HAYATI
Journal of Biosciences

Vol. 32 No. 6, November 2025 1437-1446
DOI:10.4308/hjb.32.6.1437-1446
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Intermittent Hypobaric Hypoxia Preconditioning Ameliorates Kidney Damage Compared to Acute Hypoxia

Abdul Halim Sadikin[1,2,3], Sri Widia Azraki Jusman[2,3], Wawan Mulyawan[4,5], Ani Retno Prijanti[1,2,3*]

[1]*Doctoral Program in Biomedical Sciences, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*
[2]*Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*
[3]*Center of Hypoxia and Oxidative Stress Studies, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*
[4]*Indonesian Air Force Institute of Aviation Medicine (Lembaga Kesehatan Penerbangan dan Ruang Angkasa dr. Saryanto), Jakarta 12770, Indonesia*
[5]*Aviation Medicine Program, Departement of Community Medicine, Faculty of Medicine, Universitas Indonesia, Jakarta 10430, Indonesia*

ARTICLE INFO

Article history:
Received December 1, 2023
Received in revised form November 4, 2024
Accepted June 11, 2025
Available Online July 29, 2025

KEYWORDS:
Kidney,
Kidney function,
HIF-1α,
Intermittent hypobaric hypoxia,
Nrf2

ABSTRACT

Many studies have reported that intermittent hypobaric hypoxia (IHH) can cause oxidative stress and tissue damage in the kidneys, which can lead to impaired kidney function. However, a study using simulated intermittent hypobaric hypoxia was considered to have a protective role for the kidneys. This study aims to determine the effect of IHH on kidney adaptation to oxidative stress and kidney damage. Twenty-five rats were divided into five groups: control, one-time hypoxia (AHH), two-time hypoxia (IHH 1), three-time hypoxia (IHH 2), and four-time hypoxia (IHH 3). The intermittent interval was one week, and at the time of treatment, the rats were placed in a hypoxic chamber. At the end of treatment, the rats were sacrificed, and the kidneys, urine, and blood were collected. The kidney tissues were used for protein assay, ELISA (HIF-1α and VEGF), qPCR (cytoglobin (Cygb) and renin), SOD and GPx activity assay. Blood was used for creatinine and urea assay, and urine was used for KIM-1 ELISA. Formalin-submerged tissues were used for histopathological analysis. The level of HIF-1α and VEGF increased significantly from AHH to IHH 1. Cygb and renin expressions peaked at AHH and decreased at subsequent IHH groups. The Nrf2 level and GPx activity didn't show any difference, but SOD activity peaked at IHH 1. The creatinine level only peaked at IHH 2; other groups remained the same as the control. Urea levels decreased with more IHH sessions, and KIM-1 didn't show any difference. Our findings exhibit that hypoxia preconditioning by IHH treatment leads to the kidney's adaptability to hypoxia and does not cause kidney damage.

1. Introduction

The kidneys are vital organs responsible for maintaining the body's fluid balance, filtering waste products, and regulating blood pressure (Levey and James 2017). However, various environmental factors, such as high altitude, can have adverse effects on kidney function. High altitude can commonly occur in air travel, mountain climbing, and military operations. In these occurrences, the individual is exposed to alternating periods of low oxygen pressure and normal atmospheric pressure, a phenomenon known as intermittent hypobaric hypoxia (IHH).

Recent studies have reported that IHH can cause oxidative stress and tissue damage in various organs, including the kidney (Rathi *et al.* 2023). Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses. This can lead to cellular damage and contribute to the development of diseases, including kidney disease (Popolo *et al.* 2013), which in turn causes tissue damage and can impair kidney function.(Webster *et al.* 2017). Oxidative stress caused

* Corresponding Author
E-mail Address: aniretno@gmail.com

by hypoxia has been shown to activate pro-inflammatory pathways, induce apoptosis, and impair renal functions (Keshtkar *et al.* 2019). Vascularization is essential for maintaining healthy kidney function, as the kidney relies on oxygenated blood via the bloodstream (Domenech *et al.* 2017). Hypoxia can also affect vascularization in the kidney (Vilaseca *et al.* 2017). However, IHH can be simulated in a hypobaric chamber and has been shown to have health benefits (Coimbra-Costa *et al.* 2021). Studies suggested that IHH may also have protective effects on the kidney by reducing oxidative stress and affecting vascularization.(Vilaseca *et al.* 2017)

Therefore, it is important to investigate the impact of IHH on the kidney in a controlled and simulated environment. This study aims to determine the effect of IHH kidney adaptation by measuring the levels of Hypoxia-Inducible Factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF), as well as the expression of cytoglobin (Cygb) and renin. We also aim to determine the effect of IHH on oxidative stress in the kidney by measuring the levels of nuclear factor-erythroid-2-related factor 2 (Nrf2) and the activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx). Furthermore, we aim to investigate whether IHH contributes to kidney damage by measuring the levels of creatinine, urea, and kidney injury molecule-1 (KIM-1).

2. Materials and Methods

This research was an experimental study of intermittent hypobaric hypoxia (IHH) using male Sprague-Dawley rats. Twenty-five rats were obtained from the Indonesian FDA Laboratory Services (INFALABS), with ethical approval granted by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (approval number: 787/UN2.F1/ETIK/PPM.00.02/2022). The rats were divided into five groups: C (control), AHH (acute hypobaric hypoxia, exposed to hypobaric hypoxia one time), IHH 1 (exposed to two-time intermittent hypobaric hypoxia), IHH 2 (exposed to three-time intermittent hypobaric hypoxia), and IHH 3 (exposed to four-time intermittent hypobaric hypoxia).

2.1. Hypobaric Hypoxia Treatment

The rats were 8 weeks of age, with an average body weight of 200 g. The intermittent interval was one week, and at the time of treatment, the rats were exposed to hypobaric hypoxia in a hypoxic chamber. The hypobaric profile was as follows: the chamber's pressure was decreased to an equivalent of 25,000 ft altitude with an increment of 3,000 ft per minute. After the pressure reached 25,000 ft, it remained constant for 5 minutes, and then the chamber's pressure was returned to normal at a rate of 3,000 ft/min (Figure 1). After treatment, the designated group was sacrificed, and the kidneys were taken, and urine collected along with blood for further examination. Hypobaric hypoxia treatments were conducted at the Indonesian Air Force Dr. Saryanto Institute of Aviation Medicine (LAKESPRA Dr. Saryanto: *Lembaga Kedokteran Penerbangan dan Ruang Angkasa*), and measurement of markers was conducted at the Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia.

