

Original Article

The effects of chronic hypoxia on the endocrine-related parameters in elderly rats with type 2 diabetes mellitus

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Abstract: Objective: This study aimed to investigate the effects of chronic hypoxia on the endocrine-related parameters in elderly rats with type 2 diabetes mellitus. Methods: Sixty, 55-week-old, female SD rats were studied, among which 20 were randomly divided into a blank control group (the BCG), and 40 were intraperitoneally injected with low-dose streptozotocin (STZ) for modeling and divided into a model control group (the MCG, n=20) and a hypoxic group (the HG, n=20). The BCG and the MCG were fed routinely under normoxia, while the HG was fed in the hypoxic environment of an OxyCycler Model A84XO hypoxic chamber. After 12 weeks of intervention, the body weights, the fasting blood glucose (FBG) levels, the fasting insulin (FINS) levels, the blood lipid metabolism levels, the bone densities, the fresh weights of the femurs, the biomechanical properties of the femurs, the inflammatory factor levels, the H&E staining of the liver tissue, and the oil red O staining were compared. Results: The increases in weight gain and the FBG and FINS levels in the HG were lower than the corresponding levels in the MCG and were higher than the levels in the BCG ($P<0.05$). The TC, TG, and LDL-C levels in the HG were lower than they were in the MCG and higher than they were in the BCG ($P<0.05$). The bone density and fresh weight of the femurs at 12 weeks after the intervention in the HG were higher than they were in the MCG and lower than they were in the BCG ($P<0.05$). The maximum stress, maximum load, fracture load, and elastic modulus in the HG were higher than they were in the MCG and lower than they were in the BCG ($P<0.05$). The TNF- α , IL-6, PAI-1, and CRP levels in the HG were lower than they were in the MCG and higher than they were in the BCG ($P<0.05$). Conclusion: Chronic hypoxia can improve the endocrine parameters and insulin resistance, improve the insulin sensitivity and the femoral biomechanics, reduce the inflammatory factors levels, and improve the glucose and lipid metabolism levels and liver function in elderly rats with type 2 diabetes.

Keywords: Chronic hypoxia, type 2 diabetes, endocrine parameters

Introduction

Type 2 diabetes, also known as adult onset diabetes, occurs in people aged 35-40 years and accounts for more than 90.0% of diabetes cases [1]. However, patients with type 2 diabetes do not completely lose insulin secretion capacity, but are in a relatively deficient state [2]. Studies have shown [3] that type 2 diabetes is more common in obese, middle-aged and elderly people and people with a family history of diabetes, with clinical manifestations such as polyphagia, polyuria, fatigue, etc. It is called the "third biggest killer" threatening human health after tumors and cardiovascular and cerebrovascular diseases [4]. Persistent hyperglycemia in patients with type

2 diabetes not only affects their health, but it also increases the burdens on society and family, as well as the rates of hyperlipidemia, obesity, and atherosclerosis [5]. At present, there is still a lack of radical treatment for diabetes mellitus, and diet control, medication, and exercise are recognized as the three major effective measures to prevent and treat diabetes. Studies have shown [6] that non-drug therapy has better compliance and is a more economical and side-effect-free intervention than long-term drug therapy, which may lead to side effects and dependence. However, elderly patients have a poor long-term prognosis due to older age and the associated underlying diseases.

Chronic hypoxia and the endocrine levels in elderly diabetic rats

Table 1. The instruments and reagents

Instruments and reagents	Manufacture
High-speed refrigerated centrifuge (5810R)	High-speed refrigerated centrifuge (5810R)
RM223 5 Paraffin Wax Slicer	Leica, Germany
Low temperature refrigerator	Thermo Forma, USA
Neutral gum polyformaldehyde	Shanghai Model Factory, China
Sumac (H8070)	Fuzhou Dingguo Biotechnology Co.
Anhydrous ethanol	Solarbio China
DH3 600 1 B Electrically heated thermostatic incubator	Shanghai Bioengineering & Biological Corporation, China
Enzyme-linked immunosorbent assay kits	Tianjin Tester, China
FINNPIPTE Adjustable Pipettes	Beijing Zhong Shan Jin Qiao Biotechnology Co.
Hypoxic chamber, OxyCycler Model A84XO	Thermo Scientific Inc., USA
Dual-energy X-ray bone densitometer	USA
Material testing machine, SUN1000 V669	Faxitron Ultrafocus 100, USA
	GALDABINI, Italy

