DOI: 10.1002/vms3.639

ORIGINAL ARTICLE

WILEY

Vascular characteristics and expression of hypoxia genes in Tibetan pigs' hearts

Yanan Yang[1] | Caixia Gao[2] | Tianliang Yang[1] | Yuzhu Sha[1] | Yuan Cai[1] | Xinrong Wang[1] | Qiaoli Yang[1] | Chengze Liu[1] | Biao Wang[1] | Shengguo Zhao[1]

[1] College of Animal Science & Technology, Gansu Agricultural University, Lanzhou, P.R. China

[2] State Key Laboratory of Veterinary Biotechnology, Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Harbin, P.R. China

Correspondence
Shengguo Zhao, College of Animal Science & Technology, Gansu Agricultural University, Lanzhou Gansu, P.R. China.
Email: zhaosg@gsau.edu.cn; zhaoshengguo0628@hotmail.com

Funding information
National Natural Science Foundation of China, Grant/Award Numbers: 31760644, 32060730

Abstract

Background: Tibetan pigs have exhibited unique characteristics from low-altitudes pigs and adapted well to the Qinghai-Tibet Plateau.

Objectives: The current study was undertaken to investigate the hypoxic adaptation of heart in Tibetan pigs.

Methods: The hearts of Tibetan pigs and Landrace pigs raised at high or low altitudes were compared using 3D casting technology, scanning electron microscopy and real-time quantitative PCR (qRT-PCR).

Results: We found that the ratio of the major axis to the minor axis and the density of the heart were significantly higher in Tibetan pigs than in Landrace pigs ($p < 0.05$). Tibetan pigs had larger diameters and higher densities of arterioles than Landrace pigs ($p < 0.05$), and these features have a similar variation with the expression of vascular endothelial growth factor (*VEGF*). The cardiac expression levels of hypoxia-inducible factor-1α (*HIF-1α*) and endothelial nitric oxide synthase (eNOS) were significantly higher in pigs reared at high altitudes than in those reared at low altitudes ($p < 0.05$). In contrast, Egl nine homolog 1 (*EGLN1*) had the opposite trend with respect to *HIF-1α* and *eNOS* and was related to red blood cell (RBC) counts. Notably, the expressions of erythropoietin (*EPO*) and endothelial PAS domain-containing protein 1 (*EPAS1*) were significantly higher in Landrace pigs kept at high altitudes than in the others ($p < 0.05$) and were associated with haemoglobin.

Conclusions: These findings show that the regulation of the heart function of Tibetan pigs in a hypoxic environment is manifested at various levels to ensure the circulation of blood under extreme environmental conditions.

KEYWORDS
hearts, hypoxic genes, Tibetan pigs, vascular morphology

1 | BACKGROUND

The hypoxic environment at high altitude imposes extreme physiological challenges in pigs (Ma et al., 2019; B. Zhang et al., 2017; B. Zhang et al., 2019). Hypoxia response is characterized by systemic changes in organs, tissues and cells (Alexandra et al., 2019; Hernandez & Alverdy, 2017; Vozdek et al., 2018; Z. Xu & Rothstein, 2018). The Tibetan pigs have evolved physiological adaptations to high-altitude hypoxia, with larger and stronger hearts and a less intense erythropoietic response than other pig populations (Ai et al., 2014; Qi et al., 2019). The heart is the primary and most dynamic organ of the circulatory system, and it is vital for maintaining the stability of the internal environment (Defaria Yeh et al., 2018; Meilhac & Buckingham, 2018). In a hypoxic environment, the decrease in the oxygen transport capacity of the body can be compensated by enhancing the heart's blood-pumping function, increasing the heart rate, raising the haemoglobin (HGB) content of the blood and redistributing the blood supply (Faulhaber et al., 2015). The cardiovascular response to hypoxia is a dynamic process that begins immediately upon exposure and evolves over days, weeks and years with prolonged exposure. As hypoxia becomes sustained, arterial oxygen content increases secondary to haemoconcentration, ventilatory acclimatization and increases in red cell mass (Ai et al., 2014).

Hypoxia-inducible factor (HIF) is the master regulator of the transcriptional response to hypoxia, and the expression levels of HIF can dramatically change the expression of hypoxia-related genes (Bhattarai et al., 2018; Shakhsheer et al., 2019). Based on the findings of these studies, it might be interesting to explore whether the genesis and development of blood vessels also impact the physiological characteristics of the heart. In the present study, we comprehensively compared the features of the cardiac arteries, the changes in blood biochemical and physiological indexes and the regulatory mechanisms of hypoxia-induced related genes between Tibetan pigs and Landrace pigs reared at different altitudes to understand the adaptive changes in heart tissue in the hypoxic response process.

