clopidogrel therapy [98]	Myocardial Infarction	No significant effect in the MI incidence reduction in prospective observation of the CPAP treatment patients [58] (2020, randomized controlled trial, 2551 patients), [111] (2016, randomized controlled trial, 2717 patients)

6. Erythrocytes

The cause of an increased cardiovascular morbidity in OSA patients could be found in functional and structural changes of erythrocytes. OSA is associated with an increased erythrocyte adhesion measured by the optic protocols on glass slide samples. Erythrocyte adhesiveness and aggregation correlate with an increase of inflammatory acute phase proteins [112].

Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) are endogenous inhibitors of NO synthesis whose plasma concentrations were demonstrated to be elevated in OSA patients. Plasma ADMA levels were associated with a lower membrane fluidity of erythrocytes, suggesting that ADMA might have a close correlation with the rheologic behavior of erythrocytes and the microcirculation [113]. ADMA and the non-specific pharmacological NOS inhibitor, L-NAME, independently reduced the deformability of red blood cells (RBC) obtained from rabbits treated with a high cholesterol diet. The effect was reversed with activators of the NO pathway. These results suggest that NO plays an important role in improving the microcirculation by restoring RBC deformability. Impaired erythrocyte deformability may be partially dependent upon the accumulation of ADMA in RBC [114]. Red blood cells contain a nitric oxide synthase (RBC-NOS) which produces NO modifying RBC deformability through direct S-nitrosylation of cytoskeleton proteins including spectrin-α, spectrin-β [115]. Other cytosolic erythrocytic NG-dimethylated proteins like protein 4.1 could also play a role in changing the stability of the erythrocyte membrane.

NO binds cooperatively to βCys93 in oxygenated Hb creating s-nitrosyloheamoglobin (SNO-Hb). In the deoxygenated state of hemoglobin reactions of NO with heme-iron are favored over thiol. Only a small fraction of the NO carried by Hb is released from RBCs, but transition from high to low oxygen tension in the peripheral arterioles and capillaries promotes its release as SNO-based vasodilatory activity [116]. No data was found on the role of OSA and ROS in the hemoglobin S-nitrosylation.

Moreover, RBCs contain the arginine-rich proteins which could be a potential source of inhibitory methylarginines (Figure 4). Protein-arginine methyltransferases (PRMTs) catalyze the methylation of proteinic L-arginine to produce the monomethyl and dimethylarginine proteins. Bollenbach et al. observed that main sources of methylarginines include proteins responsible for the stability of erythrocyte membrane spectrin-α, spectrin-β and protein 4.1. The same study observed that methylated arginine can change protein function and some peptide chains with methylated arginine induced platelet aggregation [117].

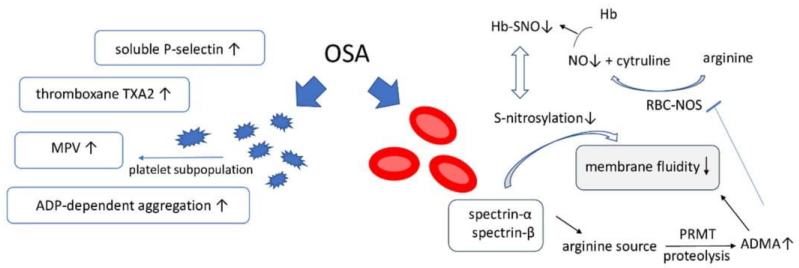

Figure 4. OSA effect on platelets and erythrocytes. OSA: obstructive sleep apnea; MPV-mean platelet volume; ADMA: asymmetric dimethylarginine, PMRT: protein-arginine methyl transferase, Hb-SNO: s-nitrosyloheamoglobin; RBC-NOS: red bleed cell nitric oxide synthase. ↑: increased; ↓: decreased.

In vitro, upon lysis, erythrocytes are able to release pathologically relevant quantities of free ADMA. After 2 h of incubation free ADMA level increased sevenfold [118]. However, another study suggests that relevant physiological rate of in vivo hemolysis is unlikely to increase significantly human plasma concentration of free ADMA [119]. Further studies need to be undertaken to examine the role of RBC in generation and storage of

ADMA, bioavailability and synthesis of NO and its contribution to the pathogenesis of endothelial dysfunction.

DDAH is an enzyme responsible for hydrolysis of both ADMA and L-NMMA, which was found in human RBCs by immunoprecipitation using a specific monoclonal antibody to human DDAH [120]. DDAH activity can be inhibited by SH-specific agents such as inorganic and organic mercury compounds, and by S-nitrosothiols which block the SH group of DDAH that is essential for its hydrolytic activity [121].

7. Conclusions

Numerous studies on pathophysiological mechanisms of sleep apnea contributing to an increased cardiovascular risk were conducted. Some clinical studies have shown the importance of molecular changes caused by intermittent hypoxia leading to the endothelial dysfunction. Sleep apnea and all pathophysiological changes could also affect platelets and erythrocytes, which may result in a hypercoagulative state and higher risk of atherothrombotic events.

The enthusiasm for potential clinical usefulness of CPAP therapy is lowered by a deficiency of strong randomized studies confirming its effectiveness in cardiovascular risk reduction. The CPAP improves endothelial function. Nevertheless, when compared with the natural history of OSA, the use of CPAP seems to result in an insignificant reduction in the cardiovascular events incidence, as demonstrated in more than one large clinical trials [111,122]. It is noteworthy that some trend for lowering the risk of stroke for those subjects with good CPAP adherence therapy ($\geq$4 h per night) has been demonstrated, as results from one study [123]. This review proves CPAP effectiveness in different subpopulations of OSA patients and its additional positive effects in reduction of arrythmias, lowering diabetes incidence and better hypertension treatment.

The discrepancies in the results of clinical studies and some translative difficulties with transposition of the basic research results to clinical setting, may stem from simultaneous role of both, endothelium and blood components in the pathophysiology of the OSA-related thromboembolic complications as well as indicate their common role as the therapeutic target in the course of CPAP treatment.

Therefore, future prospective studies are needed in order to define novel therapeutic strategies aimed at minimizing the cardiovascular risk in subjects with obstructive sleep apnea.

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References

1. Senaratna, C.V.; Perret, J.L.; Lodge, C.J.; Lowe, A.J.; Campbell, B.E.; Matheson, M.C.; Hamilton, G.S.; Dharmage, S.C. Prevalence of obstructive sleep apnea in the general population: A systematic review. *Sleep Med. Rev.* **2017**, *34*, 70–81. [CrossRef] [PubMed]
2. Lee, C.H.; Sethi, R.; Li, R.; Ho, H.H.; Hein, T.; Jim, M.H.; Loo, G.; Koo, C.Y.; Gao, X.F.; Chandra, S.; et al. Obstructive sleep apnea and cardiovascular events after percutaneous coronary intervention. *Circulation* **2016**, *133*, 2008–2017. [CrossRef]
3. Dewan, N.A.; Nieto, F.J.; Somers, V.K. Intermittent hypoxemia and OSA: Implications for comorbidities. *Chest* **2015**, *147*, 266–274. [CrossRef] [PubMed]

4. Turrens, J.F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* **2003**, *552*, 335–344. [CrossRef] [PubMed]
5. Picón-Pagès, P.; Garcia-Buendia, J.; Muñoz, F.J. Functions and dysfunctions of nitric oxide in brain. *Biochim. Biophys. Acta Mol. Basis Dis.* **2019**, *1865*, 1949–1967. [CrossRef] [PubMed]
6. Kooy, N.W.; Lewis, S.J.; Royall, J.A.; Ye, Y.Z.; Kelly, D.R.; Beckman, J.S. Extensive tyrosine nitration in human myocardial inflammation: Evidence for the presence of peroxynitrite. *Crit. Care Med.* **1997**, *25*, 812–819. [CrossRef]
7. Roggensack, A.M.; Zhang, Y.; Davidge, S.T. Evidence for Peroxynitrite Formation in the Vasculature of Women with Preeclampsia. *Hypertension* **1999**, *33*, 83–89. [CrossRef]
8. Ohmori, H.; Kanayama, N. Immunogenicity of an inflammation-associated product, tyrosine nitrated self-proteins. *Autoimmun. Rev.* **2005**, *4*, 224–229. [CrossRef]
9. De Pascali, F.; Hemann, C.; Samons, K.; Chen, C.A.; Zweier, J.L. Hypoxia and reoxygenation induce endothelial nitric oxide synthase uncoupling in endothelial cells through tetrahydrobiopterin depletion and S-glutathionylation. *Biochemistry* **2014**, *53*, 3679–3688. [CrossRef]
10. Alonso-Fernandez, A.; Garcia-Rio, F.; Arias, M.A.; Hernanz, A.; de la Pena, M.; Pierola, J.; Barcelo, A.; Lopez-Collazo, E.; Agusti, A. Effects of CPAP on oxidative stress and nitrate efficiency in sleep apnoea: A randomised trial. *Thorax* **2009**, *64*, 581–586. [CrossRef]
11. Tuleta, I.; França, C.N.; Wenzel, D.; Fleischmann, B.; Nickenig, G.; Werner, N.; Skowasch, D. Intermittent Hypoxia Impairs Endothelial Function in Early Preatherosclerosis. In *Advances in Experimental Medicine and Biology*; Springer: Cham, Switzerland, 2015; Volume 858, pp. 1–7.
12. Wang, J.; Chen, S.; Ma, X.; Cheng, C.; Xiao, X.; Chen, J.; Liu, S.; Zhao, B.; Chen, Y. Effects of endothelial progenitor cell-derived microvesicles on hypoxia/reoxygenation-induced endothelial dysfunction and apoptosis. *Oxid. Med. Cell. Longev.* **2013**, *2013*, 572729. [CrossRef] [PubMed]
13. Zhang, Q.; Shang, M.; Zhang, M.; Wang, Y.; Chen, Y.; Wu, Y.; Liu, M.; Song, J.; Liu, Y. Microvesicles derived from hypoxia/reoxygenation-treated human umbilical vein endothelial cells promote apoptosis and oxidative stress in H9c2 cardiomyocytes. *BMC Cell Biol.* **2016**, *17*, 25. [CrossRef] [PubMed]
14. Bhattacharjee, R.; Khalyfa, A.; Khalyfa, A.A.; Mokhlesi, B.; Kheirandish-Gozal, L.; Almendros, I.; Peris, E.; Malhotra, A.; Gozal, D. Exosomal cargo properties, endothelial function and treatment of obesity hypoventilation syndrome: A proof of concept study. *J. Clin. Sleep Med.* **2018**, *14*, 797–807. [CrossRef]
15. Priou, P.; Gagnadoux, F.; Tesse, A.; Mastronardi, M.L.; Agouni, A.; Meslier, N.; Racineux, J.L.; Martinez, M.C.; Trzepizur, W.; Andriantsitohaina, R. Endothelial dysfunction and circulating microparticles from patients with obstructive sleep apnea. *Am. J. Pathol.* **2010**, *177*, 974–983. [CrossRef]
16. Sharma, P.; Dong, Y.; Somers, V.K.; Peterson, T.E.; Zhang, Y.; Wang, S.; Li, G.; Singh, P. Intermittent hypoxia regulates vasoactive molecules and alters insulin-signaling in vascular endothelial cells. *Sci. Rep.* **2018**, *8*, 14110. [CrossRef]
17. Kaczmarek, E.; Bakker, J.P.; Clarke, D.N.; Csizmadia, E.; Kocher, O.; Veves, A.; Tecilazich, F.; O'Donnell, C.P.; Ferran, C.; Malhotra, A. Molecular Biomarkers of Vascular Dysfunction in Obstructive Sleep Apnea. *PLoS ONE* **2013**, *8*, e70559. [CrossRef]
18. Sun, H.; Zhang, H.; Li, K.; Wu, H.; Zhan, X.; Fang, F.; Qin, Y.; Wei, Y. ESM-1 promotes adhesion between monocytes and endothelial cells under intermittent hypoxia. *J. Cell. Physiol.* **2019**, *234*, 1512–1521. [CrossRef] [PubMed]
19. Zhou, J.; Bai, W.; Liu, Q.; Cui, J.; Zhang, W. Intermittent Hypoxia Enhances THP-1 Monocyte Adhesion and Chemotaxis and Promotes M1 Macrophage Polarization via RAGE. *Biomed Res. Int.* **2018**, *2018*, 1650456. [CrossRef]
20. Chuang, L.P.; Chen, N.H.; Lin, Y.; Ko, W.S.; Pang, J.H.S. Increased MCP-1 gene expression in monocytes of severe OSA patients and under intermittent hypoxia. *Sleep Breath.* **2016**, *20*, 425–433. [CrossRef]
21. Gileles-Hillel, A.; Almendros, I.; Khalyfa, A.; Zhang, S.X.; Wang, Y.; Gozal, D. Early intermittent hypoxia induces proatherogenic changes in aortic wall macrophages in a murine model of obstructive sleep apnea. *Am. J. Respir. Crit. Care Med.* **2014**, *190*, 958–961. [CrossRef]
22. Gautier-Veyret, E.; Bäck, M.; Arnaud, C.; Belaïdi, E.; Tamisier, R.; Lévy, P.; Arnol, N.; Perrin, M.; Pépin, J.L.; Stanke-Labesque, F. Cysteinyl-leukotriene pathway as a new therapeutic target for the treatment of atherosclerosis related to obstructive sleep apnea syndrome. *Pharmacol. Res.* **2018**, *134*, 311–319. [CrossRef] [PubMed]
23. Olejarz, W.; Głuszko, A.; Cyran, A.; Bednarek-Rajewska, K.; Proczka, R.; Smith, D.F.; Ishman, S.L.; Migacz, E.; Kukwa, W. TLRs and RAGE are elevated in carotid plaques from patients with moderate-to-severe obstructive sleep apnea syndrome. *Sleep Breath.* **2020**, *24*, 1573–1580. [CrossRef] [PubMed]
24. Williams, A.; Scharf, S.M. Obstructive sleep apnea, cardiovascular disease, and inflammation—Is NF-κB the key? *Sleep Breath.* **2007**, *11*, 69–76. [CrossRef]
25. Kyotani, Y.; Takasawa, S.; Yoshizumi, M. Proliferative pathways of vascular smooth muscle cells in response to intermittent hypoxia. *Int. J. Mol. Sci.* **2019**, *20*, 2706. [CrossRef] [PubMed]
26. Bao, Q.; Zhang, B.; Suo, Y.; Liu, C.; Yang, Q.; Zhang, K.; Yuan, M.; Yuan, M.; Zhang, Y.; Li, G. Intermittent hypoxia mediated by TSP1 dependent on STAT3 induces cardiac fibroblast activation and cardiac fibrosis. *eLife* **2020**, *9*, e49923. [CrossRef]
27. Panoutsopoulos, A.; Kallianos, A.; Kostopoulos, K.; Seretis, C.; Koufogiorga, E.; Protogerou, A.; Trakada, G.; Kostopoulos, C.; Zakopoulos, N.; Nikolopoulos, I. Effect of CPAP treatment on endothelial function and plasma CRP levels in patients with sleep apnea. *Med. Sci. Monit.* **2012**, *18*, CR747–CR751. [CrossRef]