The occurrence and development of type 2 diabetes is a multifactorial process, a process mainly related to genetic factors, environmental factors, age, and lifestyles, etc., among which environmental factors play a key role [7]. Chronic hypoxia refers to the long-term and continuous exposure to a certain degree of hypoxia in humans or animals. A study has shown [8] that chronic hypoxia improves the hypoxic tolerance of tissues and is beneficial to many tissues in other organs, including the heart, liver, and nerves. A study has found [9] that chronic hypoxia can improve the myocardial resistance to ischemia and hypoxia and play a good cardioprotective role. Meanwhile, chronic hypoxia can improve the conditions of immune diseases (including allergic dermatitis and bronchial asthma in children), and promote the stability and recovery of immune function in patients with allergic diseases. Moreover, chronic hypoxia can be used to enhance the body's tolerance to hypoxia and it is widely used in chronic diseases, such as type 2 diabetes [10]. Therefore, the present study was conducted on 55-week-old female SD rats to investigate the effects of chronic hypoxia on the endocrine-related parameters of elderly rats with type 2 diabetes (ERT2D).

Materials and methods

Animal data

Sixty female 55-week-old, SD rats, with body weights ranging from 180-220 g, and with an average weight of (200.00±10.00) g, were selected as the experimental animals. Animal

certificate number: SCXX-2005-0001. Among the rats, 20 were included in a blank control group (the BCG), and 40 were fed a high-fat diet, and type 2 diabetes was induced using intraperitoneal injections of low-dose streptozotocin (STZ). The success of the modeling was determined by using glucose tests and glucose tolerance tests. Next, the 40 model rats were randomized into a model control group (the MCG, n=20) and a hypoxic group (the HG, n=20). These animals were placed in the animal chamber and were routinely fed at 24-26°C in a 12 h light and 12 h dark cycle, and they could eat and drink freely.

Instruments and reagents

The instruments and reagents are listed in **Table 1**.

Methods

(1) Modeling method. The twenty rats in the blank control group were not involved in the modeling. Forty rats were modeled with low-dose STZ injections to induce type 2 diabetes. After one week of adaptive feeding, the rats were modeled from the second week. The rats were continuously fed a high-sugar and high-fat diet (20 KJ/g), including 65% basic feed, 25% refined lard, 5% sucrose, and 5% milk powder, for 6 weeks, and they received a single dose of 30 mg/kg STZ (Qingdao Jie Shi Kang Co., Ltd.). The STZ was dissolved in a 0.1 mmol/L, pH 4.4 citric acid-sodium citrate buffer, and the final concentration of the solution was adjusted to 1% [11]. (2) Modeling success criteria. The suc-

Chronic hypoxia and the endocrine levels in elderly diabetic rats

Table 2. Comparison of the weight gain and the FBG and FINS levels ($\bar{x} \pm s$)

Group	Number	Weight gain (g)	FBG (mmol/L)	FINS (mIU/L)
Hypoxia group	20	25.79±3.96 ^{a,b}	13.26±2.31 ^{a,b}	39.44±4.31 ^{a,b}
Model control group	20	35.94±4.85 ^a	20.69±4.51 ^a	56.78±6.39 ^a
Blank control group	20	20.29±3.23	8.45±0.45	35.69±3.22

Compared with the blank control group, ^a $P < 0.05$; compared with the model control group, ^b $P < 0.05$.

cess of the modeling was determined through blood glucose tests and random blood glucose level checks. At 72 h after the STZ injections, a random blood glucose level ≥ 16.67 mmol/L or a fasting blood glucose ≥ 11.1 mmol/L indicated that the modeling was successful. (3) Post-modeling grouping and processing methods. They were randomized into the MCG ($n=20$) and the HG ($n=20$) following the successful modeling. The BCG and the MCG were routinely fed under normoxia (protein 21%, fat 4.5%, carbohydrates 62.6%, fiber and other 11.9%). The HG was raised in an OxyCycler Model A84XO hypoxia chamber (oxygen fraction of 16%, equivalent to 2400 m altitude). Each group completed 12 weeks of intervention. This study was approved by the Animal Ethics Committee of Taizhou Hospital of Integrated Traditional Chinese and Western Medicine.

