

## Original Article

# The effect of traditional Tibetan guozhuang dance on vascular health in elderly individuals living at high altitudes

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**Abstract:** To evaluate the effect of dance on vascular-related factors and cerebral hemodynamics in elderly individuals in Qinghai-Tibetan plateau regions (mean altitude  $\geq 2,300$  m). Thirty elderly individuals, who practiced traditional Tibetan Guozhuang dance or did not, were enrolled, respectively. Serum PGC-1 $\alpha$ , HCY, FSTL-1, VEGF and HIF-1 $\alpha$  were measured by ELISA assays. Carotid artery stenosis and plaque, IMT, extracranial internal carotid artery stenosis and cerebral arteriosclerosis were evaluated using CUS and TCD. Body weight, BMI, heart rate, systolic pressure, and diastolic pressure, serum BGS, TC, LDL, HIF-1 $\alpha$ , VEGF, and HCY in the dance group were significantly lower than the no-dance group. FSTL-1 levels, SO<sub>2</sub> and SO<sub>2</sub>/heart rate ratio in the dance group were significantly higher than the no-dance group. Incidence of extracranial internal carotid artery stenosis, carotid stenosis and plaque in the dance group was significantly lower than the no-dance group. IMT was a significant positive correlation between PGC-1 $\alpha$  and HCY in the no-dance group. Elderly individuals who regularly practiced Tibetan dance had improved blood vessel functionality and cerebral hemodynamic at high altitudes.

**Keywords:** High altitude, Tibetan dance, vascular-related factors, carotid artery, transcranial Doppler ultrasound, carotid ultrasound

## Introduction

With the rapid increase in the aging population, the incidence of chronic disease has also increased. Cardiovascular and cerebrovascular diseases are the most common chronic diseases in elderly individuals. Ischemic heart disease and stroke accounted for 15 million deaths worldwide and was the leading cause of mortality in 2015. Hence, early intervention to delay atherosclerosis may prevent cardiovascular and cerebrovascular related diseases, as effective therapies to increase the physical condition of elderly individuals are presently lacking.

Qinghai-Tibetan Plateau is the largest and highest plateau and has the largest high-altitude

population worldwide. There are hypoxic, low pressure, high ultraviolet radiation, sunshine, hypothermic, and become harsher with increasing altitude of the environment in this area [1, 2]. Individuals living long term in these environments have extreme physiological changes compared to individuals residing in the area with sea-level [3]. A hypoxic environment affects angiogenic factors, such as vascular endothelial cell growth factor (VEGF) and hypoxia-related factor-1 $\alpha$  (HIF-1 $\alpha$ ). HIF-1 $\alpha$  is the central mediator of the hypoxic response and regulates several hundred genes. VEGF is one of the primary target genes of HIF-1 $\alpha$  and is an important regulator of angiogenesis. Previous studies have shown that elderly individuals who jogged, fast-walked, performed tai chi, mountain-climbed, or took part in other regular moderate-inten-

sity aerobic exercise could strengthen their cardiovascular and cerebrovascular function [4]. Since the 1960s, training at high altitudes has been one of the principal means of developing aerobic endurance in athletes. Blood oxygen saturation ( $SO_2$ ) and  $SO_2$ /heart rate (exercise intensity index) are easy-to-obtain and straightforward indices for assessing the effect of altitude training [5]. Tibetan dance, known as Guozhuang dance, consists of walking, jogging, singing, and dancing with musical instrument. Tibetan dance has been reported to promote cardiopulmonary function in the human body [6, 7]. A previous study demonstrated that HIF-1 $\alpha$  influences training-induced VEGF gene expression. Both HIF-1 $\alpha$  and VEGF expression are co-regulated at the transcriptional level in human skeletal muscles [8]. Studies have demonstrated that regular exercise reduced plasma homocysteine (HCY) level and that high expression of follistatin-related protein 1 (FSTL-1) under conditions of myocardial ischemia could protect myocardial cells and improve endothelial function [9, 10]. Also, physical exercise improves the regulatory role of skeletal muscle proliferate-activated receptor gamma coactivator1-alpha (PGC-1 $\alpha$ ) in fat metabolism, which is an independent indicator for assessing dietary glycemic index [11].

