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# Chronic intermittent hypoxia affects the expression of IRS – 2/p – Akt/GSK – 3 in the liver of SD rats and its impact on glucose metabolism

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## Abstract

**Background** Epidemiological studies indicate a strong association between OSA and type 2 diabetes. Currently, the insulin signal transduction pathway and its associated effector proteins have emerged as a focal point in type 2 diabetes research. However, the underlying mechanisms in OSA remain elusive. We have established an experimental model of chronic intermittent hypoxia in SD rats and conducted measurements of their fasting blood glucose, fasting plasma insulin levels, as well as the insulin signaling pathway effector proteins IRS-2, P-Akt, and GSK-3.

**Method** In the experiment, the gas path control system connected to a sealed glass container regulated the delivery of oxygen and nitrogen, ensuring a minimum oxygen concentration of 6%—12% within the cabin. Forty male Sprague–Dawley rats were divided into five groups ($n = 8$) and exposed to chronic intermittent hypoxia or normal air environment for 2, 4, 6, and 8 weeks, respectively. Upon completion of the experiment, the rats were anesthetized and euthanized. Immediately thereafter, their fasting blood glucose was measured, and their fasting insulin levels were determined using radioimmunoassay. Finally, the insulin resistance index (HOMA-IR) was calculated based on the steady-state model evaluation method. HE staining was employed to observe the morpho- logical changes of liver cells in each group of rats. Immunohistochemistry was utilized to detect the expression of insulin signaling pathway-related effector proteins, namely IRS-2, p-Akt, and GSK-3, in the liver, with their expression levels expressed as average grayscale values.

**Result** With the extension of intermittent hypoxia exposure duration, compared to the normal control group, the fasting blood glucose, fasting insulin, and insulin resistance index of rats in each experimental group increased ($n = 8$, $P < 0.05$). Additionally, the liver cells of rats exhibited damage and morphological changes. The expression of liver pathway proteins IRS-2 and P-Akt decreased ($n = 8$, $P < 0.05$), whereas the expression of GSK-3 protein increased ($n = 8$, $P < 0.05$).

**Conclusion** Chronic intermittent hypoxia activates the proteins IRS-2, P-Akt, and GSK-3 in the hepatic insulin signaling pathway, leading to liver cell damage, insulin resistance, and glucose metabolism disorders.

**Keywords** Chronic intermittent hypoxia · Insulin resistance · Insulin receptor base-2 · Phosphorylated protein kinase · Glycogen synthase kinase-3

## Introduction

OSA is a common respiratory sleep disorder, characterized by recurrent upper airway obstruction during sleep, leading to chronic intermittent hypoxia, fragmented sleep, and daytime sleepiness. OSA has a high incidence rate worldwide. A report states that nearly 1 billion adults aged 30–69 worldwide suffer from obstructive sleep apnea, with prevalence rates in some countries even exceeding 50% [1]. Recent studies indicate that OSA is an independent risk factor for the development of diabetes [2]. Recent clinical and epidemiological research has further reinforced the notion that obesity is a primary common risk factor for both diabetes

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and OSA [3, 4]. Related reports also suggest that it is not associated with central obesity, age, or other confounding factors that interfere with glucose metabolism [5, 6].

Chronic intermittent hypoxia is the primary pathological feature of OSA. Despite being a major factor in OSA, many animal experiments still utilize chronic intermit- tent hypoxia to study OSA. The mechanism by which chronic intermittent hypoxia leads to glucose metabolism disorders primarily involves alterations in intrinsic mecha- nisms such as sympathetic nervous system activation, hypothalamic–pituitary–adrenal axis excitation, oxidative stress, adipokine activation, inflammatory response, and pancreatic signaling pathway stimulation [7, 8]. There is also a positive correlation between the severity of OSA and the risk of type 2 diabetes. More specifically, mild AHI increases the risk of type 2 diabetes by 23%, while severe AHI increases this risk by a higher odds ratio [9].

The activation of the insulin-mediated PI3 K/AKT signaling pathway and alter- ations in related transcription factors may play a significant role in the glucose metabolism disorder triggered by chronic intermittent hypoxia. In the PI3 K/AKT pathway, after insulin resistance develops in the body, the expression of the key effector protein IRS becomes insufficient or its phosphorylation level significantly decreases, leading to a notable reduction in PI3 K activation and impeding insulin signaling transduction [10]. IRS expression increases during oxidative stress, while antioxidant stress compounds decrease IRS phosphorylation and the phosphorylation of its down- stream key protein, Akt, thereby impeding the development of insulin resistance [11]. Additionally, in the inflammatory response, inflammatory factors such as TNF-α can affect IRS phosphorylation, reduce the expression of glucose transporter-4, inhibit glucose uptake by adipocytes, and ultimately lead to insulin resistance [12]. GSK- 3 is located downstream of Akt and is directly regulated by its negative feedback mechanism, regulating glycogen synthesis and influencing glucose metabolism.

Therefore, it remains unclear whether chronic intermittent hypoxia can regulate glucose metabolism by influencing the insulin signaling pathway. If this is the case, the expression of effector proteins associated with the insulin signaling pathway would be altered. Our primary research objective is to explore the potential mechanisms behind the expression changes of IRS-2/p-Akt/GSK-3 proteins by observing their alterations.

## Materials and methods

During the experiment, the average weight of healthy male SD rats was (190 ± 9) g, and the experimental protocol was officially approved by the Ethics Committee of Hunan Aerospace Hospital. The 125I rat insulin radioimmunoassay kit, IRS-2, GSK-3 reagents, and DAB colorimetric kit were purchased from Wuhan Bode Biotechnology Co., Ltd. in China. P-Akt, 5% BSA blocking solution, biotinylated goat anti-rabbit IgG, and SABC (streptavidin–biotin peroxidase complex) were sourced from Beijing BioSense Biotechnology Co., Ltd.

