Sleep Medicine 15 (2014) 596–602

Contents lists available at ScienceDirect

Sleep Medicine

journal homepage: www.elsevier.com/locate/sleep

Original Article

Chronic intermittent hypoxia causes endothelial dysfunction in a mouse model of diet-induced obesity

Mohammad Badran [a], Saeid Golbidi [a], Angela Devlin [b], Najib Ayas [c,d,e,f], Ismail Laher [a,*]

[a] Department of Anesthesiology, Pharmacology and Therapeutics, University of British Columbia, Vancouver, Canada
[b] Department of Pediatrics, University of British Columbia, Child & Family Research Institute, Vancouver, Canada
[c] Division of Critical Care, Department of Medicine, University of British Columbia, Vancouver, BC, Canada
[d] Division of Respiratory Medicine, Department of Medicine, University of British Columbia, Vancouver, BC, Canada
[e] Sleep Disorders Program, UBC Hospital, Vancouver, BC, Canada
[f] Division of Critical Care Medicine, Providence Healthcare, Vancouver, BC, Canada

ARTICLE INFO

Article history:
Received 19 October 2013
Received in revised form 25 January 2014
Accepted 30 January 2014
Available online 19 February 2014

Keywords:
Sleep apnea
Intermittent hypoxia
Oxidative stress
Inflammation
Obesity
Vascular endothelium

ABSTRACT

Background: Obstructive sleep apnea (OSA) is a common disorder characterized by chronic intermittent hypoxia (CIH). OSA is prevalent in obese subjects and is associated with endothelial dysfunction and cardiovascular disorders. We tested the hypothesis that the deleterious effects of IH could be further modulated by diet-induced obesity.

Design: Thirty adult (8–10 weeks) male C57BL/6J mice were divided into four groups. Mice were subjected to CIH or intermittent air (IA) for 12 h a day and fed either a high fat (HF) or a low fat control diet (CD) for 6 weeks. We analyzed endothelial function using a wire myograph, and measured markers of oxidative stress (plasma malondialdehyde (MDA) and total antioxidant capacity (TAC)) using colorimetrical assays. We also measured C-reactive protein (CRP) using ELISA and endothelial nitric oxide (eNOS) gene expression using real time PCR.

Results: Stimulated endothelial dependent dilation was significantly impaired only in the group fed high fat diet and subjected to CIH (E_{max}: HFIH 78 ± 2%, $p < 0.0001$) when compared to the other groups (E_{max}: HFIA 95 ± 0.7%, CDIH 94 ± 2%, CDIA 97 ± 1%). Also basal endothelial dependant dilation was attenuated in the HFIH group compared to the HFIA group (E_{max}: HFIH: 179 ± 10% vs. HFIA: 149 ± 11% in the presence of L-NAME). Levels of MDA were elevated in the CDIH group when compared to CDIA (0.68 ± 0.04 vs. 0.41 ± 0.03 μM, $p < 0.05$) but were greatest in the HFIH group (0.83 ± 0.08 μM, $p < 0.05$). However, there was no significant increase in MDA levels in the HFIA group (0.45 ± 0.03 μM, p = NS) when compared to all other groups. Similar effects were observed with CRP levels; CRP levels were significantly higher in the CDIH group compared with intermittent air (10.39 ± 0.38 vs. 8.70 ± 0.21 μg/ml, $p < 0.05$) but the HFIH had the greatest levels of CRP (11.87 ± 0.31 μg/ml, $p < 0.05$). In the HFIA group, CRP levels were not elevated (9.96 ± 0.37 μg/ml, p = NS). Nevertheless, total antioxidant capacity and eNOS gene expression were not significantly different in the groups.

Conclusion: CIH caused endothelial dysfunction in mice fed an obesogenic diet. Inflammation and oxidative stress were increased in CIH and an obesogenic diet exacerbated these effects.