Outcome measurement

(1) Three groups of body mass, FBG, and FINS. At 12 weeks after the intervention, the three groups were weighed. Five rats were taken from each group, and 4 mL of blood was drawn from their abdominal aortas, and their FBG levels were determined using the test-paper after centrifugation. The rats' FINS levels were determined using enzyme-linked immunosorbent assay [12, 13]. (2) Lipid metabolism levels. The above isolated serum samples were collected. The total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-C), and low-density lipoprotein (LDL-C) levels [14] were measured with an automatic biochemical analyzer. (3) Bone density and fresh weights of the femurs. The bone density was measured using a dual-energy X-ray bone densitometer before and at 12 weeks after the intervention. Five rats were taken from each group and sacrificed by breaking their necks, and their femurs were taken and accurately weighed using a balance [15, 16]. (4) Femoral biomechanical level. Five rats were taken from each group and sacrificed by cervical dislocation. Their femurs were taken

to measure the maximum stress, maximum load, fracture load, and elastic modulus levels [17, 18]. (5) Inflammatory factor levels. The TNF- α , IL-6, and PAI-1 levels were calculated using an enzyme-linked immunosorbent assay. The C-reactive protein (CRP) levels were determined using the immunoturbidimetric method [19]. (6) H&E staining and oil red O staining of liver. The liver tissues were obtained and placed in a 10.0% formalin solution, and 5 μ m sections were prepared routinely after paraffin embedding. The H&E staining and Oil Red O staining were performed to observe the distribution of the fat droplets and blood vessels in the liver tissues [20].

Statistical analysis

The data were processed using SPSS 18.0. The count data were expressed as n (%) and were examined using χ^2 tests, and the measurement data was expressed as ($\bar{x} \pm s$) and were compared using t tests. $P < 0.05$ indicated a significant difference.

Results

Comparison of the weight gain and the FBG and FINS levels among the three groups

The weight gain and the FBG and FINS levels in the HG were lower than they were in the MCG and higher than they were in the BCG ($P < 0.05$). The weight gain and the FBG and FINS levels in the BCG were lower than they were in the MCG ($P < 0.05$, **Table 2**).

Comparison of the lipid metabolism levels among the three groups

The HDL-C levels did not differ significantly among the three groups ($P > 0.05$). The TC, TG and LDL-C levels in the HG were lower than they were in the MCG but higher than they were in the BCG ($P < 0.05$). The TC, TG and LDL-C levels in the BCG were lower than they were in the MCG ($P < 0.05$, **Table 3**).

Chronic hypoxia and the endocrine levels in elderly diabetic rats

Table 3. Comparison of the lipid metabolism levels (mmol/L, $\bar{x} \pm s$)

Group	Number	TC	TG	HDL-C	LDL-C
Hypoxia group	20	2.04±0.47 ^{a,b}	1.35±0.36 ^{a,b}	1.20±0.25	0.69±0.15 ^{a,b}
Model control group	20	2.96±0.69 ^a	2.04±0.41 ^a	1.19±0.25 ^a	1.06±0.21 ^a
Blank control group	20	1.95±0.41	1.12±0.32	1.18±0.24	0.38±0.08

Compared with the blank control group, ^a $P < 0.05$; compared with the model control group, ^b $P < 0.05$.

