

Original Article

The anti-hypoxic effects of oat (*Avena sativa* L.) oligopeptides in mice

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Abstract: Objective: To explore the anti-hypoxic effects of oat oligopeptides (OOPs) in mice. Methods: We randomly divided mice into six groups, including a vehicle control group, a whey protein group (0.50 g/kg), and four OOPs-treated groups (0.25, 0.50, 1.00, and 2.00 g/kg). The test substances were administered by gavage once a day for 30 days. The normobaric hypoxia, sodium nitrite toxicosis, and acute cerebral ischemia survival times were recorded. Also, the MDA content, the lactate levels, the LDH activity, and the mRNA levels of HIF-1 α and VEGF in the brains were measured. We performed a whole blood cell analysis using a blood analyzer. Results: The OOPs significantly extended the survival times of normobaric hypoxia, sodium nitrite toxicosis, and acute cerebral ischemia. Notably, the OOPs enhanced the RBC, Hb, and Hct levels, decreased the malonaldehyde (MDA) and lactate content in the brain, enhanced the brain lactate dehydrogenase (LDH) activity, and increased the hypoxia-inducible factor 1 α (HIF1 α) mRNA and the vascular endothelial growth factor (VEGF) mRNA expression levels. Conclusion: OOPs have anti-hypoxic effects, and the mechanism may involve improving the blood's oxygen carrying capacity and oxygen utilization rate minimizing the lipid peroxidation lesions, increasing the brain's ability to buffer against lactic acidosis in mice, and promoting angiogenesis and regulating the hypoxic response.

Keywords: Oat, oligopeptides, anti-hypoxia, mice

Introduction

Hypoxia is defined as a pathological process in which tissue cell metabolism and even morphological structure changes abnormally because of a lack of oxygen or obstacles to the use of oxygen. The symptoms generally include a rapid heartbeat, a dry mouth, and even nausea, vomiting, diarrhea, palpitations, shortness of breath, and even worse, malaise, coma, shock, etc. [1]. Hypoxia not only damages the physiological functions of various systems such as the nerves, digestion, respiration, urination, and endocrine function, but it also affects the metabolism of carbohydrates, proteins, lipids, water, and electrolytes, and ultimately endangers health. Thus, it is very necessary and significant to find safe and effective methods for the prevention of hypoxia. Currently, the standard vasodilating agents - acetazolamide and nifedipine - are used to reduce the incidence and severity of hypoxia [2, 3]. However, multiple adverse effects, such as headache and cardio-

palms, have been observed in clinical practice [4].

In recent years, nutrition intervention has received increasing attention, and a large number of studies have shown the safety and effectiveness of anti-hypoxic natural food ingredients [5-7]. To date, researchers have isolated various bioactive peptides from plants, animals and microorganisms and confirmed that they have diverse biological activities, such as antimicrobial, antioxidant, anti-hypoxic, cholesterol-lowering, and cyto- or immunomodulatory activities [8-12]. Bioactive peptides are considered to have great potential in the medical and health fields. In 2015, the share of peptide therapy in the global market was 17.5 billion US dollars, and this share is expected to reach 47 billion US dollars by 2025 [13]. As of February 2016, over 60 peptide-related drugs have been approved by the U.S. Food and Drug Administration (FDA) [14], and more than 400 of them are in preclinical or clinical trials [13].

Oat belongs to the Poaceae family and is an annual grass, believed to have originated in Asia. Among the cultivated oats, *Avena sativa* L. (common oat) is the most important variety [15]. Oat has been generally recognized as a healthy grain since the mid-1980s and is well accepted now in the world [15, 16]. Numerous studies have indicated the beneficial effects of oat products, including its anti-inflammatory effect [17], its blood sugar level regulation [18], its hypolipidemic capacity [19], its immunoregulation [20], its antioxidant effect [20] and so on. Whole oat groat contains numerous functional components, such as unsaturated fatty acids, soluble fibers, vitamins, minerals, phytochemicals, β -glucan, proteins and peptides [15, 21]. The biological activities of phytochemicals [22], β -glucan [23-26], and fiber [27, 28] have been demonstrated. What's more, protein hydrolysates of oat also have a variety of activities including a hypolipidemic capacity [29] and antifatigue [16]. However, oligopeptides, an important biologically-active ingredient in walnuts, have a small molecular weight, easy absorption, and high bioavailability, but their anti-hypoxic effects are rarely reported. We hypothesize that oat oligopeptides (OOPs) have anti-hypoxic effects. Therefore, this study aimed to investigate OOPs' anti-hypoxic effects in mice.

Materials and methods

OOP extraction

OOPs were derived from oats (*Avena sativa* L.) using enzymatic hydrolysis. In brief, we washed the walnuts, chopped them, homogenized them in distilled water, adjusted the pH to 8.0, and then, the most critical step, we treated them with compound protease at 40°C for 3 hours. Next, nanofiltration, freeze concentration, decolorization, purification, and spray drying were performed to obtain WOP powder.

A Phenomenex C18 column (10 mm \times 250 mm) was used to purify the sample. The measurement results showed that the WOP content was 87.83%, and the amino acid content was 3.92%, and the relative molecular weights were between 180 Da and 1000 Da.

Chemicals and reagents

Malondialdehyde (MDA) and lactate detection kits were purchased from Beyotime Biotech-

nology (Shanghai, China). A lactate dehydrogenase (LDH) assay kit was obtained from Yingke Xinchuang Technology Co., Ltd. (Macao, China). Medical soda lime and sodium nitrite were purchased Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All reagents used in the study were analytical grade.

