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Differential metabolic and inflammatory responses to intermittent hypoxia in substrains of lean and obese C57BL/6 mice

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ABSTRACT

Aims: This study was to investigate the degree of susceptibility to intermittent hypoxia (IH), a hallmark of obstructive sleep apnea (OSA), between the two mice inbred lines C57BL/6N (6N) and C57BL/6J (6J).

Materials and methods: Four-week old male mice of 6N and 6J substrains (n = 8) were randomized to standard diet (SD) group or high fat (HF) diet group. At the age of 13-week, all two groups of mice were subjected to either air or IH (IH30; thirty hypoxic events per hour) for one week.

Key findings: All mice fed with HF diet exhibited obesity with more body weight and fat mass (percentage to body weight) gain. IH reduced serum LDL, HDL and total cholesterol levels in lean 6J mice. In obese mice, IH lowered obesity-induced serum total cholesterol level in 6J substrain but raised further in 6N substrain. Furthermore, IH caused elevation of serum FFA and MDA levels, and pro-inflammatory cytokines MCP-1 and IL-6 levels in subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) of lean 6J but not lean 6N mice. There was reduced number of adipocytes and elevation of macrophages in SAT and VAT of HF-induced obese mice of both substrains. IH led to increased number of adipocytes and macrophages in SAT of lean 6J mice.

Significance: The genetic difference between 6N and 6J mice may have direct impact on metabolic and inflammatory responses after IH. Therefore, attention must be given for the selection of C57BL mice substrains in the experimental IH-exposed mouse model.

1. Introduction

Obstructive sleep apnea (OSA) refers to the sleep-related breathing disorder characterized by recurrent episodes of upper airway collapse, leading to increased negative intrathoracic pressure, sleep fragmentation and intermittent hypoxia (IH) during sleep [1]. There is growing evidence linking OSA independently to the development of various cardio-metabolic diseases [2–4]. IH during sleep causes several pathological responses including oxidative stress and inflammation [1,5]. Clinical studies have shown elevations of circulating pro-inflammatory cytokine levels and increased levels of biomarkers of oxidative stress such as lipid peroxidation in subjects with OSA compared with controls [6]. Obesity is an important risk factor for OSA as majority of patients with OSA are obese [5]. Apart from direct influence on metabolic pathways by the process of overweight, OSA may interact with obesity to propagate or amplify adverse metabolic effects, which may run in a vicious cycle [1,5,7]. Previous animal studies have reported that IH-induced oxidative stress and inflammation were exacerbated in high-fat diet-induced obesity, but the modulation of the expression or the function of the adipocytokines within the process of OSA remains unclear [8].

Rodent models have been established to investigate the molecular mechanisms involved in the process of diet-induced obesity and IH. For metabolic studies, the most commonly used mouse model has been the C57BL/6 strain because of its high susceptibility to develop obesity and hyperglycemia when fed with a high-fat (HF) diet compared to other strains [9]. Both C57BL/6N (6N) and C57BL/6J (6J) mice are widely used substrains for biomedical research purpose. Unfortunately, some studies do not properly state which C57BL/6 mice substrains have been used, making data interpretation difficult [8,10]. However, subtle genetic differences have developed in C57BL/6 mice substrains from different sources, which can have a substantial impact on types of

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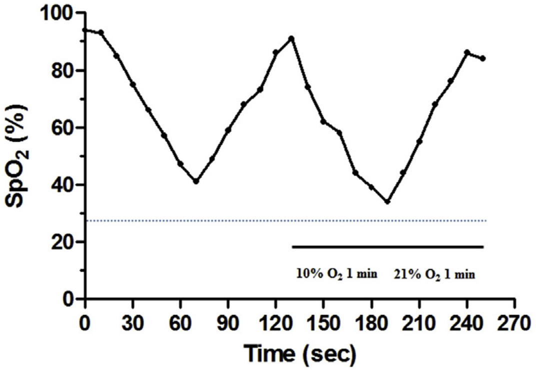

Fig. 1. Blood oxygen saturation (SpO_2) fluctuation over time in a mouse exposed to IH30 (10% O_2 for 1 min followed by 21% O_2 for 1 min; 30 cycles/h) measured by MouseVent G500 (Kent Scientific Corporation, Torrington, CT, USA).

experiments [11]. Our previous findings in 6N mice led to conflicting results compared to other studies using 6J mice [12], which raised the concern that some problems with reproducibility of data could come from the unrecognized mismatch of different background substrains. Therefore, we aimed to perform a comparison between 6N and 6J mice on the effects of HF diet and IH exposure.

2. Materials and methods

2.1. Animal protocols

Four-week old male C57BL/6N mice (Strain code: 027; Charles River, NCI Research Models and Services, Frederick, Maryland, USA) and C57BL/6J mice (Stock #000664; Jackson Laboratories, Bar Harbor, Maine, USA) were randomly divided into standard diet group and HF diet (45% of calories; Research Diets, New Brunswick, NJ, USA; diet D12451) group. The consumption of diets and the gain of body weight were examined every week. At the age of 13-week, mice from both substrains were subjected to air or/intermittent hypoxia (IH). The IH protocol was performed using OxyCycler Model A84XOV (BioSpherix, NY, USA). Each IH cycle comprised 60-s 10% oxygen levels followed by another 60-s 21% oxygen levels (i.e. 30 cycles/h) for 8 h per day. This hypoxic profile was associated with arterial oxyhemoglobin saturation (SpO_2) which fluctuated between a peak of 86–94%, gradually decreasing to a nadir of 34–41%, for 30 times per hour, as measured using a pulse oximeter (MouseVentG500; Kent Scientific; Torrington, CT, USA) (Fig. 1). Animals in control group were kept in an identical environment receiving air instead. All mice were sacrificed after one week of exposure. Total fat mass and total lean mass were measured by the Minispec LF90 Body Composition Analyzer (Bruker, Billerica, MA, USA) before sacrifice. Mice were sacrificed with overdose of sodium pentobarbital anesthesia (100 mg/kg, i.p.) after fasting overnight. Serum samples, anterior (i.e. at the front) subcutaneous and epididymal (as visceral) adipose tissues were collected at the time after sacrifice. All animal procedures conformed to the guidelines from Directive 2010/63/EU of the European Parliament. Animal care and experimental protocol for the study were approved by the Committee on the Use of Live Animals in Teaching and Research of The University of Hong Kong (CULATR 3795–15).

