

Effect of high-altitude hypoxia environment on uric acid excretion, Desmin protein level in podocytes, and Na⁺-K⁺-ATPase activity

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ABSTRACT

To investigate the oxidative stress and adaptive compensation of kidneys in rats in high-altitude hypoxia environments, 20 Wistar rats (3 months) were randomly and equally grouped. The rats in the test group were fed in a low-pressure oxygen chamber, and those in the control group (controls) were fed in a normal environment. On the 5th, 10th, 20th, and 30th day, the excretion of uric acid in rats was detected by a biochemical analyzer, the level of desmin protein in rat podocytes was detected by immunohistochemistry, and the activity of Na⁺-K⁺-ATPase in rat proximal tubular epithelial cells was measured by liquid scintillation method. The results showed that with the increased time, the level of uric acid in the blood of rats in the test group increased dramatically ($P<0.05$). On the 30th day, the blood uric acid content of the test group was 52.33 μmol/L, and that of the control group was 38.43 μmol/L. The blood uric acid content in the test group was dramatically increased relative to the control group. Immunohistochemistry showed that the desmin protein in podocytes of the test group (0.14) was considerably higher than that in the control group ($P<0.05$). The Na⁺-K⁺-ATPase activity of proximal renal tubular epithelial cells in the test group was 611.2 pmol pi/mg protein/h, which was considerably lower than the versus control group ($P<0.05$). In summary, in high altitude hypoxia environment, uric acid accumulated in the body, and renal filtration and excretion ability was limited.

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Introduction

In the plateau, the oxygen environment is different from other plain areas, and the oxygen content is low, which is a special low oxygen environment, which has a series of impacts on the body's hemorheology, blood biochemistry, blood gas, and organ function, and even pathological changes (1, 2). The hypoxic environment on the plateau will cause the rapid decline of oxygen partial pressure or oxygen content in the circulating blood of the human body, which will lead to the inability of body tissues and cells to obtain enough oxygen to maintain their normal functional activities, and the body will show various pathological states (3-5). After the appearance of altitude hypoxia, it will cause the disorder of the normal function of various systems of the body, and at the same time, there will be many stress changes. Among many organs in the human body, the kidney is one of the most vulnerable organs, and the altitude hypoxia environment will cause a series of damage to the kidney (6-8). Acute hypoxia at altitude will lead to kidney damage, and its continued development will lead to acute renal dysfunction and even multiple organ dysfunction or failure in severe cases (9-11). As one of the important organs, the kidney plays a crucial role in maintaining the internal environment of the body. The kidney is very sensitive to the environment of hypoxia. When the

body is in a hypoxic state, the blood flow and plasma flow of the kidney will be considerably reduced, and the permeability of renal glomerular capillaries will be enhanced accordingly, resulting in the change in glomerular filtration rate. In addition, pathological changes also occur in renal tubules in various renal tissues, and the secretion, absorption, and excretion functions of renal tubule epithelial cells are considerably affected (12-15). Hypoxia leads to abnormal secretion of various hormones, such as catecholamine and antidiuretic hormone, resulting in the release of renin, which in turn promotes the formation of angiotensin II. The effect of angiotensin II on blood vessels can cause the contraction of renal arteries, which leads to the continuous narrowing of renal blood perfusion and the obvious ischemia and hypoxia of renal tissue structure. Then, the glomerular filtration rate continues to decrease, the urine volume of the body decreases, and the glomerulus and renal tubules in the kidney are severely damaged (16-19).

Indicators of renal dysfunction vary, including small molecules such as urea nitrogen and creatinine, which are normal products of nitrogen metabolism. When kidney function is normal, it can be completely filtered out of the body through the glomerulus, but when kidney function decreases or is damaged, urea nitrogen and creatinine can't be filtered out through the glomerulus normally, and