### Experimental animals and grouping

We selected 40 healthy male Sprague–Dawley (SD) rats (animal batch number: SCXK (Jin) 2009–0001) with an average weight of (190 ± 9) g. These rats were pro- vided by the Experimental Animal Center of Shanxi Medical University. During the experiment, these rats were housed in the laboratory of the Respiratory Department of Shanxi Medical University, following standard feeding practices, allowing for free access to food and water, and kept at a room temperature of 25 ˚C-27 ˚C [8]. According to the random number table method, the animals were divided into several different groups: normal control group (NC group), chronic intermittent hypoxia 2-week group (CIH2 group), chronic intermittent hypoxia 4-week group (CIH4 group), chronic inter- mittent hypoxia 6-week group (CIH6 group), and chronic intermittent hypoxia 8-week group (CIH8 group), with each group containing 8 animals.

### Preparation of the chronic intermittent hypoxia model

We have constructed an animal intermittent hypoxic chamber with a volume of 65 cm × 45 cm × 50 cm using transparent plexiglass material. The chamber is equipped with an oxygen meter from Zhejiang Xin'anjiang Analytical Instrument Factory 2 and a humidity meter from Shenzhen Ruitesi Instrument Co., Ltd., which are used to measure oxygen concentration and humidity respectively. Through a microcontroller programming system (jointly developed by the First Hospital of Shanxi Medical Uni- versity and Taiyuan University of Technology), we control the one-way solenoid valve system to supply air, and connect the transparent plastic pressure-resistant tube to the control system box, nitrogen cylinder, and oxygen cylinder outside the glass chamber.

In the established inflation protocol, nitrogen gas is initially injected into the chamber for 60 s, causing the oxygen concentration to drop from 21% to (8 ± 2)% and maintain that level for 10 s. Subsequently, oxygen is introduced for 40 s to gradually raise the oxygen concentration back to 21%, which is then maintained for an additional 10 s. This constitutes a 120-s cycle, achieving 30 low-oxygen events per hour, similar to the condition observed in severe OSAHS. A portable oxygen meter continuously monitors the oxygen concentration within the low-oxygen chamber. The moisture and CO2 produced by the rats inside the chamber are

absorbed by calcium oxide, while efforts are made to maintain the temperature within the chamber between 24–29 ˚C and the humidity between 40%−50%. According to the gas supply plan, rats from the CIH2, CIH4, CIH6, and CIH8 groups are placed in the intermittent hypoxia chambers from 8am to 5 pm daily, with free access to water and food. In contrast, rats in the NC group breathe normal air.

## Sample collection

The CIH2, CIH4, CIH6, and CIH8 groups ceased intermittent hypoxia expo- sure on days 15, 29, 43, and 57, respectively. Following a 12-h fast, the rats were weighed. Anesthesia was induced by injecting 20% urethane (5 ml/kg) into the abdom- inal cavity. The rats were then placed in a supine position on the animal experimental platform. Their noses were routinely disinfected, and 0.5 cm of the tip was surgically removed using surgical scissors. Fasting blood glucose levels were measured using a medical blood glucose meter (Johnson & Johnson Medical Devices, USA). Then, cut the abdominal wall along the midline to expose the abdominal aorta. Collect arterial blood and centrifuge it for 5 min (3000r/min, r = 190) in a centrifuge provided by Xiangyi Company in Changsha, Hunan. Take 1.0 ml of the upper serum from each group and test for insulin in a −80 ˚C freezer (Thermo-Forma, USA). Separate the liver tissue, immerse a portion of it in 4% formaldehyde solution (provided by the Pathol- ogy Department of Shanxi Medical University) for 48 h, and embed it in paraffin for immunohistochemical analysis.

## Experimental indicator testing

### Fasting blood glucose and serum insulin detection in rats

During basic fasting blood glucose testing, we excised 0.5 cm from the tail tips of rats in each group after modeling, and measured their blood glucose levels using a Johnson & Johnson OneTouch Ultra blood glucose meter. The 125I insulin radioim- munoassay method was employed to detect insulin levels in serum. Firstly, the rat serum samples were retrieved from the ultra-low temperature freezer (−80 ˚C), thawed at room temperature, and thoroughly mixed in a vortex mixer. Subsequently, the serum samples were labeled and neatly arranged on the EP tube rack in order. All reagents must be fully mixed in the serum awaiting testing. Subsequently, samples are added sequentially using a micropipette to ensure thorough mixing. Following a 15- minute incubation at room temperature, the mixture is centrifuged for 15 min at 3500r/min. Afterwards, the supernatant is discarded, and the precipitate is retained. Lastly, the count of sediment in each EP tube is measured using a fully automated radioimmunoassay analyzer located in the Endocrine Laboratory at Shanxi Medical University. All data is then automatically processed by a computer. To assess the level of insulin resistance, we utilize the steady-state model insulin resistance index (HOMA-IR), which is calculated as (fasting blood glucose × fasting insulin)/22.5.

### Hematoxylin eosin (HE) staining

Liver paraffin sections were baked in a drying machine (LEICA, Germany) for 2 h. Upon removal, they underwent dehydration and rehydration in varying con- centrations of xylene (3 × 10 min) and alcohol (3 × 2 min) in sequential order. Subsequently, they were stained with hematoxylin for 1 min, rinsed with water for 5 min, differentiated with ethanol for 1 min, rinsed again with water, stained with eosin for 1 min, dehydrated in different concentrations of alcohol (3 × 2 min), rinsed with xylene (3 × 10 min), and finally dried at room temperature. Lastly, the morphological changes in liver cells of each rat group were observed using an optical microscope CX-21 (Olympus Corporation, Japan).