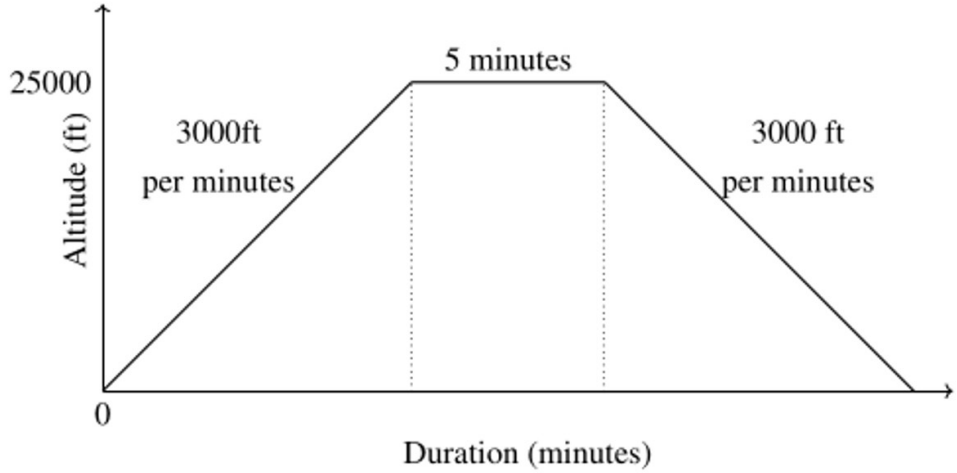


Figure 1. Hypobaric hypoxia protocol. The altitude in hypobaric chamber was increased to 25,000 feet with 3,000 ft increment/minute. The pressure stays at 25,000 feet for 5 minutes, and then decreased to normal with 3,000 ft decrement/minute

2.2. Assay of Markers

2.2.1. Tissue Homogenization

100 mg of kidney tissue was homogenized in 1 mL of 0.1 M PBS, pH 7.2. Homogenization was conducted using a micropestle in microtubes. After being thoroughly homogenized, the microtubes were centrifuged at 5,000 rpm for 10 minutes. The resulting supernatant was transferred to a new microtube and used for further assays.

2.2.2. Total Protein

To measure total protein in homogenates, bovine serum albumin (BSA) was used as a standard. Samples were diluted 100x using distilled water. Standards and samples were then measured using a spectrophotometer at $\lambda = 280$ nm. Absorbance from standards was used to generate a standard curve. Absorbance from samples was used to determine the concentration of total protein in the kidney homogenates, using the formula generated by the standard curve.

2.2.3. Measurements of HIF-1α, VEGF, Nrf2, and KIM-1

To measure the levels of KIM-1, Nrf2, VEGF, and HIF-1α, the enzyme-linked immunosorbent assay (ELISA) method was used. For VEGF, Nrf2, and HIF-1α, homogenized tissues were used, and urine samples were collected to measure KIM-1. All ELISAs were conducted using kits from Elabscience with the same protocols. Standards were used to generate a 4-point logistic curve and its formula to determine the assay concentration. For VEGF, Nrf2, and HIF-1α, the assay results were normalized to protein concentrations to determine the levels of VEGF, Nrf2, and HIF-1α per milligram of total protein.

2.2.4. Expression of Cytoglobin (Cygb) and Renin

Total RNA extraction was conducted using a kit from Zymo Research following the manufacturer's protocol. After measuring the purity and concentration of RNA, qPCR was conducted using the SensiFAST SYBR No-ROX One-Step Kit by Meridian Bioscience, following the manufacturer's protocol. Primer used as follows:

Cygb:
Forward: 5'-GAGGAAGGCGGTTCAGGCTA-3'
Reverse: 5'-TTGGCCGACGGGAAGTTCA-3'

Renin:
Forward: 5'-TCTGGGCACTCTTGTTGCTCT-3'
Reverse: 5'-CATGTCTACTCCCCGCTCCTC-3'

β-actin:
Forward: 5'- GTACAACCTTCTTGCAGCTCCTC-3'
Reverse: 5'-GACCCATACCCACCATCACAC-3'

The primers were self-designed using Primer-BLAST, available at https://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi. After the CTs were obtained, the expression values were calculated using the $2^{-\Delta\Delta Ct}$ method.

2.2.5. Activity of SOD and GPx

The activities of SOD and GPx were measured using commercial kits by RANSOD and RANSEL (Randox Laboratories Ltd., Crumlin, UK), respectively.

2.2.6. Creatinine Assay

Creatinine concentration was measured in the plasma. The method used was a colorimetric assay using 0-2 mg/ml of creatinine as the standard. Twenty microliters of standards and samples were added to 400 µL of saturated picric acid, and then 30 µL of 10% NaOH was added and mixed slowly. The mixtures were incubated at room temperature for 25 minutes. After incubation, 1550 µL of distilled water was added, and the absorbances were read using a spectrophotometer at $\lambda = 540$ nm.

2.2.7. Urea Assay

Plasma was used to measure urea concentration using the diacetyl monoxime (DAM) method. A standard of 0.1-0.5 mg/mL urea was used. There were two stages in this method: protein precipitation and urea measurement. To precipitate protein, 50 µL of standards and samples were diluted with 50 µL of distilled water. Furthermore, 950 µL of cold 5% trichloroacetic acid (TCA) was added, and the mixture was centrifuged at 5,000 rpm for 10 minutes. Twenty-five microliters of supernatant was then transferred to a new tube for urea measurement. To measure urea concentration, 500 µL of catalyst solution (a mixture of sulfuric acid and thyourea) and 500 µL of DAM were added to the supernatants and mixed. The mixtures were boiled in a water bath for 6 minutes and then cooled to room temperature. Then, it was read using a spectrophotometer at a wavelength of 525 nm.

2.2.8. Histopathology Analysis

Kidneys were submerged in formalin and then dehydrated by alcohol, followed by clearing using xylol. The kidneys are then embedded in liquid paraffin, followed by slicing using a microtome. The sliced paraffin and tissues were placed on a glass slide and

deparaffinized. The object glass, now containing only sliced tissue, was stained with hematoxylin and eosin.