28. Ning, Y.; Zhang, T.S.; Wen, W.W.; Li, K.; Yang, Y.X.; Qin, Y.W.; Zhang, H.N.; Du, Y.H.; Li, L.Y.; Yang, S.; et al. Effects of continuous positive airway pressure on cardiovascular biomarkers in patients with obstructive sleep apnea: A meta-analysis of randomized controlled trials. *Sleep Breath.* **2019**, *23*, 77–86. [CrossRef]
29. Schwarz, E.I.; Puhan, M.A.; Schlatzer, C.; Stradling, J.R.; Kohler, M. Effect of CPAP therapy on endothelial function in obstructive sleep apnoea: A systematic review and meta-analysis. *Respirology* **2015**, *20*, 889–895. [CrossRef]
30. Thunström, E.; Glantz, H.; Yucel-Lindberg, T.; Lindberg, K.; Saygin, M.; Peker, Y. Cpap does not reduce inflammatory biomarkers in patients with coronary artery disease and nonsleepy obstructive sleep apnea: A randomized controlled trial. *Sleep* **2017**, *40*, zsx157. [CrossRef]
31. Campos-Rodriguez, F.; Asensio-Cruz, M.I.; Cordero-Guevara, J.; Jurado-Gamez, B.; Carmona-Bernal, C.; Gonzalez-Martinez, M.; Troncoso, M.F.; Sanchez-Lopez, V.; Arellano-Orden, E.; Garcia-Sanchez, M.I.; et al. Effect of continuous positive airway pressure on inflammatory, antioxidant, and depression biomarkers in women with obstructive sleep apnea: A randomized controlled trial. *Sleep* **2019**, *42*, zsz145. [CrossRef]
32. Arias, M.A.; García-Río, F.; Alonso-Fernández, A.; Hernanz, Á.; Hidalgo, R.; Martínez-Mateo, V.; Bartolomé, S.; Rodríguez-Padial, L. CPAP decreases plasma levels of soluble tumour necrosis factor-α receptor 1 in obstructive sleep apnoea. *Eur. Respir. J.* **2008**, *32*, 1009–1015. [CrossRef]
33. Khayat, R.N.; Varadharaj, S.; Porter, K.; Sow, A.; Jarjoura, D.; Gavrilin, M.A.; Zweier, J.L. Angiotensin Receptor Expression and Vascular Endothelial Dysfunction in Obstructive Sleep Apnea. *Am. J. Hypertens.* **2018**, *31*, 355–361. [CrossRef]
34. Chen, W.J.; Liaw, S.F.; Lin, C.C.; Chiu, C.H.; Lin, M.W.; Chang, F.T. Effect of Nasal CPAP on SIRT1 and Endothelial Function in Obstructive Sleep Apnea Syndrome. *Lung* **2015**, *193*, 1037–1045. [CrossRef] [PubMed]
35. López-Cano, C.; Rius, F.; Sánchez, E.; Gaeta, A.M.; Betriu, À.; Fernández, E.; Yeramian, A.; Hernández, M.; Bueno, M.; Gutiérrez-Carrasquilla, L.; et al. The influence of sleep apnea syndrome and intermittent hypoxia in carotid adventitial vasa vasorum. *PLoS ONE* **2019**, *14*, e0211742. [CrossRef] [PubMed]
36. Qi, J.C.; Zhang, L.J.; Li, H.; Zeng, H.; Ye, Y.; Wang, T.; Wu, Q.; Chen, L.; Xu, Q.; Zheng, Y.; et al. Impact of continuous positive airway pressure on vascular endothelial growth factor in patients with obstructive sleep apnea: A meta-analysis. *Sleep Breath.* **2019**, *23*, 5–12. [CrossRef] [PubMed]
37. Turnbull, C.D.; Rossi, V.A.; Santer, P.; Schwarz, E.I.; Stradling, J.R.; Petousi, N.; Kohler, M. Effect of OSA on hypoxic and inflammatory markers during CPAP withdrawal: Further evidence from three randomized control trials. *Respirology* **2017**, *22*, 793–799. [CrossRef]
38. Schulz, R.; Flötotto, C.; Jahn, A.; Eisele, H.J.; Weissmann, N.; Seeger, W.; Rose, F. Circulating adrenomedullin in obstructive sleep apnoea. *J. Sleep Res.* **2006**, *15*, 89–95. [CrossRef] [PubMed]
39. Paz, Y.; Mar, H.L.; Hazen, S.L.; Tracy, R.P.; Strohl, K.P.; Auckley, D.; Bena, J.; Wang, L.; Walia, H.K.; Patel, S.R.; et al. Effect of Continuous Positive Airway Pressure on Cardiovascular Biomarkers: The Sleep Apnea Stress Randomized Controlled Trial. *Chest* **2016**, *150*, 80–90. [CrossRef]
40. Borges, Y.G.; Cipriano, L.H.C.; Aires, R.; Zovico, P.V.C.; Campos, F.V.; de Araújo, M.T.M.; Gouvea, S.A. Oxidative stress and inflammatory profiles in obstructive sleep apnea: Are short-term CPAP or aerobic exercise therapies effective? *Sleep Breath.* **2020**, *24*, 541–549. [CrossRef]
41. Lee, M.Y.K.; Ge, G.; Fung, M.L.; Vanhoutte, P.M.; Mak, J.C.W.; Ip, M.S.M. Low but not high frequency of intermittent hypoxia suppresses endothelium-dependent, oxidative stress-mediated contractions in carotid arteries of obese mice. *J. Appl. Physiol.* **2018**, *125*, 1384–1395. [CrossRef]
42. Bozkurt, H.; Neyal, A.; Geyik, S.; Taysi, S.; Anarat, R.; Bulut, M.; Neyal, A.M. Investigation of the plasma nitrite levels and oxidant-antioxidant status in obstructive sleep apnea syndrome. *Noropsikiyatri Ars.* **2015**, *52*, 221–225. [CrossRef] [PubMed]
43. Cereda, C.W.; Tamisier, R.; Manconi, M.; Andreotti, J.; Frangi, J.; Pifferini, V.; Bassetti, C.L. Endothelial dysfunction and arterial stiffness in ischemic stroke: The role of sleep-disordered breathing. *Stroke* **2013**, *44*, 1175–1178. [CrossRef]
44. Sforza, E.; Millasseau, S.; Hupin, D.; Barthélémy, J.C.; Roche, F. Arterial stiffness alteration and obstructive sleep apnea in an elderly cohort free of cardiovascular event history: The PROOF cohort study. *Sleep Breath.* **2019**, *23*, 201–208. [CrossRef] [PubMed]
45. Joyeux-Faure, M.; Tamisier, R.; Borel, J.C.; Millasseau, S.; Galerneau, L.M.; Destors, M.; Bailly, S.; Pepin, J.L. Contribution of obstructive sleep apnoea to arterial stiffness: A meta-analysis using individual patient data. *Thorax* **2018**, *73*, 1146–1151. [CrossRef] [PubMed]
46. Mo, L.; Gupta, V.; Modi, R.; Munnur, K.; Cameron, J.D.; Seneviratne, S.; Edwards, B.A.; Landry, S.A.; Joosten, S.A.; Hamilton, G.S.; et al. Severe obstructive sleep apnea is associated with significant coronary artery plaque burden independent of traditional cardiovascular risk factors. *Int. J. Cardiovasc. Imaging* **2019**. [CrossRef] [PubMed]
47. Shpilsky, D.; Erqou, S.; Patel, S.R.; Kip, K.E.; Ajala, O.; Aiyer, A.; Strollo, P.J.; Reis, S.E.; Olafiranye, O. Association of obstructive sleep apnea with microvascular endothelial dysfunction and subclinical coronary artery disease in a community-based population. *Vasc. Med. (UK)* **2018**, *23*, 331–339. [CrossRef]
48. Kim, S.; Lee, K.Y.; Kim, N.H.; Abbott, R.D.; Kim, C.; Lee, S.K.; Kim, S.H.; Shin, C. Relationship of obstructive sleep apnoea severity and subclinical systemic atherosclerosis. *Eur. Respir. J.* **2020**, *55*, 1900959. [CrossRef] [PubMed]
49. Somuncu, M.U.; Karakurt, S.T.; Karakurt, H.; Serbest, N.G.; Cetin, M.S.; Bulut, U. The additive effects of OSA and nondipping status on early markers of subclinical atherosclerosis in normotensive patients: A cross-sectional study. *Hypertens. Res.* **2019**, *42*, 195–203. [CrossRef]

50. Kim, J.; Mohler, E.R.; Keenan, B.T.; Maislin, D.; Arnardottir, E.S.; Gislason, T.; Benediktsdottir, B.; Gudmundsdottir, S.; Sifferman, A.; Staley, B.; et al. Carotid artery wall thickness in obese and nonobese adults with obstructive sleep apnea before and following positive airway pressure treatment. *Sleep* **2017**, *40*, zsx126. [CrossRef]
51. Català, R.; Ferré, R.; Cabré, A.; Girona, J.; Porto, M.; Texidó, A.; Masana, L. Efecto a largo plazo del tratamiento con presión positiva continua de la vía aérea sobre la arteriosclerosis subclínica en el síndrome de apnea-hipopnea durante el sueño. *Med. Clin. (Barc.)* **2016**, *147*, 1–6. [CrossRef]
52. Chen, L.D.; Lin, L.; Lin, X.J.; Ou, Y.W.; Wu, Z.; Ye, Y.M.; Xu, Q.Z.; Huang, Y.P.; Cai, Z.M. Effect of continuous positive airway pressure on carotid intima-media thickness in patients with obstructive sleep apnea: A meta-analysis. *PLoS ONE* **2017**, *12*, e0184293. [CrossRef]
53. Polewicz, D.; Cadete, V.J.J.; Doroszko, A.; Hunter, B.E.; Sawicka, J.; Szczesna-Cordary, D.; Light, P.E.; Sawicki, G. Ischemia induced peroxynitrite dependent modifications of cardiomyocyte MLC1 increases its degradation by MMP-2 leading to contractile dysfunction. *J. Cell. Mol. Med.* **2011**, *15*, 1136–1147. [CrossRef] [PubMed]
54. Doroszko, A.; Polewicz, D.; Sawicka, J.; Richardson, J.S.; Sawicki, G.; Cheung, P.Y. Cardiac dysfunction in an animal model of neonatal asphyxia is associated with increased degradation of MLC1 by MMP-2. *Basic Res. Cardiol.* **2009**, *104*, 669–679. [CrossRef] [PubMed]
55. Doroszko, A.; Polewicz, D.; Cadete, V.J.J.; Sawicka, J.; Jones, M.; Szczesna-Cordary, D.; Cheung, P.Y.; Sawicki, G. Neonatal asphyxia induces the nitration of cardiac myosin light chain 2 that is associated with cardiac systolic dysfunction. *Shock* **2010**, *34*, 592–600. [CrossRef]
56. Cadete, V.J.J.; Arcand, S.A.; Chaharyn, B.M.; Doroszko, A.; Sawicka, J.; Mousseau, D.D.; Sawicki, G. Matrix metalloproteinase-2 is activated during ischemia/reperfusion in a model of myocardial infarction. *Can. J. Cardiol.* **2013**, *29*, 1495–1503. [CrossRef] [PubMed]
57. Li, Z.; Du, Y.; Jia, L.; Fan, J.; Guo, R.; Ma, X.; Wang, X.; Nie, S.; Wei, Y. Association of C1q/TNF-Related Protein-9 (CTRP9) Level with Obstructive Sleep Apnea in Patients with Coronary Artery Disease. *Mediators Inflamm.* **2020**, *2020*, 7281391. [CrossRef]
58. Sánchez-de-la-Torre, M.; Sánchez-de-la-Torre, A.; Bertran, S.; Abad, J.; Duran-Cantolla, J.; Cabriada, V.; Mediano, O.; Masdeu, M.J.; Alonso, M.L.; Masa, J.F.; et al. Effect of obstructive sleep apnoea and its treatment with continuous positive airway pressure on the prevalence of cardiovascular events in patients with acute coronary syndrome (ISAACC study): A randomised controlled trial. *Lancet Respir. Med.* **2020**, *8*, 359–367. [CrossRef]
59. Peker, Y.; Thunström, E.; Glantz, H.; Eulenburg, C. Effect of Obstructive Sleep Apnea and CPAP Treatment on Cardiovascular Outcomes in Acute Coronary Syndrome in the RICCADSA Trial. *J. Clin. Med.* **2020**, *9*, 4051. [CrossRef]
60. Gottlieb, D.J.; Yenokyan, G.; Newman, A.B.; O'Connor, G.T.; Punjabi, N.M.; Quan, S.F.; Redline, S.; Resnick, H.E.; Tong, E.K.; Diener-West, M.; et al. Prospective study of obstructive sleep apnea and incident coronary heart disease and heart failure: The sleep heart health study. *Circulation* **2010**, *122*, 352–360. [CrossRef]
61. Bakker, J.P.; Baltzis, D.; Tecilazich, F.; Chan, R.H.; Manning, W.J.; Neilan, T.G.; Wallace, M.L.; Hudson, M.; Malhotra, A.; Patel, S.R.; et al. The effect of continuous positive airway pressure on vascular function and cardiac structure in diabetes and sleep apnea: A randomized controlled trial0. *Ann. Am. Thorac. Soc.* **2020**, *17*, 474–483. [CrossRef]
62. Maripov, A.; Mamazhakypov, A.; Sartmyrzaeva, M.; Akunov, A.; Muratali Uulu, K.; Duishobaev, M.; Cholponbaeva, M.; Sydykov, A.; Sarybaev, A. Right Ventricular Remodeling and Dysfunction in Obstructive Sleep Apnea: A Systematic Review of the Literature and Meta-Analysis. *Can. Respir. J.* **2017**, *2017*, 1587865. [CrossRef]
63. Nguyen, P.K.; Katikireddy, C.K.; McConnell, M.V.; Kushida, C.; Yang, P.C. Nasal continuous positive airway pressure improves myocardial perfusion reserve and endothelial-dependent vasodilation in patients with obstructive sleep apnea. *J. Cardiovasc. Magn. Reson.* **2010**, *12*, 50. [CrossRef] [PubMed]
64. Blomster, H.; Laitinen, T.; Lyyra-Laitinen, T.; Vanninen, E.; Gylling, H.; Peltonen, M.; Martikainen, T.; Sahlman, J.; Kokkarinen, J.; Randell, J.; et al. Endothelial function is well preserved in obese patients with mild obstructive sleep apnea. *Sleep Breath.* **2014**, *18*, 177–186. [CrossRef]
65. Labarca, G.; Reyes, T.; Jorquera, J.; Dreyse, J.; Drake, L. CPAP in patients with obstructive sleep apnea and type 2 diabetes mellitus: Systematic review and meta-analysis. *Clin. Respir. J.* **2018**, *12*, 2361–2368. [CrossRef]
66. De Lima, A.M.J.; Franco, C.M.R.; de Castro, C.M.M.B.; de Andrade Bezerra, A.; Ataíde, L., Jr.; Halpern, A. Effects of Nasal Continuous Positive Airway Pressure Treatment on Oxidative Stress and Adiponectin Levels in Obese Patients with Obstructive Sleep Apnea. *Respiration* **2010**, *79*, 370–376. [CrossRef] [PubMed]
67. Iftikhar, I.H.; Hoyos, C.M.; Phillips, C.L.; Magalang, U.J. Meta-analyses of the association of sleep apnea with insulin resistance, and the effects of CPAP on HOMA-IR, adiponectin, and visceral adipose fat. *J. Clin. Sleep Med.* **2015**, *11*, 475–485. [CrossRef]
68. Xu, P.H.; Hui, C.K.M.; Lui, M.M.S.; Lam, D.C.L.; Fong, D.Y.T.; Ip, M.S.M. Incident Type 2 Diabetes in OSA and Effect of CPAP Treatment: A Retrospective Clinic Cohort Study. *Chest* **2019**, *156*, 743–753. [CrossRef]
69. Janssen, C.; Pathak, A.; Grassi, G.; Van De Borne, P. Endothelin contributes to the blood pressure rise triggered by hypoxia in severe obstructive sleep apnea. *J. Hypertens.* **2017**, *35*, 118–124. [CrossRef]
70. Hou, H.; Zhao, Y.; Yu, W.; Dong, H.; Xue, X.; Ding, J.; Xing, W.; Wang, W. Association of obstructive sleep apnea with hypertension: A systematic review and meta-analysis. *J. Glob. Health* **2018**, *8*, 010405. [CrossRef] [PubMed]