Animals

Adult male BALB/C mice, weighing 18-22 g, were purchased from the Laboratory Animal Science Department of Peking University Health Science Center. The temperature was maintained at 25°C \pm 1°C, the relative humidity was kept at 55% \pm 5%. A 12-hour:12-hour light-dark cycle was maintained. The animals could freely enjoy an AIN-93G diet and fresh water. We fed the mice adaptively without intervention for 7 days. The mice were treated in compliance with the Principle of Laboratory Animal Care (National Institutes of Health publication no. 85-23, revised 1985).

Groups and treatment

We randomly divided the mice into 4 experimental sets (n=72), namely experimental sets 1, 2, 3, and 4. Then we randomly divided each set of mice into 6 groups (n=12), including one vehicle control group, one whey protein group (0.50 g/kg), and four OOPs intervention groups (0.25 g/kg, 0.50 g/kg, 1.00 g/kg, 2.00 g/kg, namely OOPs-LG, OOPs-MG, OOPs-HG and OOPs-VHG, respectively). The vehicle control group was given the vehicle using gavage, and the whey protein group and the 4 OOPs groups were administered whey protein and corresponding doses of OOPs respectively. We intervened once a day for a total of 30 days. The animals' weight was recorded weekly.

Normobaric hypoxia assessment

The effects of OOPs on the normobaric hypoxic survival times of the mice: 60 minutes after the final dose, each mouse in experimental set 1 was placed into a 250 ml sealed container containing 5 g of medical soda lime. The oxygen deprivation survival duration times were recorded.

The effects of the OOPs on the MDA content, lactate levels, and LDH activity: After the death of each mouse, its brain was immediately separated and homogenized with physiological

Table 1. The effects of the OOPs on the weight

Weight (g)	vehicle control	whey protein	OOPs-LG	OOPs-MG	OOPs-HG	OOPs-VHG
Experimental set 1						
Initial weight	20.8±1.3	21.1±1.2	20.9±1.1	20.3±1.1	21.3±1.0	21.5±1.0
Terminal weight	25.6±1.6	26.0±1.5	25.5±1.7	25.5±1.5	25.4±1.7	25.1±1.6
Experimental set 2						
Initial weight	20.9±1.3	21.0±1.2	21.2±1.1	21.2±1.0	21.3±1.1	20.7±1.2
Terminal weight	25.4±1.5	26.1±1.5	25.5±1.5	25.6±1.6	25.5±1.7	25.9±1.4
Experimental set 3						
Initial weight	20.6±1.2	20.1±1.1	21.0±1.0	21.2±1.0	20.9±1.1	21.1±1.3
Terminal weight	25.6±1.6	26.0±1.5	25.4±1.6	25.4±1.5	25.7±1.6	25.7±1.25
Experimental set 4						
Initial weight	21.1±1.1	20.5±1.1	20.8±1.2	21.0±1.2	20.4±1.1	21.1±1.3
Terminal weight	25.4±1.5	25.3±1.4	25.7±1.4	25.5±1.3	25.4±1.4	25.2±1.5

The values are presented as the mean ± SD (n=12). OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

saline to a 10% solution at 4°C. We measured the MDA content, lactate levels, and LDH activity in the brain using the corresponding kits.

The effects of the OOPs on the HIF-1 α and VEGF mRNA levels: After the death of each mouse, its brain was immediately separated and total RNA was extracted from its brain tissue using Ribospin (GeneAll, Inc., Seoul, Korea). An ABI 7300 real-time PCR detection system was used for the real-time reverse transcription-PCR to measure the target genes' RNA expressions. We used M-MLV kits (Invitrogen) to complete a reverse transcription-polymerase chain reaction (RT-PCR) analysis of the target mRNA levels. The special primers were as follows: HIF-1 α , forward 5'-TCACCA-CAGGACAGTACAG GATGC-3' and reverse 5'-CCAGCAAAGTTAAAGCAT CAGGTTCC-3'; VEGF, forward 5'-ACG AAGTGGTGAAGTTCATGGATG-3' and reverse 5'-TTC TGTATCAGTCTTCTCGGTGAG-3'; GAPDH, forward 5'-GCCAAAGGGTCATC-ATCTC-3' and reverse 5'-GTAGAGGCAGGGATG-ATGTT-3'. After normalizing the target mRNA value to the GAPDH mRNA level, the target mRNA value was measured by comparing it with the control sample, then the comparison period threshold ($\Delta\Delta C_t$) method was used for the calculation.

Sodium nitrite toxicosis assessment

60 minutes after the final dose, each mouse in experimental set 2 was injected intraperitoneally with 240 mg/kg sodium nitrite (0.1 ml/10 g), and the survival time was recorded.

Acute cerebral ischemia assessment

60 minutes after the final dose, each mouse in experimental set 3 was killed immediately by decapitation, and the time between the decapitation and the final breath was recorded.

Whole blood analysis of the mice

60 minutes after the final dose, a blood sample was obtained in EDTA-containing tubes from the eyeballs of each mouse in experimental set 4. We used a Sysmex XT-2000i blood analyzer (Roche Diagnostics) to analyze the RBC, Hb, and Hct within 3 hours of the blood collection.

Statistical analysis

We used SPSS software version 20.0 for the statistical analysis. The homogeneity of the variances was checked. Then we performed a one-way analysis of variance test as well as the LSD method. We considered *P* less than 0.05 as a significant difference.

Results

The effects of the OOPs on the weight

No significant differences in weight were found among all the groups (*P*>0.05) (**Table 1**).