### Immunohistochemistry analysis

Liver paraffin sections were placed in a drying machine (LEICA, Germany) and baked for 2 h. Upon removal, they were immediately subjected to dewaxing and hydration in different concentrations of xylene (3 × 10 min) and alcohol (3 × 2 min) in that order. After hydration, the sections were placed in 3% H2O2 for 15 min to reduce the influence of endogenous peroxidase on the experimental results. The sections were then placed in a distilled water tank for 3 min and washed three times with PBS solution (pH = 7.2), each wash lasting for 3 min. The cleaned sections were placed in a pressure cooker containing citrate buffer (pH 6.0) for 2 min to perform thermal antigen retrieval. After natural cooling, the sections were removed and washed three times with PBS solution, each wash lasting for 5 min.

Apply a drop of 5% BSA blocking solution onto the sliced tissue, incubate it in an intelligent constant temperature incubator JCZ-GPL (Nantong Jiacheng Instrument Company, China) at 37 ˚C for 1 h to block non-specific protein staining. Remove excess liquid and add IRS-2 (1:100), P-Akt (1:200), and GSK-3 (1:100) primary anti- bodies diluted with PBS solution. After 20 min, rinse with PBS solution three times, each time for 3 min. Apply a drop of biotinylated goat anti-rabbit IgG secondary antibody onto the surface of the sliced tissue, incubate at 37 ˚C for 20 min- utes, remove and rinse with PBS solution three times, each time for 3 min. Add a drop of SABC (streptavidin–biotin peroxidase complex) onto the tissue, and after 20 min, rinse five times with PBS solution, each time for 4 min. Following rins- ing, stain with DAB reagent. Finally, rinse the slides with clean water to terminate staining, remove and immerse them in different

concentrations of alcohol (3 × 2 min- utes) for dehydration, and then rinse with xylene (3 × 10 min). Finally, air dry the slides at room temperature. An Image Pro Plus image system (Sichuan Chuanda Intelligent Software Co., Ltd., China) was used for image acquisition and grayscale quantitative analysis.

### Statistical analysis

We conducted a detailed statistical analysis of the collected data using SPSS 17.0 statistical software. For metric data conforming to a normal distribution, we employed a particular method, while the differences between groups were compared using anal- ysis of variance. For pairwise comparisons, we utilized the Least Significant Difference.

(LSD) method, and for correlation analysis, we opted for Pearson correlation analysis. When the *P*-value is less than 0.05, these differences are considered statistically significant.

## Results

### Fasting blood glucose, plasma insulin, and insulin resistance index

As the duration of intermittent hypoxia exposure increases, the levels of insulin, plasma insulin, and insulin resistance index in the CIH group gradually rise compared to the NC group. This increase is significant, with the CIH8 group exhibiting the highest levels, followed by CIH6, CIH4, CIH2, and then the NC group. Significant differences in fasting blood glucose, plasma insulin, and insulin resistance index are observed among different populations, with the differences between the two groups being statistically significant ($P < 0.001$, Table 1).

### The average grayscale values of IRS-2, P-Akt, and GSK-3 proteins in rat liver

As the duration of intermittent hypoxia exposure increased, the average gray values of IRS-2 and P-Akt proteins in the CIH groups gradually increased, with a sig- nificant increase compared to the NC group. The CIH8 group exhibited the highest levels, followed by the CIH6, CIH4, CIH2, and NC groups. The differences in the aver- age gray value levels of IRS-2 and P-Akt proteins among the groups were statistically significant ($P < 0.05$). In contrast, the average gray value of GSK-3 protein gradually decreased, with a significant decrease compared to the NC group. The CIH8 group exhibited the lowest levels, followed by the CIH6, CIH4, CIH2, and NC groups. The differences in the average gray value levels of GSK-3 protein among the groups were statistically significant ($P < 0.001$, Table 2).

### Correlation analysis between IRS-2, P-Akt, GSK-3 and HOMA-IR

The average grayscale values of HMOA-IR were positively correlated with IRS-2 ($r = 0.867$ $P < 0.001$), P-AKT ($r = 0.903$ $P < 0.001$), and negatively correlated with GSK-3 ($r = -0.855$ $P < 0.001$), as shown in Table 3.

### Morphological changes of rat liver cells (HE staining)

The morphology of liver cells in each group is shown in Fig. 1. Under light microscopy, the liver cells in the NC group exhibited relatively normal size, clear results, uniform cytoplasm, and no obvious vacuoles. As the duration of chronic inter- mittent hypoxia exposure increased, few normal-shaped liver cells were observed, with most cells showing vesicular degeneration and steatosis, accompanied by infiltration of surrounding inflammatory cells. These changes were particularly evident in the CIH8 group.

**Table 1** Effects of CIH on fasting blood glucose, serum insulin, and HOMA-IR in each group of rats

| Number of group | cases | Blood glucose (mmol/L) | Insulin (mIU/L) | HOMA-IR |
|---|---|---|---|---|
| NC Group | 8 | 3.11 ± 0.32a | 2.88 ± 0.36b | 0.40 ± 0.08c |
| CIH2 Group | 8 | 3.55 ± 0.44a | 3.51 ± 0.26b | 0.56 ± 0.10c |
| CIH4 Group | 8 | 4.45 ± 0.28a | 4.05 ± 0.38b | 0.80 ± 0.12c |
| CIH6 Group | 8 | 5.80 ± 0.19a | 4.60 ± 0.35b | 1.19 ± 0.09c |
| CIH8 Group | 8 | 6.68 ± 0.61a | 6.19 ± 0.97b | 1.83 ± 0.29c |
| F value | | 116.185 | 45.189 | 110.876 |
| *P* value | | < 0.001 | < 0.001 | < 0.001 |

*NC group* normal control group, *CIH2 group* Chronic intermittent hypoxia 2- week group, *CIH4 group* Chronic intermittent hypoxia 4-week group, CIH6 chronic intermittent hypoxia 6-week group, *CIH8* Chronic intermittent hypoxia 8-week group, *HOMA-IR* Insulin Resistance Index; Pairwise comparison of blood glucose levels between groups, $^{a}P < 0.05$; Comparison of insulin levels between groups with $^{b}P < 0.05$; Comparison between HOMA-IR groups pairwise, $^{c}P < 0.05$.