2.2. Measurement of lipid parameters, peroxidation, adiponectin and leptin levels

Serum levels of high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol and triglyceride were measured with kits from EKF Diagnostics Company (Stanbio, Boerne, TX, USA). Malondialdehyde (MDA; Nanjing Juancheng Institute of Bioengineering, Nanjing, Jiangsu, China) and free fatty acid (FFA) levels (Roche, Mannheim, Germany) were applied to assess lipid peroxidation as a marker of oxidative stress in the circulation. Serum adiponectin (Antibody and Immunoassay Services, HKU, Hong Kong) and leptin (Peprotech, Rocky Hill, NJ, USA) levels were measured by using ELISA kits according to the manufacturer's instructions.

2.3. Preparation of adipose tissue protein

A small piece of subcutaneous or visceral adipose tissues from each mouse was homogenized in ice-cold T-PER™ Tissue Protein Extraction Reagent (Cat. # 78510; ThermoFisher Scientific, Rockford, IL, USA)

Table 1
Metabolic characteristics of experimental mice for both sub-strains.

Parameter: Mean ± SEM		Air	IH	HF	HF + IH
Initial body weight (g)	6N	15.0 ± 0.6	15.9 ± 0.6	15.4 ± 1.0	16.2 ± 0.7
	6J	15.3 ± 0.5	15.9 ± 0.4	15.8 ± 0.5	16.3 ± 0.6
Body weight gain (g)	6N	13.4 ± 0.9	11.8 ± 0.7	**24.4 ± 0.8**[b]	**23.4 ± 1.1**[a]
	6J	12.4 ± 0.9	11.7 ± 0.5	**17.6 ± 1.1** [a g]	**18.5 ± 0.8** [b f]
Fat mass (% of body weight)	6N	16.6 ± 0.9	**12.0 ± 0.4**[a]	**36.8 ± 1.0** [c]	**35.5 ± 1.7** [c]
	6J	12.9 ± 0.3	11.1 ± 0.3	**25.5 ± 0.7** [c g]	**26.7 ± 0.8** [c f]
Food consumption (g/week)	6N	26.4 ± 0.5	26.2 ± 0.6	19.8 ± 0.5	20.0 ± 0.4
	6J	27.5 ± 0.3	26.4 ± 0.3	18.5 ± 0.4	20.3 ± 0.4
Serum LDL cholesterol (mg/dl)	6N	28.3 ± 3.1	30.8 ± 2.5	**92.8 ± 10.9** [c]	**103.7 ± 9.9** [b]
	6J	39.9 ± 2.4	**23.1 ± 1.2** [a e]	**70.0 ± 5.1** [c]	**64.9 ± 6.5** [b f]
Serum HDL cholesterol (mg/dl)	6N	51.1 ± 2.9	50.9 ± 3.4	**76.2 ± 3.6** [c]	**71.9 ± 3.9** [b]
	6J	55.8 ± 0.7	**49.6 ± 1.4** [a]	**80.7 ± 2.2** [c]	**75.8 ± 1.4** [c]
Serum total cholesterol (mg/dl)	6N	66.1 ± 4.1	59.4 ± 3.3	**130.6 ± 7.6** [c]	**151.1 ± 5.1** [c d]
	6J	80.3 ± 4.9	**58.6 ± 1.1** [a]	**123.6 ± 6.7** [c]	**103.0 ± 5.8** [a d g]
Serum triglyceride (mg/dl)	6N	15.3 ± 1.7	17.1 ± 1.9	**32.9 ± 3.5** [c]	**33.3 ± 2.4** [c]
	6J	15.6 ± 1.0	17.3 ± 1.3	**26.0 ± 1.6** [c]	**24.8 ± 1.3** [b,e]

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

[a] $p < 0.05$.

[b] $p < 0.01$.

[c] $p < 0.001$ compared to relative Air group.

[d] $p < 0.05$ compared to relative HF group.

[e] $p < 0.05$.

[f] $p < 0.01$.

[g] $p < 0.001$ compared to relative group of 6N substrain; n = 8 for each group.