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their blood concentration increases accordingly (20). Uric acid is one of the metabolites of the human body and the product of purine metabolism. Uric acid is excreted in the body through the kidney and the intestine, in which the kidney plays a major role, and about two-thirds of uric acid needs to be excreted through kidney filtration (21). Various conditions can lead to the increase of uric acid in the blood of the body, mainly including the reduction of glomerular filtration rate caused by kidney injury and the inability to expel uric acid. Abnormal proximal renal tubules increase the reabsorption of uric acid, and the purine metabolism and energy metabolism of the body are abnormal (22, 23). The microscopic structure of podocytes shows that there are about nano-scale cracks, called hiatus, between two adjacent podocytes. Its surface is covered with a stretched-out membrane, which is the last barrier for blood to filter out and for fluid to enter the podocyte. In addition, numerous foot processes of podocytes can contract and expand, thus changing the size of the hiatus and the area of the filtration membrane, then regulating the filtration function of the glomerulus (24, 25). Podocyte desmin protein is a kind of intermediate silk protein in podocyte skeleton proteins. Under normal circumstances, desmin protein is not expressed in podocytes, and occasionally a small amount of desmin protein can be seen in glomerular mesangial cells. When the glomerulus is injured, desmin is expressed and has become a marker protein of podocyte injury (26-28). The normal activity of Na⁺-K⁺-ATPase plays a crucial role in maintaining the normal physiological functions of cells (29). Studies suggested that the reduction of Na⁺-K⁺-ATPase activity often predicts the occurrence of disease, and its abnormality is often positively correlated with the severity of the disease (30, 31). Under a hypoxic environment, the activity of Na⁺-K⁺-ATPase decreases, which affects glomerular filtration and tubular reabsorption (32).

In this experiment, a total of 20 3-month-old Wistar rats were purchased and randomly rolled into the test group and control group, with 10 rats in each group. The rats in the test group were reared in a low-pressure cage to replicate the hypoxic plateau environment, while the rats in the control group were reared in a normal environment. At different time points on the 5th, 10th, 20th, and 30th days, the excretion of uric acid in rats was detected by a biochemical analyzer, and the level of desmin protein in rat podocytes was detected by immunohistochemistry. The changes of Na⁺-K⁺-ATPase activity in rat proximal tubular epithelial cells were measured by the liquid scintillation method, and the effects of high-altitude hypoxia on uric acid excretion, podocyte desmin protein level, and Na⁺-K⁺-ATPase activity were investigated.

Materials and Methods

Experimental animals

The Wistar rats used in this experiment were all purchased, all male, weighing about 300g. There were 20 Wistar rats in total, which were rolled into the test group and control group, with 10 rats in each group. Rats in the test group were fed in low-pressure cages to replicate the hypoxic plateau environment, while rats in the control group were fed in a normal environment. All animals were free to move and eat and drink within certain limits. The water change frequency was 2 d/ time, the pad change frequency was 5 d/ time, the indoor temperature was kept at about

23°C, the relative humidity was set at about 55%, and the lighting time of the feeding environment was kept at 12 h/ day. During the experiment, all treatments of Wistar rats were strictly carried out in accordance with the national experimental animal regulations. This animal experiment had been approved by the *Ethical Committee of Laboratory Animals*.

Main reagents

Hematoxylin dye solution and hematoxylin blue return solution were purchased from Shanghai Yuanye Biotechnology Co., LTD. 3,3'-diaminobenzidine (DAB) chromogenic kit and neutral gum were purchased from Changzhou Lier Chemical Co., LTD. Rabbit desmin antibody and anti-rabbit secondary antibody were purchased from Sigma Company. Incubation solution A: 5 KCl, 10 MgCl₂, 50 NaCl, 1 ethylene glycol diethyl ether diamine tetraacetic acid, 100 trihydroxymethylaminomethane hydrochloride, 10 Na₂ATP, (r-³²P) ATP, and (r-³²P) ATP (-5 NCi/μL), and the adjusted pH was 7.4. Incubation solution B: 10 MgCl₂, 1 ethylene glycol diethyl ether diamine tetraacetic acid, 10 Na₂ATP, 150 trihydroxymethylaminomethane hydrochloride, (r-³²P) ATP (-5 NCi/μL), and 4 Ouabain, with adjusted pH at 7.4.

Detection of hematuria index in rats

On the 5th, 10th, 20th, and 30th days after the beginning of the experiment, the urine of Wistar rats in the test group and control group was collected after the fasting and water restriction at different time points. The urine protein of rats was detected by an automatic biochemical analyzer. At different time points on the 5th, 10th, 20th, and 30th days after the start of the experiment, the blood of Wistar rats in the test group and control group was collected by means of blood collection from the orbital venous plexus. The blood was incubated at 37°C for 2 h in the laboratory and then left in the refrigerator at 4°C overnight. The blood was centrifuged at 4,500 rpm for 5 min, and the supernatant was absorbed to obtain the serum of Wistar rats in the test group and control group. The serum uric acid, serum creatinine, and blood urea nitrogen of rats were measured by an automatic biochemical analyzer.