**Table 2** The effect of CIH on the average grayscale values of IRS-2, P-Akt, and GSK-3 proteins in each group of rats ($\bar{x} \pm s$)

| Number of group | cases | IRS-2 | P-Akt | GSK- 3 |
|---|---|---|---|---|
| NC Group | 8 | 166.60 ±3.95a | 162.49 ±2.62b | 197.30 ±4.23c |
| CIH2 Group | 8 | 172.07 ±4.91a | 172.62 ±3.12 b | 184.22 ±4.19c |
| CIH4 Group | 8 | 177.77 ±5.31a | 180.89 ±3.38b | 180.54 ±2.33c |
| CIH6 Group | 8 | 185.43 ±6.14a | 189.02 ±3.34b | 175.01 ±3.62c |
| CIH8 Group | 8 | 195.40 ±4.27a | 197.11 ±5.8b | 163.79 ±3.22c |
| F value | | 41.370 | 99.5509 | 94.019 |
| *P* value | | < 0.001 | < 0.001 | < 0.001 |

*IRS-2* Insulin receptor base-2, *P-AKT* phosphorylated protein kinase B, *GSK-3* Glycogen synthase kinase-3, *NC group* Normal control group, *CIH2 group* Chronic intermittent hypoxia 2-week group, *CIH4 group* Chronic inter- mittent hypoxia 4-week group, *CIH6* Chronic intermittent hypoxia 6-week group, *CIH8* Chronic intermittent hypoxia 8-week group; Pairwise comparison of grayscale values between IRS-2 groups, $^{a}P <$ ¡0.05; The pairwise comparison of grayscale values between P-AKT groups is $^{b}P < 0.05$; Comparison of gray val- ues between GSK-3 groups with$^{c}P < 0.05$.

**Table 3** Correlation analysis between HOMA-IR and average grayscale values of IRS-2, P-Akt, and GSK-3

| Group | Pearson correlation coefficient (r) | P value |
|---|---|---|
| HOMA-IR and IRS-2 | 0.867 | < 0.001 |
| HOMA-IR and P-Akt | 0.903 | < 0.001 |
| HOMA-IR and GSK-3 | −0.855 | < 0.001 |

*HOMA-IR* Insulin Resistance Index, *IRS-2* Insulin receptor base-2, *P-AKT* phosphorylated protein kinase B, *GSK-3* Glycogen synthase kinase-3

## Immunohistochemistry technique

### Expression of IRS-2

Immunohistochemical analysis under light microscopy revealed that IRS-2 was expressed in the cytoplasm of rat liver cells, appearing as scattered or focal brown granules. The expression of IRS-2 was more pronounced in the cytoplasm of endothelial cells in the portal area (Fig. 2).

Quantitative analysis of the images showed that compared to the control group, the average grayscale value of IRS-2 protein in liver cells of each experimental group gradually increased with the increase in intermittent hypoxia exposure time. The CIH8 group exhibited the highest grayscale value, followed by the CIH6, CIH4, CIH2, and NC groups. Protein expression decreased slightly, and this difference was significant ($P < 0.05$).

### Expression of P-Akt

Immunohistochemical analysis under light microscopy demonstrated that P-Akt was expressed in the cytoplasm of liver cells in the portal area of rats (Fig. 3. Quantitative analysis of the images revealed differences between each experimental group and the control group. With the increase in intermittent hypoxia exposure time, the average gray value of P-Akt protein in liver cells of each group gradually increased. The CIH8 group exhibited the highest levels, followed by the CIH6, CIH4, CIH2, and NC groups. Protein expression decreased slightly, and this difference was significant ($P < 0.05$).