The effects of the OOPs on the normobaric hypoxia survival times

No significant differences in the normobaric hypoxia survival times were found between the

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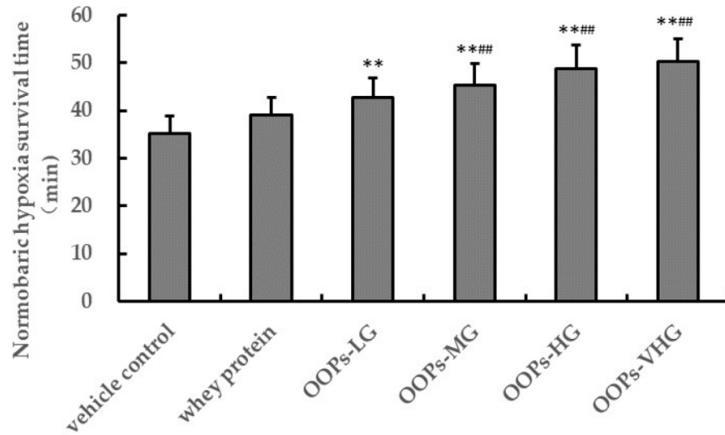


Figure 1. The effects of the OOPs on the normobaric hypoxia survival times. ** $P < 0.01$ versus the vehicle control group; ## $P < 0.01$ versus the whey protein group. OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

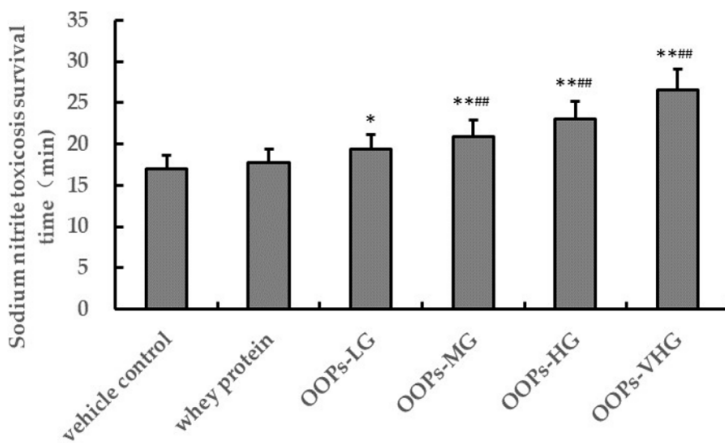


Figure 2. The effects of the OOPs on the sodium nitrite toxicosis survival times.

vehicle control group and the whey protein group ($P > 0.05$). The normobaric hypoxia survival times in OOPs-LG, OOPs-MG, OOPs-HG, and OOPs-VHG were 21.15%, 28.32%, 38.37%, and 42.68% higher than the vehicle control group, respectively ($P < 0.01$). The normobaric hypoxia survival times of the OOPs-MG, OOPs-HG, and OOPs-VHG were significantly extended when compared with the whey protein group ($P < 0.01$) (Figure 1).

The effects of the OOPs on the sodium nitrite toxicosis survival times

No significant differences in the sodium nitrite toxicosis survival times were found between the vehicle control group and the whey protein group ($P > 0.05$). The sodium nitrite toxicosis

survival times in OOPs-LG, OOPs-MG, OOPs-HG and OOPs-VHG were 13.97%, 22.79%, 35.29%, and 56.62% higher than the vehicle control group, respectively ($P < 0.05$ for OOPs-LG, $P < 0.01$ for OOPs-MG, OOPs-HG, and OOPs-VHG). The sodium nitrite toxicosis survival times of OOPs-MG, OOPs-HG, and OOPs-VHG were significantly extended when compared to the whey protein group ($P < 0.01$) (Figure 2).

The effects of the OOPs on the acute cerebral ischemia survival times

No significant differences in the acute cerebral ischemia survival times were found between the vehicle control group and the whey protein group ($P > 0.05$). The acute cerebral ischemia survival times in OOPs-MG, OOPs-HG and OOPs-VHG were 9.28%, 11.92%, and 15.87% longer than the vehicle control group, respectively ($P < 0.05$ for OOPs-MG, $P < 0.01$ for OOPs-HG and OOPs-VHG). The acute cerebral ischemia survival times in OOPs-MG, OOPs-HG and OOPs-VHG were significantly extended when compared to the whey protein group ($P < 0.05$ for OOPs-MG, $P < 0.01$ for OOPs-HG and OOPs-VHG) (Figure 3).

The effects of the OOPs on the RBC, Hb, and Hct

No significant differences in the RBC, Hb, or Hct were found between the vehicle control group and the whey protein group ($P > 0.05$). In comparison with the vehicle control group, the RBC and Hct in the OOPs-MG, OOPs-HG, and OOPs-VHG were significantly enhanced ($P < 0.01$), Hb in OOPs-LG, OOPs-MG, OOPs-HG and OOPs-VHG was significantly increased ($P < 0.05$ for OOPs-LG, $P < 0.01$ for OOPs-MG, OOPs-HG and OOPs-VHG). Moreover, compared with the whey protein group, the RBC, Hct, and Hb of the OOPs-MG were significantly enhanced ($P < 0.05$); the RBC, Hct, and Hb in OOPs-HG were significantly increased ($P < 0.05$ for RBC