In recent years, transcranial Doppler ultrasonography (TCD) and carotid ultrasound (CUS) has become reliable methods for accurate diagnosis and early detection of cerebrovascular diseases [12]. Measuring the Intima-media thickness (IMT) is useful for determining carotid atherosclerosis. In the present study, we have determined if there are beneficial effects of Tibetan dance on vascular function in elderly individuals living in high plateau regions. We evaluated the impact of dance on blood vessel function and cerebral hemodynamics using vascular-related indices in peripheral blood as well as through CUS and TCD ultrasound methods.

### Materials and methods

#### *Study population*

Dance group: Between September 2011 and September 2013, 30 retired elderly over 60 years of age (60-75 years old) from Xining City (mean altitude  $\geq 2,300$  m) of Qinghai Province were enrolled in the study. There were 14

women (46.7%) and 16 men (53.3%). The inclusion criteria were as follows: 5 days per week; greater than 120 minutes per Week of daily traditional Tibetan Guozhuang dance for at least five years, heart rate maintained at 120-150 beats/min during this activity (60% to 80% maximum heart rate; moderate or medium intensity) and normal finding on a physical examination, electrocardiogram and chest X-rays, as well as healthy liver and kidney function. Individuals were excluded if they had any of the following: diabetes, hypertension, coronary heart disease, hyperthyroidism, history of drinking or smoking, and cerebrovascular diseases, including stroke, vascular dementia, and hypertensive encephalopathy.

No-dance group: Comprised of 30 retired elderly over 60 years of age (60-75 years old) recruited from a physical examination center at Qinghai Provincial People's Hospital during the same period as individuals enrolled in the dance group. There were 12 women (40.0%) and 18 men (60.0%). The inclusion criteria were as follows: Less than 10 minutes a day of morning walk and sitting or relaxing for long periods at home. The other inclusion criteria were similar to those of the dance group. Individuals were excluded if they had any of the following: aphasia, severe hearing or a visual impairment, a history of mental illness or mental retardation, any infectious diseases, a history of diabetes, hypertension, and coronary heart disease or hyperthyroidism.

#### *Investigational method*

Demographic data that was collected included age, gender, education level, nationality, occupation, body weight, height, body mass index (BMI), and residence status. Blood pressure and heart rate were measured using an HBP-1300 (OMRON Healthcare, Dalian, China).  $SO_2$  was measured using a GE Datex Ohmeda Tuffsat Handheld Pulse Oximeter (Illinois, U.S.A). Details on exercise included the method, daily duration and history, and dietary intake. Individuals and/or their guardians agreed to participate in this study and consented to blood and related examinations by signing the informed consent form. The project was approved by the ethics committee of Qinghai Provincial People's Hospital (number: 2013-0525-006).

**Table 1.** Baseline characteristics of participants in the dance and no-dance groups (N, %)

Group	N	Gender		Nationality		
		Male	Female	Han	Tibetan	Hui
Dance	30	16 (53.30)	14 (46.70)	27 (90.00)	2 (6.67)	1 (3.33)
No-dance	30	18 (60.00)	12 (40.00)	26 (86.67)	3 (10.0)	1 (3.33)
X <sup>2</sup> -test		0.271		0.352		
P-value*		0.602		0.839		

Note: \*A chi-squared test. *P* values of <0.05 were considered statistically significant.

#### Determination of blood indicators

10 mls of fasting venous blood were collected to analyze blood cell (RBC) counts, mean corpuscular volume (MCV), hemoglobin (HB) levels, blood glucose (BGS) levels, creatinine (CR), uric acid (UA), lactate dehydrogenase (LDH) and blood lipids (triglyceride [TG], cholesterol [TC] and low-density lipoprotein [LDL]) using an automatic biochemical analyzer (Toshiba, Japan). Serum levels of angiogenesis-related factors, which included PGC-1 $\alpha$ , HCY, FSTL-1, VEGF, and HIF-1 $\alpha$  were measured using an enzyme-linked immunosorbent assay (ELISA) (Wksu-BIO, Shanghai, China).