2 | METHODS

2.1 | Ethics statement

All animal experiments were conducted according to the guidelines for the care and use of experimental animals established by the Ministry of Science and Technology of the People's Republic of China (approval number: 2006–398), and the work was approved by Gansu Agricultural University (GSAU-Eth-AST-2021-023), Lanzhou, China.

2.2 | Collection of hearts and treatment

The hearts were collected from Tibetan pigs (Hezuo pigs, Figure 1) inhabiting Hezuo county, Gannan (Tibetan male piglets from the highlands (TH), 3000 m) and Jingchuan (Tibetan male piglets migrated to lowlands (TL), 1000 m) as well as Landrace pigs inhabiting Jingchuan (Landrace male piglets from the lowlands (LL), 1000 m) and Yongdeng (Landrace male piglets migrated to highlands (LH), 2500 m) in Gansu province; these four groups contained an average of seven pigs each, for a total sample size of 28 animals. All the pigs were male and adopted the same feeding management method in farms, which were sacrificed at 1 year of age. The animals were anesthetized quickly with an intramuscular injection of ketamine (10-15 mg/kg) for euthanasia. After removal, 12 hearts (three per group) were immersed in liquid nitrogen for RNA extraction. The rest of the hearts (four per group) were cleaned to create 3D casts of the cardiac arteries and arterioles. Venous blood was also collected.

2.3 | Procedure for creating vascular casts

First, the morphological indexes of the heart were measured with a ruler and a Vernier caliper. Second, 7% acrylonitrile butadiene styrene (ABS; Kumho Industry, Korea) perfusion solution mixed with Sudan III dye (Guangfu Industry, China) was injected continuously into the cardiac artery to make the casts. Finally, the perfused hearts were immersed in 40% concentrated hydrochloric acid to dissolve the tissue, and the casts were flushed with water once they had been denuded of tissue.

2.4 | Observing the characteristics of the casts and measuring the diameters of the arteries

The anatomical characteristics of the cardiac arteries and their branches were observed with a dissecting microscope, and

FIGURE 1 Tibetan pigs in the present study. (a) A group of Tibetan pigs. (b) A profile shot of a Tibetan pig. (c) A front shot of a Tibetan pig

photographs of the casts were taken with a digital camera. The vascular diameters at several sites along the cardiac arteries and arterioles were measured using a digital Vernier caliper (Hengliang, China). The ultrastructural morphology of the arteriolar endothelium was examined in samples that had been obtained randomly from the cardiac vascular casts, washed with an ultrasonic cleaner (Dakang Industry, China), dried and sputter coated with a layer of palladium/gold using a 3500 VA sputter coater (JMB, Japan). Images of the arteriolar endothelium were obtained with a scanning electron microscope (Hitachi S-3400N; Japan) at an accelerating voltage of 15 kV. The ultrastructural morphology of the arteriolar endothelium was studied, and morphological differences among the pigs were determined with the Image J analysis system.

2.5 | Measurement of blood physiological indexes

A haematology analyzer (GRT-6008) was used to measure the blood parameters of 12 pigs.

2.6 | Real-time quantitative PCR analysis of the transcript abundance of hypoxia-related genes

Total RNA was extracted with TRIzol and subjected to quantitative and qualitative detection; the samples with the best integrity were selected for the following experiments. A HiScript II 1st-strand cDNA synthesis kit (Vazyme, China) was used to synthesize cDNA, which was stored at −20°C. Then, ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) was used for quantitative real-time fluorescence analysis. Primer sequences for the genes *HIF-1α*, *EPAS1*, *EPO*, *VEGF*, *eNOS*, *EGLN1* and *GAPDH* (reference gene) were used for real-time quantitative PCR (qRT-PCR) (Table S1). The data were analyzed with the $2^{-\triangle\triangle Ct}$ method.

2.7 | Statistical analysis

The significance of the differences in the various parameters between the Tibetan pigs and Landrace pigs at different altitudes was determined by multivariate analysis of variance (ANOVA) using SPSS 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used for multiple comparisons. The differences in cardiac arteries between Tibetan pigs and Landrace pigs were analyzed; $p < 0.05$ was taken to indicate a significant difference.