Detection of rat podocyte proteins

Immunohistochemical staining was performed to detect the content of kidney podocyte specific protein desmin in experimental and control Wistar rats. Wistar rats were sacrificed at different time points on the 5th, 10th, 20th, and 30th days after the beginning of the experiment, and the kidney tissues of the rats were collected and treated in formaldehyde solution. The paraffin-embedded specimens were cut into 5μm thick slices in the slicer, and the 5μm thick slices were dewaxed into water. The sections were soaked in citric acid antigen repair buffer and boiled in an induction cooker for about 15 min. To block endogenous peroxidase, the slices were incubated in 3% H₂O₂ for about 30 min under dark conditions. Then, they were sealed with serum for about 45 min, added with diluted primary antibody at a ratio of 1:1,000 and incubated overnight at 4°C, and incubated with secondary antibody at room temperature for 1 h. DAB chromogenic solution was added for 10 min, hematoxylin was used to redye the slices for about 2 min, hematoxylin differentiation solution was used for 10 s, and hematoxylin blue-returning solution was used for

blue-returning. After dehydration, the slices were sealed with neutral gum and observed under a microscope. The expression of desmin in kidney tissue was quantified by software and recorded and compared.

Western blot was adopted to detect the content of kidney podocyte-specific protein desmin in experimental and control Wistar rats. Wistar rats were sacrificed at different time points on the 5th, 10th, 20th, and 30th days after the beginning of the experiment. The renal cortical histone of the renal cortex of rats was taken, and its concentration was measured. After the protein was extracted, the sample loading buffer was added, and the induction cooker was boiled with water for 10 min. 100 μ L sample was added to each well of the gel, and protein electrophoresis was performed. After protein separation, the membrane was transferred with a membrane transfer apparatus. After 1 h, the nitrocellulose membrane was removed and sealed with skim milk at room temperature for 2 h. The primary antibody was diluted at a ratio of 1:1,000 and incubated overnight at 4°C, then cleaned for 4 times. The second antibody was incubated at room temperature for 1 h and cleaned 4 times. ECL was used for color rendering and membrane scanning was used for analysis.

Detection of Na⁺-K⁺-ATPase activity in rat

Wistar rats were sacrificed at different time points on 5th, 10th, 20th, and 30th days after the beginning of the experiment. Renal tubular epithelial cells were isolated from rat kidney tissues and inoculated into 24-well plates. Trypsin was used for digestion, and then the cells were carefully absorbed with a pipette gun and transferred to a centrifuge tube. 100 μ L distilled water was added in it and mixed gently, refrigerated at -80°C, and then transferred to a water bath at 37°C for rewarming. After freezing and thawing twice, 30 μ L sample was transferred to tinfoil paper. At the same time, distilled water blank control group was set, and incubation solutions A and B were successively added to measure the activities of magnesium ATPase and total ATPase. The mixture was put into 37°C water bath for 15 min, transferred to ice cubes for pre-cooling, and then added with 5 μ L 4°C 5% trichloroacetic acid to stop the reaction. It was transferred to activated carbon suspension, mixed gently, and centrifuged at 1,500 rpm for 10 min. After centrifugation, 500 μ L of supernatant was transferred with a pipette gun to a liquid flash counting bottle, and its own counter was used to calculate the pulse value. Na⁺-K⁺-ATPase activity was the total ATPase activity minus Mg ATPase activity. Meanwhile, protein quantification of samples was required to achieve standardization, which was calculated according to the following equation:

$$X = \frac{A-B}{N} \times V \times T \times SRA$$

X is the activity of Na⁺-K⁺-ATPase, A is the pulse number of the sample to be tested, B is the pulse number of the blank control group, V is the volume of the sample, T is the incubation time of the sample, and SRA (phosphorus) is the specific activity of ATP.

SPSS was used for statistical analysis of data, and the data in line with the normal distribution were expressed as mean \pm standard deviation (mean \pm S). The t -test was used to express measurement data, and the chi-square (χ^2) test was used to express count data, with $P < 0.05$ indicating a statistical difference.