Table 4. Comparison of the bone densities and fresh femur weights ($\bar{x} \pm s$)

Group	Number	Bone density (g/cm ²)		Weight of fresh femur (g)
		Pre-intervention	12 weeks after intervention	
Hypoxia group	20	0.245±0.037	0.221±0.032 ^{a,b}	1.13±0.22 ^{a,b}
Model control group	20	0.246±0.037	0.186±0.026 ^a	0.96±0.20 ^a
Blank control group	20	0.245±0.036	0.243±0.034	1.20±0.25

Compared with the blank control group, ^a $P < 0.05$; compared with the model control group, ^b $P < 0.05$.

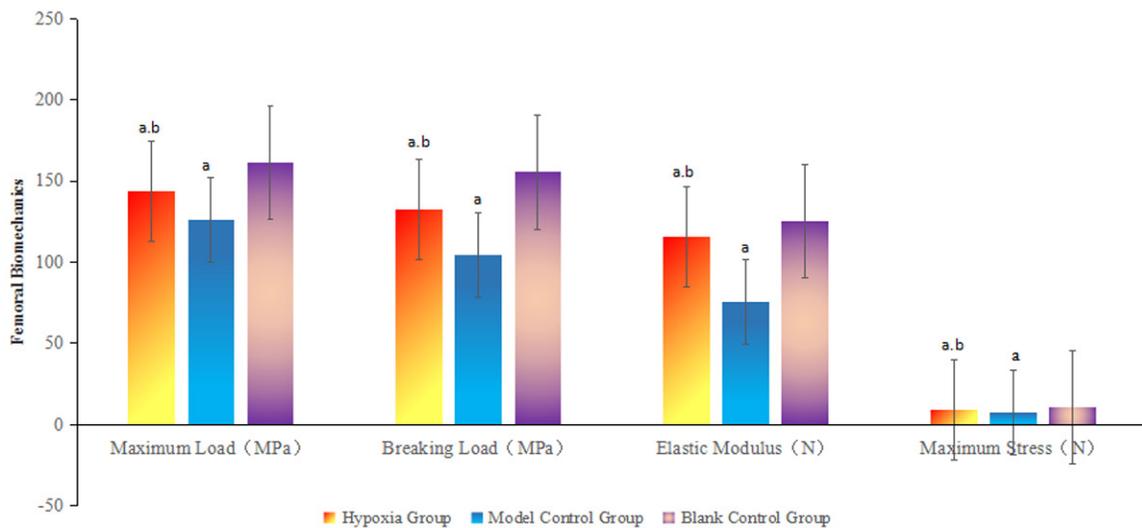


Figure 1. Comparison of the femoral biomechanical levels.

Comparison of bone densities and fresh femur weights

The bone density levels in the three groups showed no significant differences before the intervention ($P > 0.05$). At 12 weeks after the intervention, the bone density and fresh femur weights in the HG were higher than they were in the MCG and lower than they were in the BCG ($P < 0.05$), and they were lower in the MCG than they were in the BCG ($P < 0.05$, **Table 4**).

Comparison of the biomechanical properties of the femurs

The maximum stresses, maximum loads, fracture loads, and elastic moduli of the HG were higher than they were in the MCG and lower

than they were in the BCG ($P < 0.05$), and they were higher in the BCG than they were in the MCG ($P < 0.05$, **Figure 1**).

Comparison of the inflammatory factors

The TNF- α , IL-6, and PAI-1 levels and the CRP in the HG were lower than they were in the MCG and higher than they were in the BCG ($P < 0.05$). The TNF- α , IL-6, and PAI-1 levels and the CRP in the BCG were lower than they were in the MCG ($P < 0.05$, **Table 5**).

Comparison of the hepatic H&E and the Oil Red O staining

The MCG had different sizes of fat droplets and a few capillaries. The HG had a few fat droplets

Chronic hypoxia and the endocrine levels in elderly diabetic rats

Table 5. Comparison of the inflammatory factors ($\bar{x} \pm s$)

Group	Number	TNF- α (ng/L)	PAI-1 (ug/L)	CRP (mg/L)	IL-6 (ug/L)
Hypoxia group	20	80.39 \pm 4.34 ^{a,b}	9.47 \pm 0.69 ^{a,b}	30.23 \pm 1.21 ^{a,b}	2.53 \pm 0.39 ^{a,b}
Model control group	20	97.49 \pm 6.56 ^a	15.39 \pm 3.12 ^a	36.69 \pm 4.31 ^a	3.23 \pm 0.44 ^a
Blank control group	20	65.23 \pm 5.39	5.41 \pm 0.78	12.45 \pm 1.21	1.76 \pm 0.32

Compared with the blank control group, ^a $P < 0.05$; compared with the model control group, ^b $P < 0.05$.