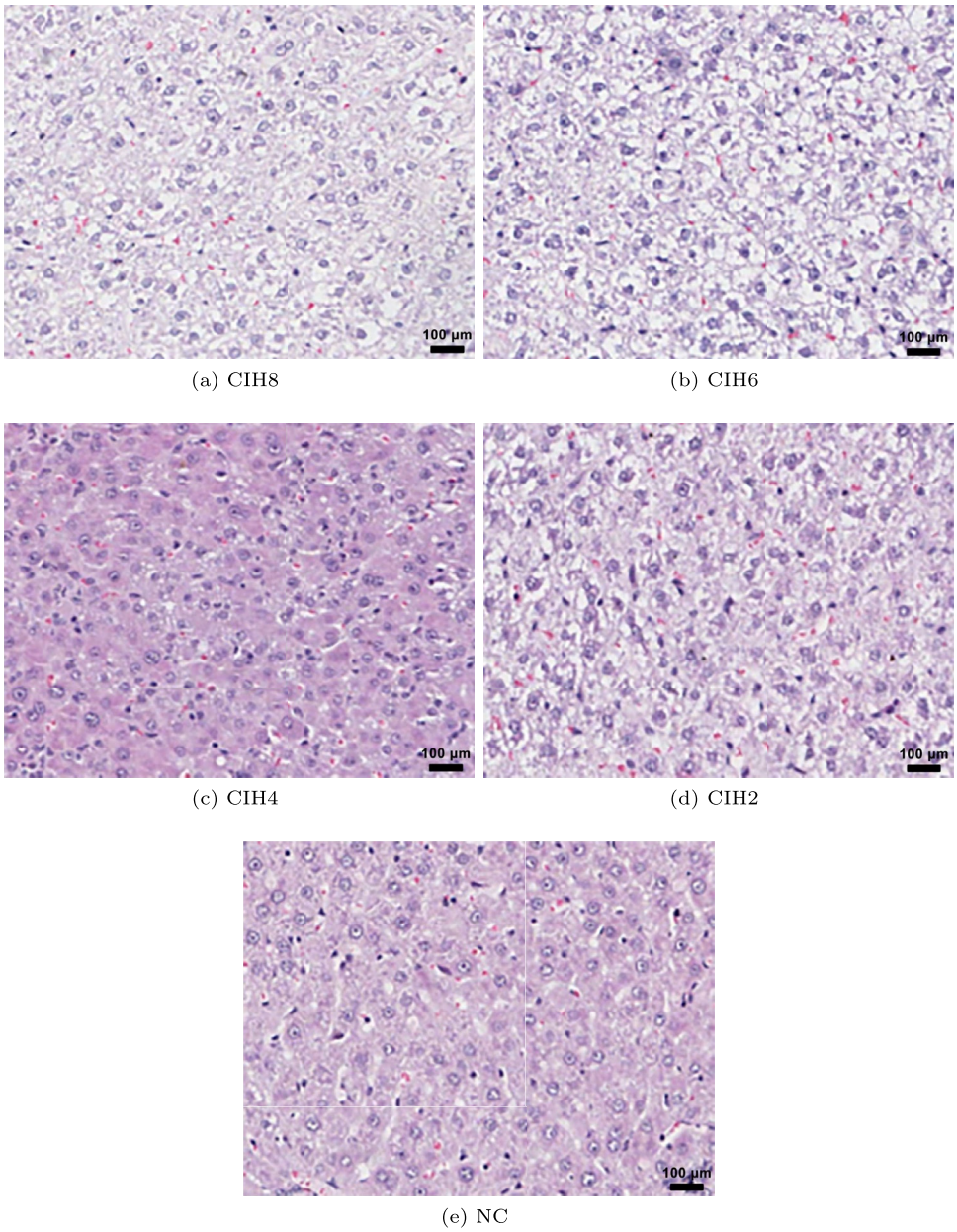
### Expression of GSK-3

Under light microscopy, immunohistochemistry results revealed that GSK-3 was expressed in the nuclei of liver cells, with scattered or focal brownish-yellow granules present in the nucleus. A small amount of scattered brownish-yellow granules was also expressed in the cytoplasm, as shown in Fig. 4. Quantitative analysis of the images demonstrated that, compared to the control group, the average gray value of GSK-3 protein in liver cells gradually decreased with increasing intermittent hypoxia exposure time. Specifically, the protein expression significantly increased in the following order: CIH8 grou$P <$ CIH6 grou$P <$ CIH4 grou$P <$ CIH2 grou$P <$ NC group ($P < 0.05$).

## Discussion

Currently, OSAHS is closely associated with diabetes. Approximately 15%−30% of OSAS patients suffer from T2DM, and most T2DM patients also exhibit obstructive sleep apnea [6, 13]. Experimental studies have confirmed the negative impact of OSA or chronic intermittent hypoxia on glucose metabolism, which is linked to insulin resistance [14]. Furthermore, the severity of OSAHS positively correlates with insulin resistance [15, 16], while prolonged hypoxia exceeding 6 weeks solely affects fasting blood glucose levels without influencing insulin sensitivity or glucose tolerance [17]. In our study, as demonstrated in Table 1, compared to the control group, with extended exposure time of SD rats, fasting blood glucose and insulin concentration levels rose, leading to insulin resistance, which was notably pronounced in the CIH8-week group.

How does chronic intermittent hypoxia induce glucose metabolism disorders and lead to insulin resistance? Some studies have confirmed that chronic intermittent hypoxia