#### Assessment by CUS and TCD

Carotid ultrasound (CUS) was performed using the Philips IE33 ultrasound machine (New York, USA) to assess carotid intima-media thickness (IMT), presence of atherosclerotic plaques in the lumen, size, morphology and luminal stenosis. Extracranial internal carotid artery stenosis and cerebral arteriosclerosis were determined using a DWL Transcranial Doppler Detector Multi-Dop BOX San Juan Capistrano, CA, USA). Ultrasonography was performed by certified ultrasound technicians at the Institute of Geriatrics. TCD was also used to measure the bilateral middle cerebral arteries (MCAS) and the basal artery (BA). The five cerebral haemodynamic parameters were as follows: the mean velocity (Vm), systolic velocity (SPV), diastolic velocity (EDV), pulsation index (PI), and resistance index (RI). The mean parameters of the two sides were used for the statistical analyses. The degree of carotid stenosis was categorized as follows: mild stenosis (<50%), moderate stenosis (50%-69%), severe stenosis (70%-99%), and vascular occlusion (no color Doppler blood flow signals) [13].

#### Quality control of data gathered

All Investigators involved in this project should have at least a Master's degree in geriatric diseases from the Institute of Geriatrics of Qinghai Provincial People's Hospital. Investigators were meticulously trained before initiating the study. Quality control was performed on-site during the study and after completion of the questionnaires, one enrolled individual at a time. When discrepancies were observed, corrective measures were performed, with timely follow-ups. Two investigators were responsible for data input, with 10% of all data undergoing quality checks.

#### Statistical analysis

Statistical analysis was performed using the SPSS24.0 software from International Business Machines Corporation (IBM) (version 24.0, SPSS Inc., Chicago, IL, USA). Student *t*-test was used to compare the aerobic exercise and no-exercise groups. A chi-squared test ( $\chi^2$ -test) was used to determine significant differences between the expected frequencies and the observed frequencies in one or more categories. Linear correlation analysis was performed for the IMT of the carotid artery and serum levels of angiogenesis-related factors. *P* values of <0.05 were considered statistically significant.

## Results

#### Socio-demographic data

The 60 enrolled individuals consisted of three ethnic groups and included Tibetan, Hui and Han, with a mean age of 65 years. The majority of enrolled study participants had primary education, with a mean education period of 4.67 years. The baseline characteristics of the groups were not significantly different (*P*>0.05) (Table 1).

## Vascular-related factors, cerebral hemodynamic, Tibetan dance

**Table 2.** General data show on participants in the dance and no-dance groups at a high altitude

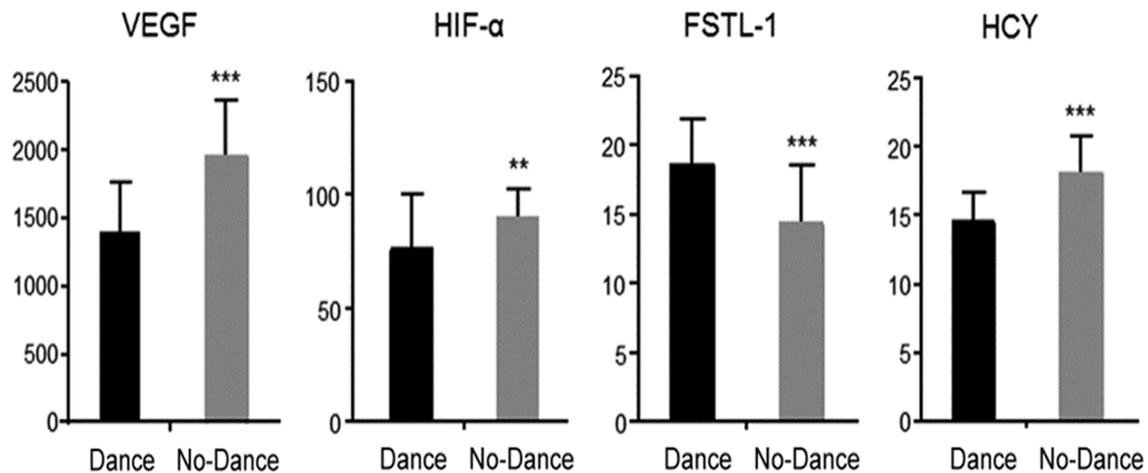
Group	N	Age (years)	Education (years)	Dance history (years)	Daily exercise duration (h)	Body weight (kg)	Body Mass Index (kg/m <sup>2</sup> )	Heart rate (b/min)	Systolic pressure (mmHg)	Diastolic pressure (mmHg)	SO <sub>2</sub> (%)	SO <sub>2</sub> /Heart rate
Dance	30	64.30±11.91	4.69±3.89	7.46±7.30**	1.41±1.55**	64.97±11.30*	24.34±3.27**	67.60±10.33*	123.50±16.94*	76.67±9.51*	94.04±2.51**	1.40±15.25*
No-dance	30	64.96±10.84	4.58±3.38	1.28±1.14	0.15±0.33	70.15±12.74	29.66±3.28	76.33±10.54	130.61±10.03	82.52±8.16	91.83±3.23	1.25±20.28
t-test		1.523	0.260	6.996	14.345	2.279	6.186	2.099	2.475	2.295	2.907	2.210
P-value		0.133	0.796	0.000	0.000	0.026	0.000	0.034	0.016	0.025	0.005	0.031