Results

Rat hematuria index results

The measurement results of blood uric acid are shown in Figure 1. The uric acid content of the test group and the control group on the 5th, 10th, 20th, and 30th days were counted and compared, and it was found that there was no considerable difference in blood uric acid between the two groups of rats on the 5th and 10th days ($P > 0.05$). On the 20th day, there was a considerable difference in blood uric acid content between the test group and the control group, and the rats in the test group increased dramatically ($P < 0.05$). On the 30th day, the blood uric acid content of the test group was 52.33 μ mol/L, and the blood uric acid content of the control group was 38.43 μ mol/L. The difference between the two groups was more obvious, with statistical significance ($P < 0.05$).

The measurement results of urine protein are shown in Figure 2. Statistical and comparison of the urine protein content of the rats in the test group and the control group on the 5th, 10th, 20th, and 30th days showed that there was no considerable difference in the urine between the two groups of rats on the 5th and 10th days ($P > 0.05$). On the 20th day, there was a considerable difference in the urine protein content between the test group and the control group, and the rats in the test group increased dramatically ($P < 0.05$). The difference between the two groups continued to expand on the 30th day.

The blood creatinine measurement results are shown in Figure 3. The blood creatinine content of the test group and the control group on the 5th, 10th, 20th, and 30th days were counted and compared, and it was found that there was no considerable difference in the blood of the two groups of rats on the 5th day ($P > 0.05$). On the 10th, 20th, and 30th days, the blood creatinine content of the test group and the control group was considerably different, and the rats in the test group increased dramatically ($P < 0.05$).

The blood urea nitrogen measurement results are

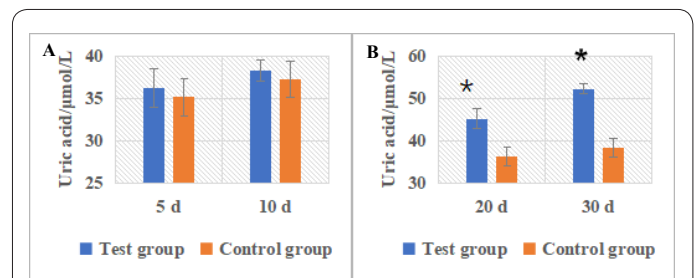


Figure 1. Comparison of blood uric acid levels in two groups of rats at different times (A: 5d and 10d; B: 20d and 30d). Note: * meant $P < 0.05$, with statistically substantial difference.

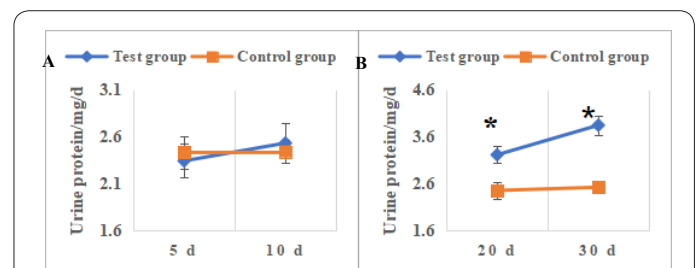


Figure 2. Comparison results of the urinary protein content of two groups of rats at a different times (A: 5d and 10d; B: 20d and 30d). Note: * meant $P < 0.05$, with a statistically substantial difference.

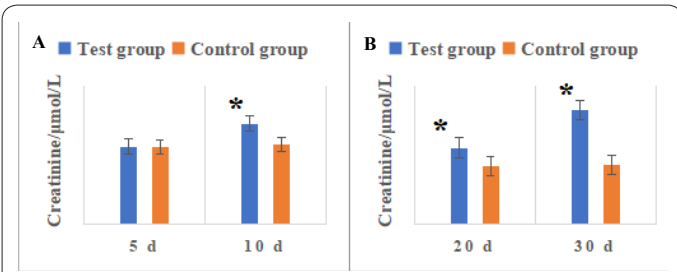


Figure 3. Comparison of serum creatinine levels in two groups of rats at different times (A: 5d and 10d; B: 20d and 30d). Note: * meant $P<0.05$, with a statistically substantial difference.

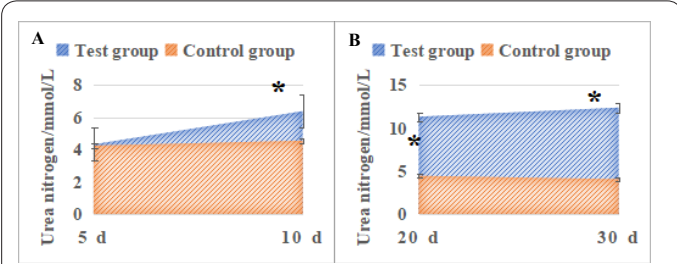


Figure 4. Comparison of blood urea nitrogen content in two groups of rats at a different times (A: 5d and 10d; B: 20d and 30d). Note: * meant $P<0.05$, with a statistically substantial difference.