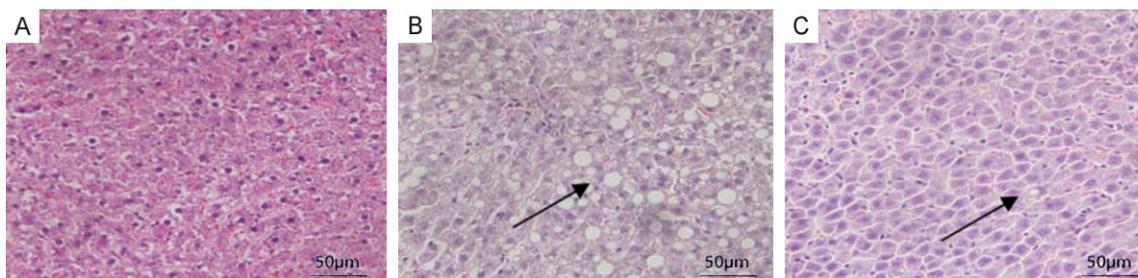


Figure 2. Comparison of the H&E staining results ($\times 200$). Note: A shows the H&E staining results of the blank control group; B shows the H&E staining results of the model control group; C shows the H&E staining results of the hypoxic group (the arrows in the figure point to fat droplets).

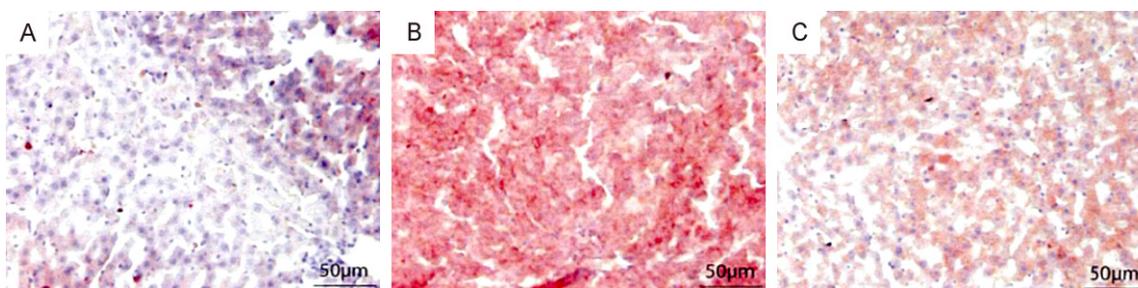


Figure 3. Comparison of the Oil Red O staining results of the three groups ($\times 200$). Note: A shows the results of the Oil Red O staining in the blank control group; B shows the results of the Oil Red O staining in the model control group; C shows the results of the Oil Red O staining in the hypoxia group.

and a large number of capillaries. No fat droplets and a large number of capillaries were observed in the BCG (**Figures 2 and 3**).

Discussion

Diabetes mellitus is a serious threat to human health as a non-infectious disease. It is clinically manifested as insufficient insulin secretions and/or defective insulin action, leading to metabolic disorders and persistent hyperglycemia [21]. A study has shown [22] that patients with diabetes mellitus will develop severe macrovascular and microvascular complications as the disease progresses, leading to organ dysfunction and metabolic disorders, especially structural abnormalities of the bone tissue and dysfunction of the bone metabolism. Therefore,

the early prevention and treatment of type 2 diabetes is of great significance for improving patient prognosis [23, 24].