Note: Data are given as the mean ± SD. \*P<0.05, \*\*P<0.01 (t-test) relative to dance group.

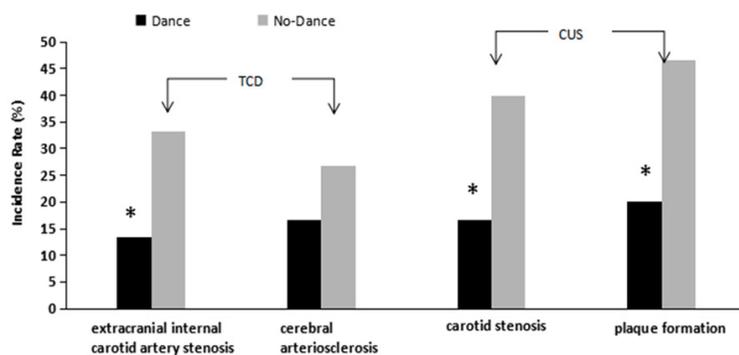
**Table 3.** The blood vascular-related factors of participants

Group	N	RBC (×10 <sup>12</sup> /L)	MCV (fL)	HB (g/L)	BGS (mmol/L)	CR (mmol/L)	UA (mmol/L)	LDH (mmol/L)	TC (mmol/L)	TG (mmol/L)	LDL (mmol/L)
Dance	30	4.97±0.59**	95.90±4.95	148.31±20.80	4.97±0.90*	71.0±14.60	316.1±72.31	191.12±18.63*	4.57±0.74**	1.86±1.18	2.59±0.51*
No-dance	30	4.56±0.68	96.88±5.20	155.00±15.23	5.72±1.10	73.4±14.00	333.1±94.11	172.21±20.10	5.72±1.10	1.90±1.70	3.04±0.78
t-test		2.696	1.420	1.408	2.323	-0.650	-0.788	2.118	2.855	0.624	2.622
P-value		0.009	0.161	0.164	0.024	0.519	0.434	0.032	0.006	0.535	0.011

Note: Data are given as the mean ± SD. \*P<0.05, \*\*P<0.01 (t-test) relative to dance group. Abbreviation: RBC, red blood cell; MCV, mean corpuscular volume; HB, hemoglobin; BGS, blood glucose; CR, creatinine; UA, uric acid; LDH, lactate dehydrogenase; TC, cholesterol; TG, triglyceride; LDL, low-density lipoprotein.



**Figure 1.** Serum levels of angiogenesis-related factors among the study participants (µg/L). The levels of serum vascular endothelial cell growth factor (VEGF), hypoxia-related factor (HIF-1α), follistatin-related protein-1 (FSTL-1), and homocysteine (HCY) for the dance group compared to the no-dance group at high altitude. Data is presented as mean ± SD. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (t-test) relative to dance group.



**Figure 2.** Data demonstrates the change in incidence rates of extracranial internal carotid artery stenosis, carotid stenosis and plaque formation between the two groups. No significant differences were observed for cerebral arteriosclerosis. Data is presented as the incidence rates (%) \* $P < 0.05$ . Chi-squared test was used to determine significant differences between the dance and no-dance groups.

The average dance history and daily dance time in the dance group were significantly higher compared to the no-dance group ( $P < 0.01$ ), while the body weight, BMI, heart rate, systolic pressure, and diastolic pressure were significantly lower ( $P < 0.05$ ), while the  $SO_2$  and  $SO_2$ /heart rate ratio were significantly higher ( $P < 0.01$ ,  $P < 0.05$ ) (Table 2).