71. Daniels, F.; De Freitas, S.; Smyth, A.; Garvey, J.; Judge, C.; Gilmartin, J.J.; Sharif, F. Effects of renal sympathetic denervation on blood pressure, sleep apnoea severity and metabolic indices: A prospective cohort study. *Sleep Med.* **2017**, *30*, 180–184. [CrossRef] [PubMed]
72. De Souza, F.; Muxfeldt, E.S.; Margallo, V.; Cortez, A.F.; Cavalcanti, A.H.; Salles, G.F. Effects of continuous positive airway pressure treatment on aldosterone excretion in patients with obstructive sleep apnoea and resistant hypertension: A randomized controlled trial. *J. Hypertens.* **2017**, *35*, 837–844. [CrossRef]
73. Muxfeldt, E.S.; Margallo, V.; Costa, L.M.S.; Guimarães, G.; Cavalcante, A.H.; Azevedo, J.C.M.; De Souza, F.; Cardoso, C.R.L.; Salles, G.F. Effects of continuous positive airway pressure treatment on clinic and ambulatory blood pressures in patients with obstructive sleep apnea and resistant hypertension: A randomized controlled trial. *Hypertension* **2015**, *65*, 736–742. [CrossRef]
74. Hu, X.; Fan, J.; Chen, S.; Yin, Y.; Zrenner, B. The Role of Continuous Positive Airway Pressure in Blood Pressure Control for Patients with Obstructive Sleep Apnea and Hypertension: A Meta-Analysis of Randomized Controlled Trials. *J. Clin. Hypertens.* **2015**, *17*, 215–222. [CrossRef]
75. Lei, Q.; Lv, Y.; Li, K.; Ma, L.; Du, G.; Xiang, Y.; Li, X. Efeitos da pressão positiva contínua nas vias aéreas na pressão arterial em pacientes com hipertensão resistente e apneia obstrutiva do sono: Revisão sistemática e meta-análise de seis ensaios clínicos controlados aleatórios. *J. Bras. Pneumol.* **2017**, *43*, 373–379. [CrossRef]
76. Pengo, M.F.; Soranna, D.; Giontella, A.; Perger, E.; Mattaliano, P.; Schwarz, E.I.; Lombardi, C.; Bilo, G.; Zambon, A.; Steier, J.; et al. Obstructive sleep apnoea treatment and blood pressure: Which phenotypes predict a response? A systematic review and meta-analysis. *Eur. Respir. J.* **2020**, *55*, 1901945. [CrossRef] [PubMed]
77. Schwarz, E.I.; Schlatzer, C.; Stehli, J.; Kaufmann, P.A.; Bloch, K.E.; Stradling, J.R.; Kohler, M. Effect of CPAP Withdrawal on myocardial perfusion in OSA: A randomized controlled trial. *Respirology* **2016**, *21*, 1126–1133. [CrossRef] [PubMed]
78. Turnbull, C.D.; Sen, D.; Kohler, M.; Petousi, N.; Stradling, J.R. Effect of supplemental oxygen on blood pressure in obstructive sleep apnea (SOX) a randomized continuous positive airway pressure withdrawal trial. *Am. J. Respir. Crit. Care Med.* **2019**, *199*, 211–219. [CrossRef] [PubMed]
79. Wong, H.S.; Williams, A.J.; Mok, Y. The relationship between pulmonary hypertension and obstructive sleep apnea. *Curr. Opin. Pulm. Med.* **2017**, *23*, 517–521. [CrossRef]
80. Sommer, N.; Strielkov, I.; Pak, O.; Weissmann, N. Oxygen sensing and signal transduction in hypoxic pulmonary vasoconstriction. *Eur. Respir. J.* **2016**, *47*, 288–303. [CrossRef] [PubMed]
81. Wang, Y.; Chai, Y.; He, X.; Ai, L.; Sun, X.; Huang, Y.; Li, Y. Intermittent hypoxia simulating obstructive sleep apnea causes pulmonary inflammation and activates the Nrf2/HO-1 pathway. *Exp. Ther. Med.* **2017**, *14*, 3463–3470. [CrossRef]
82. Norton, C.E.; Jernigan, N.L.; Kanagy, N.L.; Walker, B.R.; Resta, T.C. Intermittent hypoxia augments pulmonary vascular smooth muscle reactivity to NO: Regulation by reactive oxygen species. *J. Appl. Physiol.* **2011**, *111*, 980–988. [CrossRef] [PubMed]
83. Imran, T.F.; Ghazipura, M.; Liu, S.; Hossain, T.; Ashtyani, H.; Kim, B.; Michael Gaziano, J.; Djoussé, L. Effect of continuous positive airway pressure treatment on pulmonary artery pressure in patients with isolated obstructive sleep apnea: A meta-analysis. *Heart Fail. Rev.* **2016**, *21*, 591–598. [CrossRef]
84. Huang, B.; Liu, H.; Scherlag, B.J.; Sun, L.; Xing, S.; Xu, J.; Luo, M.; Guo, Y.; Cao, G.; Jiang, H. Atrial fibrillation in obstructive sleep apnea: Neural mechanisms and emerging therapies. *Trends Cardiovasc. Med.* **2020**, *31*, 127–132. [CrossRef] [PubMed]
85. Xu, H.; Wang, J.; Yuan, J.; Hu, F.; Yang, W.; Guo, C.; Luo, X.; Liu, R.; Cui, J.; Gao, X.; et al. Implication of Apnea-Hypopnea Index, a Measure of Obstructive Sleep Apnea Severity, for Atrial Fibrillation in Patients With Hypertrophic Cardiomyopathy. *J. Am. Heart Assoc.* **2020**, *9*, e015013. [CrossRef] [PubMed]
86. Yang, X.; Zhang, L.; Liu, H.; Shao, Y.; Zhang, S. Cardiac sympathetic denervation suppresses atrial fibrillation and blood pressure in a chronic intermittent hypoxia rat model of obstructive sleep apnea. *J. Am. Heart Assoc.* **2019**, *8*, e010254. [CrossRef] [PubMed]
87. Varga, P.; Rosianu, H.; Vesa, S.; Hancu, B.; Beyer, R.; Pop, C. The impact of continuous positive airway pressure on cardiac arrhythmias in patients with sleep apnea. *J. Res. Med. Sci.* **2020**, *25*. [CrossRef]
88. Abumuamar, A.M.; Newman, D.; Dorian, P.; Shapiro, C.M. Cardiac effects of CPAP treatment in patients with obstructive sleep apnea and atrial fibrillation. *J. Interv. Card. Electrophysiol.* **2019**, *54*, 289–297. [CrossRef] [PubMed]
89. Müller, P.; Grabowski, C.; Schiedat, F.; Shin, D.I.; Dietrich, J.W.; Mügge, A.; Deneke, T.; Walther, J.W.; Kara, K. Reverse Remodelling of the Atria After Treatment of Obstructive Sleep Apnoea with Continuous Positive Airway Pressure: Evidence from Electro-mechanical and Endocrine Markers. *Hear. Lung Circ.* **2016**, *25*, 53–60. [CrossRef] [PubMed]
90. Fein, A.S.; Shvilkin, A.; Shah, D.; Haffajee, C.I.; Das, S.; Kumar, K.; Kramer, D.B.; Zimetbaum, P.J.; Buxton, A.E.; Josephson, M.E.; et al. Treatment of obstructive sleep apnea reduces the risk of atrial fibrillation recurrence after catheter ablation. *J. Am. Coll. Cardiol.* **2013**, *62*, 300–305. [CrossRef]
91. Naruse, Y.; Tada, H.; Satoh, M.; Yanagihara, M.; Tsuneoka, H.; Hirata, Y.; Ito, Y.; Kuroki, K.; Machino, T.; Yamasaki, H.; et al. Concomitant obstructive sleep apnea increases the recurrence of atrial fibrillation following radiofrequency catheter ablation of atrial fibrillation: Clinical impact of continuous positive airway pressure therapy. *Hear. Rhythm* **2013**, *10*, 331–337. [CrossRef]
92. Caples, S.M.; Mansukhani, M.P.; Friedman, P.A.; Somers, V.K. The impact of continuous positive airway pressure treatment on the recurrence of atrial fibrillation post cardioversion: A randomized controlled trial. *Int. J. Cardiol.* **2019**, *278*, 133–136. [CrossRef]
93. Korostovtseva, L.S.; Zvartau, N.E.; Rotar, O.P.; Sviryaev, Y.V.; Konradi, A.O. Predictors of heart rhythm disturbances in hypertensive obese patients with obstructive sleep apnea. *J. Geriatr. Cardiol.* **2017**, *14*, 553–562. [CrossRef]

94. Brunetti, L.; Francavilla, R.; Scicchitano, P.; Tranchino, V.; Loscialpo, M.; Gesualdo, M.; Zito, A.; Fornarelli, F.; Sassara, M.; Giordano, P.; et al. Impact of sleep respiratory disorders on endothelial function in children. *Sci. World J.* **2013**, *2013*, 719456. [CrossRef] [PubMed]
95. Loffredo, L.; Zicari, A.M.; Occasi, F.; Perri, L.; Carnevale, R.; Angelico, F.; Del Ben, M.; Martino, F.; Nocella, C.; Savastano, V.; et al. Endothelial dysfunction and oxidative stress in children with sleep disordered breathing: Role of NADPH oxidase. *Atherosclerosis* **2015**, *240*, 222–227. [CrossRef] [PubMed]
96. Perikleous, E.; Steiropoulos, P.; Tzouvelekis, A.; Nena, E.; Koffa, M.; Paraskakis, E. DNA methylation in pediatric obstructive sleep apnea: An overview of preliminary findings. *Front. Pediatr.* **2018**, *6*, 154. [CrossRef]
97. Patinkin, Z.W.; Feinn, R.; Santos, M. Metabolic consequences of obstructive sleep apnea in adolescents with obesity: A systematic literature review and meta-analysis. *Child. Obes.* **2017**, *13*, 102–110. [CrossRef]
98. Gong, W.; Wang, X.; Fan, J.; Nie, S.; Wei, Y. Impact of obstructive sleep apnea on platelet function profiles in patients with acute coronary syndrome taking dual antiplatelet therapy. *J. Am. Heart Assoc.* **2018**, *7*, e008808. [CrossRef] [PubMed]
99. Hong, S.N.; Yun, H.C.; Yoo, J.H.; Lee, S.H. Association between hypercoagulability and severe obstructive sleep apnea. *JAMA Otolaryngol. Head Neck Surg.* **2017**, *143*, 996–1002. [CrossRef]
100. Jiang, X.M.; Qian, X.S.; Gao, X.F.; Ge, Z.; Tian, N.L.; Kan, J.; Zhang, J.J. Obstructive Sleep Apnea Affecting Platelet Reactivity in Patients Undergoing Percutaneous Coronary Intervention. *Chin. Med. J. (Engl.)* **2018**, *131*, 1023–1029. [CrossRef]
101. Basili, S.; Pignatelli, P.; Tanzilli, G.; Mangieri, E.; Carnevale, R.; Nocella, C.; Di Santo, S.; Pastori, D.; Ferroni, P.; Violi, F. Anoxia-reoxygenation enhances platelet thromboxane A2 production via reactive oxygen species-generated NOX2: Effect in patients undergoing elective percutaneous coronary intervention. *Arterioscler. Thromb. Vasc. Biol.* **2011**, *31*, 1766–1771. [CrossRef] [PubMed]
102. Cheung, P.Y.; Stevens, J.P.; Haase, E.; Stang, L.; Bigam, D.L.; Etches, W.; Radomski, M.W. Platelet dysfunction in asphyxiated newborn piglets resuscitated with 21% and 100% oxygen. *Pediatr. Res.* **2006**, *59*, 636–640. [CrossRef] [PubMed]
103. Oga, T.; Chin, K.; Tabuchi, A.; Kawato, M.; Morimoto, T.; Takahashi, K.; Handa, T.; Takahashi, K.; Taniguchi, R.; Kondo, H.; et al. Effects of obstructive sleep apnea with intermittent hypoxia on platelet aggregability. *J. Atheroscler. Thromb.* **2009**, *16*, 862–869. [CrossRef] [PubMed]
104. Rahangdale, S.; Yeh, S.Y.; Novack, V.; Stevenson, K.; Barnard, M.R.; Furman, M.I.; Frelinger, A.L.; Michelson, A.D.; Malhotra, A. The influence of intermittent hypoxemia on platelet activation in obese patients with obstructive sleep apnea. *J. Clin. Sleep Med.* **2011**, *7*, 172–178. [CrossRef]
105. Cofta, S.; Wysocka, E.; Dziegielewska-Gesiak, S.; Michalak, S.; Piorunek, T.; Batura-Gabryel, H.; Torlinski, L. Plasma Selectins in Patients with Obstructive Sleep Apnea. In *Advances in Experimental Medicine and Biology*; Springer: Dordrecht, The Netherlands, 2013; Volume 756, pp. 113–119.
106. Winiarska, H.M.; Cofta, S.; Bielawska, L.; Płóciniczak, A.; Piorunek, T.; Wysocka, E. Circulating P-Selectin and Its Glycoprotein Ligand in Nondiabetic Obstructive Sleep Apnea Patients. In *Advances in Experimental Medicine and Biology*; Springer: Cham, Switzerland, 2020; Volume 1279, pp. 61–69.
107. Minoguchi, K.; Yokoe, T.; Tazaki, T.; Minoguchi, H.; Oda, N.; Tanaka, A.; Yamamoto, M.; Ohta, S.; O'Donnell, C.P.; Adachi, M. Silent brain infarction and platelet activation in obstructive sleep apnea. *Am. J. Respir. Crit. Care Med.* **2007**, *175*, 612–617. [CrossRef] [PubMed]
108. Krieger, A.C.; Anand, R.; Hernandez-Rosa, E.; Maidman, A.; Milrad, S.; DeGrazia, M.Q.; Choi, A.J.; Oromendia, C.; Marcus, A.J.; Drosopoulos, J.H.F. Increased platelet activation in sleep apnea subjects with intermittent hypoxemia. *Sleep Breath.* **2020**, *24*, 1537–1547. [CrossRef] [PubMed]
109. Jóźwik-Plebanek, K.; Prejbisz, A.; Wypasek, E.; Pręgowska-Chwała, B.; Hanus, K.; Kaszuba, A.M.; Januszewicz, M.; Bieleń, P.; Kabat, M.; Kruk, M.; et al. Altered plasma fibrin clot properties in hypertensive patients with obstructive sleep apnoea are improved by continuous positive airway pressure treatment. *J. Hypertens.* **2017**, *35*, 1035–1043. [CrossRef]
110. Wickwire, E.M.; Bailey, M.D.; Somers, V.K.; Srivastava, M.C.; Scharf, S.M.; Johnson, A.M.; Albrecht, J.S. CPAP adherence is associated with reduced risk for stroke among older adult Medicare beneficiaries with obstructive sleep apnea. *J. Clin. Sleep Med.* **2021**. [CrossRef]
111. McEvoy, R.D.; Antic, N.A.; Heeley, E.; Luo, Y.; Ou, Q.; Zhang, X.; Mediano, O.; Chen, R.; Drager, L.F.; Liu, Z.; et al. CPAP for Prevention of Cardiovascular Events in Obstructive Sleep Apnea. *N. Engl. J. Med.* **2016**, *375*, 919–931. [CrossRef]
112. Peled, N.; Kassirer, M.; Kramer, M.R.; Rogowski, O.; Shlomi, D.; Fox, B.; Berliner, A.S.; Shitrit, D. Increased erythrocyte adhesiveness and aggregation in obstructive sleep apnea syndrome. *Thromb. Res.* **2008**, *121*, 631–636. [CrossRef]
113. Tsuda, K.; Nishio, I. An association between plasma asymmetric dimethylarginine and membrane fluidity of erythrocytes in hypertensive and normotensive men: An electron paramagnetic resonance investigation. *Am. J. Hypertens.* **2005**, *18*, 1243–1248. [CrossRef]
114. Kuwai, T.; Hayashi, J. Nitric oxide pathway activation and impaired red blood cell deformability with hypercholesterolemia. *J. Atheroscler. Thromb.* **2006**, *13*, 286–294. [CrossRef]
115. Grau, M.; Pauly, S.; Ali, J.; Walpurgis, K.; Thevis, M.; Bloch, W.; Suhr, F. RBC-NOS-Dependent S-Nitrosylation of Cytoskeletal Proteins Improves RBC Deformability. *PLoS ONE* **2013**, *8*, e56759. [CrossRef] [PubMed]
116. Premont, R.T.; Reynolds, J.D.; Zhang, R.; Stamler, J.S. Role of Nitric Oxide Carried by Hemoglobin in Cardiovascular Physiology: Developments on a Three-Gas Respiratory Cycle. *Circ. Res.* **2020**, *126*, 129–158. [CrossRef]