2.2.9. Data Analysis

All data were analysed using GraphPad Prism version 10.0. Normality tests were conducted using the Shapiro-Wilk test. Parametric tests were conducted using the ANOVA test, followed by a post-hoc test to determine differences between treatment groups. Nonparametric tests were conducted using the Kruskal-Wallis test, followed by a post-hoc test to determine differences between treatment groups.

3. Results

3.1. Kidney Adaptation to IHH: HIF-1α, and Expressions of Its Downstream Target Proteins, VEGF, Cygb, and Renin in Kidney Tissue

Analysis of HIF-1α showed a significant difference between groups ($p = 0.05$). Post-hoc analysis reveals a significant increase from AHH to IHH. The highest level of HIF-1α was observed in IHH 1, and subsequent treatments remained unchanged from the control (Figure 2A). Analysis of VEGF showed a significant difference between groups ($p = 0.003$). Post-hoc analysis revealed a significant increase from AHH to IHH 1 ($p = 0.0399$) (Figure 2B).

Analysis of Cygb expression showed the highest expression in the acute group (AHH) and a significant decrease in subsequent IHH treatments. Renin expression showed the same pattern, with the highest expression in the acute group and a decrease at subsequent treatments (Figure 2C and D).

3.2. Kidney Adaptation to Oxidative Stress in Intermittent Hypobaric Hypoxia Treatment

Analysis on Nrf2 didn't show any significant differences between groups ($p = 0.13$). However, it shows that it peaked at IHH 1, with a slight increase from AHH, and then descended at IHH 2 (Figure 3A). Glutathione Peroxidase activity also didn't show any significant difference between groups ($p = 0.817$) (Figure 3B). However, SOD activity showed significant difference between groups ($p = 0.001$). SOD activity peaked at IHH 1 and decreased at the following intermittent treatments (Figure 3C).

3.3. Analysis of Kidney Functions

Analysis of creatinine showed a significant increase in creatinine levels ($p = 0.038$). Creatinine levels were shown to peak at IHH 2, and remained the same as normal in the other groups (Figure 4A). Analysis on urea showed a significant difference among groups ($p = 0.00002$). Urea levels were shown to decrease with more intermittent sessions of IHH (Figure 4B). On the other hand, KIM-1 analysis showed a significant difference between groups ($p = 0.04$); however, the post-hoc test didn't show significant differences among groups. The chart showed that the concentration of KIM−1 tends to increase along the duration of IHH (Figure 4C). Histopathology analysis of the kidney tissue did not show any damage. AHH group showed some thickening at the Bowman's capsule and lumen, and it did not occur in subsequent treatment of intermittent hypoxia.

4. Discussion

Continuous systemic hypoxia has been shown to increase HIF-1α expression in various organs, including the kidneys. In the kidneys, increased expression of HIF-1α is associated with increased expression of renin, which can lead to hypertension (Prijanti *et al.* 2012). Continuous hypoxia is also proven to cause structural damage to the kidneys. In contrast to constant hypoxia, which persists continuously, intermittent hypobaric hypoxia (IHH) involves exposure to hypobaric hypoxia over a specific period, followed by exposure to normoxia between hypobaric hypoxia treatments. Intermittent exposure to hypoxia aims to enable organs to adapt to hypoxic conditions. As a form of adaptation, HIF-1α is stabilized due to low oxygen pressure. HIF-1 is a transcription factor that plays a critical role in cellular responses to low oxygen levels, such as hypobaric hypoxia (Schuler *et al.* 2005). Under normoxic conditions, HIF-1α is degraded by the ubiquitin-proteasome system; however, under hypoxic conditions, it is stabilized due to the low oxygen level, which inhibits prolyl hydroxylase enzyme activity (Chowdhury *et al.* 2016). Then, HIF-1α forms a heterodimer with HIF-1β in the cytoplasm. Furthermore, this complex translocates to the nucleus and binds to hypoxia-responsive elements