1. Introduction

Obstructive sleep apnea (OSA) is a common disorder characterized by recurrent collapse of the upper airway at night leading to repetitive episodes of asphyxia and chronic intermittent hypoxia (CIH). OSA is being implicated increasingly as an independent risk factor for cardiovascular disease, and this effect is likely predominately due to the intermittent hypoxia and reoxygenation associated with OSA [1]. Furthermore, OSA is strongly associated with the development of endothelial dysfunction, a pathologic state of the vascular endothelium characterized by impaired vasodilatation in response to specific stimuli such as acetylcholine; importantly, endothelial dysfunction is a precursor of atherosclerosis in human and animal studies [2–5]. Endothelial dysfunction observed in

* Corresponding author. Address: Department of Pharmacology and Therapeutics, Faculty of Medicine, University of British Columbia, Vancouver, BC V6T 1Z3, Canada. Tel.: +1 (604) 822 5882; fax: +1 (604) 224 5142.
E-mail address: ilaher@interchange.ubc.ca (I. Laher).

http://dx.doi.org/10.1016/j.sleep.2014.01.013
1389-9457/

patients with OSA and CIH can occur through many mechanisms including oxidative stress and activation of inflammation [6,7].

Obesity is a very strong risk factor for OSA. Nearly 60–90% of OSA patients are obese [8] and weight gain worsens severity of OSA, while losing weight improves it [9]. Adipose tissue is also metabolically active, and may contribute to systemic inflammation and the development of vascular disease through production of many pro-inflammatory cytokines such as tumor necrosis factor-α, interleukin (IL)-4, IL-6, and others [10].

We hypothesized that obesity may worsen the deleterious effect of CIH on vascular endothelial function. Specifically, we used a validated mouse model of CIH and fed animals either a low fat diet or obesogenic diet; we then assessed endothelial function, oxidative stress, and inflammatory markers after 6 weeks.

2. Methods

2.1. Animal model

The animal care and experimental protocols used in this study were approved by the Animal Care Committee at the University of British Columbia, Canada (Animal Care Certificate A06-0308). Male C57BL/6 mice that were 8–10 weeks old were purchased from Jackson Laboratory (n = 5–10 per group; Bar Harbor, ME) and housed in the University Animal Facility with a 12-h: 12-h dark/light cycle and allowed free access to water and food. The animals were either fed a high-fat diet or a low-fat control formula (purchased from Research Diets, New Brunswick, NJ). The high fat diet consisted of 60% kcal of fat (225 kcal soybean oil and 2205 kcal lard), 20% carbohydrates (500 kcal maltodextrin 10 and 275 kcal sucrose) and 20% protein (800 kcal casein and 3 g/kg L-cystine) with a total of 4057 kcal/773 g (Catalogue no. D12492). The control diet consisted of 10% fat (225 kcal soybean and 180 kcal lard), 70% carbohydrates (1260 kcal corn starch, 140 kcal maltodextrin 10 and 1400 kcal sucrose) and 20% protein (800 kcal casein and 12 kcal L-cystine) with a total of 4057 kcal/1055 g (Catalogue no. D12450B).

Animals were divided into four groups: (1) intermittent air with low-fat control diet (CDIA), (2) chronic intermittent hypoxia with control diet (CDIH), (3) obesogenic high fat diet and intermittent air (HFIA) and (4) obesogenic diet and intermittent hypoxia (HFIH).