#### Effect of Guozhuang dance on peripheral blood vascular-related factors

RBC counts and LDH activity in the dance group were significantly higher compared to the no-dance group ( $P < 0.05$ ), while the levels of

serum BGS, TC and LDL were significantly lower ( $P < 0.05$ ). No significant differences were observed in MCV, HB, CR, UA or TG between the two groups (Table 3).

#### Effect of Guozhuang dance on serum angiogenesis-related factors

As shown in the serum levels of HIF-1α, VEGF and HCY in the dance group were significantly lower compared to the no-dance group ( $P < 0.01$ ,  $P < 0.001$ ), while FSTL-1 levels were significantly higher ( $P < 0.001$ ). No significant differences were observed in PGC-α serum levels between the dance and the no-dance groups ( $3.20 \pm 0.54$  versus  $3.44 \pm 0.71$ ;  $P = 0.369$ ) (Figure 1).

#### Effect of Guozhuang dance on cerebral hemodynamics

As shown individuals of the dance and the no-dance groups underwent both TCD and CUS. As demonstrated by TCD, the incidence rate of extracranial internal carotid artery stenosis in the dance group (13.33%) was lower compared to the no-dance group (33.33%) ( $P < 0.05$ ). However, there were no significant

**Table 4.** A comparison of the cerebral hemodynamic parameters between the exercise and no-exercise groups at high altitude

Parameters	NO	Middle cerebral arteries		t-test	P-value	Basal arteries		t-test	P-value
		Dance	No-dance			Dance	No-dance		
Vm (cm/s)	30	49.67±9.26**	41.87±7.31	3.620	0.001	38.73±9.38*	32.03±9.38	2.465	0.017
PSV (cm/s)	30	76.67±12.49**	68.83±9.52	2.733	0.008	55.90±13.82*	47.40±12.99	2.454	0.017
EDV (cm/s)	30	68.03±12.93**	56.17±13.99	3.412	0.001	99.97±11.13*	91.67±14.99	2.437	0.018
PI	30	0.94±0.13	1.02±0.25	1.259	0.213	0.87±0.17	0.92±0.17	1.066	0.205
RD	30	0.57±0.023*	0.72±0.22	2.566	0.013	0.52±0.36*	0.72±0.32	2.224	0.029

Note: Data are given as the mean ± SD. \* $P < 0.05$ , \*\* $P < 0.01$  (t-test) relative to dance group. Abbreviation: Vm, mean velocity; PSV, systolic velocity; EDV, diastolic velocity; PI, pulsation index; RD, resistance index.  $V_m = (PSV + EDV \times 2) / 3$ ;  $PI = (PSV - EDV) / [\text{mean}(PSV + EDV)]$ ;  $RD = (PSV - EDV) / PSV$ .

differences in the rate of cerebral arteriosclerosis in two groups ( $P > 0.05$ ). CUS demonstrated that the incidence rate of carotid stenosis (16.67%) and plaque formation (20.00%) in the dance group was significantly lower compared to the no-dance group (40.00%, 46.67%) ( $P < 0.05$ ) (Figure 2).

The IMT of the bilateral mean carotid arteries in the dance group was significantly lower compared to the no-dance group (0.923±0.29 versus 1.03±0.35;  $P < 0.001$ ). The Vm, SPV, EDV and RD of MCAs and BAs were significantly higher in the dance group than in the no-dance group ( $P < 0.01$ ,  $P < 0.05$ ); however, there were no significant differences of the PI in two groups ( $P > 0.05$ ) (Table 4).