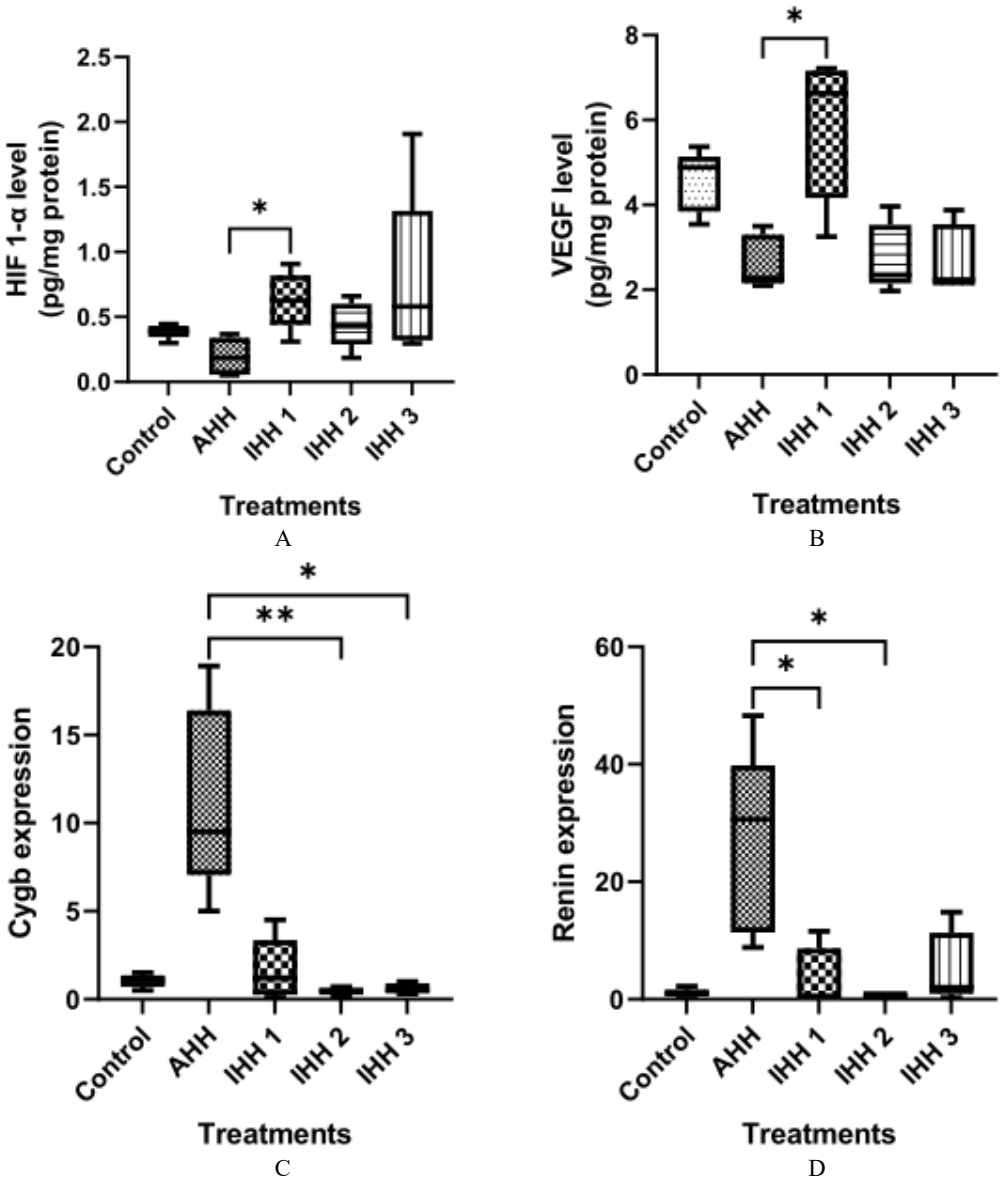
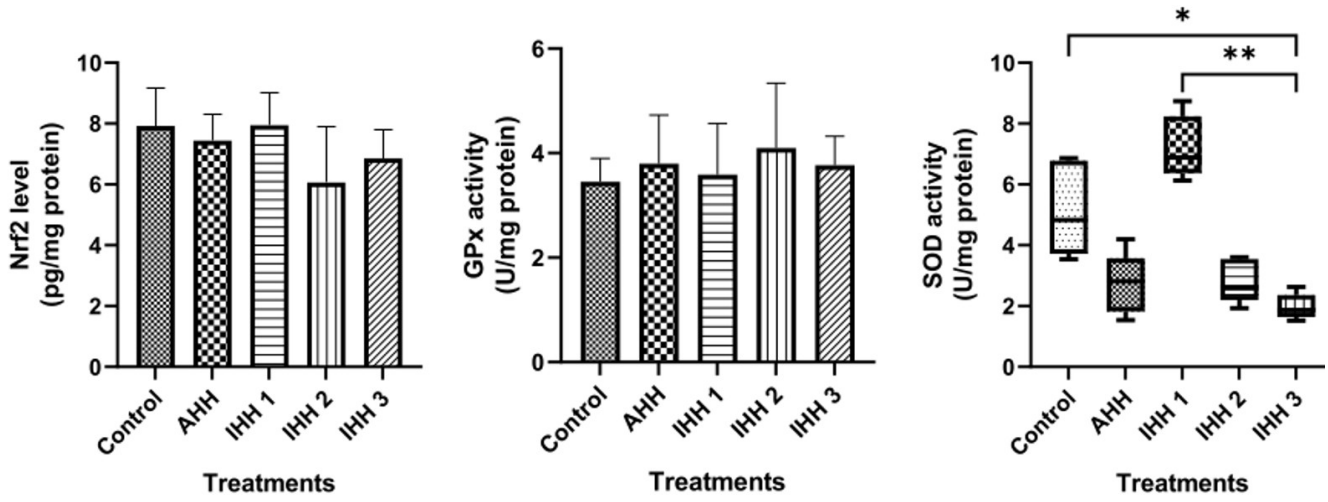


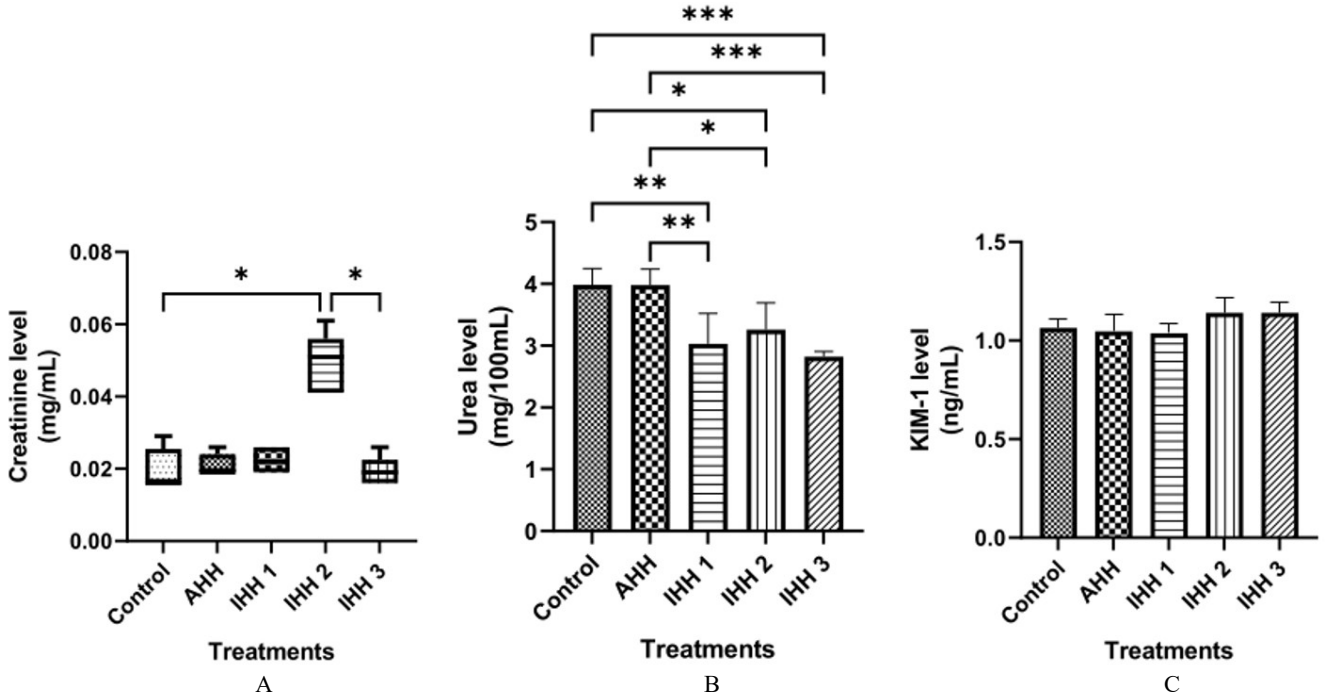
Figure 2. Kidney adaptation to hypoxia in IHH treatment (preconditioning). (A) HIF-1α protein level, (B) VEGF protein, (C) Cytoglobin mRNA, and (D) renin mRNA. (Control = control group, at normoxia; AHH = acute hypobaric hypoxia, subjected to hypoxia only once; IHH 1 = intermittent hypobaric hypoxia 1, subjected to hypoxia twice, with normoxia in between; IHH 2 = intermittent hypobaric hypoxia 2, subjected to hypoxia 3 times, with normoxia in between; IHH 3 = intermittent hypobaric hypoxia 3, subjected to hypoxia 4 times, with normoxia in between)