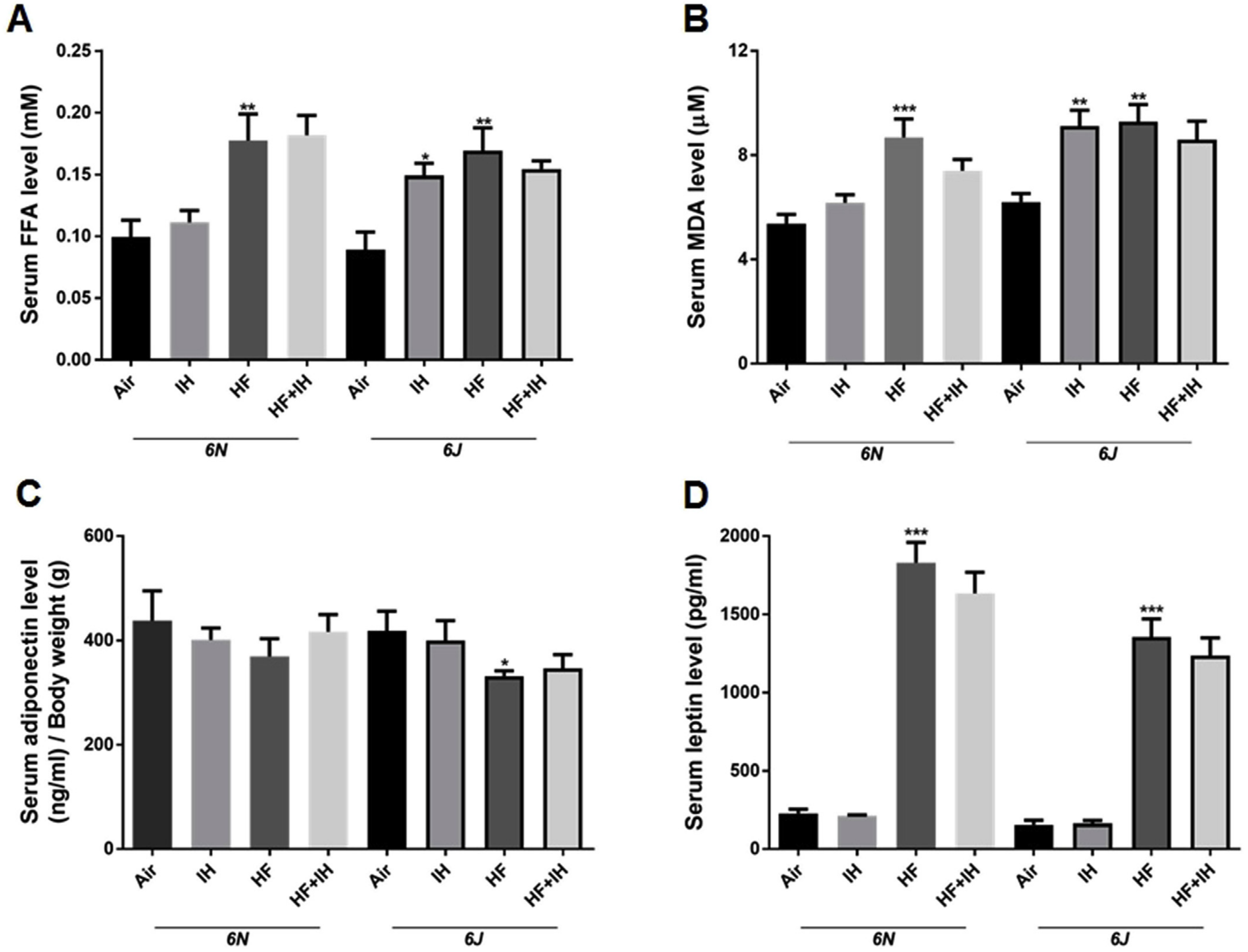

Fig. 2. Serum oxidative and inflammatory markers. HF elevated serum FFA (A), MDA (B) and leptin (D) level significantly in mice of both sub-strains; suppressed serum adiponectin level adjusted by body weight (C) reaching significance in HF mice for 6J substrain; One week exposure of IH induced a non-significant increase in serum FFA (A) and MDA (B) level for lean 6N mice, but reached significance for lean 6J mice. Values were expressed as mean ± SEM (n = 8 for each group). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared to relative Air group.

supplemented with 1/100 (v/v) cocktail of protease and phosphatase inhibitors (Cat. # 78440; ThermoFisher Scientific) before sonication. The suspensions were centrifuged at 14,000 × *g* for 15 min at 4 °C and the supernatants were collected as total protein. The protein concentration was measured by Bradford protein assay (BioRad Laboratories, Hercules, California, USA).

2.4. Assessment of inflammatory cytokines

Supernatants collected from adipose tissue homogenates were used to analyze in duplicate for the presence of interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1) (eBioscience, San Diego, CA, USA) and adiponectin (Antibody and Immunoassay Services, HKU, Hong Kong). The expression levels were normalized to total protein concentrations.

2.5. Haematoxylin and eosin (H & E) staining

Both small pieces of subcutaneous and visceral adipose tissues were fixed immediately after dissection and embedded in paraffin. Samples were dewaxed with xylene and rehydrated with ethanol prior to H & E staining. Images were visualised using a light microscope (Nikon eclipse, Nikon, Melville, NY, USA) and photomicrographs were taken of each slide using SPOT RT3 colour camera (Diagnostic Instruments, Sterling Heights, USA). Photomicrographs were taken at 20× magnification, and the results expressed as cells/mm^2. Number of adipocytes within the analysed area was quantified by the open source software Adiposoft (ImageJ, NIH, Bethesda, MD) [13].

2.6. Immunohistochemical (IHC) staining

After fixing with paraformaldehyde, both subcutaneous and visceral adipose tissue were used for IHC staining with anti-mouse F4/80 antibody (Santa Cruz, USA) to detect the total macrophages. Images were taken by SPOT RT3 colour camera (Diagnostic Instruments) connected with microscope (Nikon eclipse) under 40× magnification. The total number of adipocytes per mm^2 analysed area was quantified by Adiposoft (ImageJ) as above. The F4/80 density was obtained by counting the total number of F4/80-positive cells in the same whole analysed area as for adipocytes on each section, and was expressed as percentage of F4/80 positive cells over total number of adipocytes.

2.7. Statistical analysis

Data were presented as mean ± SEM. One-way ANOVA test with post hoc analysis (Tukey) was used to compare multiple groups.