#### Relationship between serums PGC- $\alpha$ and HCY levels and IMT of the bilateral carotid arteries

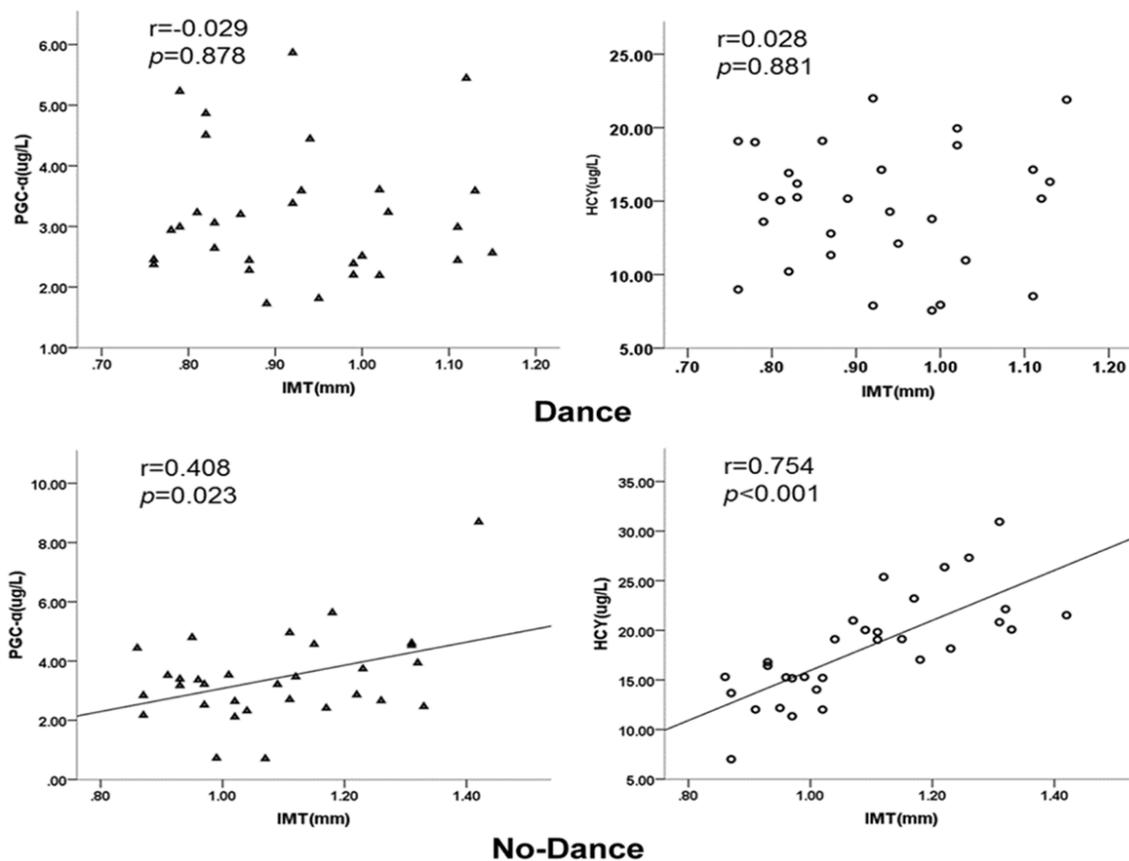
To demonstrate the effects of inactivity on serum angiogenesis-related factors and cerebral hemodynamics, we analyzed the correlation between the serum levels of PGC- $\alpha$ , HCY and the IMT of the bilateral mean carotid arteries in the two groups. As shown in (Figure 3), there was a positive correlation between serum levels of PGC- $\alpha$ , HCY and IMT scores in the no-dance group ( $r = 0.408$ ,  $P < 0.05$ ;  $r = 0.754$ ,  $P < 0.01$ ). However, no significant correlations were observed in the dance group ( $r = 0.029$ ,  $P > 0.05$ ;  $r = 0.028$ ,  $P > 0.05$ ). These results indicate that higher serum PGC- $\alpha$ , HCY levels were associated with higher IMT scores in the no-dance group in individuals living in high altitudes.

#### Discussion

Moderate-intensity aerobic exercise increases the heart rate and promotes increased oxygen

uptake to improve the overall health of an individual. Examples include running, swimming, jumping, dancing, jogging, fast walking, tai chi, mountain climbing, and bicycling. An aerobic exercise is a form of physical exercise that combines rhythmic aerobic exercise with stretching and strength training routines to improve all elements of fitness (flexibility, muscular strength, and cardio-vascular fitness) [14]. Compared to walking, or playing an instrument, dancing has the advantage of combining physical activity with social, emotional interaction, and sensory stimulation, and has been shown to have health benefits [15]. Traditional Guozhuang dance is now prevalent among people living in the Qinghai-Tibet plateau regions. It consists of "singing coupled with joyful dancing in a circle" and is performed in public places as a way of relaxation and exercise after supper in Tibet. In the icy environment of the plateau, people unconsciously formed the habit of stomping their feet to keep warm. A previous study reported that Tibetan dance improves mental and physical health and is suitable for the elderly [16]. It increases the stamina and has the benefits of both sports and fitness regimens [17]. The present study found that RBC counts and LDH activity were increased in elderly individuals who regularly practiced Tibetan dance in high plateau. Improving the aerobic capacity by dancing affects the RBC, enzymes, maximum oxygen uptake, and lactic acid tolerance, and has been beneficial to one's overall health. However, no significant differences in MCV or HB levels were observed between individuals in the dance and no-dance groups. These results may be Tibetan dance can stimulate peripheral chemoreceptor to elevate RBC counts, because in the high-altitude hypoxic environment can stimulate the kidneys to generate more erythropoietin, which