(HREs) in the promoter regions of target genes (Lee *et al.* 2004). Our data showed that the levels of HIF-1α were significantly increased at IHH 1, following twice exposure to hypobaric hypoxia, compared to the acute group (AHH) (Figure 1A). It was also observed that with increasing IHH treatment (IHH 2 and 3), HIF-1α expression decreased, but not to the same extent as the acute group, similar to the normal group. It is considered that at three- and four-times exposure to hypoxia (IHH 2 and 3), the kidneys have already adapted to hypoxia; hence, HIF-1α expression no longer increases. On the other hand, when the kidneys are continuously exposed to hypoxia, the level of HIF-1α gradually increases until the 7th day and then



A B C

Figure 3. Kidney respon to oxidative stress during hypobaric hypoxia. (A) Nrf2 protein, (B) glutathione peroxidase (GPx) activity, and (C) superoxide dismutase (SOD) activity. (Control = control group, at normoxia; AHH = acute hypobaric hypoxia, subjected to hypoxia only once; IHH 1 = intermittent hypobaric hypoxia 1, subjected to hypoxia twice, with normoxia in between; IHH 2 = intermittent hypobaric hypoxia 2, subjected to hypoxia 3 times, with normoxia in between; IHH 3 = intermittent hypobaric hypoxia 3, subjected to hypoxia 4 times, with normoxia in between)



A B C

Figure 4. Analysis of kidney damage. (A) Plasma creatinine level, (B) Plasma urea level, and (C) KIM-1 level in urine. (Control = control group, at normoxia; AHH = acute hypobaric hypoxia, subjected to hypoxia only once; IHH 1 = intermittent hypobaric hypoxia 1, subjected to hypoxia twice, with normoxia in between; IHH 2 = intermittent hypobaric hypoxia 2, subjected to hypoxia 3 times, with normoxia in between; IHH 3 = intermittent hypobaric hypoxia 3, subjected to hypoxia 4 times, with normoxia in between)