3 | RESULTS

3.1 | Differences in cardiac morphology

The analysis of cardiac morphology revealed some differences in shape between the hearts of Tibetan pigs (TH and TL) and Landrace pigs (LH and LL) (Table 1). Although the hearts of Tibetan pigs were markedly smaller than those of Landrace pigs, adult Tibetan pigs also weighed less than Landrace pigs; thus, we used the ratio (heart tissue weight/body weight) to represent the relative weight of the heart. Surprisingly, the ratio of the major axis to the minor axis was significantly higher in Tibetan pigs than in Landrace pigs ($p < 0.05$). The heart density of Tibetan pigs was significantly higher than that of Landrace pigs ($p < 0.05$); in contrast, the ratio of the major axis to the minor axis was lower in Tibetan pigs than in Landrace pigs. These findings indicated that the hearts of Tibetan pigs were heavier and wider than those of Landrace pigs.

3.2 | Differences in the anatomical architecture and diameter of the cardiac arteries

In observing cast specimens, we found that the coronary arteries branched repeatedly in the heart wall until they reached the capillaries (Figure 2, Table 2). Several flexible arterioles branched off the arteries at different angles; the first branch exhibited the smallest angle, and the arterioles were shaped like tree roots, forming an almost 90° angle.

We compared the diameters of the cardiac arteries and branches relative to body weight in Tibetan pigs and Landrace pigs reared at different altitudes; the results showed similar distributions and variations in arterial diameter among all groups, and the diameter decreased as the branch level. The major/minor axis ratio of Tibetan pig hearts was lower than that of Landrace pig hearts. Tibetan pigs had more and denser arterioles than Landrace pigs; additionally, the average diameters of the main artery and branches were significantly larger in the former breed ($p < 0.05$), and the diameters of seven parts were significantly larger in TH than in TL ($p < 0.05$). The vessels of the high-altitude pigs were larger in diameter than those of the low-altitude pigs by a nonsignificant margin ($p > 0.05$).

3.3 | Characteristics of the arteriolar epithelium observed by scanning electron microscopy

The rough surfaces of the cardiac arteries were observed in Tibetan pigs and Landrace pigs using a scanning electron microscope (Figure 3). A three-dimensional analysis revealed that the main trunk of the coronary artery followed a bent course and became increasingly fine in diameter as it proceeded. Each trunk gave out many branches in different directions, covering the surface of the heart or extending inwards and had an increasing number of branches from the aorta to the arterioles. The uneven distribution of the cardiac arteriole surfaces resembled tree bark, and the arterioles ended with obtuse-angled branches measuring 40–168 μm in diameter. Endothelial nucleus indentations were distributed on the surfaces of the cardiac arterioles, and the length and width were approximately 19.88 and 2.31 μm, respectively. Interestingly, the surfaces of the arterioles had plicated endothelial nucleus indentations shaped like ripples in water; these indentations were distributed uniformly, and their length and width were

TABLE 1 Cardiac morphological indicators

Ratio	Breeds TH	TL	LH	LL
Heart weight/body weight	$0.0033^{a} \pm 0.0001$	$0.0034^{a} \pm 0.0002$	$0.0026^{b} \pm 0.0002$	$0.0026^{b} \pm 0.0001$
Density	$1.4802^{a} \pm 0.0214$	$1.4163^{b} \pm 0.0286$	$1.1000^{c} \pm 0.0214$	$1.0404^{d} \pm 0.0077$
Major axis/minor axis of the heart	$1.1400^{b} \pm 0.0470$	$1.1708^{b} \pm 0.1340$	$1.5300^{a} \pm 0.0321$	$1.5573^{a} \pm 0.0365$

Notes: Because the Tibetan and Landrace pigs exhibited major differences in body weight, we compared the ratio of heart weight to body weight. Among the TH, LH, TL and LL groups, different letters in the same column indicate a significant difference ($p < 0.05$), while the same letters in the same column indicate no significant difference ($p > 0.05$).

Abbreviations: LH, Landrace male piglets migrated to highlands; LL, Landrace male piglets from the lowlands; TH, Tibetan male piglets from the highlands; TL, Tibetan male piglets migrated to lowlands.