A study indicated [25] that the incidence rate of diabetes is relatively low in residents who live in high altitude areas for long periods of time. Scholars have found the incidence of type 2 diabetes in the Aymara people who live at high altitudes low. A study also showed [26] that hiking in the highlands helps reduce the fasting glucose, fasting insulin, and cholesterol levels in patients with type 2 diabetes. All of these findings suggest that hypoxia in the plateau may help improve the symptoms of type 2 diabetes, but this conclusion has not been confirmed. In this study, chronic hypoxia intervention was given to type 2 diabetic rats, and the

Chronic hypoxia and the endocrine levels in elderly diabetic rats

results showed that the weight gain, FBG, and FINS levels in the HG were lower than they were in the MCG and higher than they were in the BCG ($P<0.05$). The HDL-C levels exhibited no significant differences among the three groups ($P>0.05$). The TC, TG, and LDL-C levels in the HG were all lower than they were in the MCG and higher than they were in the BCG ($P<0.05$), indicating that chronic hypoxia can reduce blood glucose levels, improve lipid levels, and result in a good prognosis. Previous studies have shown that sustained chronic hypoxia can improve the hypoxia tolerance of tissues and help improve cardiac, neurological, and liver functions [27]. At the same time, sustained chronic hypoxia can improve the myocardial anti-ischemia and hypoxia abilities and play a good role in myocardial protection. However, a chronic hypoxic environment can enable rats to adapt to the new environment, regulate the glucose metabolism and improve their blood glucose tolerance. A study revealed [28] that blood glucose is sourced from intestinal absorption, the breakdown of glycogen, and gluconeogenesis. Chronic sustained hypoxia can increase GLUT4 expressions on the cell membranes of a striated muscle fiber cells, increase the rate of glucose uptake, and increase the level of glucose in the skeleton, contributing to the enhancement of glucose metabolism [29]. In this study, the bone density and fresh femur weight levels at 12 weeks after the intervention in the HG were higher than they were in the MG and lower than they were in the BCG. The maximum stress, maximum load, fracture load, and elastic modulus in the HG were higher than they were in the MCG and lower than they were in the BCG, indicating that the effect of chronic hypoxia on the air-bone density and biomechanics in the elderly rats with type 2 diabetes was relatively small.

The effects of hypoxia on the human body are determined by many factors, such as the degree, duration, and form of the hypoxia, which is often accompanied by a co-involvement of the inflammatory factors [30]. In the present study, the TNF- α , IL-6, and PAI-1 levels and the CRP in the HG were lower than they were in the MCG and higher than they were in the BCG ($P<0.05$), indicating that high expressions of the inflammatory factors often accompany the process in ERT2D, and their expression levels are related to the severity of the

disease condition in rats. The CRP levels are elevated due to body tissue damage or inflammatory stimulation, which is a kind of acute phase protein produced by the liver, while TNF- α and IL-6 affect the CRP levels. Studies have shown that CRP can directly induce endothelial cells to produce PAI-1 and increase the expression of its active nitric oxide synthase, which helps to accelerate atherosclerosis. Persistent hypertension will induce the endothelial cells to produce PAI-1 in vivo, increase its activity, and inhibit the expression levels of nitric oxide synthase [31]. From the results of this study, it can be seen that chronic hypoxia can improve the endocrine indexes of ERT2D and reduce the inflammatory factor levels, which can fundamentally control the occurrence and development of the disease and provide new ideas for the treatment of diabetes. In this study, through H&E staining and nuclear Oil Red O staining, we found that the chronic hypoxic environment helps to improve the liver function and reduce fat accumulation in the liver. However, there are also many limitations to this study. On the one hand, the number of included rats was relatively small, so the results need to be further verified by experiments using a larger sample size. On the other hand, there are large human errors in rat modeling and data processing, so our findings need to be further studied and discussed.

In summary, chronic hypoxia can improve the endocrine indexes and insulin resistance and improve the insulin sensitivity and femoral biomechanics in ERT2D without affecting the bone density, reduce the inflammatory factors, promote glucose lipid metabolism and liver function, and provide new strategies for the prevention and treatment of type 2 diabetes.

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Disclosure of conflict of interest

None.

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Chronic hypoxia and the endocrine levels in elderly diabetic rats

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Chronic hypoxia and the endocrine levels in elderly diabetic rats

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