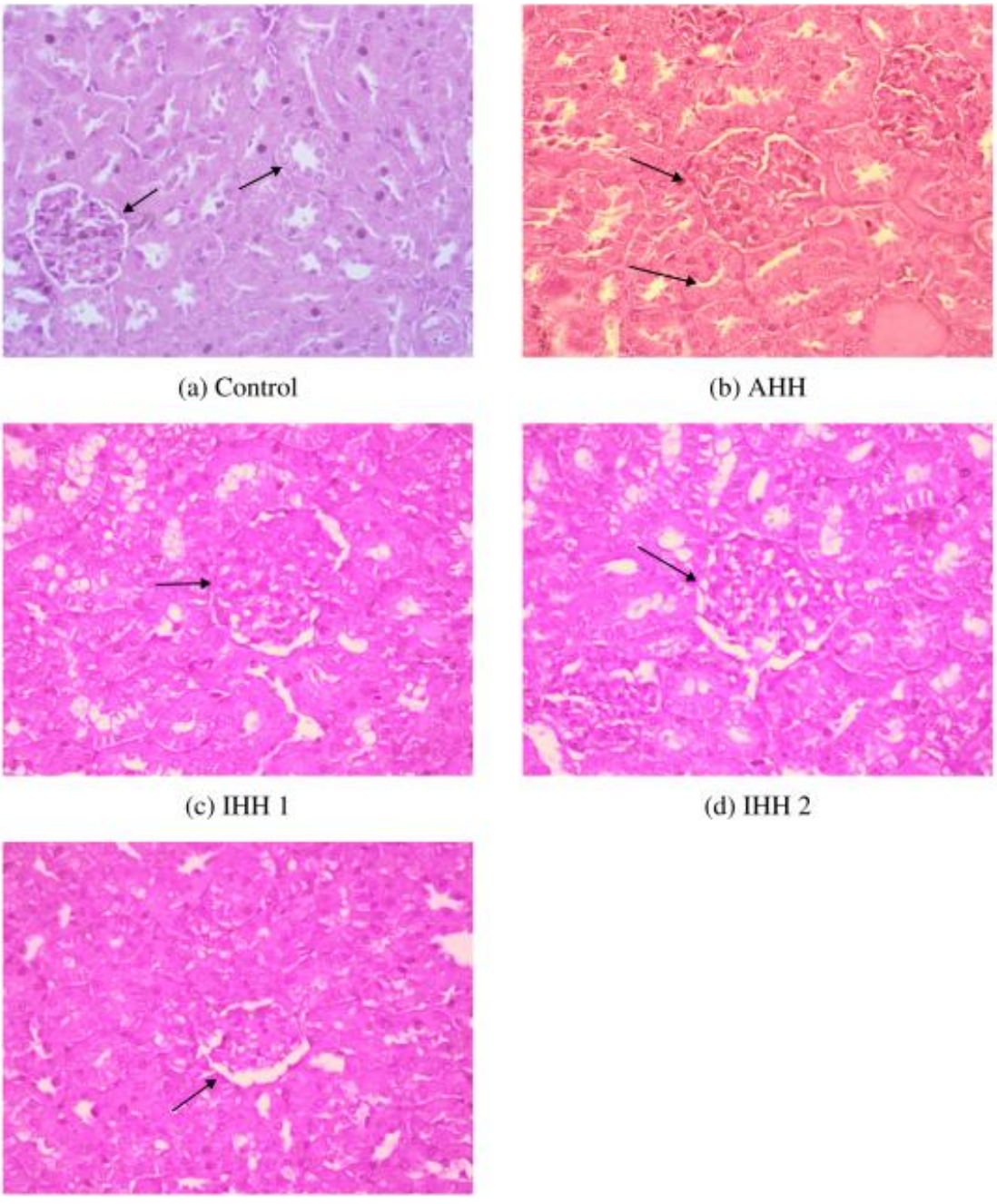


Figure 5. Histopatology examination of kidney tissue treated with acute hypobaric hypoxia and hypobaric hypoxia preconditioning (IHH). Hematoxillin-Eosin staining, 400x magnification. Scale in µm. (Control: control group, at normoxia; AHH: acute hypobaric hypoxia, subjected to hypoxia only once; IHH 1: intermittent hypobaric hypoxia 1, subjected to hypoxia twice, with normoxia in between; IHH 2: intermittent hypobaric hypoxia 2, subjected to hypoxia 3 times, with normoxia in between; IHH 3: intermittent hypobaric hypoxia 3, subjected to hypoxia 4 times, with normoxia in between. The arrows showed that in AHH group, there was some thickening at bowman's capsule and lumen, and it did not occur in subsequent groups)

decreases at the 14th day (Prijanti *et al.* 2019). The differences in the pattern demonstrate that our model of intermittent hypoxia is more effective in the kidneys' adaptability to hypoxia. Other researchers have shown that in heart tissues, the effect of IHH increased the HIF-1α level to reach its peak in the acute group and declined with more sessions of hypobaric hypoxia (Herawati *et al.* 2017). This finding differs from our previous finding, where the level of HIF-1α was at its lowest in the acute group. This discrepancy between the heart and the kidney indicates that the kidney adapts to hypoxia more slowly compared to the heart tissue. The reason for this is that kidneys are poorly oxygenated, even in normoxic conditions (Lübbers and Baumgärtl 1997). Conversely, the heart muscle is predominantly aerobic, and it prefers to use fatty acids as its primary energy source during metabolism (Vargas-Delgado *et al.* 2023).

As a transcription factor, HIF-1 controls the expression of various genes necessary for adapting to hypoxia. In this research, hypobaric hypoxia at first exposure did not increase HIF-1α; this may be due to the short exposure time to hypobaric hypoxia, only five minutes at 25,000 ft (7,620 m) altitude. However, in the group with twice the exposure, a response was observed, increasing the level of HIF-1α. HIF-1 regulates the expression of various genes essential for adaptation to hypoxia, including VEGF and cytoglobin (Cygb). Both play a role in increasing oxygen supply. Both VEGF and Cygb were known to be regulated by HIF-1, and their regulation during hypoxia has been established in various organs (Masengi *et al.* 2018; Daruningrum *et al.* 2020). In addition to stabilizing HIF-1, hypoxia also increases the production of reactive oxygen species (ROS). This causes oxidative stress, which can lead to cell damage and the development of diseases, including kidney diseases (Popolo *et al.* 2013), resulting in tissue damage and disrupting kidney function. (Webster *et al.* 2017).

Our data showed that VEGF expression was significantly increased after one session of HH but decreased drastically to normal levels after two and three sessions of IHH. In the acute exposure group, the VEGF level was similar to that of normal individuals and significantly lower than in the second exposure group. It is possible that, in acute hypoxia, the kidneys are unable to cope with the lack of oxygen and thus express VEGF (Figure 2B). In this research, it is also demonstrated that VEGF is regulated by the HIF complex, a finding that aligns with other studies (Lin *et al.* 2004).

Cygb mRNA expression showed a significant increase in the acute group, and decreased drastically in subsequent IHH treatment (Figure 1C). Another study of the effects of IHH on Cygb expression in the brain showed a significant increase in Cygb expression with increasing IHH sessions (Masengi *et al.* 2018). This difference in results is likely due to the better oxygen supply to the brain than to the kidneys. Cygb expression in our study also suggests that in more intermittent sessions, the kidneys have adapted and no longer need to express Cygb. Other previous studies concerning Cygb in the kidney were conducted in a continuous model of hypoxia, and it showed that its mRNA and protein peaked at the 3rd day of hypoxia (Daruningrum *et al.* 2020) and declined at longer durations of hypoxia, and also showed a parallel increment and decrement in both Cygb mRNA and protein. This Daruningrum study showed direct regulation of the HIF-1α complex on Cygb expression. The difference in our model showed that exposure to 25,000 ft intermittently caused Cygb in the kidney to be expressed earlier than in continuous hypoxia. Increasing ROS during hypoxia (HH) can stimulate the expression of Cygb (Li *et al.* 2007).

Another protein regulated by HIF-1 is renin. Renin plays an important role in regulating blood pressure and electrolyte balance in the body through its involvement in the renin-angiotensin-aldosterone system (RAAS) (MacGregor *et al.* 1981). We want to know if there is any adaptation to protect the kidney through renin expression during HH. It had been previously studied that the expression of renin is under HIF-1 control (Prijanti *et al.* 2012). Our findings are similar to those of other investigations using the normobaric continuous model of hypoxia (Prijanti *et al.* 2019). The results showed that renin expression increased significantly in the acute group and tended to decrease in the IHH group. In the acute group, renin was highly expressed to adapt to hypoxia by increasing blood pressure. At IHH 2 and 3, renin expression decreased. This is possibly due to a sufficient oxygen supply to the kidneys, which decreases renin expression (Figure 1D).

Nrf2 is a protein that acts as a response to oxidative stress because it increases antioxidant synthesis as oxidative stress occurs. Our study found that Nrf2 activation was not significantly affected by IHH. However, the results showed that Nrf2 expression tended to decrease in later IHH sessions (H2 and H3), although it was not statistically significant. This decrease in Nrf2 is likely due to the kidneys' adaptation to oxidative stress, as shown in Figure 2A.