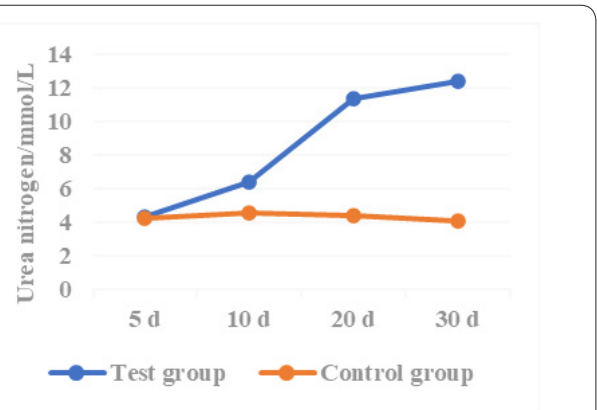


Figure 5. Changes of blood urea nitrogen in two groups of rats with time.

shown in Figures 4 and 5. The blood urea nitrogen content of the rats in the test group and the control group on the 5th, 10th, 20th, and 30th days were counted and compared. It was found that the blood urea nitrogen content of the rats in the test group increased slowly and then gradually stabilized, while the blood urea nitrogen content of the rats in the control group did not change considerably. The comparison results between the two groups showed that there was no considerable difference in blood urea nitrogen on the 5th day ($P>0.05$). On the 10th and 20th days, the blood urea nitrogen content of the test group and the control group had a considerable difference, and the test group was dramatically increased ($P<0.05$). On the 30th day, there was no considerable difference between the two groups ($P>0.05$).

Detection results of desmin protein in rat podocytes

Immunohistochemical detection results of desmin protein in rat podocytes in Figure 6 showed that the desmin protein of the podocytes in the test group and the control group was not detected on the 5th and 10th days, while the desmin protein in the test group was dramatically increased on the 20th and 30th days, and the difference was significant ($P<0.05$).

Western blot was used to detect desmin protein in rat podocytes. Figure 7 showed that desmin protein was not detected in podocytes on the 5th and 10th days of the test group and the control group. On the 20th and 30th days, the desmin protein of the test group was dramatically increased, and the difference was significant ($P<0.05$).

Detection results of Na⁺-K⁺-ATPase activity in rats

The Na⁺-K⁺-ATPase activity of rats in the test group and the control group on the 5th, 10th, 20th, and 30th days were counted and compared. Figure 8 showed that there was no considerable difference in Na⁺-K⁺-ATPase activity on the 5th day ($P>0.05$). On the 10th, 20th, and 30th days, the Na⁺-K⁺-ATPase activity between the test group and the control group was considerably different. On the 30th

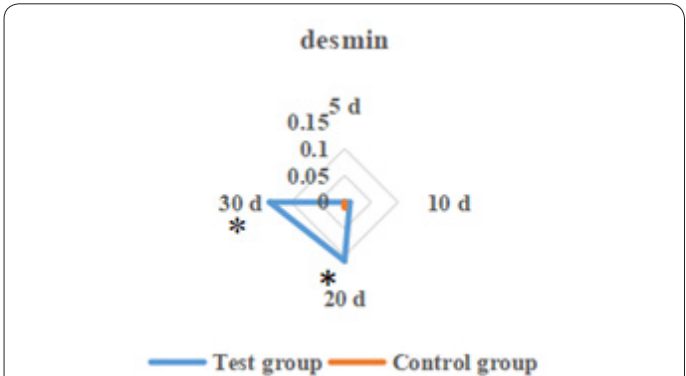


Figure 6. Immunohistochemical detection results of desmin protein content in podocytes of two groups of rats. Note: * meant $P<0.05$, with a statistically substantial difference.