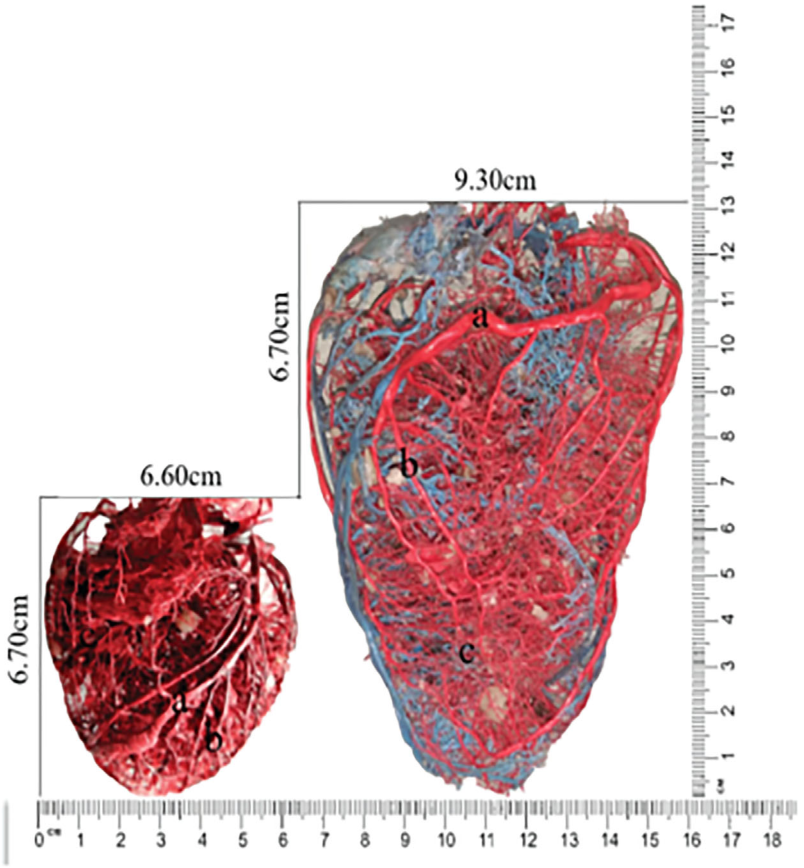

FIGURE 2 Morphological features of the cardiac arteries. The images show casts of the cardiac arteries in two pig breeds: Tibetan pigs (left), and Landrace pigs (right). (a) Coronary artery. (b) First-level branch. (c) Arterioles

approximately 27.94 and 1.97 μm, respectively. The surfaces of the cardiac arterioles had more and deeper bark-like endothelial cell indentations and circumferential constrictions in Tibetan pigs, particularly TH, than in Landrace pigs. Meanwhile, the bark-like endothelial cell indentations and circumferential constriction were visible mainly on the terminal arterioles in LH.

3.4 | Correlation analysis of hypoxia-related genes

Our previous studies of blood biochemical criteria have shown that there are significant differences in red blood cell (RBC) counts, HGB, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and red cell volume distribution width-coefficient of variation among the four groups (Figure 4).

The analysis of gene expression revealed that there were significant differences in the expression levels of *HIF-1α*, *VEGF*, *eNOS*, *EPO*, *EPAS1* and *EGLN1* among the four groups (Figure 5). The cardiac expression levels of *HIF-1α* and *eNOS* were significantly higher in pigs reared at high altitude than in those reared at low altitude ($p < 0.05$), and the highest levels of these genes were found in the TH group. In contrast, *EGLN1* had the opposite trend with respect to *HIF-1α* and *eNOS* and was related to RBCs. The expression of *VEGF* in Tibetan pigs was significantly higher than that in Landrace pigs ($p < 0.05$) and exhibited a

TABLE 2 Diameters of the cardiac arteries

Part	Branches	Average diameter/weight			
		TH (3000 m)	LH (2500 m)	TL (1000 m)	LL (1000 m)
Ratio (major/minor axis)		1.0898	1.5814	1.1006	1.5807
Left coronary artery	Trunk	$0.0451^{a} \pm 0.0009$	$0.0112^{b} \pm 0.0002$	$0.0446^{a} \pm 0.0007$	$0.0112^{b} \pm 0.0003$
	First-level branch	$0.0387^{a} \pm 0.0010$	$0.0097^{c} \pm 0.0002$	$0.0372^{b} \pm 0.0003$	$0.0095^{c} \pm 0.0001$
	Second-level branch	$0.0186^{a} \pm 0.0006$	$0.0048^{c} \pm 0.0002$	$0.0152^{b} \pm 0.0016$	$0.0042^{c} \pm 0.0002$
	Third-level branch	$0.0163^{a} \pm 0.0006$	$0.0043^{c} \pm 0.0003$	$0.0140^{b} \pm 0.0009$	$0.0041^{c} \pm 0.0001$
Right coronary artery	Trunk	$0.0457^{a} \pm 0.0008$	$0.0111^{c} \pm 0.0001$	$0.0428^{b} \pm 0.0008$	$0.0107^{c} \pm 0.0004$
	First-level branch	$0.0387^{a} \pm 0.0010$	$0.0102^{c} \pm 0.0002$	$0.0356^{b} \pm 0.0009$	$0.0097^{c} \pm 0.0003$
	Second-level branch	$0.0185^{a} \pm 0.0006$	$0.0050^{c} \pm 0.0001$	$0.0153^{b} \pm 0.0009$	$0.0043^{c} \pm 0.0001$
	Third-level branch	$0.0156^{a} \pm 0.0006$	$0.0044^{c} \pm 0.0002$	$0.0124^{b} \pm 0.0004$	$0.0041^{c} \pm 0.0001$