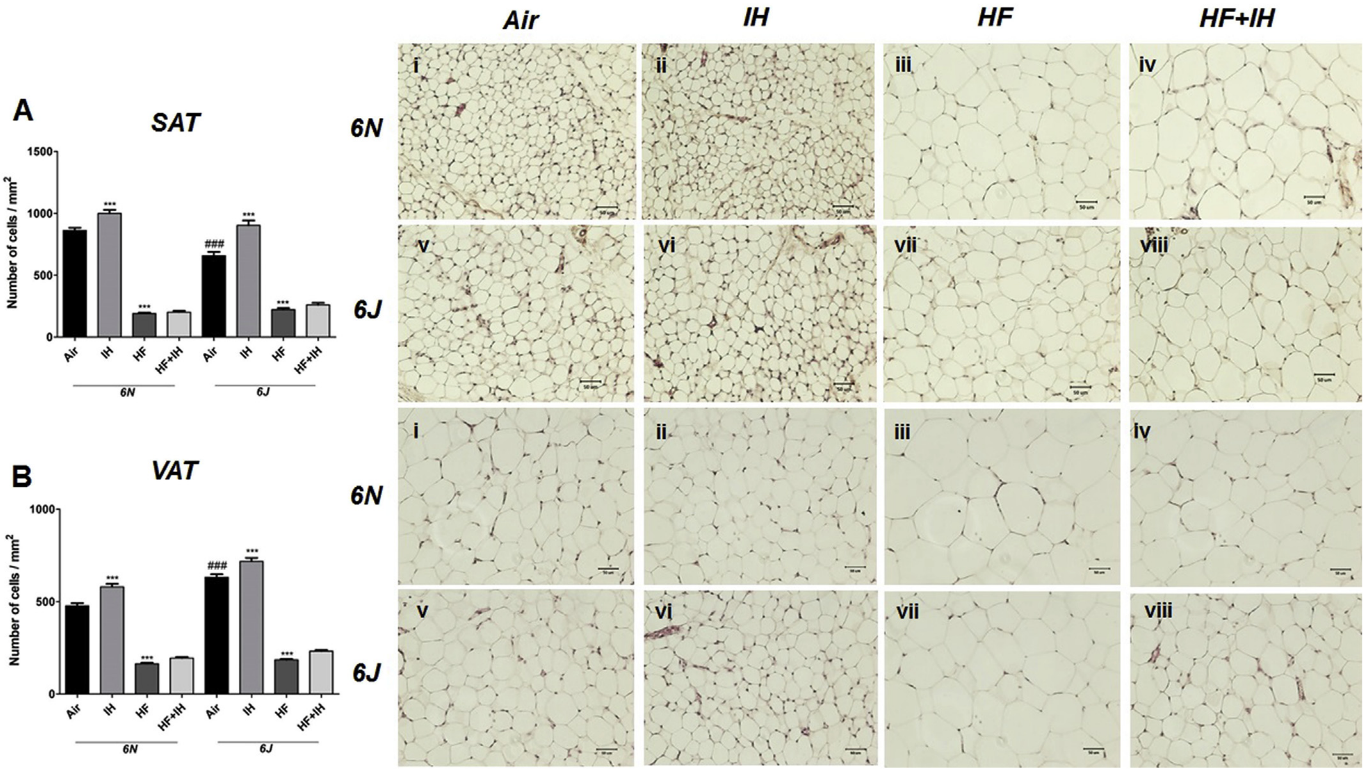

Fig. 3. Haematoxylin & Eosin staining on the sections of adipose tissues. Both SAT (A) and VAT (B) from Air (i, v), IH (ii, vi), HF (iii, vii) and HF + IH (vi, viii) animals of 6N and 6J substrains were stained with haematoxylin and eosin to determine number of adipocytes. Representative images were obtained at ×20 magnification, scale bar represents 50 μm. Numbers of adipocytes increased after IH treatment for both substrains of mice, and was significantly reduced in HF group. Values were expressed as mean ± SEM (n = 8 for each group). $^{***}p < 0.001$ compared to relative Air group. $^{\#\#\#}p < 0.001$ compared to relative group of 6N substrain.

Student's *t*-test was also applied for variables measured at a single time point where appropriate. All the statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software Inc, San Diego, CA, USA). Significance was achieved if p value < 0.05.

3. Results

3.1. Metabolic characteristics of experimental groups

The body weight gain and food consumption was comparable in lean mice from both substrains with or without IH treatment. Mice fed with HF diet exhibited obesity with a significant increase in body weight and fat mass gain. Overall, there was less body weight gain in obese mice from 6J substrain than those from 6N substrain. Serum levels of total cholesterol, HDL cholesterol (HDL-C), LDL cholesterol (LDL-C) and triglyceride (TG) significantly elevated in obese mice compared to lean mice from both substrains. IH exposure caused significant reduction in serum levels of total cholesterol, HDL-C and LDL-C in lean 6J mice but not in lean 6N mice. In obese mice, IH lowered obesity-induced serum total cholesterol level in 6J substrain but raised further in 6N substrain (Table 1).

3.2. Serum free fatty acid (FFA), malondialdehyde (MDA), adiponectin and leptin levels

In lean mice, IH exposure caused significant elevation in serum levels of FFA and MDA in 6J sub-strain but not in 6N substrain (Fig. 2A and B). In diet-induced obese mice, there were significant elevation in serum levels of FFA, MDA and leptin from both 6N and 6J substrains (Fig. 2A, B, 2D), and significant suppression in serum level of adiponectin (adjusted by body weight) in 6J substrain but not reaching significance in 6N sub-strain (Fig. 2C). IH exposure caused no effect in diet-induced obese mice of both 6N and 6J substrains.

3.3. Morphological changes of adipocytes in adipose tissues

Adipocytes number within subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) significantly dropped in diet-induced obese mice of both 6N and 6J substrains, in consistent with the expansion of SAT and VAT as a hallmark of obesity. IH exposure caused significant increase in number of adipocytes in SAT and VAT in lean mice but not in diet-induced obese mice of both 6N and 6J substrains. In SAT, there was significant lower number of adipocytes in lean 6J than in lean 6N mice, and vice versa in VAT (Fig. 3).

3.4. Macrophages accumulation in adipose tissues

The accumulation of macrophages in SAT and VAT was identified as brown colour by F4/80 IHC staining. The lean 6J mice had less F4/80 stained positive cells in SAT and VAT compared to lean 6N mice, indicating lower basal inflammatory level in adipose tissue. IH exposure caused significant elevation of number in F4/80 stained positive cells in SAT of lean 6J mice and in VAT of both 6N and 6J substrains. Diet-induced obesity also caused significant increase in F4/80 stained positive cells in SAT and VAT of both 6N and 6J substrains, in which IH exposure had no effect (Fig. 4).

3.5. Adiponectin, MCP-1 and IL-6 levels in adipose tissues

The basal levels of adiponectin and inflammatory cytokines such as MCP-1 and IL-6 in SAT of lean 6J mice were significantly lower than in SAT of lean 6N mice (Fig. 5A, B and 5C). In agreement with the morphological changes of adipocytes, diet-induced obesity caused significant elevation of adiponectin and MCP-1 levels in SAT and VAT of