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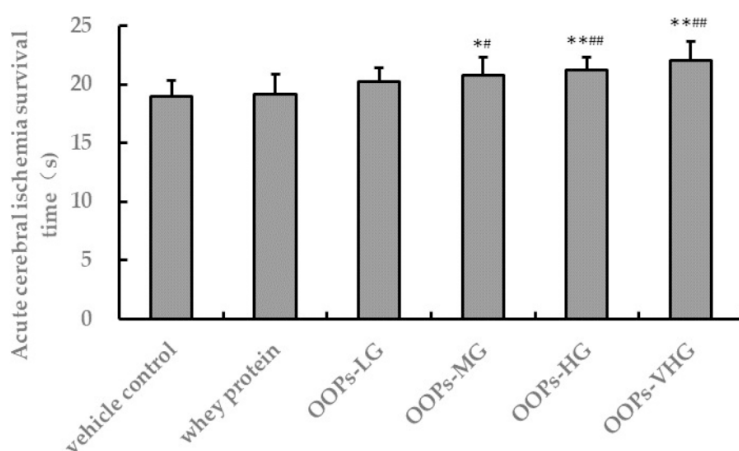


Figure 3. The effects of the OOPs on the acute cerebral ischemia survival times. * $P < 0.05$, ** $P < 0.01$ versus vehicle control group; # $P < 0.05$, ## $P < 0.01$ versus the whey protein group. OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

and Hct, $P < 0.01$ for Hb); RBC, the Hct and Hb in the OOPs-VHG were significantly enhanced ($P < 0.05$ for RBC, $P < 0.01$ for Hct and Hb) (Figure 4).

Effects of the OOPs on the brain MDA content

No significant difference in the brain MDA content was found between the vehicle control group and the whey protein group ($P > 0.05$). In comparison with the vehicle control group, the brain MDA content of the OOPs-LG, OOPs-MG, OOPs-HG and OOPs-VHG were significantly decreased ($P < 0.01$). The brain MDA content in OOPs-MG, OOPs-HG and OOPs-VHG was lower than it was in the whey protein group ($P < 0.01$) (Figure 5).

The effects of the OOPs on the brain lactate levels and LDH activity

There was no significant difference in the brain lactate levels and the LDH activity between the vehicle control group and the whey protein group ($P > 0.05$). In comparison with the vehicle control group, the brain lactate levels were significantly decreased and the brain LDH activity was significantly enhanced in the OOPs-LG, OOPs-MG, OOPs-HG, and OOPs-VHG ($P < 0.05$ for OOPs-LG, $P < 0.01$ for OOPs-MG, OOPs-HG and OOPs-VHG). Compared with the whey protein group, the brain lactate levels were significantly decreased and the brain LDH activity was higher in OOPs-MG, OOPs-HG and OOPs-

VHG ($P < 0.05$ for OOPs-MG, $P < 0.01$ for OOPs-HG and OOPs-VHG) (Figure 6).

The effects of the OOPs on HIF-1 α and VEGF mRNA levels in the brains

No significant differences in the HIF-1 α and VEGF mRNA levels were found between the vehicle control group and the whey protein group ($P > 0.05$). The HIF-1 α and VEGF mRNA levels in OOPs-LG, OOPs-MG, OOPs-HG and OOPs-VHG were higher than they were in the vehicle control group ($P < 0.05$ for OOPs-LG and OOPs-MG, $P < 0.01$ for OOPs-HG and OOPs-VHG). In addition, compared with the whey protein group, the HIF-1 α

and VEGF mRNA levels in OOPs-MG, OOPs-HG and OOPs-VHG were significantly enhanced ($P < 0.05$ for OOPs-MG, $P < 0.01$ for OOPs-HG and OOPs-VHG) (Figure 7).

Discussion

Hypoxia is a stressor to the body, disturbing its normal metabolic processes, especially the antioxidant function, and even leading to death due to an insufficient energy supply to the main organs such as the heart and brain. In this study, we evaluated the anti-hypoxic effects of OOPS for the first time in mice.

Whey protein is a protein extracted from milk using advanced technology. It has high bio-availability and a variety of biological activities, such as anti-oxidation, immunomodulation, anti-fatigue, anti-viral and anti-bacterial [30]. In order to rule out false positive results that may be caused by protein supplements, we used whey protein as a protein control. Under our experimental conditions, the effects of whey protein against hypoxia were not observed.

In the normobaric hypoxia assessment, insufficient oxygen supply severely reduced the intracellular oxygen partial pressure, leading to mitochondrial dysfunction and affecting the energy metabolism. In the sodium nitrite toxicosis assessment, sodium nitrite converted bivalent hemoglobin into trivalent hemoglobin, dis-