The insignificance of Nrf2 levels in our findings may be caused by a short period of HH (5 minutes) and a long period of intervals (7 days between exposures), so that the kidneys are able to recuperate.

Glutathione peroxidase (GPx) and superoxide dismutase (SOD) are both antioxidant enzymes whose synthesis is regulated by Nrf2. The results showed that GPx activity did not change significantly. However, SOD activity decreased significantly in the acute group and reached its peak after twice exposure to hypoxia, then decreased significantly at subsequent treatments. It is possible that to overcome oxidative stress due to hypoxia in the kidneys, SOD plays a more critical role than GPx. SOD activity was low in the acute group and peaked after twice-exposure to hypoxia, showing the same pattern as Nrf2. Interestingly, Cygb expression showed an opposite pattern to that of SOD and Nrf2. It is known that Cygb also plays a protective role in oxidative stress (Li *et al.* 2007). These results indicate that in the kidney, during the early (acute) stage of hypobaric hypoxia, Cygb plays a more active role in dealing with oxidative stress. In contrast, during intermittent hypobaric hypoxia, this role is taken over by SOD.

Vasoconstriction can be caused by increased renin expression, which further has the potency to cause kidney tissue damage. Therefore, we wanted to know whether injury and impairment of kidney function occurred due to exposure to intermittent hypobaric hypoxia. Figure 3A shows that creatinine levels stabilize with increasing IHH sessions. Creatinine is a product of creatine phosphate degradation in muscle tissue and is filtered from the blood through the kidneys into the urine (Salazar 2014). Therefore, blood creatinine levels are often used as an indicator of kidney function, and higher creatinine levels indicate impaired kidney function. (Tynkevich *et al.* 2014)

Previous studies have shown that hypoxia can cause kidney tissue damage (Rathi *et al.* 2023). However, the results of our research showed that creatinine levels were relatively stable except in the IHH 2 group, which increased significantly. This indicates that when the kidneys experienced intermittent hypoxia for four weeks, kidney function remained stable. This also demonstrates the kidney's ability to adapt to hypoxia. These results are supported by plasma urea levels in this study. Figure 3B shows that as the number of intermittent hypoxia sessions increases, urea levels tend to decrease. Urea is a byproduct of protein breakdown in the body and is excreted through the kidneys in urine. Therefore, plasma urea levels are often used as an indicator of kidney function.

Higher urea concentrations indicate impaired kidney function (Levey *et al.* 2002). The results of observations on plasma urea levels support our suspicion that renal adaptation occurs with increasing IHH sessions. Histopathology analysis of the cortex tissue also supports the notion of renal adaptation in IHH. As with increasing IHH sessions, tissue damage did not occur, as evidenced by the preservation of the glomeruli and tubules.

Furthermore, we found no significant difference between groups in KIM-1 levels in urine (Figure 3C). KIM−1 is a protein expressed in the kidney in response to kidney injury and is considered a sensitive biomarker of kidney damage (Bonventre 2008). Thus, the trend of KIM-1 not increasing significantly suggests that IHH treatment does not cause kidney function impairment, as indicated by the results of plasma creatinine and urea. It is interesting to note that after three and four times exposure to hypoxia, KIM-1 levels increased slightly, although not significantly. It is not yet known whether intermittent hypoxia, carried out with growing sessions, will result in a significant increase in KIM-1.

In general, our findings indicate that although kidneys are poorly oxygenated organs, they exhibit good adaptability to IHH. It is demonstrated that HIF-1α is stabilized faster in kidneys exposed to intermittent hypoxia (IHH) than in kidneys exposed to continuous hypoxia. We also discovered that in IHH, Cygb and renin in the kidneys were not directly under the influence of HIF-1. In the case of Cygb, it could be influenced by oxidative stress, where it is also shown that Nrf2 and GPx didn't significantly change. SOD activity however, increased after twice exposure to hypoxia. Here, we also demonstrate that after undergoing IHH for 4 times, the kidneys are not damaged, as shown by the results of creatinine, urea, and KIM-1.

In conclusion, IHH treatment leads to the kidneys' adaptability to hypoxia and does not cause kidney damage. As we have demonstrated in this model that Cygb plays an important role in overcoming oxidative stress in the early stages of IHH, it is imperative to conduct further studies on the interaction and dynamics of Cygb, HIF-1α, Nrf2, and SOD in a hypoxic environment. In this research, we didn't measure the glomerular filtration rate (GFR) due to our limitation of not obtaining a proper metabolic cage.

Acknowledgements

We would like to thank the staff at LAKESPRA, especially Major Dr. Wardaya SSi., M.Biomed., who assisted us in conducting the hypobaric hypoxia experiment at their facility.

References

Bonventre, J.V., 2008. Kidney injury molecule-1 (KIM-1): a specific and sensitive biomarker of kidney injury. *Scand. J. Clin. Lab. Invest.* 68, 78-83. https://doi.org/10.1080/00365510802145059

Chowdhury, R., Leung, I.K., Tian, Y.-M., Abboud, M.I., Ge, W., Domene, C., Cantrelle, F.-X., Landrieu, I., Hardy, A.P., Pugh, C.W., others, 2016. Structural basis for oxygen degradation domain selectivity of the HIF prolyl hydroxylases. *Nat. Commun.* 7, 1-10. https://doi.org/10.1038/ncomms12673

Coimbra-Costa, D., Garzón, F., Alva, N., Pinto, T.C., Aguado, F., Torrella, J.R., Carbonell, T., Rama, R., 2021. Intermittent hypobaric hypoxic preconditioning provides neuroprotection by increasing antioxidant activity, erythropoietin expression and preventing apoptosis and astrogliosis in the brain of adult rats exposed to acute severe hypoxia. *Int. J. Mol. Sci.* 22, 5272. https://doi.org/10.3390/ijms22105272

Daruningrum, I.S., Prijanti, A.R., Mudjihartini, N., Sadikin, M., Jusman, S.W.A., 2020. Cytoglobin expression in rat kidney during exposure to systemic chronic hypoxia. *Acta Biochim. Indones.* 3, 30-36. https://doi.org/10.32889/actabioina.v3i1.55

Domenech, P., Perez, T., Saldarini, A., Uad, P., Musso, C.G., 2017. Kidney-lung pathophysiological crosstalk: its characteristics and importance. *Int. Urol. Nephrol.* 49, 1211-1215. https://doi.org/10.1007/s11255-017-1585-z