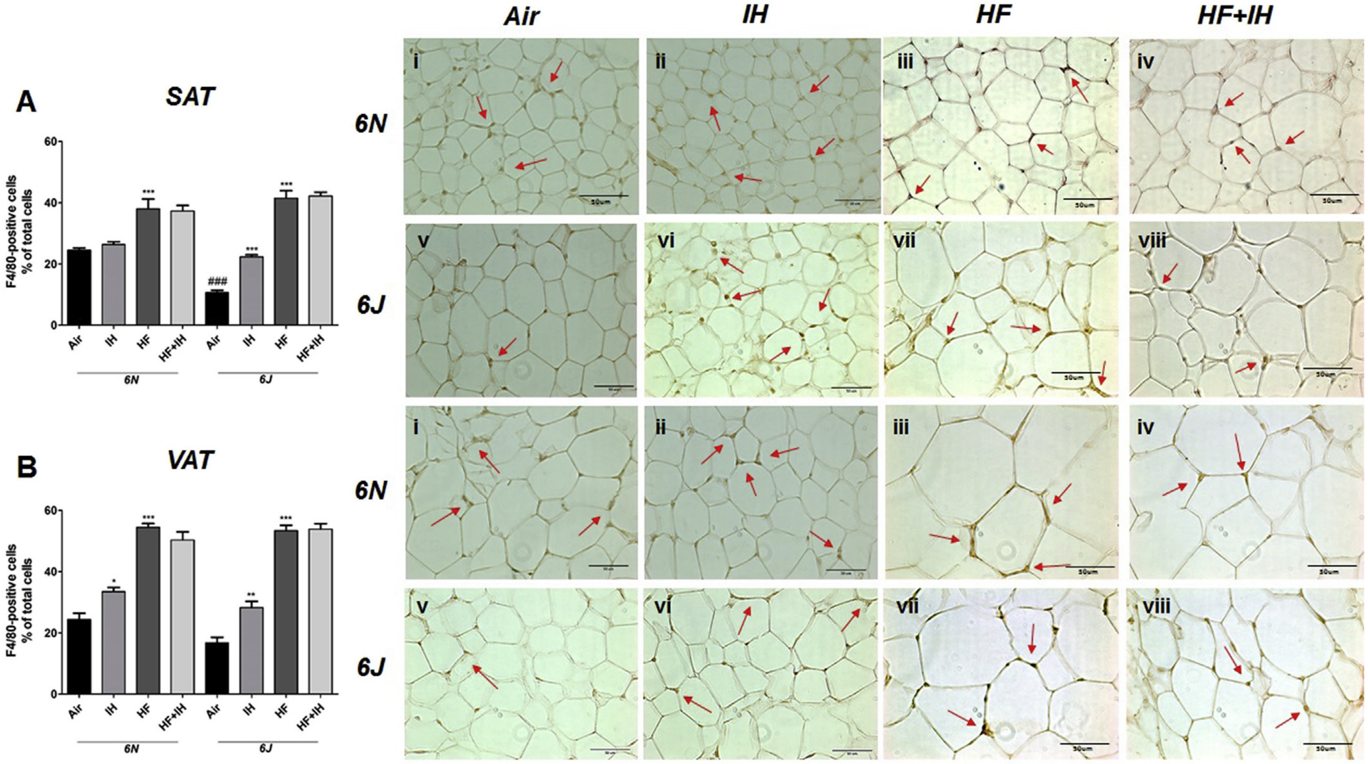

Fig. 4. Immunohistochemical staining on the sections of adipose tissues. Both SAT (A) and VAT (B) from animals of 6N and 6J substrains were stained with F4/80 (red arrows) to determine total macrophages. Representative images were obtained at ×40 magnification, scale bar represents 50 μm. Macrophages are stained brown. For lean animals, F4/80 expressing cells were small and rarely seen in both SAT and VAT; IH induced macrophages aggregation were observed (ii, vi), while 6J mice have rather less number of total macrophages than 6N mice in Air group (i, v). The fraction of F4/80 expressing cells was much greater in obese mice (iii, iv, vii, viii). Quantification of macrophage infiltration for different treatment was performed. The F4/80 positive cells percentage was expressed by counting the total number of F4/80 positive cells in each section compared with the total number of adipocytes. Values were expressed as mean ± SEM (n = 8 for each group), $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to relative Air group. $^{\#\#\#}p < 0.001$ compared to relative group of 6N substrain. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

both 6N and 6J substrains (Fig. 5A, B, 5D and 5E). In lean mice, IH exposure caused significant elevation of MCP-1 and IL-6 levels in SAT and VAT of 6J mice only, indicating inflammatory status (Fig. 5B, C, 5E and 5F). IH had no effects on any of the parameters in obese mice of either sub-strains.

4. Discussion

To the best of our knowledge, this was the first time that the 6J substrain from Jackson Laboratories and 6N substrain from Charles River for IH exposure were used in the same study for comparison. The main finding of our study was that the genetic difference between 6N and 6J substrains had direct impact on IH treatment.

Both 6N and 6J substrains gained body weight significantly and exhibited obesity after fed with HF diet, but 6J mice experienced less body weight and fat mass gain than 6N mice. Adipocyte hypertrophy was observed in both SAT and VAT of diet-induced obese mice of both 6N and 6J substrains. In general, the adipocyte size of SAT and VAT was larger in 6N mice than in 6J mice reflecting on the level of adiponectin, in agreement with the previous report [14]. VAT had greater adipocyte size than SAT in both substrains of diet-induced obese mice, indicating expansion of SAT and VAT, a hallmark of obesity.

In lean mice of 6N and 6J substrains, IH exposure led to significant adipocyte hyperplasia in SAT and VAT. The adipocyte size of SAT is larger in 6J mice than in 6N mice, and vice versa in VAT. Adipose tissue grows by two mechanisms: hyperplasia (increase in cell number) and hypertrophy (increase in cell size). Hyperplasia represents a healthy expansion of the adipose tissue while adipocyte hypertrophy leads to lipid-loaded, dysfunctional adipocytes that mediate inflammation [15]. Consistent to the morphological changes of adipocytes in SAT from lean 6J mice, the basal SAT levels of pro-inflammatory cytokines MCP-1 and IL-6 were found to be significant lower than the levels in lean 6N mice, suggesting the presence of lower inflammatory status. The 6J substrain displayed more sensitive to IH than 6N substrain with significant elevation of inflammatory cytokines MCP-1 and IL-6 levels in both SAT and VAT.

It is well recognized that obesity increases morbidity and mortality significantly. Obesity is a strong risk factor for many cardiometabolic disorders as well as OSA [16,17]. Whether and how IH alters metabolism and interacts with adipokines remains unclear. Previous animal studies suggest that IH exacerbated pre-existing insulin resistance and glucose intolerance in diet-induced obese mice, but no dramatic impact on lean mice was observed [18]. In this study, for 6N and 6J substrains, diet-induced obesity caused oxidative stress as indicated by the elevation of serum MDA level. Furthermore, our immunohistochemical detection of the macrophage-specific antigen F4/80 suggested that obesity was clearly associated with adipose tissue macrophage (ATM) accumulation, which is consistent with previous studies [19,20]. In agreement with the lower basal level of inflammation in SAT from lean 6J mice, we also found less F4/80 stained positive cells in lean 6J mice than in 6N mice. However, metabolic dysfunction has been reported previously when diet-induced obese mice were exposed to IH [18]. It is unclear why IH effect is observed only in lean 6J mice, but not in diet-induced obese 6J mice in this study. This may be due to the differences in frequencies, magnitudes and durations of IH regimens. Oxidative stress and the release of pro-inflammatory cytokines and adipokines are closely associated with IH, while the impact is proportionally associated with the severity of IH as well as the type of mice substrain.