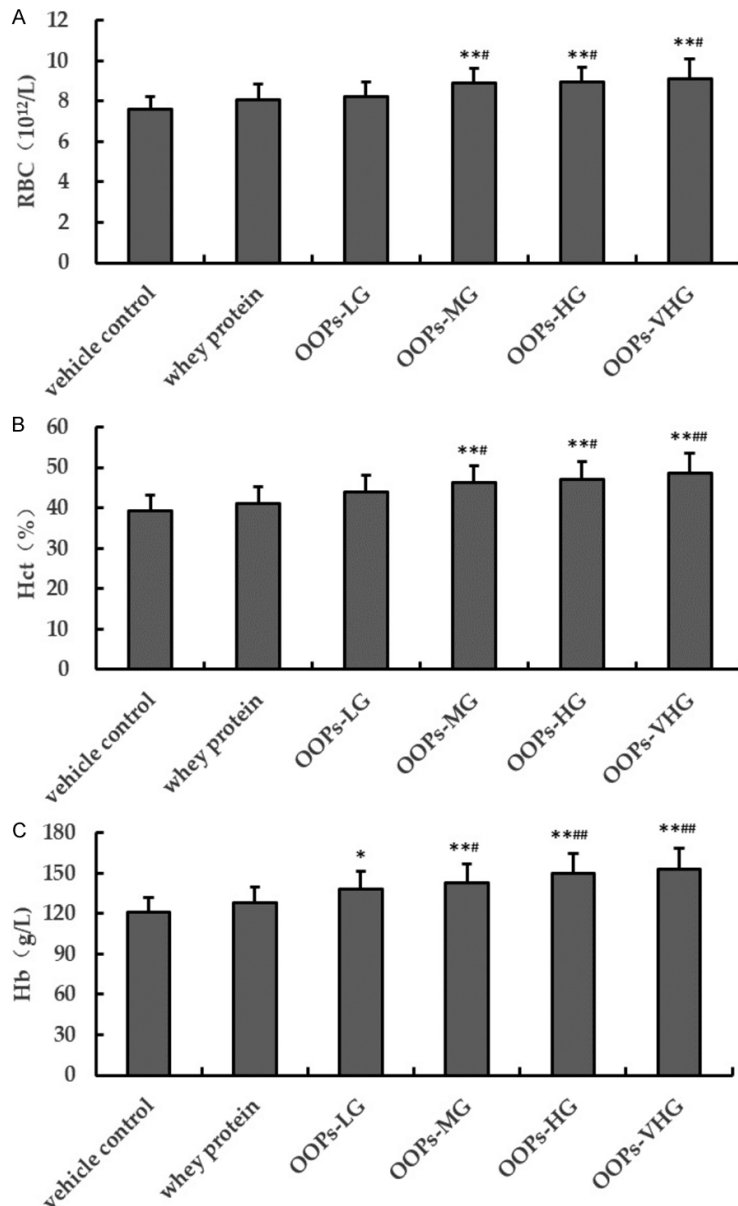


Figure 4. The effects of the OOPs on RBC, Hb and Hct. * $P < 0.05$, ** $P < 0.01$ versus the vehicle control group; # $P < 0.05$, ## $P < 0.01$ versus the whey protein group. OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

rupting the oxygen-carrying capacity of hemoglobin and resulting in tissue hypoxia. In the acute cerebral ischemia assessment, the decapitation terminated the blood supply to the brain, but the brain could still work for a short time, showing regular mouth gasping. The gasp time could be used as a crucial indicator to evaluate the protective effect of the tested samples on cerebral ischemic anoxia. The above results indicated that WOP intervention

significantly extended the survival durations in the above three assessments. Hence, OOPs have the function of improving anoxia tolerance.

Red blood cells make up the largest number of blood corpuscles and act as the major media for transporting oxygen in the blood [31]. The number of red blood cells directly reflects the blood's oxygen carrying capacity. Hct refers to the volume ratio of red blood cells to whole blood, indirectly indicating the number and size of red blood cells. Oxygen combines with iron atoms and is transported through the blood. Hb is easily combined with oxygen in areas with high oxygen content and is readily separated from oxygen in places with low oxygen content. This characteristic enables erythrocytes to function as oxygen carriers [32]. The results in the present study suggest that OOPs can enhance the RBC, Hb, and Hct levels, thus improving the oxygen carrying capacity of blood and increasing the oxygen utilization rate of mice.

During hypoxia, oxygen cannot be completely reduced to water by the function of mitochondrial cytochrome oxidase. Therefore, reduced equivalents are accumulated in the respiratory chain, leading to ROS formation due to the autooxidation of mitochondrial complexes [33]. When ROS production exceeds the capacity of cellular antioxidant systems, oxidative stress occurs. Membrane lipids in the brain contain abundant polyunsaturated fatty acids, which are crucial targets for free radical attacks [34, 35]. Furthermore, the brain has lower levels of antioxidant enzymes than other organs [36, 37]. The above two factors make the brain very susceptible to lipid peroxidation, in the process of which a mixture of alkenes, epoxy-fatty acids, alkanes, alkenals, alkanals, and aldehydes

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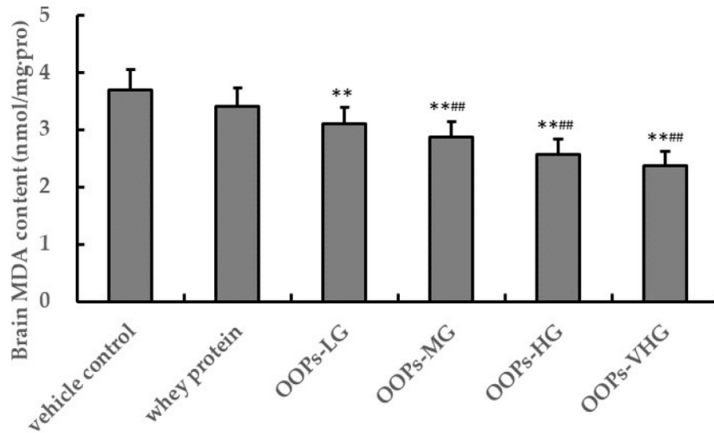


Figure 5. The effects of the OOPs on the brain MDA content. ** $P < 0.01$ versus the vehicle control group; ## $P < 0.01$ versus the whey protein group. OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