Herawati, M., Mulyawan, W., Farhan, F.S., Ferdinal, F., Jusman, S.W.A., Sadikin, M., others, 2017. Expression of hypoxia-inducible Factor-1α and myoglobin in rat heart as adaptive response to intermittent hypobaric hypoxia exposure. *HAYATI J. Biosci.* 24, 131-135. https://doi.org/10.1016/j.hjb.2017.08.010

Keshtkar, S., Kaviani, M., Jabbarpour, Z., Geramizadeh, B., Motevaseli, E., Nikeghbalian, S., Shamsaeefar, A., Motazedian, N., Al-Abdullah, I.H., Ghahremani, M.H., others, 2019. Protective effect of nobiletin on isolated human islets survival and function against hypoxia and oxidative stress-induced apoptosis. *Sci. Rep.* 9, 1-13. https://doi.org/10.1038/s41598-019-48262-6

Lee, J.W., Bae, S.H., Jeong, J.W., Kim, S.H., Kim, K.W., 2004. Hypoxia-inducible factor (HIF-1) α: its protein stability and biological functions. *Exp. Mol. Med.* 36, 1. https://doi.org/10.1038/emm.2004.1

Levey, A.S., Coresh, J., Bolton, K., Culleton, B., Harvey, K.S., Ikizler, T.A., Johnson, C.A., Kausz, A., Kimmel, P.L., Kusek, J., others, 2002. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am. J. Kidney Dis.* 39, 1-226.

Levey, A.S., James, M.T., 2017. Acute kidney injury. *Ann. Intern. Med.* 167, 66-80. https://doi.org/10.7326/L18-0017

Li, D., Chen, X.Q., Li, W.-J., Yang, Y.-H., Wang, J.-Z., Yu, A.C.H., 2007. Cytoglobin up-regulated by hydrogen peroxide plays a protective role in oxidative stress. *Neurochem. Res.* 32, 1375-1380. https://doi.org/10.1007/s11064-007-9317-x

Lin, C., McGough, R., Aswad, B., Block, J.A., Terek, R., 2004. Hypoxia induces HIF-1α and VEGF expression in chondrosarcoma cells and chondrocytes. *J. Orthop. Res.* 22, 1175-1181. https://doi.org/10.1016/j.orthres.2004.03.002

Lübbers, D.W., Baumgärtl, H., 1997. Heterogeneities and profiles of oxygen pressure in brain and kidney as examples of the pO_2 distribution in the living tissue. *Kidney Int.* 51, 372-380. https://doi.org/10.1038/ki.1997.49

MacGregor, G., Markandu, N., Roulston, J., Jones, J., Morton, J., 1981. Maintenance of blood pressure by the renin-angiotensin system in normal man. *Nature.* 291, 329-331. https://doi.org/10.1038/291329a0

Masengi, A.S.R., Farhan, F.S., Mulyawan, W., Sadikin, M., Mudjihartini, N., Jusman, S.W.A., 2018. Cytoglobin, neuroglobin, and acetylcholinesterase activity in rat brain as adaptation responses to intermittent hypobaric hypoxia. *F1000Research.* 7, 426. https://doi.org/10.12688/f1000research.13592.1

Popolo, A., Autore, G., Pinto, A., Marzocco, S., 2013. Oxidative stress in patients with cardiovascular disease and chronic renal failure. *Free Radic. Res.* 47, 346-356. https://doi.org/10.3109/10715762.2013.779373

Prijanti, A.R., Ranasasmita, R., Sandra, Y., Wanandi, S.I., 2012. Correlation between hypoxia inducible factor -1α and renin expression in rats kidney induced by cobalt chloride. *Med. J. Indones.* 21, 128-32. https://doi.org/10.13181/mji.v21i3.491

Prijanti, A.R., Iswanti, F., Ferdinal, F., Jusman, S., Soegianto, R., Wanandi, S.I., Sadikin, M., 2019. Hypoxia increased malondialdehyde from membrane damages is highly correlated to HIF-1α but not to renin expression in rat kidney, *IOP Conf. Ser.: Earth Environ. Sci.* 217, 012062 https://doi.org/10.1088/1755-1315/217/1/012062

Rathi, V., Tiwari, I., Kulshreshtha, R., SK Sagi, S., 2023. Hypobaric hypoxia induced renal injury in rats: prophylactic amelioration by quercetin supplementation. *Plos One.* 18, e0279304. https://doi.org/10.1371/journal.pone.0279304

Salazar, J.H., 2014. Overview of urea and creatinine. *Lab. Med.* 45, e19-e20. https://doi.org/10.1309/LM920SBNZPJRJGUT

Schuler, B., Lundby, C., Gassmann, M., 2005. HIF-1 and the adaptation of man to high altitude. *Schweiz. Z. Sportmed. Sporttraumatologie.* 53, 82.

Tynkevich, E., Flamant, M., Haymann, J.-P., Metzger, M., Thervet, E., Boffa, J.-J., Vrtovsnik, F., Houillier, P., Froissart, M., Stengel, B., others, 2014. Decrease in urinary creatinine excretion in early stage chronic kidney disease. *PloS One.* 9, e111949. https://doi.org/10.1371/journal.pone.0111949

Vargas-Delgado, A.P., Arteaga Herrera, E., Tumbaco Mite, C., Delgado Cedeno, P., Van Loon, M.C., Badimon, J.J., 2023. Renal and cardiovascular metabolic impact caused by ketogenesis of the SGLT2 inhibitors. *Int. J. Mol. Sci.* 24, 4144. https://doi.org/10.3390/ijms24044144

Vilaseca, A., Campillo, N., Torres, M., Musquera, M., Gozal, D., Montserrat, J.M., Alcaraz, A., Touijer, K.A., Farre, R., Almendros, I., 2017. Intermittent hypoxia increases kidney tumor vascularization in a murine model of sleep apnea. *PLoS One.* 12, e0179444. https://doi.org/10.1371/journal.pone.0179444

Webster, A.C., Nagler, E.V., Morton, R.L., Masson, P., 2017. Chronic kidney disease. *The lancet.* 389, 1238-1252. https://doi.org/10.1016/S0140-6736(16)32064-5