Note: Among the TH, LH, TL and LL groups, different letters in the same column indicate a highly significant difference ($p < 0.05$), and the same letters in the same column indicate no significant difference ($p > 0.05$).
Abbreviations: LH, Landrace male piglets migrated to highlands; LL, Landrace male piglets from the lowlands; TH, Tibetan male piglets from the highlands; TL, Tibetan male piglets migrated to lowlands.

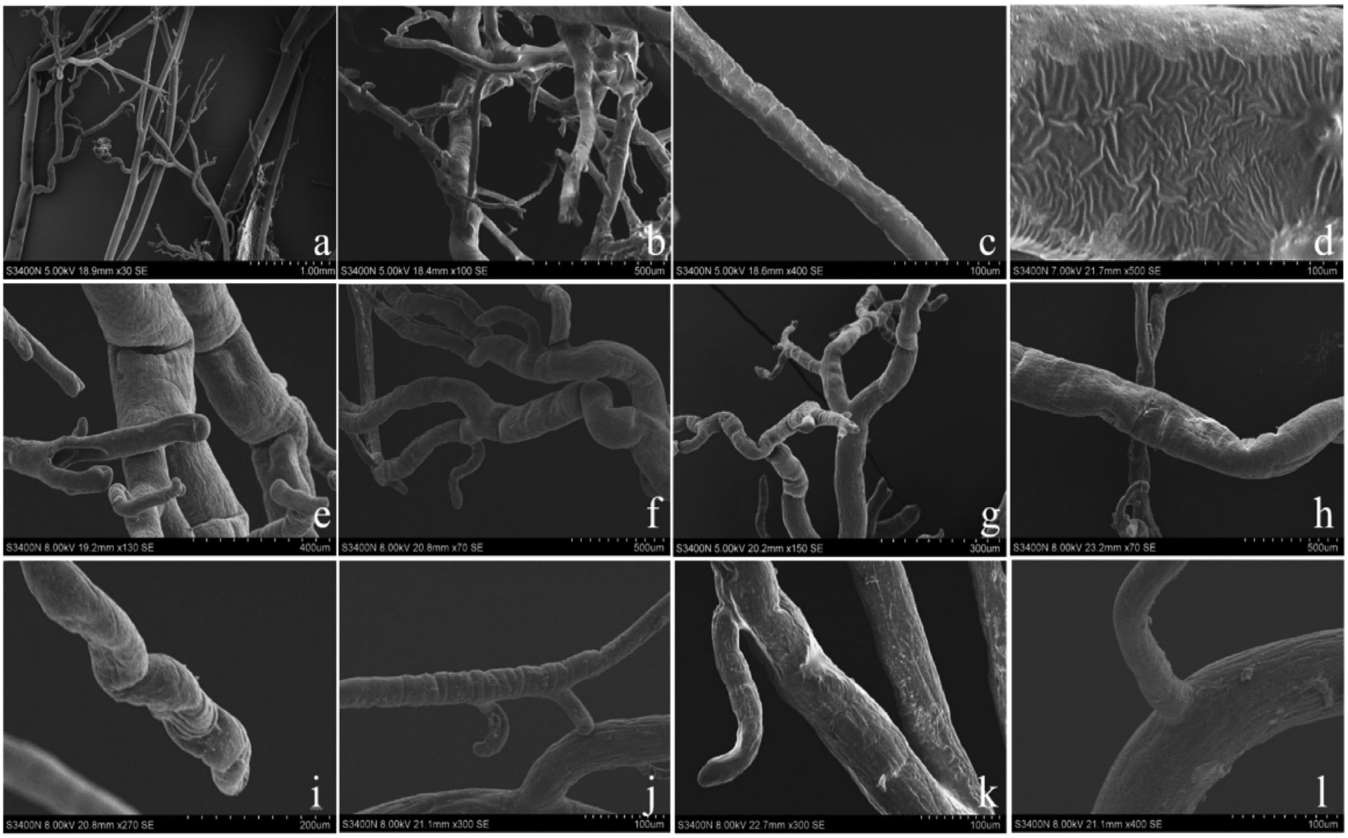

FIGURE 3 Distribution and superficial features of the arterioles in the heart. (a) The arteriole distribution of the left atrium (×30). (b) The arteriole distribution of the atrium (×100). (c) The arteriole distribution of the ventricle (×400). (d) The arteriole distribution of the ventricle (×500). (e) Imprints of the endothelial nuclei of the TH group (×130). (f) Imprints of the endothelial nuclei of the LH group (×70). (g) Imprints of the endothelial nuclei of the TL group (×150). (h) Imprints of the endothelial nuclei of the LL group (×70). (i) The distribution of annular narrowing in the TH group (×270). (j) The distribution of annular narrowing in the LH group (×300). (k) The distribution of annular narrowing in the TL group (×300). (l) The distribution of annular narrowing in the TL group (×400)

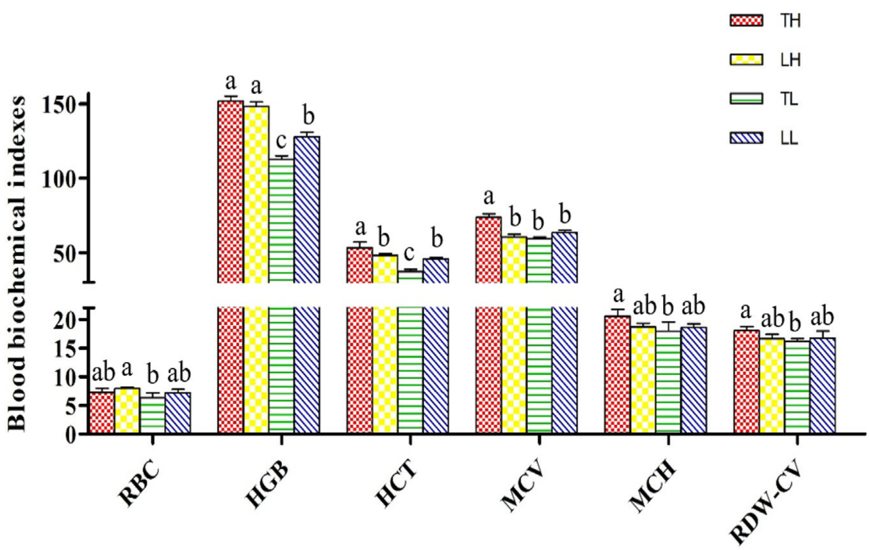

FIGURE 4 Expression levels of blood biochemical indexes in the four groups of pigs. Note: Different superscripts indicate that the expression levels differed significantly ($p < 0.05$, mean ± SE) between groups, and matching superscripts indicate that the levels did not differ significantly ($p > 0.05$, mean ± SE)