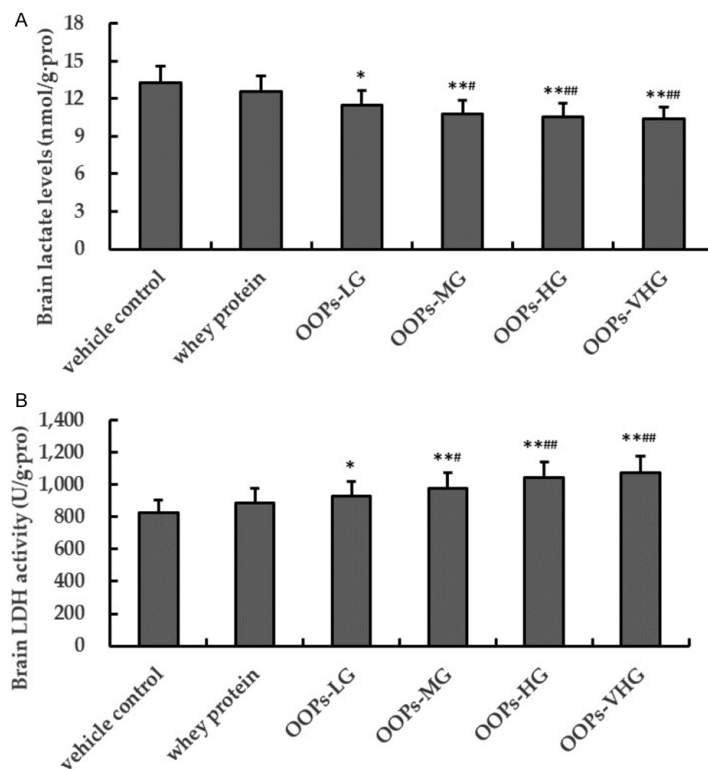


Figure 6. The effects of the OOPs on the brain lactate levels and LDH activity. * $P < 0.05$, ** $P < 0.01$ versus the vehicle control group; # $P < 0.05$, ## $P < 0.01$ versus the whey protein group. OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

lipid peroxidation [39-41]. In the present study, OOPs significantly decreased the brain MDA content of the mice, thus minimizing the lesion of lipid peroxidation.

In the process of hypoxia, energy produced by aerobic respiration is not enough to meet the tissue's needs. Under these circumstances, due to anaerobic respiration, excessive lactic acid will be produced, lowering the pH value, affecting the related enzymes' activities, and causing intracellular acidosis [42]. Because LDH is a key enzyme in this process, changes in the quantity and activity of LDH directly affect the energy metabolism in vivo. The results of this study suggest that OOPs can reduce lactate levels and enhance LDH activity in brain, consequently increasing the brain's ability to buffer against lactic acidosis in mice.

Blood vessels are an important source of oxygen for tissue cells. Vascular endothelial growth factor (VEGF) is an important regulator that can stimulate vascular endothelial proliferation and migration, change vascular permeability, and promote angiogenesis. It is an important marker of angiogenesis [43]. Hypoxia-inducible factor 1 α (HIF-1 α) is considered a key transcription factor involved in the hypoxic response [44, 45]. It can regulate the transcription of a variety of genes. The transcription product of HIF-1 α can reduce the oxygen consumption of cells or increase the oxygen supply of hypoxic tissues, thereby alleviating the contradiction between oxygen supply and demand and maintaining the stability of the internal environment. HIF-1 α is also the core regulator of angiogenesis under hypoxia,

and it plays a key role in the angiogenic process of hypoxic damaged tissues. Studies have

including MDA are yielded [38]. In general, the MDA content is used as a crucial indicator to

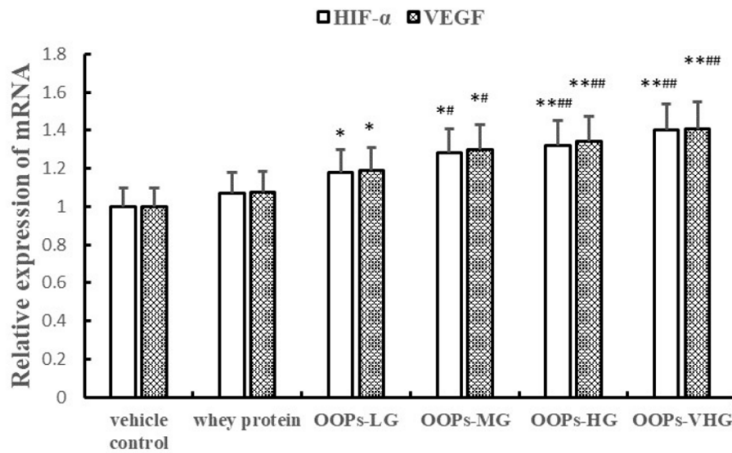


Figure 7. The effects of the OOPs on the HIF-1 α and VEGF mRNA levels. * P <0.05, ** P <0.01 versus the vehicle control group; # P <0.05, ## P <0.01 versus the whey protein group. OOPs, small molecule oligopeptides isolated from oat; OOPs-LG, 0.25 g/kg OOPs group; OOPs-MG, 0.50 g/kg OOPs group; OOPs-HG, 1.00 g/kg OOPs group; OOPs-VHG, 2.00 g/kg OOPs group.

shown [46] that in a normal aerobic environment, HIF-1 α is at a low concentration due to increased degradation and transcriptional inhibition; when tissue cells are hypoxic, HIF-1 α is rapidly activated and highly expressed by the stimulation of various hypoxia response genes. The results of this study show that OOPs can induce an increase in the expression levels of VEGF mRNA and HIF-1 α mRNA in the process of brain hypoxic injury, suggesting that the body initiates its own protective mechanism under hypoxic stimulation, and the high mRNA expression of HIF-1 α promotes it by up-regulating the downstream VEGF expression. Angiogenesis induces an adaptation of the local tissues to hypoxia and prevents further tissue deterioration.

The present study demonstrated the anti-hypoxic effects of OOPs in mice for the first time. The effects might work in the following ways: one is to improve the blood's oxygen-carrying capacity and oxygen-utilization rate, the other is to minimize the lesion of lipid peroxidation, the third is to increase the brain's ability to buffer against lactic acidosis of mice, and the fourth is to promote angiogenesis and regulate the hypoxic response. We look forward to more in-depth research on the mechanism.