(a) CIH8 (b) CIH6

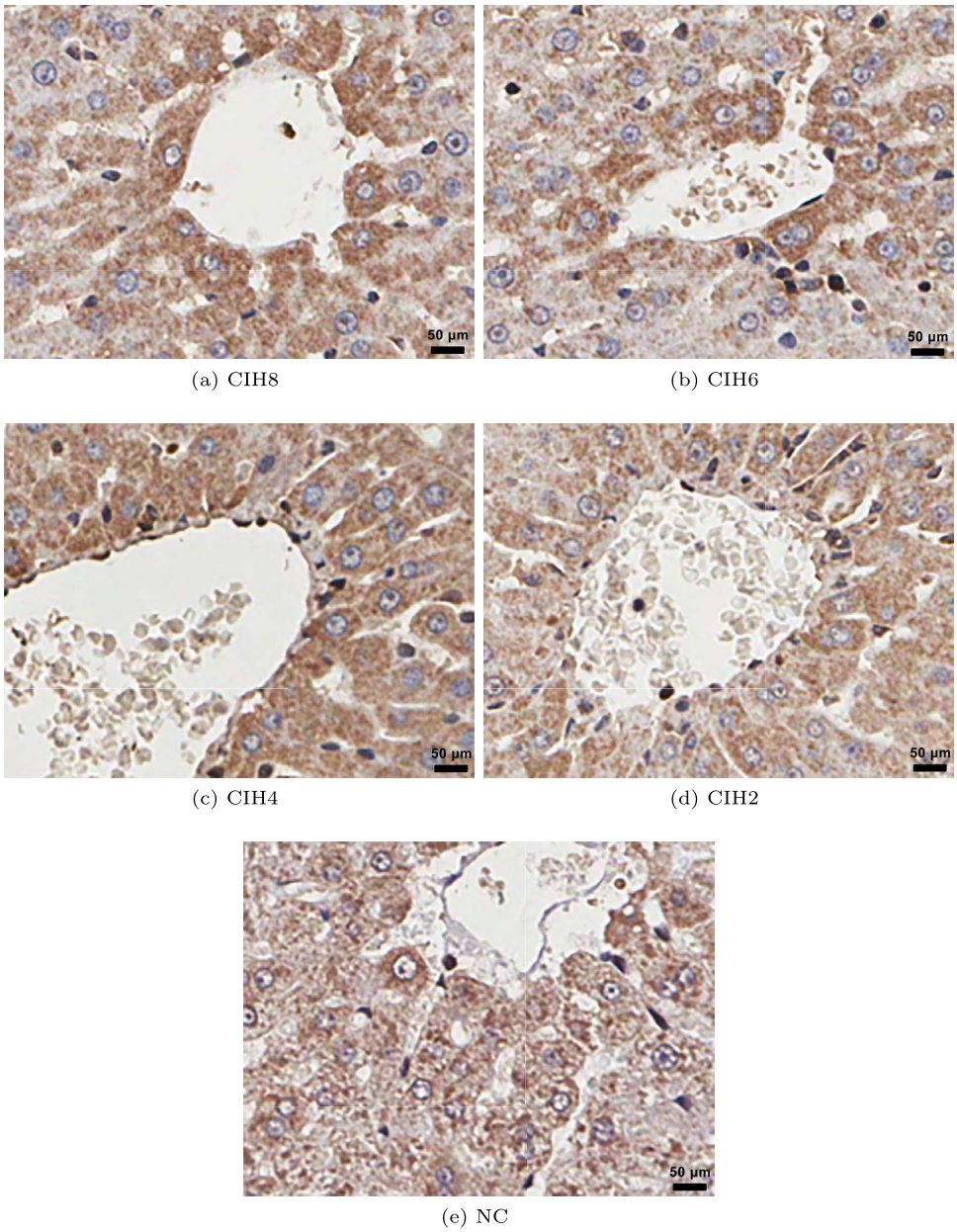
(c) CIH4 (d) CIH2

(e) NC

**Fig. 1** Observation of morphological changes in liver cells of rats in each group using HE staining method Under the light microscope, the morphology of liver cells in each group can be seen by HE staining ×20

repeatedly induces hypoxia and reoxygenation, generating a large amount of oxidative products and systemic oxidative stress. This alters the function of biofilms, potentially damaging insulin-sensitive target tissue cells, resulting in insulin cell dys- function and ultimately diabetes [18, 19]. Savransky et al. [20]. discovered that after 12 weeks of exposure to chronic intermittent hypoxia in rats, liver cells suffered dam- age, as evidenced by an increase in the damage marker ALT. They hypothesized that the accumulation of liver glycogen contributed to a second round of liver cell dam- age. Recent studies have shown that CIH can cause liver enzyme damage, steatosis, inflammatory response, and liver fibrosis through various mechanisms [21–23]. This aligns with our research findings, as illustrated in Fig. 1. We observed significant liver cell membrane damage, cytoplasmic looseness, cell edema, and inflammatory cell infil- tration with prolonged exposure to intermittent hypoxia, particularly evident in the CIH8 week group.

Current research from abroad has revealed that intermittent hypoxia can induce glucose metabolism disorders. Consequently, we examined alterations in proteins related to the insulin signaling pathway under intermittent hypoxia and explored their potential mechanisms. The insulin-mediated PI3 K/AKT signaling pathway serves as the primary postreceptor transduction pathway and plays a crucial role in the compensatory function of pancreatic beta cells. Recent studies have identi- fied the PI3 K/AKT signaling pathway as a critical component in regulating glucose metabolism [24]