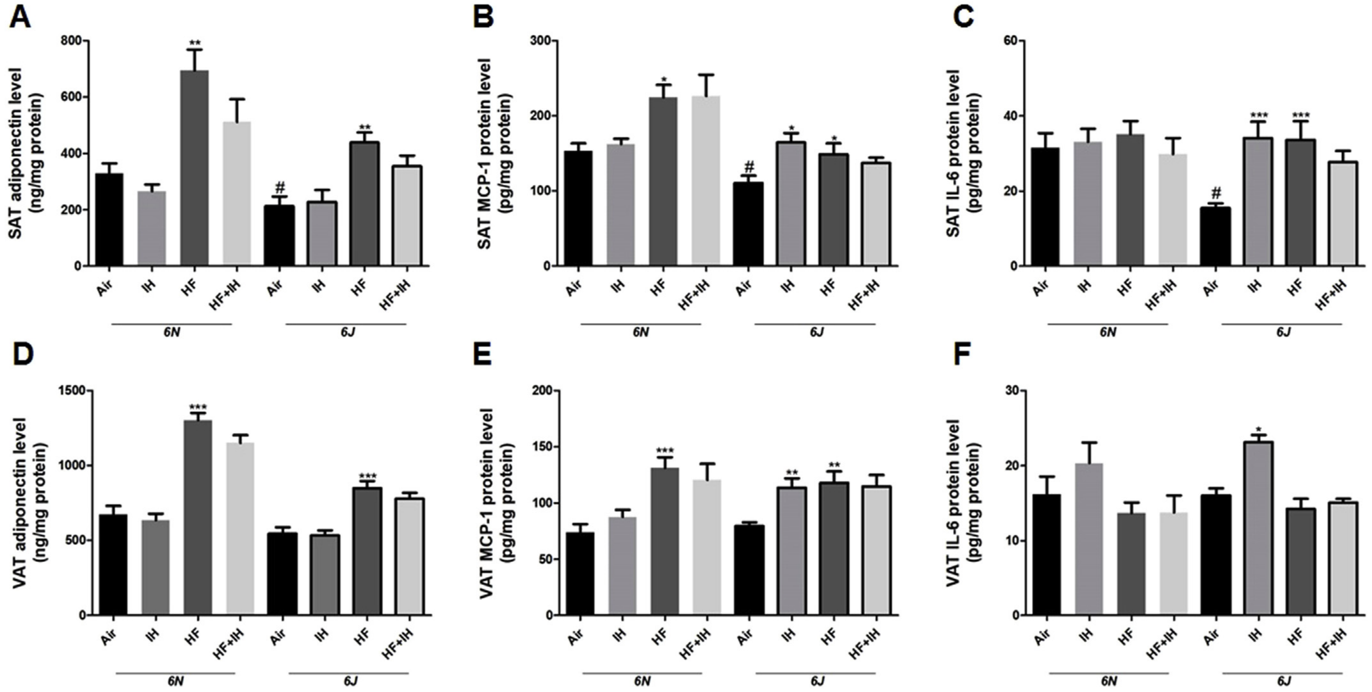

Fig. 5. SAT and VAT inflammatory markers. In SAT, the basal level (Air group) of adiponectin **(**A**)** and inflammatory cytokines **(**B, C**)** is lower in 6J mice than in 6N mice. HF increased adiponectin **(**A**)** level and pro-inflammatory cytokine MCP-1 **(**B**)** protein level significantly in mice of both substrains, while significant increase of IL-6 **(**C**)** level only observed in 6J mice; IH led to upregulation of inflammatory cytokines **(**B, C**)** in lean 6J mice. Similarly, in VAT, HF increased adiponectin **(**D**)** and MCP-1 **(**E**)** protein level significantly in both substrains of mice; In lean mice, after IH treatment, inflammatory cytokines **(**E, F**)** showed a trend of increase in 6N mice and attained significance in 6J mice. Values were expressed as mean ± SEM (n = 8 for each group).). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to relative Air group; $^{\#}p < 0.05$ compared to relative group of 6N substrain.

The current findings on serum lipid levels are also in contrary to one previous study using 6J mice [21], in which high-frequency IH (60 cycles/h) was found to increase in fasting serum total cholesterol, HDL cholesterol (HDL-C) and TG levels compared with mice exposed to air; whereas changes in fasting levels of LDL cholesterol (LDL-C) and free fatty acids (FFAs) did not reach statistical significance. In this study, we did not observe IH-induced hyperlipidemia but found significant reduction in fasting serum total cholesterol, HDL-C and LDL-C and elevation of fasting serum FFAs in lean 6J mice only. The exhibition of opposite responses could be explained by the differences in frequencies, magnitudes and durations of IH regimens. To date, less is known about phenotypic differences between inbred sub-strains of mice that comprise only subtle genetic variability. The C57BL/6J mice strain supplied by the Jackson Laboratory lacks NNT activity due to a Nnt gene mutation. Therefore, the C57BL/6J mitochondria are unable to properly metabolize exogenous peroxide, in which the NNT enzyme is a source of NADPH in the mitochondrial matrix [22]. On the contrary, the C57BL/6N mice do not harbour such mutation [23,24].

In agreement with the previous observations on the body weight gain and increased levels of lipid markers in diet-induced obese mice for both substrains, the differences in IH effects may link to a spontaneous loss-of-function mutation in Nnt which has been associated in some studies with impaired glucose-stimulated insulin secretion (GSIS) and glucose intolerance [23,25–27]. Our study also indicated that 6J mice are more susceptible to IH exposure, yet this cannot be explained by the impaired GSIS because the significance was found in lean 6J mice only. More detailed investigation are needed to evaluate the mechanisms of the susceptibilities in IH-exposed models between 6N and 6J mice, through which we can further explore the exact relationship between IH and obesity. In fact, there is evidence to suggest that pathological responses of adipose tissue to obesity and IH may be quite similar [28]. IH has been reported to be a pro-inflammatory stimulus [29]. Indeed, the cardioprotective effect of IH has been summarized by many research groups [30,31]. In this study, beneficial effect of short-term exposure of IH30 for 7 days on blood lipid reduction was reported in both lean and diet-induced obese 6J substrain mice, suggesting an effective multipronged therapeutic approach for the treatment of sleep apnea. These findings were clearly contradictory with those reported by others [18,21,32], which might be due to the efficacy of IH that is dependent upon five key variables [33]: (i) the intensity of hypoxia; (ii) the duration of hypoxia; (iii) the number of cycles; (iv) the pattern of IH presentation; and (v) the total protocol duration. In human subjects, one recent study demonstrated intermittent normobaric hypoxia as a potential non-pharmacological strategy for high altitude acclimatization [34]. On the other hand, normbaric intermittent hypoxia over 8 months showed no effects on reduction of body weight and metabolic risk factors [35].