117. Bollenbach, A.; Gambaryan, S.; Mindukshev, I.; Pich, A.; Tsikas, D. GC-MS and LC-MS/MS pilot studies on the guanidine (NG)-dimethylation in native, asymmetrically and symmetrically NG-dimethylated arginine-vasopressin peptides and proteins in human red blood cells. *J. Chromatogr. B Anal. Technol. Biomed. Life Sci.* **2020**, *1141*, 122024. [CrossRef] [PubMed]
118. Zwemer, C.F.; Davenport, R.D.; Gomez-Espina, J.; Blanco-Gonzalez, E.; Whitesall, S.E.; D'Alecy, L.G. Packed red blood cells are an abundant and proximate potential source of Nitric oxide synthase inhibition. *PLoS ONE* **2015**, *10*, e0119991. [CrossRef]
119. Tsikas, D.; Böhmer, A.; Großkopf, H.; Beckmann, B.; Dreißigacker, U.; Jordan, J.; Maassen, N. Clinical-chemistry laboratory relevant hemolysis is unlikely to compromise human plasma concentration of free asymmetric dimethylarginine (ADMA). *Clin. Biochem.* **2012**, *45*, 1536–1538. [CrossRef]
120. Kang, E.S.; Cates, T.B.; Harper, D.N.; Chiang, T.M.; Myers, L.K.; Acchiardo, S.R.; Kimoto, M. An enzyme hydrolyzing methylated inhibitors of nitric oxide synthase is present in circulating human red blood cells. *Free Radic. Res.* **2001**, *35*, 693–707. [CrossRef] [PubMed]
121. Bollenbach, A.; Tsikas, D. Pharmacological activation of dimethylarginine dimethylaminohydrolase (DDAH) activity by inorganic nitrate and DDAH inhibition by N^{G}-hydroxy-l-arginine, N^{ω},N^{ω}-dimethyl-l-citrulline and N^{ω},N^{ω}-dimethyl-N^{δ}-hydroxy-l-citrulline: Results and overview. *Amino Acids* **2019**, *51*, 483–494. [CrossRef] [PubMed]
122. Barbé, F.; Durán-Cantolla, J.; Sánchez-De-La-Torre, M.; Martínez-Alonso, M.; Carmona, C.; Barceló, A.; Chiner, E.; Masa, J.F.; Gonzalez, M.; Marín, J.M.; et al. Effect of continuous positive airway pressure on the incidence of hypertension and cardiovascular events in nonsleepy patients with obstructive sleep apnea: A randomized controlled trial. *JAMA J. Am. Med. Assoc.* **2012**, *307*, 2161–2168. [CrossRef]
123. Qiu, Z.H.; Luo, Y.M.; McEvoy, R.D. The Sleep Apnea Cardiovascular Endpoints (SAVE) study: Implications for health services and sleep research in China and elsewhere. *J. Thorac. Dis.* **2017**, *9*, 2217–2220. [CrossRef]

5. PRACA NR 2:

Effect of Obstructive Sleep Apnea and CPAP Treatment on the Bioavailability of Erythrocyte and Plasma Nitric Oxide

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Effect of Obstructive Sleep Apnea and CPAP Treatment on the Bioavailability of Erythrocyte and Plasma Nitric Oxide

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Abstract: Introduction: Endothelial dysfunction resulting from decreased nitric oxide (NO) bioavailability is an important mechanism that increases cardiovascular risk in subjects with obstructive sleep apnea (OSA). NO is produced by nitric oxide synthase (NOS) in a reaction that converts L-arginine to L-citrulline. Asymmetric-dimethylarginine (ADMA) is created by L-arginine and is a naturally occurring competitive inhibitor of nitric oxide synthase (NOS). The aim of our study was to verify if erythrocytes could play a role in the storage and accumulation of ADMA in OSA patients. The crosstalk between erythrocyte-ADMA, SDMA, L-arginine, and L-citrulline levels and endothelial function was investigated in OSA subjects both at baseline and prospectively following 1-year CPAP (continuous positive airway pressure) treatment. Material and Methods: A total of 46 subjects with OSA were enrolled in this study and divided into two groups: those with moderate-to-severe OSA and those with mild or no OSA. A physical examination was followed by blood collection for the assessment of biochemical cardiovascular risk factors and the nitric oxide bioavailability parameters both in plasma and erythrocytes. Vasodilative endothelial function was assessed using Laser Doppler Flowmetry (LDF). Results: No significant changes regarding the NO pathway metabolites were noted apart from the plasma L-citrulline concentration, which was decreased in patients with OSA (26.9 ± 7.4 vs. 33.1 ± 9.4 μM, $p < 0.05$). The erythrocyte ADMA concentration was lower than in plasma irrespective of the presence of OSA (0.33 ± 0.12 vs. 0.45 ± 0.08 μM in OSA, $p < 0.05$ and 0.33 ± 0.1 vs. 0.45 ± 0.07 μM in the control, $p < 0.05$). No significant changes regarding the LDF were found. CPAP treatment did not change the levels of NO metabolites in the erythrocytes. Conclusions: The erythrocyte pool of the NO metabolic pathway intermediates does not depend on OSA and its treatment, whereas the erythrocytes could constitute a high-volume buffer in their storage Hence, the results from this prospective study are a step forward in understanding the role of the erythrocyte compartment and the intra-erythrocyte pathways regulating NO bioavailability and paracrine endothelial function in the hypoxia-reoxygenation setting, such as obstructive sleep apnea.

Keywords: obstructive sleep apnea; endothelial dysfunction; oxidative stress; nitric oxide; asymmetric dimethylarginine

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1. Introduction

Obstructive sleep apnea (OSA) is recognized as an independent cardiovascular risk factor and is estimated to affect close to one billion patients worldwide [1]. Increased intra-thoracic pressure, activation of the adrenergic system, increased release of inflammatory cytokines, and oxidative stress, all leading to endothelial dysfunction [2,3] are among the pivotal pathogenic factors that contribute to OSA-related target organ damage. The

impairment of mitochondrial oxidative phosphorylation during hypoxia, subsequently followed by reoxygenation, induces the production of reactive oxygen species (ROS) and the uncontrolled phosphorylation of numerous proteins (Figure 1). ROS generation involves the mitochondrial respiratory chain and numerous enzyme complexes, including NADPH oxidase and xanthine oxidase (XO) [4]. ROS are capable of limiting NO bioavailability by reacting with NO synthase (NOS) cofactors such as tetrahydrobiopterin. Superoxide can easily react with NO itself, creating a highly toxic peroxynitrite ($ONOO^-$), a source of nitrosative stress leading to post-translational modifications of numerous proteins (nitration and S-nitrosylation) [5]. As far as the literature is concerned, it has been shown that the expression of superoxide dismutase (SOD), a key antioxidant enzyme, is significantly limited in subjects with OSA [6], whereas malondialdehyde (MDA), a commonly investigated marker of lipid peroxidation, is significantly increased in the course of OSA [7].

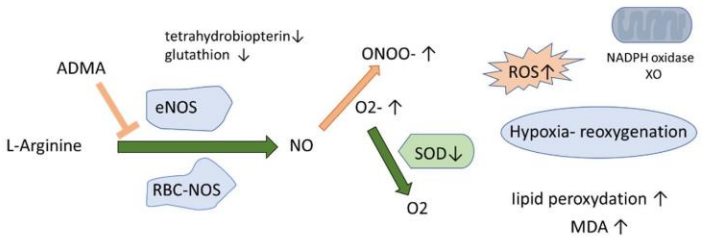


Figure 1. Oxidative stress in OSA. Abbreviations: RBC-NOS: red blood cell nitric oxide synthase; ADMA: asymmetric dimethylarginine SOD: superoxide dismutase; XOD: xanthine oxidase; $ONOO^-$: peroxynitrite; O_2^-: superoxide; ROS: reactive oxygen species; MDA: malonyldialdehyde; eNOS: endothelial nitric oxide synthase, RBC-NOS: nitric oxide synthase in the erythrocyte.

Alterations in the nitric oxide (NO) metabolic pathway and subsequent impairment of endothelial vasodilative function may play a pivotal role in the pathogenesis of increased cardiovascular risk in OSA. NO is not only an important vasodilator, but it also reduces platelet degranulation and decreases platelet and leukocyte adhesion to the endothelium [8]. Asymmetric dimethylarginine (ADMA) is the most important competitive inhibitor of nitric oxide synthase (NOS), thus regulating the bioavailability of NO. Elevated plasma levels of ADMA have already been shown to be an early predictor of increased cardiovascular risk [9]. ADMA is present not only in plasma but also in the intracellular compartment, including endothelial cells and human erythrocytes [10]. Erythrocytes, due to their high contribution to the hematocrit, could play an important role in the storage and accumulation of ADMA [11], thus modulating the plasmatic concentration and finally endothelial function by blocking nitric oxide synthase. ADMA is a highly regulated molecule, both in terms of its biosynthesis, degradation, metabolism, and transport. Its biosynthesis in intraerythrocytic cells is possible by protein-arginine methyltransferases (PRMTs) catalyzing the methylation of proteinic L-arginine [12]. RBCs contain arginine-rich proteins, derived mostly from lysis of the nucleus during erythropoiesis, which could be a potential source of inhibitory methylarginines [12]. Interestingly, increased methylation of the L-arginine residue by PRMT has been shown to be increased in hypoxia [13]. In vitro, lysis of erythrocytes results in the release of pathologically relevant quantities of free ADMA, which after 2 h of incubation increased sevenfold [14]. Nevertheless, another study suggests that in vivo hemolysis is unlikely to significantly increase human plasma concentrations of free ADMA [15].

Furthermore, the degradation of ADMA by the dimethylarginine dimethylaminohydrolases (DDAH1 and DDAH2) was decreased in the animal models of hypoxia [16]. Interestingly, the expression of the DDAH enzyme, was also identified in human RBCs [17,18].

Noteworthy, in addition to the cellular production and degradation of ADMA, the transmembrane transport of ADMA by CATs (cationic amino-acid transporters) determines

its intracellular concentration. The transport of arginine and ADMA was examined in animal models and cell cultures. CAT-1, CAT-2A, CAT-2B, and the system y + L amino acid transporters were shown to be more relevant for transmembrane transport of arginine and ADMA [19]. CAT-1 expression is involved in erythroid hematopoiesis through importing L-arginine, which appears to be essential for the differentiation of RBCs [20]. CAT-1 is expressed in all tissues and organs except for the liver, where CAT-2A is expressed strongly and s the quantitative uptake of L-arginine and ADMA at high concentrations [19]. CAT-2B is induced by pro-inflammatory cytokines in numerous tissues, along with arginase and NOS [21]. In its physiological concentration range, ADMA is unlikely to impair CAT1-mediated transport of L-arginine. Conversely, high L-arginine concentrations inhibit the CAT1-mediated cellular uptake of ADMA [22].