(a) CIH8 (b) CIH6

(c) CIH4 (d) CIH2

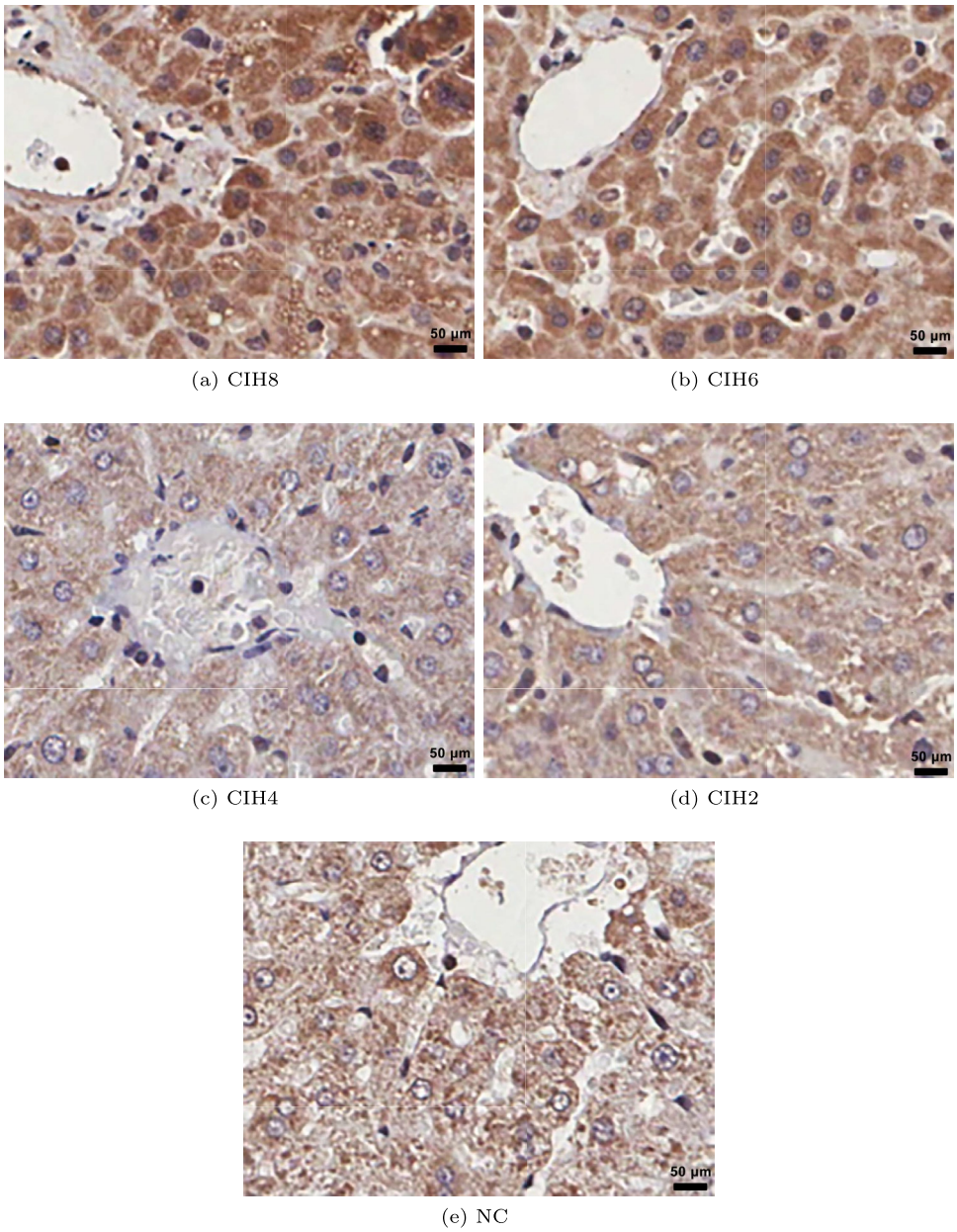
(e) NC

**Fig. 2** Expression and distribution of IRS-2 Under light microscopy, IRS-2 expression in liver cells of each group can be observed. Immunohistochemical staining was performed at a magnification of ×40

and forming the foundation for maintaining insulin levels and blood glucose balance. This pathway encompasses a series of signaling cascades.

Initially, insulin binds to the insulin receptor (IRS), phosphorylating tyrosine residues on downstream substrate molecules. The IRS family (IRS-1, 2, 3, 4) pre- dominantly contributes to insulin-regulated metabolic signal transduction. Chronic intermittent hypoxia has been demonstrated to decrease the phosphorylation level of IRS2 via multiple pathways, including oxidative stress, disruption of mitochondrial function, and endoplasmic reticulum stress. Upon phosphorylation, the IRS protein becomes capable of binding to proteins with SH2 domains, such as Grb2, SHP2 pro- tein tyrosine phosphatase, and phosphatidylinositol 3-kinase (PI3 K). The activation of PI3 K phosphorylation subsequently leads to the activation of the key downstream protein AKT in the PI3 K signaling pathway [25, 26]. The PI3 K/AKT signaling path- way plays a crucial role in the insulin signaling cascade and glucose transport [27]. Recent research has indicated a significant reduction in PI3 K activity in the skeletal muscle of patients with type 2 diabetes [28, 29].

Our study revealed that in rats exposed to intermittent hypoxia, as the duration of intermittent hypoxia increased, the expression of insulin signaling pathway effec- tor proteins IRS-2 and P-Akt in the liver decreased compared to the control group. The expression levels followed the order of CIH8 grou$P$ < CIH6 grou$P$ < CIH4 group < CIH2 grou$P$ < NC group, with significant differences ($P$ < 0.001).