## Vascular-related factors, cerebral hemodynamic, Tibetan dance



**Figure 3.** Correlation between the serum levels of proliferate-activated receptor gamma coactivator1-alpha (PGC-1 $\alpha$ ) ( $\mu\text{g/L}$ ) and homocysteine (HCY) ( $\mu\text{g/L}$ ) and intima-media thickness (IMT) (mm) of the bilateral carotid arteries in the dance and no-dance groups. There was a positive correlation between serum levels of PGC- $\alpha$ , HCY and IMT scores in the no-dance group ( $r = 0.408$ ,  $P < 0.05$ ;  $r = 0.754$ ,  $P < 0.001$ ). However, no significant correlations were observed in the dance group ( $r = 0.029$ ,  $P > 0.05$ ;  $r = 0.028$ ,  $P > 0.05$ ).

can induce the redistribution of blood to maintain oxygen homeostasis and to produce more RBC counts [18]. Studies have shown that regular low-strength endurance exercise in the elderly promotes cholesterol metabolism and catabolism. It accelerates the removal of LDL deposits on the vessel wall. Such exercises also improve the utilization of sugar, increase the sensitivity of insulin, reduce insulin resistance, and lower blood glucose levels [19]. The results demonstrated that the levels of BGS, TC, and LDL significantly decreased in the dance group compared to the no-dance group. Studies have shown that long-term and regularly practiced Tibetan dance in elderly individuals living at high altitudes could reduce the levels of blood lipids and glucose, increase RBC counts and the LDH activity, improve muscle strength, flexibility fitness, and psychological situations.

Study has found that  $\text{SO}_2$  and heart rate influences fatigue recovery, as observed in swimmers from Shanghai training at high altitudes (1,888 m) [20]. The results from the present study showed that the  $\text{SO}_2$  and ratio of  $\text{SO}_2$ /heart rate were significantly higher in the dance group compared to the no-dance group, thus indicating that Tibetan dance can be used as a fitness model for elderly by enhancing  $\text{SO}_2$  and  $\text{SO}_2$ /heart rates which were important indicators for heart functions in the elderly with hypoxia conditions. The strenuous exercise may regulate the immune function and prevent sudden death in hypoxia environments. Tyagi reported that physical activity and aerobic fitness had a positive effect on the heart rate in obese adults [21]. We previously reported that exercise in elderly individuals improved heart rate and cardiac autonomic function in hypoxic environments [22]. The results of the present

study demonstrated that body weight, BMI, heart rate, and blood pressure were significantly lower in individuals who partook in Tibetan Guozhuang dance compared to those who did not. Hence, long-term and regularly practiced Tibetan dance for training to adopt a healthy lifestyle, guiding their appropriate physical exercise, that resulting in improving vascular function in elderly individuals living at high altitudes.

HIF-1 $\alpha$  is a major regulator of oxygen homeostasis and plays a role in tissue remodeling during physiological adaptation to moderate levels of high-altitude hypoxia [23, 24]. HIF-1 $\alpha$  mediates the body's response to hypoxia, including erythropoiesis, glycolysis, the production of a variety of cytokines for angiogenesis, and the regulation of blood vessel tone. It has been well accepted that hypoxia involves in inflammatory process. Many oxygen-dependent genes are changes downstream of HIF-1 $\alpha$  during hypoxia and vascular endothelial growth factor (VEGF) is one of the primary target ones. It has been known that VEGF production is vitally dependent on HIF-1 $\alpha$  during hypoxia or ischemia [25, 26]. In the present study, we found that VEGF in serum are significantly increased in the dance group under the hypoxic conditions, indicating the Tibetan dance may improve the vascular protective abilities under the hypoxic condition. In addition, the Tibetan dance increased VEGF in serum may beneficial for suppressing the up-regulated systemic inflammation during hypoxia surrounding [27], because VEGF has been shown to downregulate cell adhesion molecule expression and reduce platelet aggregation. Further studies need to evidence the effects of Tibetan dance on systemic inflammation in elderly individuals during hypoxia condition, because HIF-1 $\alpha$  itself is a known immunomodulator which crosstalk with NF- $\kappa$ B, the critical transcription factors critical regulators of genes that function in inflammation [28].