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Disclosure of conflict of interest

None.

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References

- [1] Houston CS, Sutton JR, Cymerman A and Reeves JT. Operation Everest II: man at extreme altitude. *J Appl Physiol* (1985) 1987; 63: 877-82.
- [2] Wang J, Ke T, Zhang X, Chen Y, Liu M, Chen J and Luo W. Effects of acetazolamide on cognitive performance during high-altitude exposure. *Neurotoxicol Teratol* 2013; 35: 28-33.
- [3] Kss S, Veeramohan PH and Mathew T. Nifedipine inhibits hypoxia induced transvascular leakage through down regulation of NF κ B. *Respir Physiol Neurobiol* 2012; 183: 26-34.
- [4] Fagenholz PJ, Gutman JA, Murray AF and Harris NS. Treatment of high altitude pulmonary edema at 4240 m in Nepal. *High Alt Med Biol* 2007; 8: 139-46.
- [5] Wang J, Li LZ, Liu YG, Teng LR, Lu JH, Xie J, Hu WJ, Liu Y, Liu Y, Wang D and Teng LS. Investigations on the antifatigue and antihypoxic effects of *Paecilomyces hepiali* extract. *Mol Med Rep* 2016; 13: 1861-8.
- [6] Mo WL, Chai CZ, Kou JP, Yan YQ and Yu BY. Sheng-Mai-San attenuates contractile dysfunction and structural damage induced by chronic intermittent hypoxia in mice. *Chin J Nat Med* 2015; 13: 743-50.
- [7] Lee CS, Choi EY, Lee SC, Koh HJ, Lee JH and Chung JH. Resveratrol inhibits hypoxia-induced vascular endothelial growth factor expression and pathological neovascularization. *Yonsei Med J* 2015; 56: 1678-85.
- [8] Hartmann R and Meisel H. Food-derived peptides with biological activity: from research to food applications. *Curr Opin Biotechnol* 2007; 18: 163-9.

- [9] Murray BA and FitzGerald RJ. Angiotensin converting enzyme inhibitory peptides derived from food proteins: biochemistry, bioactivity and production. *Curr Pharm Des* 2007; 13: 773-91.
- [10] He LX, Zhang ZF, Sun B, Chen QH, Liu R, Ren JW, Wang JB and Li Y. Sea cucumber (*Codonopsis pilosula*) oligopeptides: immunomodulatory effects based on stimulating Th cells, cytokine secretion and antibody production. *Food Funct* 2016; 7: 1208-16.
- [11] Cheung RC, Ng TB and Wong JH. Marine peptides: bioactivities and applications. *Mar Drugs* 2015; 13: 4006-43.
- [12] Gao X, Zhang H, Zhuang W, Yuan G, Sun T, Jiang X, Zhou Z, Yuan H, Zhang Z and Dong H. PEDF and PEDF-derived peptide 44mer protect cardiomyocytes against hypoxia-induced apoptosis and necroptosis via anti-oxidative effect. *Sci Rep* 2014; 4: 5637.
- [13] Ghosh S. Future market insights, healthcare and medical devices, peptide therapeutics market: forecast and analysis 2015-2025.
- [14] Fosgerau K and Hoffmann T. Peptide therapeutics: current status and future directions. *Drug Discov Today* 2015; 20: 122-8.
- [15] Sadiq BM, Tahir-Nadeem M, Khan MK, Shabir R and Butt MS. Oat: unique among the cereals. *Eur J Nutr* 2008; 47: 68-79.
- [16] Xu C, Lv J, You S, Zhao Q, Chen X and Hu X. Supplementation with oat protein ameliorates exercise-induced fatigue in mice. *Food Funct* 2013; 4: 303-9.
- [17] Zhang X, McGeoch SC, Megson IL, MacRury SM, Johnstone AM, Abraham P, Pearson DW, de Roos B, Holtrop G, O'Kennedy N and Lobley GE. Oat-enriched diet reduces inflammatory status assessed by circulating cell-derived microparticle concentrations in type 2 diabetes. *Mol Nutr Food Res* 2014; 58: 1322-32.
- [18] Shen RL, Cai FL, Dong JL and Hu XZ. Hypoglycemic effects and biochemical mechanisms of oat products on streptozotocin-induced diabetic mice. *J Agric Food Chem* 2011; 59: 8895-900.
- [19] Raimondi de Souza S, Moraes de Oliveira GM, Raggio Luiz R and Rosa G. Effects of oat bran and nutrition counseling on the lipid and glucose profile and anthropometric parameters of hypercholesterolemia patients. *Nutr Hosp* 2016; 33: 123-130.
- [20] Davis JM, Murphy EA, Brown AS, Carmichael MD, Ghaffar A and Mayer EP. Effects of moderate exercise and oat beta-glucan on innate immune function and susceptibility to respiratory infection. *Am J Physiol Regul Integr Comp Physiol* 2004; 286: R366-72.
- [21] Comino I, Bernardo D, Bancel E, de Lourdes MM, Sanchez B, Barro F, Suligoi T, Ciclitira PJ, Cebolla A, Knight SC, Branlard G and Sousa C. Identification and molecular characterization of oat peptides implicated on coeliac immune response. *Food Nutr Res* 2016; 60: 30324.
- [22] Sawicki CM, McKay DL, McKeown NM, Dallal G, Chen C and Blumberg JB. Phytochemical pharmacokinetics and bioactivity of oat and barley flour: a randomized crossover trial. *Nutrients* 2016; 8: 813.
- [23] Nwachukwu ID, Devassy JG, Aluko RE and Jones PJ. Cholesterol-lowering properties of oat beta-glucan and the promotion of cardiovascular health: did Health Canada make the right call? *Appl Physiol Nutr Metab* 2015; 40: 535-42.
- [24] Wang Q and Ellis PR. Oat beta-glucan: physico-chemical characteristics in relation to its blood-glucose and cholesterol-lowering properties. *Br J Nutr* 2014; 112 Suppl 2: S4-S13.
- [25] Whitehead A, Beck EJ, Tosh S and Wolever TM. Cholesterol-lowering effects of oat beta-glucan: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2014; 100: 1413-21.
- [26] Song X and Hubbe MA. TEMPO-mediated oxidation of oat beta-D-glucan and its influences on paper properties. *Carbohydr Polym* 2014; 99: 617-23.
- [27] Rebello CJ, O'Neil CE and Greenway FL. Dietary fiber and satiety: the effects of oats on satiety. *Nutr Rev* 2016; 74: 131-47.
- [28] Sayanjali S, Sanguansri L, Buckow R, Gras S and Augustin MA. Oat fiber as a carrier for curcuminoids. *J Agric Food Chem* 2014; 62: 12172-7.
- [29] Tong LT, Guo L, Zhou X, Qiu J, Liu L, Zhong K and Zhou S. Effects of dietary oat proteins on cholesterol metabolism of hypercholesterolaemic hamsters. *J Sci Food Agric* 2016; 96: 1396-401.
- [30] He LX, Ren JW, Liu R, Chen QH, Zhao J, Wu X, Zhang ZF, Wang JB, Pettinato G and Li Y. Ginseng (*Panax ginseng* Meyer) oligopeptides regulate innate and adaptive immune responses in mice via increased macrophage phagocytosis capacity, NK cell activity and Th cells secretion. *Food Funct* 2017; 8: 3523-3532.
- [31] Li Q, Zhou M, Liu H, Wang Y and Guo F. Red blood cell count automation using microscopic hyperspectral imaging technology. *Appl Spectrosc* 2015; 69: 1372-80.
- [32] Oder E, Safo MK, Abdulmalik O and Kato GJ. New developments in anti-sickling agents: can drugs directly prevent the polymerization of sickle haemoglobin in vivo? *Br J Haematol* 2016; 175: 24-30.