Recently, it has been shown that increased cardiovascular morbidity in OSA patients could be related to the functional and structural changes in erythrocytes [23]. The red blood cells (RBCs) express a nitric oxide synthase (RBC-NOS, structurally identical with the endothelial isoform—eNOS), which, by producing NO, modifies the erythrocytes' deformability through direct S-nitrosylation of cytoskeleton proteins, including spectrin-α and spectrin-β [24]. ADMA, by limiting NO production, may decrease the membrane fluidity of RBCs, suggesting that ADMA might have a close correlation with the rheologic behavior of erythrocytes in the microcirculation [25].

Hence, the goal of our study was to verify if ADMA concentrations within RBCs depend on the severity of OSA. Secondly, we aimed to verify if erythrocytes could play a role in the storage and accumulation of asymmetric dimethylarginine (ADMA) in OSA patients. The correlations between the erythrocyte nitric oxide metabolic pathway intermediates and the other risk factors and endothelial vasodilative function at the level of microcirculation were also investigated.

2. Materials and Methods

2.1. Bioethics Approval

The study protocol has been approved by the Bioethics Committee of Wroclaw Medical University: protocol nr KB-335/2018 from 29 May 2018 for studies involving humans. All the volunteers agreed to participate in this project by signing a written informed consent on the form previously verified by the Bioethics Committee. The project procedures are consistent with the principles of the Declaration of Helsinki (Seventh Revision, 64th World Medical Association meeting, Fortaleza, Brazil, 2013).

2.2. Subject Recruitment

Subjects suspected of OSA who were admitted to the hospital between January 2019–November 2020 for a polysomnography examination were considered for participation in this study. The initial inclusion criteria for these subjects were: suspicion of OSA based on signs and symptoms assessed by questionnaires (Berlin, Germany, STOP-BANG, Epworth scale); age between 30–70 years; and written informed consent. The exclusion criteria were the presence of diabetes, developed vascular disease such as past stroke, past myocardial infarction, previous revascularization, anemia, polycythemia, and malignancies. Subjects with a history of premature cardiovascular death in family history (men < 55 years old, women < 65 years old), comorbidity with rheumatic diseases, and taking drugs such as anticoagulants, antiplatelets, anti-inflammatory, immunosuppressants, as well as nebivolol (due to interference with the nitric oxide metabolism), were also excluded. After a careful analysis, a total of 47 subjects were enrolled in this study, but one subject was excluded because of untreated newly diagnosed diabetes mellitus.

2.3. Measurements

About 40 mL of peripheral venous blood was collected in the morning for the fasting condition, directly after the polysomnographic examination. In the hospital laboratory, we provided biochemical blood tests assessing cardiovascular risk, which were performed ad

hoc from the obtained serum samples. The samples for biochemical analysis of selected intermediates involved in the nitric oxide biotransformation pathway were prepared simultaneously and stored at −80 °C until subsequent analyses.

Assessment of endothelial vasodilatory function using Laser Doppler Flowmetry (LDF) was performed in both groups, as described below.

One night, polysomnography was performed using the NOX-A1 system (Nox Medical, Reykjavik, Iceland). Polysomnograms were described following the standard sleep criteria introduced by the American Academy of Sleep Medicine Task Force [26].

Abnormal respiratory events were scored from the standardized airflow signal:

- apneas—defined as the absence of airflow for ≥10 s,
- hypopneas (a reduction in the amplitude of breathing by ≥30% for ≥10 s with a ≥3% decline in blood oxygen saturation SpO_2) or followed by arousal.

2.4. Subgroups

After polysomnographic examination, the patients were divided into groups depending on the severity of their sleep apnea, based on the calculated apnea-hypopnea index (AHI) [27]. Patients with moderate and severe sleep apnea for whom CPAP treatment was indicated and recommended were collected in the OSA group. The patients with mild OSA as well as those with excluded OSA following the diagnostics formed the control group, without CPAP treatment. Such selection of the control group allowed for the matching of clinically similar patients, initially suspected with OSA, where the PSG result was the only one criterion assigning the subjects to a particular subgroup.

From the OSA group, only these subjects with compliance to the therapy and willingness to participate in the follow-up were invited for the second evaluation, one year after the diagnosis and beginning of the treatment (Figure 2).

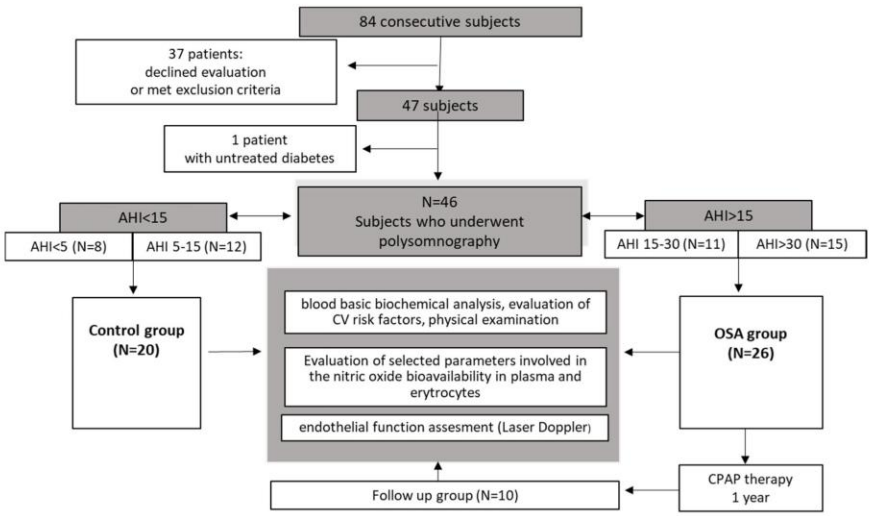


Figure 2. A flow chart of the study protocol. Abbreviations: OSA: Obstructive sleep apnea; AHI: apnea hypopnea index; CV: cardiovascular; CPAP: continuous positive airway pressure.

2.5. Biochemical Analysis

The blood was collected using an S-Monovettes, (SARSTEDT, Sarstedt Germany). Whole blood for RBC isolation was drawn into the tube containing sodium citrate at a ratio of 1:10 (one part citrate to nine parts blood). The erythrocytes were obtained by removing the plasma and buffy coat of the blood by centrifugation at 1000× *g* for 10 min at

room temperature and subsequently washing four-times with phosphate buffered saline (PBS). RBC count as well as contamination by leucocytes (WBC) and platelets (PLT) were assessed using the Sysmex XT-2000i. Purified cells were finally adjusted to a concentration of 2.5×10^9 in the sample and stored at −80 °C until subsequent analyses. Each sample contained the same number of cells: 2.5×10^9 erythrocytes/sample. As a result, all measurements of the intra-erythrocyte metabolites were conducted in the same number of pooled-down RBCs.

RBC metabolites extraction: RBC samples were thawed on ice, and afterwards, 10 µL of an internal standard solution and 1200 µL of a cold extraction solution of methanol, acetonitrile, and water (5:3:2) were added and vortexed (for 15 min, at 1200× *g*, at 4 °C). Samples were then centrifuged (15 min at 2200× *g*, at 4 °C) and the supernatants were transferred into the microcentrifuge tubes. Afterwards, the samples were dried at 50 °C.

The derivatization of amino acids was performed using benzoyl chloride (BCl) as reagent. Dried samples were dissolved in 100 µL of water and vortexed (5 min, 1200× *g*, 25 °C). Subsequently, 50µL of borate buffer (0.025 M $Na_2B_4O_7 \times 10\ H_2O$, 1.77 mM NaOH, pH = 9.2), 400 µL of acetonitrile, and 10 µL of 10% BCl in acetonitrile were added and vortexed again (10 min, 1200× *g*, 25 °C). Following this procedure, the samples were dried at 45 °C with a SpeedVac-Vacuum-Concentrator and subsequently reconstituted in 50 µL of a 3% solution of methanol in water and centrifuged (10 min at 15,000× *g*, 4 °C). The supernatant was transferred into a chromatographic polypropylene vial with 200 µL of insert.

The liquid chromatography–mass spectrometry analysis was conducted using the SYNAPT-G2 Si mass spectrometer, which was coupled with an Acquity I-Class ultra-performance liquid chromatography system (Waters, Milford, MA, USA). The mass spectrometer contained an electrospray ionization source. Data was acquired by a MassLynx 4.1 software (Waters) for the following ions (m/z): 237.1239, 243.1339, 263.1090, 267.1382, 279.1457, 286.1897, 307.1770, and 314.2209 for, ornithine, D6-ornithine, citrulline, D4-cytrulline, arginine, D7-arginine, ADMA, SDMA, and D7-ADMA, respectively.

The plasma concentrations of the NO-pathway metabolites were measured, as previously described by Fleszar et al. 2018 [28]. Afterwards, the samples were centrifuged (for 7 min, at 4 °C, using 22,000× *g*) and 100 µL of supernatant was diluted 4× with water, transferred to chromatographic glass vials for subsequent analysis, which was performed using the equipment and methods described above.

2.6. Endothelial Function Assessment

The NO-dependent vasodilatory function of the endothelium was measured by Laser Doppler Flowmetry (LDF), which enables the dynamic measurement of changes in the superficial microcirculation following application of a local thermal stimulus. Standard recording in the course of local heating consists of two main phases: the peak phase (lasting a few minutes), which depends on the stimulation of local sensory nerves leading to substance P excretion, and the plateau phase (after 20 min), conditioned mostly by nitric oxide but also by noradrenaline and neuropeptide Y [29]. The probe of the laser doppler flowmeter (Periflux 5000, Perimed, Järfälla, Sweden) was located on the forearm skin without any visible superficial vasculature. The studied arm was immobilized using a vacuum pillow, following the manufacturer's recommendations. After 10 min of baseline recording at 33° C, heating was increased to 44° C for the next half hour. In order to prevent the effect of the baseline flow variability, the results are presented as the hyperemia index (HI = perfusion following 20min of heating divided by the perfusion before heating) (Figure 3).

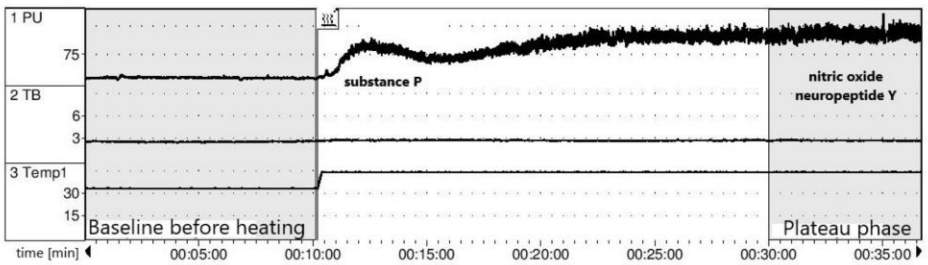


Figure 3. The Laser Doppler Flowmetry (LDF). Abbreviations: PU: perfusion unit; TB: total backscatter (the amount of light returned to the detector), Temp1: temperature of the probe 33–44 °C.

2.7. Statistical Analysis

The differences between two continuous variables were assessed using the Mann–Whitney U-test or a Student's *t*-test, as appropriate. The Wilcoxon test was used to compare paired groups before and after CPAP, as appropriate. Additionally, Spearman's rank correlation coefficient was performed. The data is presented as the mean with the standard deviation (SD) or the median with the interquartile range (Q_1–Q_3), dependending on the normality of the distribution and variance homogeneity, previously assessed using the Shapiro Wilk test and Levene's test, as appropriate.

All calculations were conducted with the Statsoft® Statistica 13.3 software, Krakow, Poland, and GraphPad PRISM (9.2.0, San Diego, CA, USA).

3. Results

3.1. Baseline Characteristics

In the study healthy, controls (AHI < 5) were grouped together with the mild obstructive sleep apnea patients (AHI < 15) and compared to the group with moderate and severe OSA (AHI > 15). There were no significant differences regarding the age and sex distribution between the two groups. However, there were significant differences between the groups regarding weight, body mass index, white blood cells, CRP, uric acid, and insulin, together with HOMA-IR and QUICKI. (Table 1).

Table 1. The baseline characteristics of groups including cardiovascular risk stratification. The results are presented as mean ± SD or median ± (Q1–Q3).

Parameter	Control Group AHI < 15 *n* = 20 (Mean ± SD) or Median(Q1–Q3)	OSA Group AHI > 15 *n* = 26 (Mean ± SD) or Median(Q1–Q3)	*p*-Value
Men (n, %)	13 65%	24 92%	NS
Women (n, %)	7 35%	2 8%	NS
Age (years)	50.6 ± 7.41	50.6 ± 9.95	NS
Height (cm)	173.6 ± 11.7	174.9 ± 6.93	NS
Weight (kg)	79.2 ± 17.4	92.0 ± 16.2	<0.05
Hypertension (n, %)	4.0 20%	11.0 42%	NS
BMI (kg/m^2) median(Q1–Q3)	25.4 (23.7–28.2)	29.1 (26.4–33.1)	<0.05
RBC (mln/µL)	5.04 ± 0.36	5.05 ± 0.34	NS
WBC (k/µL) median(Q1–Q3)	5.66 (4.41–6.91)	6.66 (5.22–8.98)	<0.05
Hb (g/dL)	15.2 ± 1.13	15.3 ± 1.00	NS
PLT (k/µL)	233.7 ± 45.0	243.0 ± 62.6	NS

Table 1. *Cont.*

Parameter	Control Group AHI < 15 n = 20 (Mean ± SD) or Median(Q1–Q3)	OSA Group AHI > 15 n = 26 (Mean ± SD) or Median(Q1–Q3)	p-Value
Ht (%)	44.8 ± 3.14	45.5 ± 2.61	NS
MCV (fL)	88.9 ± 2.53	90.2 ± 4.01	NS
MCH (pg)	30.2 ± 1.11	30.3 ± 1.33	NS
MCHC (g/dL)	34.0 ± 0.83	33.6 ± 0.91	NS
HbA1c (%)	5.52 ± 0.36	5.58 ± 0.38	NS
ALT (U/L) median(Q1–Q3)	25.0 (19.5–41.5)	33.5 (22–39)	NS
LDL (mg/dL)	129.3 ± 35.7	140.0 ± 27.6	NS
Total cholesterol (mg/dL)	212.1 ± 36.9	224.6 ± 39.9	NS
HDL (mg/dL)	57.9 ± 11.3	51.6 ± 12.7	NS
Triglycerides (mg/dL) median(Q1–Q3)	124.6 (86–145)	164.9 (116–208)	NS
TSH (µlU/mL) median(Q1–Q3)	1.46 (1.04–1.64)	1.32 (0.9–1.8)	NS
Creatinine (mg/dL) median(Q1–Q3)	0.97 (0.86–1.04)	0.99 (0.89–1.08)	NS
eGFR (mL/min/1.73 m^2)	80.5 ± 11.8	86.5 ± 14.8	NS
Uric acid (mg/dL)	4.95 ± 1.05	6.38 ± 0.92	<0.05
Urea (mg/dL)	30.3 ± 6.76	31.5 ± 7.53	NS
Mg (mmol/L)	2.13 ± 0.11	2.12 ± 0.14	NS
K (mmol/L)	4.35 ± 0.29	4.28 ± 0.28	NS
Na (mmol/L)	140.4 ± 1.83	141.5 ± 1.86	NS
hsCRP (mg/L) median(Q1–Q3)	0.46 (0.28–0.73)	1.43 (0.80–3.45)	<0.05
Ca (mmol/L)	9.37 ± 0.35	9.34 ± 0.27	NS
Glucose (mg/dL)	94.9 ± 10.4	100.5 ± 9.80	NS
Insulin (µU/mL) median(Q1–Q3)	6.15 (5.0–7.3)	11.9 (6.4–14.4)	<0.05
HOMA-IR median(Q1–Q3)	1.48 (1.16–1.64)	2.93 (1.36–3.78)	<0.05
QUICKI median	0.36 ± 0.03	0.33 ± 0.03	<0.05
AHI (events/h) median(Q1–Q3)	6.1 (3.0–8.3)	36.4 (20–37)	<0.05
ODI (events/h) median(Q1–Q3)	5.0 (2.2–9.0)	34.4 (20–39)	<0.05
Mean saturation (%)	94.3 ± 1.2	92.5 ± 1.7	<0.05
Duration of desaturation < 90% (% of total sleep time) median(Q1–Q3)	0.1 (0.0–0.9)	5.1 (1.6–11.0)	<0.05

Abbreviations: NS: result not statistically significant; BMI: body mass index; RBC: red blood cells; WBC: white blood cells; MCV: mean (red blood) cell volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; PLT: platelets; eGFR: estimated glomerular filtration rate; HbA1c: glycated hemoglobin; HOMA-IR: homeostatic model assessment of insulin resistance; QUICKI: quantitative insulin sensitivity check index. HDL: high-density lipoprotein; LDL: low-density lipoprotein; hsCRP: high-sensitivity C-reactive protein; TSH: thyroid-stimulating hormone; AHI: apnea-hypopnea index; ODI: oxygen desaturation index; Hb: hemoglobin; Ht: hematocrit.