There are several limitations in the present study. Firstly, the results only represent the difference for 6N and 6J substrains. In fact, there are major differences in phenotypes among mice strains. In respect to genetic modifiers in C57BL/6J substrain, detailed investigations are needed to identify and understand mechanisms underlying differences in susceptibility to IH. Secondly, though the hypoxic regime of IH30 was taken to simulate that of moderate degree of OSA in humans, the results demonstrated here in the animal model could not be directly projected to the human scenario. In addition, the IH event lasted for one week, which was rather short period of time. Future studies will be built on a longer time points for better understanding the oxidative and inflammatory responses to IH.

In summary, our data reveal that genetic differences between 6N and 6J mice may have direct impact on their metabolic and inflammatory responses. Diet-induced obesity seems to be more obvious in 6N mice while 6J mice are more sensitive to IH. Given the abnormalities and the widespread use of C57BL/6J mice in biomedical research, attention must be given to the design of experiments using C57BL mice substrains (C57BL/6N and C57B/6J) which may determine their responses and lead to different results for the same experimental interrogation.

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Authors' contributions

MQG, JCWM and MSMI designed the study. MQG and SCY performed the study, collected the data, carried out data analyses and produced the initial draft of the manuscript. JCWM and MSMI contributed to revising the manuscript. All authors read and approved the final submitted manuscript.

Declaration of competing interest

The authors have no conflict of interest to declare.

References

[1] L.F. Drager, S.M. Togeiro, V.Y. Polotsky, G. Lorenzi-Filho, Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome, J. Am. Coll. Cardiol. 62 (2013) 569–576.

[2] J.-D.L. Lattimore, D.S. Celermajer, I. Wilcox, Obstructive sleep apnea and cardiovascular disease, J. Am. Coll. Cardiol. 41 (2003) 1429–1437.

[3] V.K. Somers, D.P. White, R. Amin, W.T. Abraham, F. Costa, A. Culebras, S. Daniels, J.S. Floras, C.E. Hunt, L.J. Olson, Sleep apnea and cardiovascular disease: an american heart association/american college of cardiology foundation scientific statement from the american heart association council for high blood pressure research professional education committee, council on clinical cardiology, stroke council, and council on cardiovascular nursing in collaboration with the national heart, lung, and blood institute national center on sleep disorders research (national institutes of health), J. Am. Coll. Cardiol. 52 (2008) 686–717.

[4] W. McNicholas, M. Bonsignore, M.C.o.E.C.A. B26, Sleep apnoea as an independent risk factor for cardiovascular disease: current evidence, basic mechanisms and research priorities, Eur. Respir. J. 29 (2007) 156–178.

[5] D.C. Lam, K.S. Lam, M.S. Ip, Obstructive sleep apnoea, insulin resistance and adipocytokines, Clin. Endocrinol. 82 (2015) 165–177.

[6] Q. Han, S.C. Yeung, M.S. Ip, J.C. Mak, Cellular mechanisms in intermittent hypoxia-induced cardiac damage in vivo, J. Physiol. Biochem. 70 (2014) 201–213.

[7] A.N. Vgontzas, Does obesity play a major role in the pathogenesis of sleep apnoea and its associated manifestations via inflammation, visceral adiposity, and insulin resistance? Arch. Physiol. Biochem. 114 (2008) 211–223.

[8] Y. Zhang, C. Zhang, H. Li, J. Hou, Down-regulation of vascular PPAR-gamma contributes to endothelial dysfunction in high-fat diet-induced obese mice exposed to chronic intermittent hypoxia, Biochem. Biophys. Res. Commun. 492 (2017) 243–248, https://doi.org/10.1016/j.bbrc.2017.08.058.

[9] E.D. Berglund, C.Y. Li, G. Poffenberger, J.E. Ayala, P.T. Fueger, S.E. Willis, M.M. Jewell, A.C. Powers, D.H. Wasserman, Glucose metabolism in vivo in four commonly used inbred mouse strains, Diabetes 57 (2008) 1790–1799.

[10] A.L. Castro-Grattoni, M. Suarez-Giron, I. Benitez, M. Torres, I. Almendros, R. Farre, J.M. Montserrat, M. Dalmases, D. Gozal, M. Sanchez-de-la-Torre, N. Spanish Sleep, Effect of Age on the Cardiovascular Remodelling Induced by Chronic Intermittent Hypoxia as a Murine Model of Sleep Apnoea, Respirology, 2019, https://doi.org/10.1111/resp.13610.

[11] M.M. Simon, S. Greenaway, J.K. White, H. Fuchs, V. Gailus-Durner, S. Wells, T. Sorg, K. Wong, E. Bedu, E.J. Cartwright, A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains, Genome Biol. 14 (2013) R82.

[12] M.Y.K. Lee, G. Ge, M.L. Fung, P.M. Vanhoutte, J.C.W. Mak, M.S.M. Ip, Low but not high frequency of intermittent hypoxia suppresses endothelium-dependent, oxidative stress-mediated contractions in carotid arteries of obese mice, J. Appl. Physiol. 125 (2018) 1384–1395, https://doi.org/10.1152/japplphysiol.00224.2018.

[13] M. Galarraga, J. Campion, A. Munoz-Barrutia, N. Boque, H. Moreno, J.A. Martinez, F. Milagro, C. Ortiz-de-Solorzano, Adiposoft: automated software for the analysis of white adipose tissue cellularity in histological sections, J. Lipid Res. 53 (2012) 2791–2796, https://doi.org/10.1194/jlr.D023788.

[14] T. Skurk, C. Alberti-Huber, C. Herder, H. Hauner, Relationship between adipocyte size and adipokine expression and secretion, J. Clin. Endocrinol. Metab. 92 (2007) 1023–1033, https://doi.org/10.1210/jc.2006-1055.

[15] J.M. Rutkowski, J.H. Stern, P.E. Scherer, The cell biology of fat expansion, J. Cell Biol. 208 (2015) 501–512, https://doi.org/10.1083/jcb.201409063.

[16] V.Y. Polotsky, S.P. Patil, V. Savransky, A. Laffan, S. Fonti, L.A. Frame, K.E. Steele, M.A. Schweizter, J.M. Clark, M.S. Torbenson, A.R. Schwartz, Obstructive sleep apnea, insulin resistance, and steatohepatitis in severe obesity, Am. J. Respir. Crit. Care Med. 179 (2009) 228–234, https://doi.org/10.1164/rccm.200804-608OC.