Recently studies have shown that genetic or nutritional deficiencies in HCY metabolism lead to hyperhomocysteinemia (HHcy), which subsequently results in endothelial dysfunction, and is a hallmark of atherosclerosis [29, 30]. HHcy is a risk factor for carotid intima-media thickening and plaque formation [31]. HHcy-counteracting strategies, such as nutrition and exercise, should be included in combinatorial treatment

regimens for effective prevention and regression of atherosclerotic plaques [32]. Studies have demonstrated that serum FSTL-1 levels in human myotubules are increased by 22% after 60 min of cycling exercise [33]. The present study showed that the levels of serum HIF-1 $\alpha$ , VEGF, and HCY were significantly decreased in the dance group compared to the no-dance group ( $P < 0.01$ ,  $P < 0.001$ ), while serum FSTL-1 levels were significantly increased ( $P < 0.001$ ). These results demonstrate that dance activates HIF-1 $\alpha$ , which subsequently initiates transcription of downstream target genes, such as VEGF, to mediate the adaptive hypoxic response and HIF-1-mediated signaling pathway in human skeletal muscle. These results showed Tibetan dance had improved oxygen metabolism, increased human myotubules, reduced cell apoptosis, and increased blood flow of elderly individuals living in high altitudes.

Vascular disease is the leading cause of death and disability in middle-aged and older people. Several vascular risk factors can cause intracranial and extracranial atherosclerosis, while stenosis or occlusion may ultimately lead to cerebral infarction [34]. Therefore, early detection, diagnosis, and treatment are essential to reduce the mortality and disability of patients with ischemic cerebrovascular disease. While digital subtraction angiography (DSA) remains the gold standard for diagnosing cerebral ischemic disease [35], CUS and TCD are very reliable non-invasive ways to evaluate the cerebral vasculature. Previous studies have shown that regular physical activity was associated with a smaller carotid diastolic diameter and decreased the risk of carotid artery stenosis in patients with vascular disease or risk factors [36, 37]. A combination of TCD and CUS demonstrated that the incidence of extracranial internal carotid artery stenosis, carotid stenosis and plaque formation in the dance group was significantly lower compared to the no-dance group ( $P < 0.05$ ). However, no significant differences were observed between the two groups for cerebral arteriosclerosis ( $P > 0.05$ ). These findings suggest that dance may have a direct positive effect on vascular structure and function in elderly individuals living at high altitudes by improving endothelial function, increasing the levels of FSTL-1 and decreasing HHcy, which has an anti-endothelial role.

A 4-year follow-up study of 65-year-old retired elderly subjects who continued to work and participate in regular exercise showed a better cognitive function and higher overall cerebral blood flow velocity than in those who did not exercise [38]. In the present study, the Vm, SPV, EDV and RD of the bilateral MCAs and BAs in the dance group were significantly higher than in the no-dance group. The dance was also associated with beneficial effects on lowered IMT scores and carotid artery stenosis and increased the cerebral blood flow velocities by reduced blood pressure, body weight, BMI, heart rate, blood lipids and glucose of the traditional risk factors for vascular disease. However, higher serum PGC- $\alpha$  and HCY levels were associated with higher IMT scores in the no-dance group at high altitudes. These results showed that physical inactivity increases the risk of vascular risk factors to induce intracranial and extracranial atherosclerosis under hypoxia conditions. The limitation of the study was the relatively small size of samples and hence needed to be validated in a larger population cohort to determine beneficial effects of Tibetan Guozhuang dance at high altitudes.

In conclusion, the elderly individuals who long-term and regularly practiced Tibetan dance in high plateau regions was associated with a lower risk of lowered IMT scores, less carotid artery stenosis and plaque formation and may be possible beneficial effects on blood vessel functionality and cerebral hemodynamics. The benefits of the simply physical approach for improving the muscle strength, flexibility fitness, decreased traditional vascular-related risk factors and psychological conditions under hypoxia conditions may open a new window for delaying aging.

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### Disclosure of conflict of interest

None.

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## Vascular-related factors, cerebral hemodynamic, Tibetan dance

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