3.2. Assessment of Baseline Endothelial Function

No significant differences in endothelial function measured by the LDF were noted. Nevertheless, the hyperemia index reached the lowest average values in non-treated OSA subjects (11.2 ± 5.9 in the control vs. 9.6 ± 5.3 in OSA before CPAP), respectively, $p > 0.05$ for each analysis (Table 2).

Table 2. Assessment of endothelial function by laser-doppler-flowmetry (LDF).

	Hyperemia Index
	Mean ± SD
Control group	11.2 ± 5.9
OSA group before CPAP	9.6 ± 5.3
OSA after 1 year of CPAP	11.6 ± 4.0

3.3. Parameters of Nitric Oxide Bioavailability in Erythrocytes and Plasma

As shown in Figure 4, no significant differences regarding metabolites of the NO pathway were found apart from the plasma citrulline concentration, which was lower in patients with OSA (26.9 ± 7.4 vs. 33.1 ± 9.4 µM, $p < 0.05$). All of the altered nitric oxide metabolites were identified at higher concentrations in plasma than in the erythrocyte compartment, which was most significantly pronounced for the arginine concentrations. The erythrocyte-ADMA concentration was lower than in plasma irrespective of the presence of OSA (0.33 ± 0.12 vs. 0.45 ± 0.08 µM in OSA, $p < 0.05$ and 0.33 ± 0.1 vs. 0.45 ± 0.07 µM in the control, $p < 0.05$).

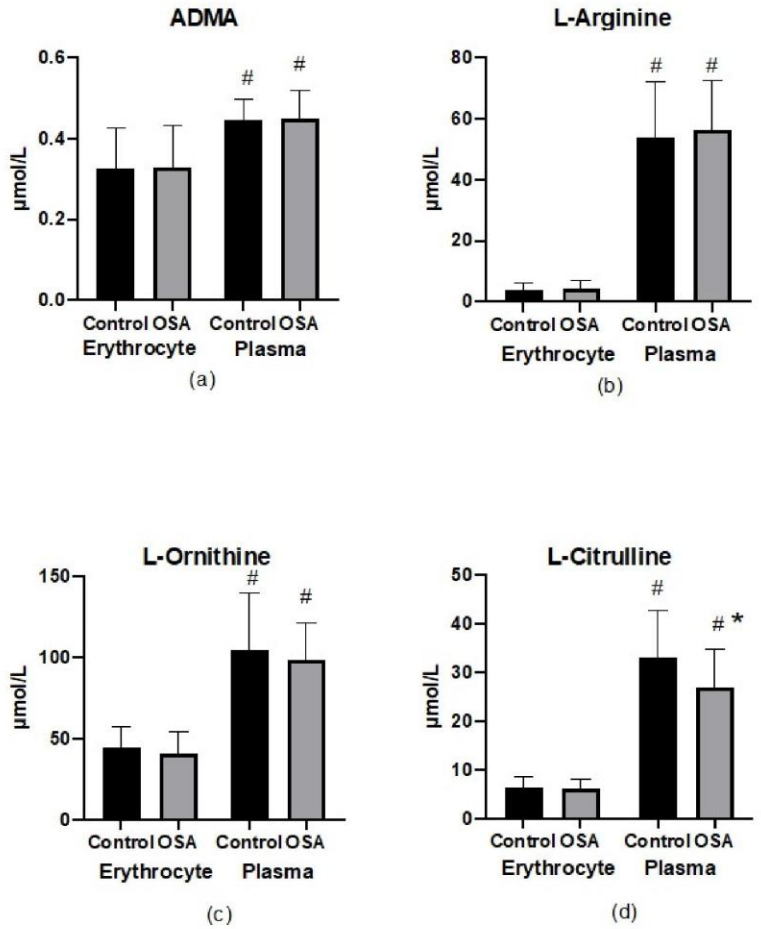


Figure 4. (**a**–**d**) Nitric oxide metabolite concentrations in different compartments. Bars and whiskers represent the mean ± SD. Abbreviations: ADMA: asymmetric dimethylarginine; OSA: obstructive sleep apnea. * denotes $p < 0.05$ vs. control in the same compartment; # denotes $p < 0.05$ between compartments in the same group.

The substrate/inhibitor ratio of nitric oxide synthase in both the plasma and erythrocyte compartments was also assessed (Figure 5). The difference between the plasma and erythrocyte nitric oxide bioavailability measured by the arginine/ADMA ratio was mainly determined by changes in the arginine concentration. There were no differences in the

arginine/ADMA ratio in both groups; nevertheless, the arginine/ADMA ratio was about 10-fold lower in the erythrocyte compartment than in the plasma.

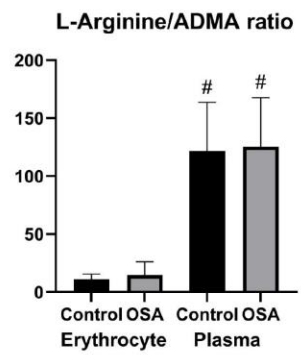


Figure 5. The NO synthase substrate (arginine) to competitive inhibitor (ADMA) ratio in plasma and erythrocytes. Bars and whiskers represent the mean ± SD. Abbreviations: ADMA: asymmetric dimethylarginine; OSA: obstructive sleep apnea. # denotes $p < 0.05$ vs. the erythrocyte compartment in the same group.

3.4. Effect of CPAP

Subjects from the OSA group who were provided with CPAP therapy and were using it regularly for about one year were asked for a second evaluation. Ten subjects met the criteria and agreed to the examination. CPAP therapy after one year of use did not show any significant changes in the bioavailability of NO metabolites' (Figure 6).

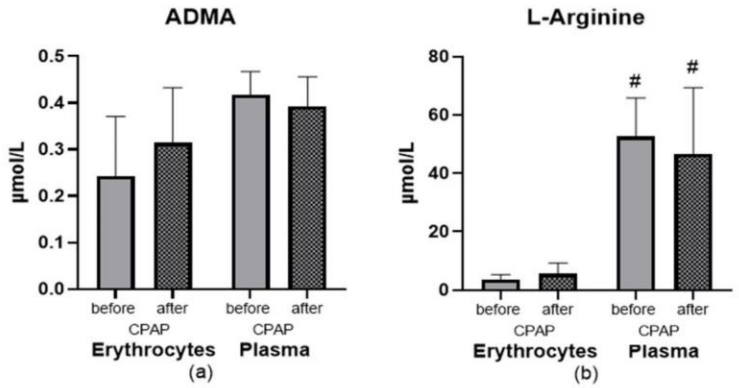
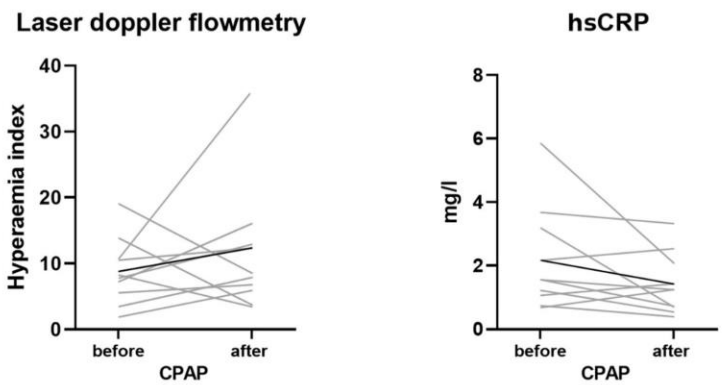


Figure 6. (**a**,**b**). The effect of 1-year CPAP therapy on the bioavailability of NO metabolites. Bars and whiskers represent the mean ± SD. Abbreviations: ADMA: asymmetric dimethylarginine; OSA: obstructive sleep apnea. # denotes $p < 0.05$ between compartments in the same group.

There was a trend for CPAP towards lowering hsCRP (Figure 7 and Table S1). Nonetheless, the hyperemia index reached greater average values in OSA subjects following CPAP. (9.6 ± 5.3 in OSA before CPAP and 11.64 ± 3.97 in OSA after 1-year CPAP, respectively, $p > 0.05$).



(a) (b)

Figure 7. (**a**,**b**) The effect of 1-year CPAP therapy on hsCRP and endothelial function measured by laser doppler flowmetry.

4. Discussion

Recent findings postulate a pivotal role for endothelial dysfunction in OSA as the first step leading to the development of target organ damage [30]. Nevertheless, the data from human studies on this matter is scarce. Moreover, the role of erythrocytes in maintaining the bioavailability of NO has yet to be proven. This is the first prospective study that evaluated the NO metabolic pathway in both plasma and erythrocyte compartments in patients with obstructive sleep apnea (OSA) as well as following 1-year CPAP treatment. Furthermore, endothelial function, assessed by laser doppler flowmetry and reflecting the vasodilative NO-dependent function of microcirculation, was measured in OSA subjects at baseline and prospectively following CPAP treatment.

4.1. NO Biotranformation in Erythrocytes and Plasma in OSA and Non-OSA Subjects

Some studies have identified a cross-talk between the occurrence of OSA and high plasma ADMA levels [31], which is not influenced by the presence or absence of conventional risk factors [32]. In our study, the plasma ADMA concentrations were similar in both groups, which has been previously demonstrated [33].

The levels of ADMA in our study measured in plasma of about 0.5 µmol/L may be below those required for competitive NOS inhibition, as the concentrations of its substrate, L-arginine, are manyfold above the Km for NOS [34]. In our study, the average concentrations of intraerythrocytic ADMA were even lower—about 0.3 µmol/L. RBC could play a more important role in the storage pool of ADMA. Interestingly, in patients undergoing hemodialysis, the urea and creatinine had a rebound ratio measured by an increase after 1 h of hemodialysis of less than 10%. In contrast, a considerable rebound ratio of 30% of ADMA was simultaneously detected [35]. Hence, we assume that ADMA may present a multicompartmental distribution in which RBCs could play a pivotal role, including in subjects with OSA.

RBCs contain eNOS and display NO-like bioactivity [36]. Noteworthy, in our study the concentration of L-arginine in RBCs was about 10-fold lower than in plasma, resulting in a lower L-arginine/ADMA ratio and lower substrate bioavailability for NOS, which is confirmed by the lower citrulline level (a side product of the NO synthesis). The L-arginine concentration is under tight control by arginase 1 (Figure 8), whose activity is regulated during ischemia-reperfusion/hypoxia-reoxygenation injuries [36]. The up-regulation of arginase activity could be caused by the peroxynitrite donor in RBCs [37]. Diminished

efflux of arginine from plasma to erythrocytes is another potential cause of 10-fold lower arginine concentrations in RBCs than in plasma.

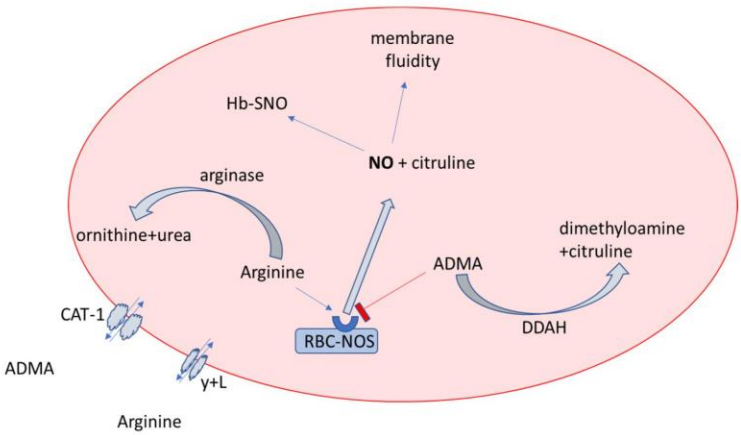


Figure 8. Nitric oxide bioavailability in erythrocytes. Abbreviations: NO: nitric oxide; RBC-NOS: nitric oxide synthase; ADMA: asymmetric dimethylarginine; CAT-1: cationic amino acid transporter-1; HB-SNO: S-nitrosohemoglobin; DDAH: dimethylarginine dimethylaminohydrolase; y + L: amino acid transporter.

The role of RBC-NOS in supporting NO production and maintaining endothelial function remains uninvestigated. Only a small amount of the nitric oxide carried by the Hb is released from the RBCs, but the transition from high to low oxygen tension in the peripheral vasculature enhances its release as SNO-based vasodilatory action [38].

4.2. Effect of CPAP

Interestingly, in a follow-up study 1-year after CPAP treatment, there was a tendency for higher concentrations of arginine in erythrocytes. This could be partially explained by the lower activity of arginase 1, because of less ROS production, which increases arginase 1 activity. Together with intraerythrocytic arginine, increased its competitive inhibitor ADMA, the Arginine/ADMA ratio was maintained at a similar level. A one-year treatment with CPAP also showed decreased CRP levels. Another study suggests that a lowered CRP might be a valuable predictor of success in OSA treatment monitoring [39].