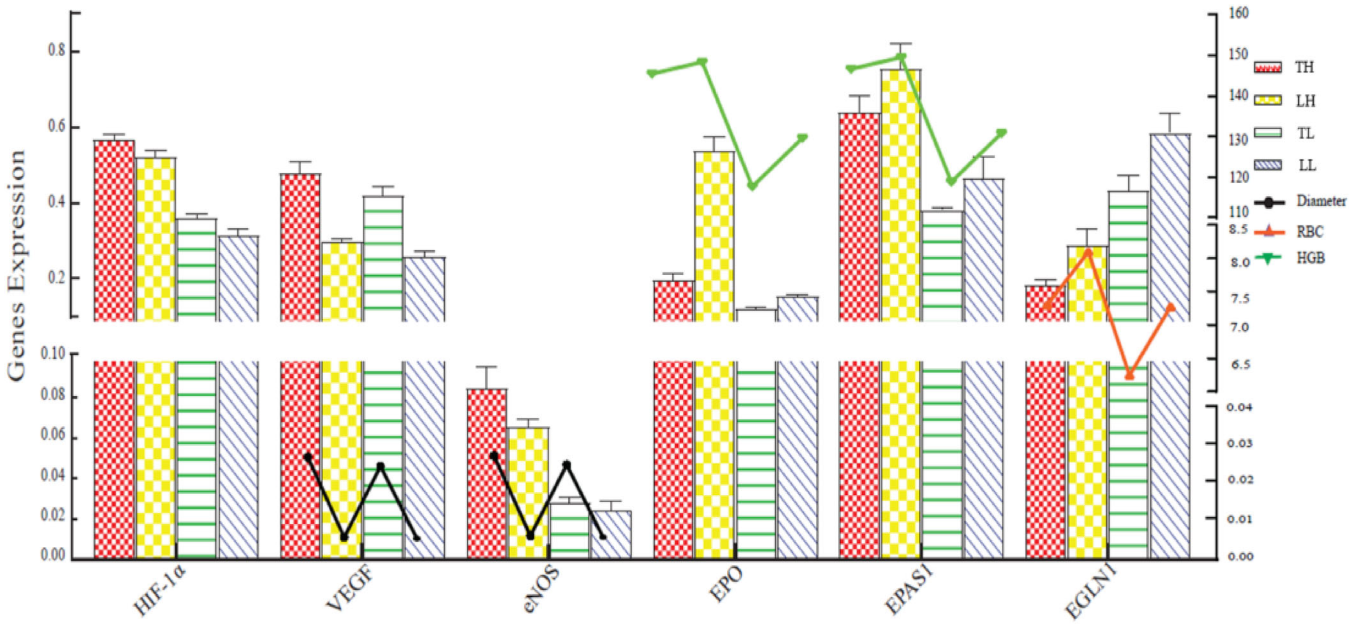

FIGURE 5 Expression levels of hypoxia-inducible factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), endothelial nitric oxide synthase (eNOS), erythropoietin (EPO), endothelial PAS domain-containing protein 1 (EPAS1) and Egl nine homolog 1 (EGLN1) in the hearts of the four groups of pigs and their relation to vessel diameter, red blood cells (RBCs) and haemoglobin (HGB). Note: Different superscripts indicate that the expression levels differed significantly ($p < 0.05$, mean ± SE),between groups, and matching superscripts indicate that the levels did not differ significantly ($p > 0.05$, mean ± SE)

similar trend to the artery diameter. Notably, the expression of *EPO* and *EPAS1* was significantly higher in LH than in the other groups ($p < 0.05$) and was associated with HGB.

4 | DISCUSSION

4.1 | Anatomical structure and arterial function

The use of oxygen by the body depends on the cardiovascular circulatory system, and the flow rate and state of blood are closely related to the oxygen concentrations (Meilhac & Buckingham, 2018). The ratio of the major axis to the minor axis was closer than 1 in Tibetan pigs than in Landrace pigs, which illustrates that the former breed has stronger heart function than the latter, enhancing the ability to adapt to a hypoxic plateau environment. The diameters of several arteries in the heart were significantly greater in Tibetan pigs than in Landrace pigs. This implies that the cardiac arterial system is more developed at higher altitudes and might more efficient to transport oxygen (Wang et al., 2020). The increased arterial diameters of Tibetan pigs might be a physiological basis for the transport of additional blood to the heart. Circulation of blood throughout the body is dependent on the efficiency of blood supply to the heart; therefore, high-altitude animals require large-diameter cardiac arterioles and branches to ensure that the blood supply meets the demand (Meilhac & Buckingham, 2018). The diameters of some of the main cardiac arteries and arteriole branches gradually increased with altitude of the Tibetan pigs to adapt to extreme environmental conditions such as high altitude and low oxygen pressure (Wang et al., 2020; Zhou et al., 2015).