[17] V.Y. Polotsky, J. Li, N.M. Punjabi, A.E. Rubin, P.L. Smith, A.R. Schwartz, C.P. O'Donnell, Intermittent hypoxia increases insulin resistance in genetically obese mice, J. Physiol. 552 (2003) 253–264, https://doi.org/10.1113/jphysiol.2003.048173.

[18] L.F. Drager, J. Li, C. Reinke, S. Bevans-Fonti, J.C. Jun, V.Y. Polotsky, Intermittent hypoxia exacerbates metabolic effects of diet-induced obesity, Obesity 19 (2011) 2167–2174, https://doi.org/10.1038/oby.2011.240.

[19] S.P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R.L. Leibel, A.W. Ferrante, Obesity is associated with macrophage accumulation in adipose tissue, J. Clin. Investig. 112 (2003) 1796–1808.

[20] C.E. Bussey, S.B. Withers, R.G. Aldous, G. Edwards, A.M. Heagerty, Obesity-related perivascular adipose tissue damage is reversed by sustained weight loss in the RatHighlights, Arterioscler. Thromb. Vasc. Biol. 36 (2016) 1377–1385.

[21] J. Li, L.N. Thorne, N.M. Punjabi, C.K. Sun, A.R. Schwartz, P.L. Smith, R.L. Marino, A. Rodriguez, W.C. Hubbard, C.P. O'Donnell, V.Y. Polotsky, Intermittent hypoxia induces hyperlipidemia in lean mice, Circ. Res. 97 (2005) 698–706, https://doi.org/10.1161/01.RES.0000183879.60089.a9.

[22] J.A. Ronchi, T.R. Figueira, F.G. Ravagnani, H.C. Oliveira, A.E. Vercesi, R.F. Castilho, A spontaneous mutation in the nicotinamide nucleotide transhydrogenase gene of C57BL/6J mice results in mitochondrial redox abnormalities, Free Radic. Biol. Med. 63 (2013) 446–456, https://doi.org/10.1016/j.freeradbiomed.2013.05.049.

[23] K. Aston-Mourney, N. Wong, M. Kebede, S. Zraika, L. Balmer, J.M. McMahon, B.C. Fam, J. Favaloro, J. Proietto, G. Morahan, Increased nicotinamide nucleotide transhydrogenase levels predispose to insulin hypersecretion in a mouse strain susceptible to diabetes, Diabetologia 50 (2007) 2476–2485.

[24] A. Toye, J. Lippiat, P. Proks, K. Shimomura, L. Bentley, A. Hugill, V. Mijat, M. Goldsworthy, L. Moir, A. Haynes, A genetic and physiological study of impaired glucose homeostasis control in C57BL/6J mice, Diabetologia 48 (2005) 675–686.

[25] H.C. Freeman, A. Hugill, N.T. Dear, F.M. Ashcroft, R.D. Cox, Deletion of nicotinamide nucleotide transhydrogenase: a new quantitive trait locus accounting for glucose intolerance in C57BL/6J mice, Diabetes 55 (2006) 2153–2156.

[26] A. Nicholson, P.C. Reifsnyder, R.D. Malcolm, C.A. Lucas, G.R. MacGregor, W. Zhang, E.H. Leiter, Diet-induced obesity in two C57BL/6 substrains with intact or mutant nicotinamide nucleotide transhydrogenase (Nnt) gene, Obesity 18 (2010) 1902–1905.

[27] G. Fergusson, M. Éthier, M. Guévremont, C. Chrétien, C. Attané, E. Joly, X. Fioramonti, M. Prentki, V. Poitout, T. Alquier, Defective insulin secretory response to intravenous glucose in C57Bl/6J compared to C57Bl/6N mice, Mol. Metab. 3 (2014) 848–854.

[28] A. Gileles-Hillel, L. Kheirandish-Gozal, D. Gozal, Biological plausibility linking sleep apnoea and metabolic dysfunction, Nat. Rev. Endocrinol. 12 (2016) 290–298, https://doi.org/10.1038/nrendo.2016.22.

[29] E. Schaefer, W. Wu, C. Mark, A. Yang, E. DiGiacomo, C. Carlton-Smith, S. Salloum, C. Brisac, W. Lin, K.E. Corey, R.T. Chung, Intermittent hypoxia is a proinflammatory stimulus resulting in IL-6 expression and M1 macrophage polarization, Hepatol. Commun. 1 (2017) 326–337, https://doi.org/10.1002/hep4.1045.

[30] A. Navarrete-Opazo, G.S. Mitchell, Therapeutic potential of intermittent hypoxia: a matter of dose, Am. J. Physiol. Regul. Integr. Comp. Physiol. 307 (2014) R1181–R1197, https://doi.org/10.1152/ajpregu.00208.2014.

[31] T.V. Serebrovskaya, L. Xi, Intermittent hypoxia training as non-pharmacologic therapy for cardiovascular diseases: practical analysis on methods and equipment, Exp. Biol. Med. 241 (2016) 1708–1723, https://doi.org/10.1177/1535370216657614.

[32] E. Olea, M.T. Agapito, T. Gallego-Martin, A. Rocher, A. Gomez-Nino, A. Obeso, C. Gonzalez, S. Yubero, Intermittent hypoxia and diet-induced obesity: effects on oxidative status, sympathetic tone, plasma glucose and insulin levels, and arterial pressure, J. Appl. Physiol. 117 (2014) 706–719, https://doi.org/10.1152/japplphysiol.00454.2014.

[33] A. Torpel, B. Peter, D. Hamacher, L. Schega, Dose-response relationship of intermittent normobaric hypoxia to stimulate erythropoietin in the context of health promotion in young and old people, Eur. J. Appl. Physiol. 119 (2019) 1065–1074, https://doi.org/10.1007/s00421-019-04096-8.

[34] Pooja A. Gangwar, K. M. Sharma, A. Singh, G. Patyal, K. Bhaumik, N.K. Bhargava, Sethy, Intermittent normobaric hypoxia facilitates high altitude acclimatization by curtailing hypoxia-induced inflammation and dyslipidemia, Pflüg. Arch. 471 (2019) 949–959, https://doi.org/10.1007/s00424-019-02273-4.

[35] H. Gatterer, S. Haacke, M. Burtscher, M. Faulhaber, A. Melmer, C. Ebenbichler, K.P. Strohl, J. Hogel, N.C. Netzer, Normobaric intermittent hypoxia over 8 Months does not reduce body weight and metabolic risk factors–a randomized, single blind, placebo-controlled study in normobaric hypoxia and normobaric sham hypoxia, Obes. Facts 8 (2015) 200–209, https://doi.org/10.1159/000431157.