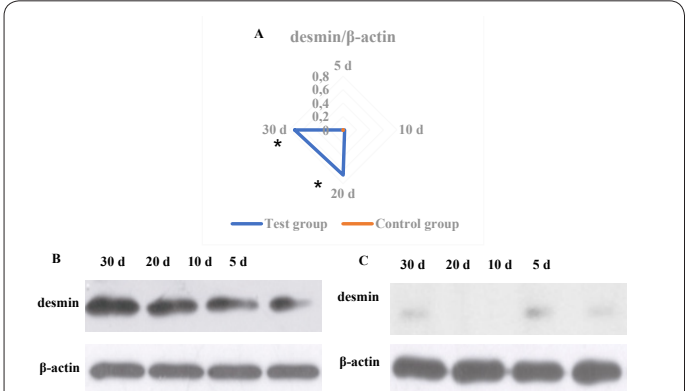


Figure 7. Western blot detection results of desmin protein content in podocytes of two groups of rats. Note: * meant $P<0.05$, with a statistically substantial difference. (Figure 7A shows the quantitative analysis of desmin in two groups of rats; Figure 7B shows the results of desmin western blotting in test group rats; Figure 7C shows the results of desmin western blotting in control group rats.)

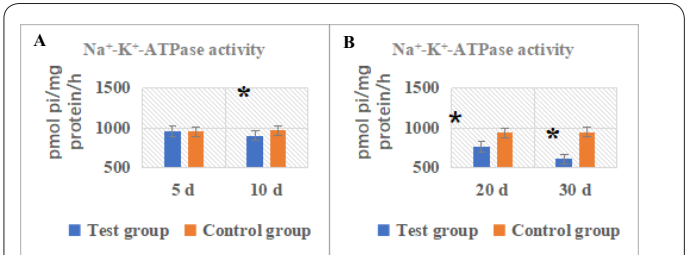


Figure 8. Comparison of Na⁺-K⁺-ATPase activity of renal tubular epithelial cells in two groups of rats at a different times (A: 5d and 10d; B: 20d and 30d). Note: * meant $P<0.05$, with a statistically substantial difference.

day, the Na⁺-K⁺-ATPase activity of rats in the test group was 611.2 pmol pi/mg protein/h, which was considerably lower than that in the control group ($P<0.05$).

Discussion

The low oxygen content in the plateau environment has a series of effects on the body's organs and tissues, including changes in blood biochemistry, rheology, and blood gas (33). The kidney, as an organ to maintain the stability of the internal environment of the body, will be damaged in the hypoxia environment, resulting in pathological changes (34). The kidney plays a key role in adapting to altitude sickness syndrome (high-altitude cerebral edema and high-altitude pulmonary edema) by regulating body fluid, electrolyte, and acid-base homeostasis (35). The kidney is sensitive to the concentration of oxygen. Once in an anoxic environment, the blood flow and plasma flow of the kidney will be considerably reduced, and the permeability of renal glomerular capillaries will be enhanced correspondingly, resulting in changes in the glomerular filtration rate (36). To explore the effect of a high-altitude hypoxic environment on the kidneys, a total of 20 3-month-old Wistar rats were purchased and randomly rolled into the test group and the control group, with 10 rats in each group. The rats in the test group were reared in a low-pressure cage to replicate the hypoxic plateau environment, while the rats in the control group were reared in a normal environment. At different time points on the 5th, 10th, 20th, and 30th days, the excretion of uric acid in rats was detected by a biochemical analyzer, and the level of desmin protein in rat podocytes was detected by immunohistochemistry. The changes of Na⁺-K⁺-ATPase activity in rat proximal tubular epithelial cells were measured by the liquid scintillation method, and the effects of high-altitude hypoxia on uric acid excretion, podocyte desmin protein level, and Na⁺-K⁺-ATPase activity were investigated.

Renal function or tissue damage can be evaluated and monitored through a variety of indicators. Urea nitrogen, urine protein, and creatinine are normal products of nitrogen metabolism. When kidney function is normal, it can be excreted by glomerular filtration, but when kidney function is reduced or damaged, blood urea nitrogen and creatinine can't be filtered out normally through the glomerulus, and its concentration in blood or urine changes correspondingly (37, 38). As a metabolite of purine, about two-thirds of uric acid needs to be excreted through the kidneys. Once the kidneys are damaged and the filtration function declines, uric acid will accumulate in the blood if it can't be excreted normally (39-41). In this experiment, the uric acid content of the test group and the control group was compared on the 5th, 10th, 20th, and 30th days. It was found that there was no considerable difference in blood uric acid between the two groups of rats on the 5th and 10th days ($P>0.05$). On the 20th day, there was a considerable difference in blood uric acid content between the test group and the control group, and the rats in the test group increased dramatically ($P<0.05$). On the 30th day, the difference between the two groups was more obvious ($P<0.05$). The comparison of urinary protein content showed that there was no considerable difference in urine between the two groups of rats on the 5th and 10th days ($P>0.05$). On the 20th day, there was a considerable difference in the urine protein content between the test group