(a) CIH8 (b) CIH6

(c) CIH4 (d) CIH2

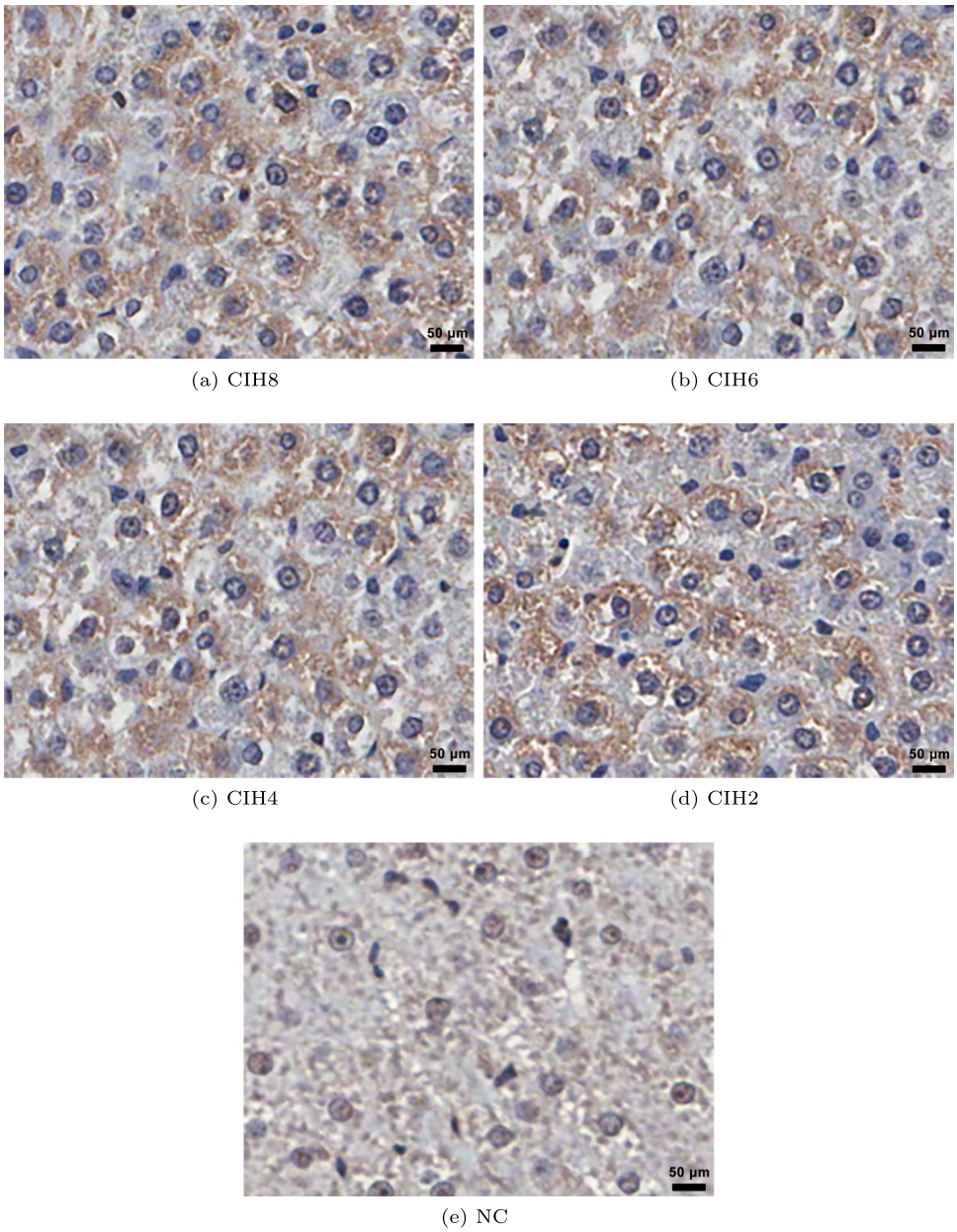
(e) NC

**Fig. 3** Expression and Distribution of P-Akt. Under light microscopy, the expression of P-Akt in liver cells was observed in each group. Immunohistochemical staining was performed at a magnification of ×40

Additionally, blood glucose levels rose and insulin resistance became evident. This suggests that intermittent hypoxia may impact liver glucose metabolism function by influencing complex signaling pathways. Correlation analysis also revealed a positive correlation ($P < 0.001$) between HOMA-IR and the average gray values of IRS-2 and P-Akt, indicating that the expression of IRS-2 and P-Akt is suppressed under CIH conditions. Taken together with the experimental findings of this study, it suggests that intermit- tent hypoxia may interfere with the phosphorylation of PI3 K/AKT signaling proteins in the insulin signaling pathway, ultimately leading to abnormal glucose metabolism.

GSK-3, as a limiting enzyme in glycogen synthesis, plays a crucial role in regulat- ing intracellular glucose transport and glycogen production. Notably, during insulin resistance, glycogen synthesis tends to decrease. Through the PI3 K/AKT signaling pathway, insulin can induce phosphorylation at specific sites on GSK-3, leading to its inactivation. Subsequently, this phosphorylation cascade effect causes the dephos- phorylation of glycogen synthase (GS), ultimately activating glycogen and protein synthesis, and reducing blood glucose levels [30]. Recent research indicates an increased expression of muscle cells or epididymal adipocytes in obese Zucker rats, ob/ob mice, diet-induced insulin resistance mice, or type 2 diabetes animal models [31, 32].

In our study, we discovered that the expression of GSK-3 protein in the liver of rats exposed to intermittent hypoxia increased with prolonged exposure time compared to the




(a) CIH8 (b) CIH6

(c) CIH4 (d) CIH2

(e) NC

**Fig. 4** Expression and Distribution of GSK-3. Under light microscopy, the expression of GSK-3 in liver cells was observed in each group. Immunohistochemical staining was performed at a magnification of ×40

control group. The expression levels were as follows: CIH8 group > CIH6 group > CIH4 group > CIH2 group > NC group, with the CIH8 group showing significant differences ($P < 0.001$). Correlation analysis further revealed a negative correlation between the average grayscale values of HOMA-IR and GSK-3, indicating that GSK-3 expression was activated in the CIH environment, accompanied by a decrease in the levels of IRS-2 and P-Akt. This suggests that CIH has disrupted the insulin signal- ing pathway, which may ultimately have adverse effects on glucose metabolism. Our research primarily focuses on in vivo animal experiments, and the number of experi- mental rats selected is limited. We need to observe the changes in PI3 K/AKT/GSK-3 over a longer period of time, and explore the factors influencing intermittent hypoxia, its corresponding protein expression, and their interrelationships in future studies.

In summary, further scientific research is needed to confirm how PI3 K/AKT/GSK-3 specifically leads to abnormal glucose metabolism under the influence of CIH.

## Conclusion

Our research revealed that CIH can damage rat liver cells, leading to glu- cose metabolism disorders and insulin resistance. The potential mechanism involves regulating the expression of the PI3 K/AKT/GSK-3 pathway mediated by insulin. Nevertheless, the role of this signaling pathway in

the pathogenesis of diabetes is still in its early stages, and its precise mechanism of action remains unclear. Further inves- tigation could shed light on the pathogenesis of OSA combined with diabetes and identify new treatment targets.

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**Author contribution** Hong Wang was involved in conceptualization, writing original draft, organizing the contents of the manuscript and reviewing. Tiantian Guo performed the writing, formating and editing.

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**Data availability** The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

## Declarations

**Conflict of interest** All authors certify that they have no affiliations with or involve- ment in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or pro- fessional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

**Ethical approval** All applicable international, national, and/or institutional guide- lines for the care and use of animals were followed. The approval number is HNHTYY20250326LLSH-04–01.



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