4.3. L-Citrulline and NO Production

Citrulline is efficiently recycled in endothelial, immune, and kidney cells and is easily converted into arginine [40]. On the other hand, citrulline is a side product in the reaction catalyzed by the eNOS as well as in the ADMA degradation, catalyzed by the DDAH, which might also prevent excessive and uncontrolled NO synthesis [40]. In our study, plasma citrulline concentrations were lower in OSA patients than in controls, which could be the first sign of diminished nitric oxide synthesis.

4.4. Other Differences in the Demographic and Biochemical Characteristics between the Groups

The comparison of groups with AHI < 15 and mild OSA with AHI > 15 showed differences in some anthropometric and metabolic parameters, including BMI, weight, fasting insulin, leukocytes, uric acid, and CRP values. Noteworthy, metabolic syndrome and all its components constitute the risk factors for OSA development, and as a result they do coexist commonly with OSA [41]. Obesity and insulin resistance, synergistically with OSA, could multiply the cardiovascular risk by increasing oxidative stress, promoting inflammation and apoptosis [42] and endothelial dysfunction, which at the early stages of atherogenesis in

clinically healthy subjects might be reversible [43]. In fact, impairment of the availability of NO could multiply the endothelial dysfunction in the comorbidity of OSA and pre-diabetes. Therefore, a subgroup analysis of the control group including only "metabolically-matched" subjects to the OSA group could provide detailed information on the direct effect of OSA on endothelial function and the NO metabolites. Nevertheless, similar values of the reactive hyperemia index (RHI), reflecting the endothelial vasodilative function of microcirculation as well as the plasma and erythrocyte levels of the NO metabolites between the groups, do not indicate that the presence of biochemical and demographical differences could blur the effect of OSA and its treatment in the study group.

5. Study Limitations

The subjects enrolled in this study suspected of having OSA had concomitant cardiovascular risk factors, such as glucose intolerance, hypertension, obesity, and smoking; therefore, the impact of OSA severity could be interfered with. Furthermore, the erythrocytes contain transmembrane proteins, whose function may affect compartment distribution and study outcomes. Nevertheless, rapid, ad hoc isolation of erythrocytes collected from the blood drawn and subsequent freezing of the RBC samples at −80 °C limited these phenomena. The small population of subjects enrolled in the study is another limitation.

6. Conclusions

The erythrocyte pool of the intermediates of the NO metabolic pathway does not depend on the severity of OSA, and erythrocytes could constitute a high-volume buffer in their storage in OSA patients. The use of CPAP for one year did not result in changes in the balance between the erythrocyte and the plasmatic pool of the NO metabolic pathway intermediates. The results from this prospective study are a step forward in understanding the role of the erythrocyte compartment and the intra-erythrocyte pathways regulating the NO bioavailability and paracrine endothelial function in the hypoxia-reoxygenation setting, such as obstructive sleep apnea. Nevertheless, future large prospective studies with precise matching of the cases in groups regarding comorbidities would limit the potentially distracting effect of concomitant disorders on the results.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijerph192214719/s1, Table S1: Baseline characteristics of subjects before and after 1 year of CPAP.

Author Contributions: Conceptualization, A.D. and J.M.; methodology, P.F., E.S.-K. and J.W.; software, J.M. and A.D. validation, A.D.; formal analysis, A.D. and H.M.; investigation, J.M.; writing—original draft preparation, A.D. and J.M.; writing—review and editing, J.G., E.S.-K. and P.R.; supervision, A.D.; project administration A.D.; funding acquisition, J.M. and A.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Wroclaw Medical University (protocol nr KB-335/2018 from 29 May 2018 for studies involving humans).

Informed Consent Statement: Written informed consent has been obtained from the patient(s) to publish this paper.

Data Availability Statement: The original data used to support the findings of this study are available upon request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

1. Benjafield, A.V.; Ayas, N.T.; Eastwood, P.R.; Heinzer, R.; Ip, M.S.M.; Morrell, M.J.; Nunez, C.M.; Patel, S.R.; Penzel, T.; Pépin, J.L.D.; et al. Estimation of the global prevalence and burden of obstructive sleep apnoea: A literature-based analysis. *Lancet Respir. Med.* **2019**, *7*, 687–698. [CrossRef]
2. Stanek, A.; Fazeli, B.; Bartuv, S.; Sutkowska, E. Editorial The Role of Endothelium in Physiological and Pathological States: New Data. *BioMed Res. Int.* **2018**, *2018*, 1098039. [CrossRef] [PubMed]
3. Stanek, A.; Brożyna-Tkaczyk, K.; Myśliński, W. Oxidative Stress Markers among Obstructive Sleep Apnea Patients. *Oxidative Med. Cell. Longev.* **2021**, *2021*, 9681595. [CrossRef]
4. Turrens, J.F. Mitochondrial formation of reactive oxygen species. *J. Physiol.* **2003**, *552*, 335–344. [CrossRef] [PubMed]
5. Dasgupta, S.; Gomez, J.-J.; Singh, I.; Khan, M. S-Nitrosylation in Regulation of Inflammation and Cell Damage. *Curr. Drug Targets* **2018**, *19*, 1831–1838. [CrossRef] [PubMed]
6. Pau, M.C.; Mangoni, A.A.; Zinellu, E.; Pintus, G.; Carru, C.; Fois, A.G.; Pirina, P.; Zinellu, A. Circulating Superoxide Dismutase Concentrations in Obstructive Sleep Apnoea (OSA): A Systematic Review and Meta-Analysis. *Antioxidants* **2021**, *10*, 1764. [CrossRef]
7. Pau, M.C.; Zinellu, E.; Fois, S.S.; Piras, B.; Pintus, G.; Carru, C.; Mangoni, A.A.; Fois, A.G.; Zinellu, A.; Pirina, P. Circulating Malondialdehyde Concentrations in Obstructive Sleep Apnea (OSA): A Systematic Review and Meta-Analysis with Meta-Regression. *Antioxidants* **2021**, *10*, 1053. [CrossRef]
8. Gao, F.; Lucke-Wold, B.P.; Li, X.; Logsdon, A.F.; Xu, L.C.; Xu, S.; LaPenna, K.B.; Wang, H.; Talukder, M.A.H.; Siedlecki, C.A.; et al. Reduction of Endothelial Nitric Oxide Increases the Adhesiveness of Constitutive Endothelial Membrane ICAM-1 through Src-Mediated Phosphorylation. *Front. Physiol.* **2018**, *8*, 1124. [CrossRef]
9. Böger, R.H.; Maas, R.; Schulze, F.; Schwedhelm, E. Asymmetric dimethylarginine (ADMA) as a prospective marker of cardiovascular disease and mortality—An update on patient populations with a wide range of cardiovascular risk. *Pharmacol. Res.* **2009**, *60*, 481–487. [CrossRef]
10. Billecke, S.S.; Kitzmiller, L.A.; Northrup, J.J.; Whitesall, S.E.; Kimoto, M.; Hinz, A.V.; D'Alecy, L.G. Contribution of whole blood to the control of plasma asymmetrical dimethylarginine. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, *291*, H1788–H1796. [CrossRef]
11. Davids, M.; van Hell, A.J.; Visser, M.; Nijveldt, R.J.; van Leeuwen, P.A.M.; Teerlink, T. Role of the human erythrocyte in generation and storage of asymmetric dimethylarginine. *Am. J. Physiol. Circ. Physiol.* **2012**, *302*, H1762–H1770. [CrossRef] [PubMed]
12. Yokoro, M.; Suzuki, M.; Murota, K.; Otsuka, C.; Yamashita, H.; Takahashi, Y.; Tsuji, H.; Kimoto, M. Asymmetric dimethylarginine, an endogenous NOS inhibitor, is actively metabolized in rat erythrocytes. *Biosci. Biotechnol. Biochem.* **2012**, *76*, 1334–1342. [CrossRef] [PubMed]
13. Yildirim, A.O.; Bulau, P.; Zakrzewicz, D.; Kitowska, K.E.; Weissmann, N.; Grimminger, F.; Morty, R.E.; Eickelberg, O. Increased protein arginine methylation in chronic hypoxia: Role of protein arginine methyltransferases. *Am. J. Respir. Cell Mol. Biol.* **2006**, *35*, 436–443. [CrossRef] [PubMed]
14. Zwemer, C.F.; Davenport, R.D.; Gomez-Espina, J.; Blanco-Gonzalez, E.; Whitesall, S.E.; D'Alecy, L.G. Packed red blood cells are an abundant and proximate potential source of Nitric oxide synthase inhibition. *PLoS ONE* **2015**, *10*, e0119991. [CrossRef] [PubMed]
15. Tsikas, D.; Böhmer, A.; Großkopf, H.; Beckmann, B.; Dreißigacker, U.; Jordan, J.; Maassen, N. Clinical-chemistry laboratory relevant hemolysis is unlikely to compromise human plasma concentration of free asymmetric dimethylarginine (ADMA). *Clin. Biochem.* **2012**, *45*, 1536–1538. [CrossRef]
16. Hannemann, J.; Zummack, J.; Hillig, J.; Böger, R. Metabolism of asymmetric dimethylarginine in hypoxia: From bench to bedside. *Pulm. Circ.* **2020**, *10*, 31–41. [CrossRef]
17. Kang, E.S.; Cates, T.B.; Harper, D.N.; Chiang, T.M.; Myers, L.K.; Acchiardo, S.R.; Kimoto, M. An enzyme hydrolyzing methylated inhibitors of nitric oxide synthase is present in circulating human red blood cells. *Free Radic. Res.* **2001**, *35*, 693–707. [CrossRef]
18. Bollenbach, A.; Tsikas, D. Pharmacological activation of dimethylarginine dimethylaminohydrolase (DDAH) activity by inorganic nitrate and DDAH inhibition by N^G-hydroxy-l-arginine, N^{ω},N'^{ω}-dimethyl-l-citrulline and N^{ω},N'^{ω}-dimethyl-N^{δ}-hydroxy-l-citrulline: Results and overview. *Amino Acids* **2019**, *51*, 483–494. [CrossRef]
19. Banjarnahor, S.; Rodionov, R.N.; König, J.; Maas, R. Transport of l-arginine related cardiovascular risk markers. *J. Clin. Med.* **2020**, *9*, 3975. [CrossRef]
20. Shima, Y.; Maeda, T.; Aizawa, S.; Tsuboi, I.; Kobayashi, D.; Kato, R.; Tamai, I. l-arginine import via cationic amino acid transporter CAT1 is essential for both differentiation and proliferation of erythrocytes. *Blood* **2006**, *107*, 1352–1356. [CrossRef]
21. Badran, M.; Golbidi, S.; Ayas, N.; Laher, I. Nitric Oxide Bioavailability in Obstructive Sleep Apnea: Interplay of Asymmetric Dimethylarginine and Free Radicals. *Sleep Disord.* **2015**, *2015*, 387801. [CrossRef] [PubMed]
22. Strobel, J.; Mieth, M.; Endreß, B.; Auge, D.; König, J.; Fromm, M.F.; Maas, R. Interaction of the cardiovascular risk marker asymmetric dimethylarginine (ADMA) with the human cationic amino acid transporter 1 (CAT1). *J. Mol. Cell. Cardiol.* **2012**, *53*, 392–400. [CrossRef]
23. Peled, N.; Kassirer, M.; Kramer, M.R.; Rogowski, O.; Shlomi, D.; Fox, B.; Berliner, A.S.; Shitrit, D. Increased erythrocyte adhesiveness and aggregation in obstructive sleep apnea syndrome. *Thromb. Res.* **2008**, *121*, 631–636. [CrossRef] [PubMed]
24. Grau, M.; Pauly, S.; Ali, J.; Walpurgis, K.; Thevis, M.; Bloch, W.; Suhr, F. RBC-NOS-Dependent S-Nitrosylation of Cytoskeletal Proteins Improves RBC Deformability. *PLoS ONE* **2013**, *8*, e56759. [CrossRef] [PubMed]