and the control group, the rats in the test group increased dramatically ($P<0.05$), and the difference between the two groups continued to expand on the 30th day. There was no considerable difference in blood creatinine and urea nitrogen between the two groups of rats on the 5th day ($P>0.05$). On the 10th, 20th, and 30th days, there was a considerable difference between the test group and the control group, and the test group increased dramatically ($P<0.05$), which indicated that the filtration function of the kidney changed pathologically in an anoxic environment, and the filtration ability decreased.

The epithelial cells of the renal follicle, with a large cell body and numerous small projections (podocytes) that expand from the cell body, are star-shaped and multi-pointed and are called podocytes. In the normal state of the kidney, the podocyte does not produce specific proteins, but once the kidney is damaged, desmin protein begins to express, which can be used as a marker to detect kidney podocyte injury. Immunohistochemical detection of desmin protein in podocytes of rats showed that no desmin protein was detected in podocytes of the test group and control group on the 5th and 10th days. On the 20th and 30th days, desmin protein in the test group was dramatically increased, and there was a considerable difference between the test group and the control group ($P<0.05$). Western blot analysis of desmin protein in podocyte of rats showed that desmin protein in podocyte of test group and control group was not detected on the 5th and 10th days. On the 20th and 30th days, desmin protein was dramatically increased in the test group and dramatically different from that in the control group ($P<0.05$), indicating that the renal tubular epithelial podocyte was damaged in the hypoxia environment, and the specific protein desmin began to express. Na⁺-K⁺-ATPase plays a crucial role in maintaining the normal physiological functions of cells, and the reduction of its activity can affect the filtration and reabsorption functions of the kidneys (42). The Na⁺-K⁺-ATPase activity of the test group and the control group was compared on the 5th, 10th, 20th, and 30th days, and the results showed that there was no considerable difference in the Na⁺-K⁺-ATPase activity on the 5th day ($P>0.05$). On the 10th, 20th, and 30th days, the Na⁺-K⁺-ATPase activity between the test group and the control group was considerably different. On the 30th day, the Na⁺-K⁺-ATPase activity of rats in the test group was 611.2 pmol pi/mg protein/h, which was considerably lower than that in the control group ($P<0.05$). Under hypoxic conditions, the activity of Na⁺-K⁺-ATPase decreased, which was related to the impairment of glomerular filtration capacity and renal tubular reabsorption capacity. Poonit et al. (2018) (43) simulated a hypoxic environment at an altitude of 5,000 meters and found that the renal filtration function of rats changed, and the levels of uric acid, creatinine, and urea nitrogen in the blood showed a trend of increasing, which is consistent with this study.

In this experiment, 20 3-month-old Wistar rats were purchased and randomly rolled into the test group and the control group, with 10 rats in each group. The rats in the test group were reared in a low-pressure cage to replicate the hypoxic plateau environment, while the rats in the control group were reared in a normal environment. At different time points on the 5th, 10th, 20th, and 30th days, the excretion of uric acid in rats was detected by a biochemical analyzer, and the level of desmin protein in rat podocytes

was detected by immunohistochemistry. The changes of Na⁺-K⁺-ATPase activity in rat proximal tubular epithelial cells were measured by the liquid scintillation method, and the effects of high-altitude hypoxia on uric acid excretion, podocyte desmin protein level, and Na⁺-K⁺-ATPase activity were investigated. It was found that in a hypoxia environment, renal uric acid, creatinine, and urea nitrogen exclusion was limited, podocyte expression of desmin protein increased, and Na⁺-K⁺-ATPase activity decreased. Hypoxia had a negative impact on renal function, resulting in decreased glomerular filtration ability. The shortcomings of this study are that the grouping is relatively single, the sample size is small, the control variables are few, and the research is not in-depth and diversified. In the next step, the sample size will be increased and more groups will be set up to further explore the significance of hypoxia time in severely impaired kidney function, whether the stress effect of kidney can compensate for partial damage and the further influence of sufficient oxygen after hypoxia on kidney damage. We will further explore the relationship between altitude hypoxia and renal function changes and provide more clinical reference value.

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