The architecture of the vascular network is closely related to blood flow and heart function, as an efficient vascular network can ensure that organs obtain blood (Avivi et al., 2005). Vascular endothelial cells are widely distributed in various tissues and are the only cells in contact with blood (Tetsuo et al., 2016). Vascular endothelial cells with a lamellar and lamelliform appearance are conducive to the absorption of liquid from plasma and its transport to tissues (X. Xu, Xu, et al., 2019). The ultrastructure of the arteriolar endothelium differed between pig breeds, in particular, the bark-like dents on the surface of the cardiac arterioles are more prominent and concentrated in Tibetan pigs than in other breeds (P. Zhang & Zuo, 1997). Some studies have found that the surfaces of the cerebral and heart arteries of yaks resemble bark, and the indentation on the surface is tapered (He, 2007). The bark dents on the surface of arterioles are made by the smooth muscle fibres of the vascular endothelium (X. Xu, Xu, et al., 2019). Vascular smooth muscle cells, which generally have a tapered shape, play an important role in regulating the rate of blood flow and maintaining vascular tension, and increased blood flow velocity leads to an increase in oxygen supply to tissues (Almohanna & Wray, 2018; Padget et al., 2019). Some studies found constriction of smooth muscle cells on the surfaces of arterioles in the lungs and the ventricular wall in yaks (He, 2007; Zhou et al., 2015). Similar structures have been observed in the cardiac arterioles in pigs, where these arterioles similarly play an active regulatory role in maintaining blood flow. In the present study, compared to the low-altitude Landrace breed, Tibetan pigs had deep and concentrated constrictions. This means that in the hearts of Tibetan pigs, the vascular network regulates and distributes blood flow in a way that is suitable for extreme environments, ensuring the normal development of the heart and systemic circulation (He, 2007). Based on these findings, blood flow velocity in Tibetan pigs is probably enhanced by the intensively developed smooth muscle fibres in the vascular endothelium of the cardiac arterioles, and this seems to be the mechanism through which normal blood pressure levels are maintained in these pigs in high-altitude and hypoxic environments (Padget et al., 2019).