For the CIH protocol, we used a validated murine model of intermittent hypoxia created by Polotsky and Tagaito [11]. Mice were housed in customized cages able to deliver either an intermittent hypoxic stimulus or intermittent room air. Ports evenly spaced near the bottom of the cages allowed the gas to enter from four sides at the level of the bedding material. A gas control delivery system regulated the flow of room air, N_2, and O_2 into the customized cages housing the mice. Programmable solenoids and flow regulators controlled the manipulation of inspired O_2 fraction (FI_{O2}) levels in each cage over a wide range of IH profiles. During the 12-h light cycle (when mice are sleeping), FI_{O2} was reduced from 20.9% to 5.0% over a 30-s period and rapidly re-oxygenated to room air levels using a burst of 100% O_2 from a medical air compressor during the following 30-s period. Room air was delivered to the cages throughout the 12-h dark cycle (when mice are active). The use of multiple inputs into the cage produced a uniform nadir FI_{O2} level throughout the cage. The fluctuating FI_{O2} levels were monitored with an O_2 analyzer (model OM11, Sensor Medics, Yorba Linda, CA). Mice were exposed for 6 weeks in either chronic IH or intermittent air (control). In mice exposed to the CIH protocol, an initial titration period was necessary over the first two days to allow the mice to adapt to IH. Initially, the nadir FI_{O2} was set to 12% and then gradually reduced over 8-h periods to 10%, 8% then to the experimental level of nadir FI_{O2} of 4.8–5% to slowly adapt the mice to hypoxia.

The control groups of mice were exposed to chronic intermittent air with flow rates and timing of solenoid valves identical to the CIH group. This would reproduce the noise associated with gas movement, but would not result in hypoxia.

2.2. Plasma and tissue collection

Pentobarbital (100 mg/kg) was used to anesthetize the mice. Blood samples were withdrawn from the inferior vena cava using heparinized syringes and then transferred to Eppendorf tubes. The blood was then centrifuged (10 min at 4 °C, 1000g) for plasma separation and the collected plasma was stored at −76 °C in aliquots after snap freezing in liquid nitrogen. Mice were euthanized after blood collection by removing the heart. Aortic blood vessels were dissected and immersed in ice-cold physiologic salt solution (PSS). The connective tissue around the blood vessels was removed using microsurgery equipment and aided by a dissecting microscope. Dissected blood vessels were either preserved in RNAlater, snap frozen in liquid nitrogen and stored at −76 °C, or mounted on the wire myograph for functional studies.

2.3. Assessment of vascular function

The cleaned aortas were cut into equal 2 mm long rings and mounted on a wire myograph DMT 620M (Danish Myotechnology, Aarhus, Denmark) for measuring isometric force. Each chamber in the wire myograph was filled with 6 ml PSS gassed with 95% O_2 and 5% CO_2. To maintain physiologic conditions, the PSS was kept at pH 7.4 and 37 °C. The aortic rings were stretched to their optimal tension (5.5 mN) according to preliminary studies in which the resting tension exhibiting the maximum force in response to 60 mM KCl was considered the optimal resting tension. The PSS was replaced every 30 min during the adjusting and equilibration periods. Aortic rings were allowed to equilibrate for 20 min and then challenged twice with 80 mM KCl before creating the concentration response curves.

2.4. Assessment of endothelium-dependant and -independent vasodilatation

The aortic rings were constricted with a submaximal concentration (producing 60–80% of the maximum response to 80 mM KCl) of the alpha1adrenoceptor agonist phenylephrine (PE). After a stable contraction was obtained, acetylcholine (ACh) was cumulatively added in half-log increments to examine endothelium-dependent vasodilatation (10^{-9}–10^{-5} M). After a 30-min washout period, the arterial rings were re-constricted with PE, and sodium nitroprusside (SNP), a direct NO donor (10^{-10}–10^{-5} M at half-log increments in a cumulative manner) was added to examine endothelium-independent vasodilatation. Responses to vasodilators (ACh and SNP) were calculated as the percent decrease in force with respect to the initial PE induced constriction (% relaxation).

2.5. Assessment of basal nitric oxide (NO)

PE concentration response curves were constructed in the absence and the presence of N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NAME, 10^{-4} M). By inhibiting NOS, L-NAME decreases basal NO production and thus eliminates its background vasorelaxant effect. Therefore, L-NAME is expected to increase PE-induced contractions. The area under the curve (AUC) for PE concentration response curve obtained in the presence of L-NAME was calculated, and divided by the AUC obtained in the absence of L-NAME. The increase in AUC due to the presence of L-NAME is