- [33] Kehrer JP and Lund LG. Cellular reducing equivalents and oxidative stress. *Free Radic Biol Med* 1994; 17: 65-75.
- [34] Tappel AL. Lipid peroxidation damage to cell components. *Fed Proc* 1973; 32: 1870-4.
- [35] Reed TT. Lipid peroxidation and neurodegenerative disease. *Free Radic Biol Med* 2011; 51: 1302-19.
- [36] Shohami E, Beit-Yannai E, Horowitz M and Kohen R. Oxidative stress in closed-head injury: brain antioxidant capacity as an indicator of functional outcome. *J Cereb Blood Flow Metab* 1997; 17: 1007-19.
- [37] Halliwell B. Role of free radicals in the neurodegenerative diseases: therapeutic implications for antioxidant treatment. *Drugs Aging* 2001; 18: 685-716.
- [38] Rauchova H, Vokurkova M and Koudelova J. Hypoxia-induced lipid peroxidation in the brain during postnatal ontogenesis. *Physiol Res* 2012; 61 Suppl 1: S89-101.
- [39] Liu Y, Tipoe GL and Fung ML. Melatonin attenuates intermittent hypoxia-induced lipid peroxidation and local inflammation in rat adrenal medulla. *Int J Mol Sci* 2014; 15: 18437-52.
- [40] Cetinkaya N, Attar R, Yildirim G, Ficicioglu C, Ozkan F, Yilmaz B and Yesildaglar N. The effects of different doses of melatonin treatment on endometrial implants in an oophorectomized rat endometriosis model. *Arch Gynecol Obstet* 2015; 291: 591-8.
- [41] Ramesh T, Sureka C, Bhuvana S and Begum VH. Oxidative stress in the brain of cigarette smoke-induced noxiousness: neuroprotective role of *Sesbania grandiflora*. *Metab Brain Dis* 2015; 30: 573-82.
- [42] Mankovskaya IN, Serebrovskaya TV, Swanson RJ, Vavilova GL and Kharlamova ON. Mechanisms of taurine antihypoxic and antioxidant action. *High Alt Med Biol* 2000; 1: 105-10.
- [43] Hu HR, Tang Y, Pang LB, Lin CL and Huang WH. Angiogenesis and full-thickness wound healing efficiency of a copper-doped borate bioactive glass/poly(lactic-co-glycolic acid) dressing loaded with vitamin E in vivo and in vitro. *ACS Appl Mater Interfaces* 2018; 10: 22939-22950.
- [44] Ishikawa H, Xu L, Sone K, Kobayashi T, Wang G and Shozu M. Hypoxia induces hypoxia-inducible factor 1 α and potential HIF-responsive gene expression in uterine leiomyoma. *Reprod Sci* 2019; 26: 428-435.
- [45] Alonso D, Serrano E, Bermejo FJ and Corral RS. HIF-1 α -regulated MIF activation and Nox2-dependent ROS generation promote leishmania amazonensis killing by macrophages under hypoxia. *Cell Immunol* 2019; 335: 15-21.
- [46] Cui HS, Joo SY, Lee DH, Yu JH and Seo CH. Low temperature plasma induces angiogenic growth factor via up-regulating hypoxia-inducible factor 1 α in human dermal fibroblasts. *Arch Biochem Biophys* 2017; 630: 9-17.