25. Tsuda, K.; Nishio, I. An association between plasma asymmetric dimethylarginine and membrane fluidity of erythrocytes in hypertensive and normotensive men: An electron paramagnetic resonance investigation. *Am. J. Hypertens.* **2005**, *18*, 1243–1248. [CrossRef] [PubMed]
26. Berry, R.B.; Brooks, R.; Gamaldo, C.; Harding, S.M.; Lloyd, R.M.; Quan, S.F.; Troester, M.T.; Vaughn, B.V. AASM Scoring Manual Updates for 2017 (Version 2.4). *J. Clin. Sleep Med.* **2017**, *13*, 665. [CrossRef]
27. Qiu, Z.H.; Luo, Y.M.; McEvoy, R.D. The Sleep Apnea Cardiovascular Endpoints (SAVE) study: Implications for health services and sleep research in China and elsewhere. *J. Thorac. Dis.* **2017**, *9*, 2217–2220. [CrossRef]
28. Fleszar, M.G.; Wiśniewski, J.; Krzystek-Korpacka, M.; Misiak, B.; Frydecka, D.; Piechowicz, J.; Lorenc-Kukuła, K.; Gamian, A. Quantitative Analysis of l-Arginine, Dimethylated Arginine Derivatives, l-Citrulline, and Dimethylamine in Human Serum Using Liquid Chromatography–Mass Spectrometric Method. *Chromatographia* **2018**, *81*, 911–921. [CrossRef]
29. Minson, C.T. Mechanisms and Modulators of Temperature Regulation: Thermal provocation to evaluate microvascular reactivity in human skin. *J. Appl. Physiol.* **2010**, *109*, 1239. [CrossRef]
30. Bironneau, V.; Tamisier, R.; Trzepizur, W.; Andriantsitohaina, R.; Berger, M.; Goupil, F.; Joyeux-Faure, M.; Jullian-Desayes, I.; Launois, S.; Le Vaillant, M.; et al. Sleep apnoea and endothelial dysfunction: An individual patient data meta-analysis. *Sleep Med. Rev.* **2020**, *52*, 101309. [CrossRef]
31. Arlouskaya, Y.; Sawicka, A.; Głowala, M.; Giebułtowicz, J.; Korytowska, N.; Tałałaj, M.; Nowicka, G.; Wrzosek, M. Asymmetric Dimethylarginine (ADMA) and Symmetric Dimethylarginine (SDMA) Concentrations in Patients with Obesity and the Risk of Obstructive Sleep Apnea (OSA). *J. Clin. Med.* **2019**, *8*, 897. [CrossRef] [PubMed]
32. Barceló, A.; De La Peña, M.; Ayllón, O.; Vega-Agapito, M.V.; Piérola, J.; Pérez, G.; González, C.; Alonso, A.; Agustí, A.G.N. Increased plasma levels of asymmetric dimethylarginine and soluble CD40 ligand in patients with sleep apnea. *Respiration.* **2009**, *77*, 85–90. [CrossRef] [PubMed]
33. Ozkan, Y.; Fırat, H.; Şimşek, B.; Torun, M.; Yardim-Akaydin, S. Circulating nitric oxide (NO), asymmetric dimethylarginine (ADMA), homocysteine, and oxidative status in obstructive sleep apnea-hypopnea syndrome (OSAHS). *Sleep Breath.* **2008**, *12*, 149–154. [CrossRef] [PubMed]
34. Teerlink, T.; Luo, Z.; Palm, F.; Wilcox, C.S. CELLULAR ADMA: REGULATION AND ACTION. *Pharmacol. Res.* **2009**, *60*, 448. [CrossRef] [PubMed]
35. Du, Q.; Gao, J.; Lu, R.; Jin, Y.; Zou, Y.; Yu, C.; Yan, Y. Asymmetric dimethylarginine compartmental behavior during high-flux hemodialysis. *Ren. Fail.* **2020**, *42*, 760–766. [CrossRef]
36. Yang, J.; Gonon, A.T.; Sjöquist, P.O.; Lundberg, J.O.; Pernow, J. Arginase regulates red blood cell nitric oxide synthase and export of cardioprotective nitric oxide bioactivity. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 15049–15054. [CrossRef]
37. Mahdi, A.; Tengbom, J.; Alvarsson, M.; Wernly, B.; Zhou, Z.; Pernow, J. Red Blood Cell Peroxynitrite Causes Endothelial Dysfunction in Type 2 Diabetes Mellitus via Arginase. *Cells* **2020**, *9*, 1712. [CrossRef]
38. Premont, R.T.; Reynolds, J.D.; Zhang, R.; Stamler, J.S. Role of Nitric Oxide Carried by Hemoglobin in Cardiovascular Physiology: Developments on a Three-Gas Respiratory Cycle. *Circ. Res.* **2020**, *126*, 129–158. [CrossRef]
39. Msaad, S.; Chaabouni, A.; Marrakchi, R.; Boudaya, M.; Kotti, A.; Feki, W.; Jamoussi, K.; Kammoun, S. Nocturnal Continuous Positive Airway Pressure (nCPAP) Decreases High-Sensitivity C-Reactive Protein (hs-CRP) in Obstructive Sleep Apnea-Hypopnea Syndrome. *Sleep Disord.* **2020**, *2020*, 8913247. [CrossRef]
40. Papadia, C.; Osowska, S.; Cynober, L.; Forbes, A. Citrulline in health and disease. Review on human studies. *Clin. Nutr.* **2018**, *37*, 1823–1828. [CrossRef]
41. Ambrosetti, M.; Lucioni, A.M.; Conti, S.; Pedretti, R.F.; Neri, M. Metabolic syndrome in obstructive sleep apnea and related cardiovascular risk. *J. Cardiovasc. Med.* **2006**, *7*, 826–829. [CrossRef] [PubMed]
42. Stanek, A.; Brożyna-Tkaczyk, K.; Myśliński, W. The role of obesity-induced perivascular adipose tissue (Pvat) dysfunction in vascular homeostasis. *Nutrients* **2021**, *13*, 3843. [CrossRef] [PubMed]
43. Stanek, A.; Wielkoszyński, T.; Bartuś, S.; Cholewka, A. Whole-body cryostimulation improves inflammatory endothelium parameters and decreases oxidative stress in healthy subjects. *Antioxidants* **2020**, *9*, 1308. [CrossRef] [PubMed]

6. OŚWIADCZENIA WSPÓŁAUTORÓW

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Cardiovascular Disorders Triggered by Obstructive Sleep Apnea—A Focus on Endothelium and Blood Components

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mój udział polegał na przeszukaniu baz danych, analizie zebranych danych, stworzeniu zasadniczej części manuskryptu wraz z rycinami.

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International Journal of Environmental Research and Public Health. 2022; 19(22):14719.

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mój udział polegał na współpracy w opracowaniu koncepcji badań, wykonaniu badań laboratoryjnych i zabezpieczeniu materiału biologicznego do dalszych analiz, zebraniu i analizie danych oraz pomocy w zredagowaniu części dotyczącej metodologii manuskryptu.

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mój udział polegał na wykonaniu oznaczeń biochemicznych zabezpieczonego materiału biologicznego oraz pomocy przy zredagowaniu części dotyczącej metodologii manuskryptu.

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mój udział polegał na analizie badań PSG oraz na nadzorze nad tekstem manuskryptu.

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mój udział polegał na analizie zebranych danych oraz nadzorze nad tekstem manuskryptu.

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mój udział polegał na opracowaniu koncepcji badań, kierowaniu ich realizacją, analizie zebranych danych oraz nadzorze nad pisaniem manuskryptu.

Adrian Doroszko

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7. STRESZCZENIE

Osoby z obturacyjnym bezdechem sennym charakteryzują się wyższym ryzykiem incydentów sercowo-naczyniowych. Terapia CPAP, zgodnie z wynikami wielu prac, zmniejsza to ryzyko, jednakże w stopniu niewystarczającym. Patofizjologia związana z obturacyjnym bezdechem sennym i nawracającymi epizodami hipoksji i reoksygenacji obejmuje szereg zmian w zakresie śródbłonka naczyniowego, jak również elementów morfotycznych krwi, a w szczególności erytrocytów. Z uwagi na obecność niezależnej syntazy tlenku azotu (NOS) i jej głównego inhibitora ADMA w erytrocytach oraz na podkreślaną rolę tlenku azotu w rozwoju dysfunkcji śródbłonka naczyniowego, postanowiono zbadać jego biodostępność w bezdechu sennym zarówno w osoczu jaki i w erytrocytach.

Do badania kwalifikowano osoby z podejrzeniem OBS w przedziale 30-70 lat. Do grupy badanej włączono 46 osób, od których pobrano około 40 ml krwi żylnej celem analizy podstawowych parametrów biochemicznych służących do oceny ryzyka sercowo-naczyniowego oraz badań biochemicznych oceniających osoczowe i wewnątrz-erytrocytarne elementy osi biotransformacji NO. Po przeprowadzeniu polisomnografii, pacjentów podzielono na podgrupy w zależności od ciężkości OBS. U każdego pacjenta wykonano nieinwazyjną ocenę wazodylatacyjnej funkcji śródbłonka naczyniowego za pomocą przepływomierza laserowego (*Laser-Doppler*). U pacjentów, u których wdrożono terapię CPAP przeprowadzono ponowną prospektywną ewaluację funkcji śródbłonka - *follow-up* w 1-rocznej obserwacji i oznaczono metabolity szlaku tlenku azotu po około roku od rozpoczęcia regularnego stosowania terapii.

W rezultacie stwierdzono brak różnic w stężeniu asymetrycznej dimetyloargininy (ADMA wewnątrz erytrocytów w zależności od ciężkości bezdechu. Stężenie ADMA w osoczu było najczęściej około 2 razy większe niż w erytrocytach, wskazując tym samym, że erytrocyty mogą stanowić wysokoobjętościowy układ buforowy dla tego inhibitora. Leczenie bezdechu sennego CPAP nie indukowało istotnej statystycznie poprawy funkcji śródbłonka, jednak wiązało się z tendencją do zwiększonej odpowiedzi wazodylatacyjnej na bodziec termiczny w badaniu *Laser Doppler Flowmetry* (LDF). L-Cytrulina, której obniżone osoczowe stężenie stwierdzono u osób ze średnim i ciężkim OBS, jako substrat w reakcji syntezy L-Argininy, może stanowić pierwszy osoczowy marker zmniejszającej się biodostępności tlenku azotu. Kardiometaboliczne markery ryzyka sercowo-naczyniowego, jak stężenie kwasu moczowego i insuliny na czczo oraz hsCRP lepiej korelowały ze stopniem ciężkości OBS niż zmiany dotyczące wykładników biodostępności tlenku azotu.

Wyniki naszego badania stanowią krok naprzód w zrozumieniu roli erytrocytów w regulacji biodostępności NO, jednak przy złożoności mechanizmów regulujących jego syntezę konieczne są dalsze szczegółowe badania. Brak różnic w stężeniach metabolitów regulujących bioaktywność NO u osób z OBS jeszcze bez zaawansowanych zmian naczyniowych jest wynikiem zwiększonej odpowiedzi mechanizmów kompensacyjnych w odpowiedzi na zwiększony stres oksydacyjny. Brak korelacji pomiędzy osoczowym i wewnątrz-erytrocytarnym stężeniem ADMA przemawia za częściową niezależnością tych dwóch kompartmentów, a także znacznej roli transportu przezbłonowego w regulacji stężeń aminokwasów i innych molekuł pomiędzy komórkami śródbłonka, osoczem i erytrocytami.

8. SUMMARY

Patients with obstructive sleep apnea are characterized by higher risk of cardiovascular events. Treatment with CPAP in a vast majority of studies was shown to reduce this risk but in an insufficient manner. The pathophysiology associated with sleep apnea and recurrent episodes of hypoxia and reoxygenation includes a number of changes in the vascular endothelium as well as morphotic elements, in particular erythrocytes. Due to the presence of an independent nitric oxide synthase (NOS) and its main inhibitor ADMA in erythrocytes, and the emphasized role of nitric oxide in the development of vascular endothelial dysfunction, it was decided to investigate its bioavailability in sleep apnea, both in plasma and in erythrocytes.

Subjects with suspected OSA aged 30-70 were qualified for the study. The study group included 46 people, from whom approximately 40 ml of venous blood was collected for the analysis of basic biochemical parameters assessing cardiovascular risk and biochemical tests assessing plasma and intra-erythrocytic elements of the NO biotransformation axis. After polysomnography, patients were divided into subgroups according to the severity of the apnea. Each patient underwent a non-invasive assessment of the endothelium function using a Laser Doppler Flowmetry (LDF). In patients with CPAP therapy, a prospective re-evaluation of endothelial function was performed - follow-up in a 1-year follow-up and the metabolites of the nitric oxide pathway were determined after about a year from the start of regular therapy.

As a result, there was no statistically significant difference in the concentration of asymmetric dimethylarginine (ADMA) - a competitive inhibitor of nitric oxide synthase - inside erythrocytes depending on the severity of apnea. The concentration of ADMA in plasma was usually about 2 times higher than in erythrocytes, hence erythrocytes may constitute a high-volume buffer system for this inhibitor. Treatment of sleep apnea with the CPAP did not induce a statistically significant improvement in endothelial function, however, it was associated with a trend to rising vasodilative response to a thermal stimulus measured by the Laser Doppler Flowmetry (LDF). L-Citrulline, a substrate in the L-Arginine synthesis, may be the first plasma marker of decreasing bioavailability of nitric oxide, as lower plasma concentration of L-Citrulline was found in subjects with moderate and severe apnea. Cardiometabolic markers of cardiovascular risk, such as uric acid, fasting insulin concentrations, and hsCRP, correlated better with the severity of sleep apnea than changes in markers of nitric oxide bioavailability.

The results from this prospective study are a step forward in understanding of the role of the erythrocyte compartment in regulation of the NO synthesis, however, due to the complexity of NO bioavailability, additional detailed studies are needed. We conclude that the lack of differences in the measured metabolites regulating NO bioactivity in subjects with sleep apnea without advanced vascular changes could be explained by increased compensatory mechanisms caused by oxidative stress. The lack of correlation between the plasma and intra-erythrocyte ADMA concentration suggests partial independence of these two compartments, as well as the important role of transmembrane transport in regulating the concentrations of amino acids and other molecules between the endothelium, plasma and erythrocytes.

9. ZGODA KOMISJI BIOETYCZNEJ

KOMISJA BIOETYCZNA
przy
Uniwersytecie Medycznym
we Wrocławiu
ul. Pasteura 1; 50-367 WROCŁAW

OPINIA KOMISJI BIOETYCZNEJ Nr KB – 335/2018

Komisja Bioetyczna przy Uniwersytecie Medycznym we Wrocławiu, powołana zarządzeniem Rektora Uniwersytetu Medycznego we Wrocławiu nr 133/XV R/2017 z dnia 21 grudnia 2017 r. oraz działająca w trybie przewidzianym rozporządzeniem Ministra Zdrowia i Opieki Społecznej z dnia 11 maja 1999 r. (Dz.U. nr 47, poz. 480) na podstawie ustawy o zawodzie lekarza z dnia 5 grudnia 1996 r. (Dz.U. nr 28 z 1997 r. poz. 152 z późniejszymi zmianami) w składzie:

dr hab. Jacek Daroszewski (endokrynologia, diabetologia)
prof. dr hab. Krzysztof Grabowski (chirurgia)
dr Henryk Kaczkowski (chirurgia szczękowa, chirurgia stomatologiczna)
mgr Irena Knabel-Krzyszowska (farmacja)
prof. dr hab. Jerzy Liebhart (choroby wewnętrzne, alergologia)
ks. dr hab. Piotr Mrzygłód (duchowny)
mgr prawa Luiza Műller (prawo)
dr hab. Sławomir Sidorowicz (psychiatria)
dr hab. Leszek Szenborn (pediatria, choroby zakaźne)
Danuta Tarkowska (pielęgniarstwo)
prof. dr hab. Anna Wiela-Hojeńska (farmakologia kliniczna)
dr hab. Andrzej Wojnar (histopatologia, dermatologia) przedstawiciel Dolnośląskiej Izby Lekarskiej)
dr hab. Jacek Zieliński (filozofia)

pod przewodnictwem
prof. dr hab. Jana Kornafela (ginekologia i położnictwo, onkologia)

Przestrzegając w działalności zasad Good Clinical Practice oraz zasad Deklaracji Helsińskiej, po zapoznaniu się z projektem badawczym pt.:

„Ocena regulacji biodostępności tlenku azotu w erytrocytach u osób z zespołem bezdechu sennego"

zgłoszonym przez **lek. Jakuba Mochola** uczestnika studiów doktoranckich w Katedrze i Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej Uniwersytetu Medycznego im. Piastów Śląskich we Wrocławiu oraz złożonymi wraz z wnioskiem dokumentami, w tajnym głosowaniu postanowiła **wyrazić zgodę** na przeprowadzenie badania w Klinice Chorób Wewnętrznych, Zawodowych, Nadciśnienia Tętniczego i Onkologii Klinicznej USK nadzorem prof. dr hab. Adriana Doroszko **pod warunkiem zachowania anonimowości uzyskanych danych**.

Uwaga: Badanie to zostało objęte ubezpieczeniem odpowiedzialności cywilnej Uniwersytetu Medycznego we Wrocławiu z tytułu prowadzonej działalności.

Pouczenie: W ciągu 14 dni od otrzymania decyzji wnioskodawcy przysługuje prawo odwołania do Komisji Odwoławczej za pośrednictwem Komisji Bioetycznej UM we Wrocławiu.

Opinia powyższa dotyczy projektu badawczego będącego podstawą rozprawy doktorskiej.

Wrocław, dnia 29 maja 2018 r.

Uniwersytet Medyczny we Wrocławiu
KOMISJA BIOETYCZNA
przewodniczący
prof. dr hab. Jan Kornafel