Tibetan pigs (TH, TL) and high-altitude pigs (TH, LH) have more developed hearts, larger vessel diameters, more abundant blood vessel surface indentations and annular constrictions, which were also found in yak hearts (He, 2007). This means that in high-altitude breeds, the heart receives abundant arterial blood flow (Wang et al., 2020). In contrast, the smaller vascular diameters of the cardiac artery and its branches in low-altitude breeds imply that the heart received less blood supply (Zhou et al., 2015).

4.2 | Blood biochemical indexes in pigs

Compared with the plain area, the plateau zokor and Tibetan antelope show lower HGB and RBC, which is beneficial to reduce blood viscosity and increase blood flow to achieve higher oxygen supply levels (Y. F. Zhang et al., 2015; W. Zhang et al., 2014). However, the HGB and RBC of Tibetan pigs and Landrace pigs in high altitude were higher than low altitude environment, and yak, Tibetan sheep and goats have similar features of HGB and RBC levels to increase the RBCs in hypoxia environment responsible for compensation by diffusion and the synthesis of HGB and increase the ability of pigs to carry oxygen (Beall et al., 2010; X. Y. Kong et al., 2014; X. Kong et al., 2019; Y. J. Xu, Gong, et al., 2019). It showed stronger oxygen release ability than hypoxic environment, thus improving the organism's tolerance to high-altitude hypoxic environment.

4.3 | Differential expression of hypoxia-related genes in the heart and their associations with the morphology and physiology of pigs

There are certain adaptive characteristics to hypoxic environment that are showed through studies on morphological characteristics of lung and heart, blood physiology and biochemistry, as well as on the evolution of genomic adaptations at the molecular level in Tibetan pigs (Yang et al., 2021; B. Zhang et al., 2017). Tibetan pigs can survive in a plateau environment due to their adaptation to hypoxic conditions, and the normal functioning of the heart is mainly regulated by angiogenesis, cellular energy metabolism and the expression of hypoxia-related genes (Ma et al., 2019; B. Zhang et al., 2019).

The expression of *HIF-1α* was highest in high-altitude Tibetan pigs than in other groups and was related to the oxygen partial pressure in the habitat as a major hypoxia-inducing factor (Amany et al., 2018; Depoix et al., 2017). *VEGF* could be positively regulated by *HIF-1α* and the expression of *VEGF* was higher in Tibetan pigs than that of Landrace pigs, which could promote endothelial cell proliferation and neovascularization, increasing vascular permeability to promote the development of cardiovascular system as a key vascular related factor (Srinivasan et al., 2015; Wang et al., 2020; Yu et al., 2018). The expression of *eNOS* and the relative vascular diameter were the highest in the high-altitude Tibetan pigs, and it is believed that *eNOS* can regulate blood pressure and vascular permeability by influencing the vascular diameter (Janaszak-Jasiecka et al., 2018; Kim et al., 2016).

EPAS1 has a potential key role in normal erythropoiesis (Peng et al., 2017; X.-H. Xu et al., 2018; Xin et al., 2020). The expression of *EPAS1* is significantly higher in the high-altitude than in the low-altitude group, which may adapt to the long-term hypoxia environment by mediating the preferential proliferation of erythrocytes under chronic hypoxia conditions (Liu et al., 2020; Peng et al., 2017; X.-H. Xu et al., 2018). *EPO* expression is rapidly increased to stimulate compensatory production of RBSs to relieve anoxic environment (El et al., 2013). The expression of *EPO* in the high-altitude group was significantly higher than that in the low-altitude group, which resulted in enhanced proliferation and differentiation of RBC precursors and excessive accumulation of RBCs to increased HGB (Amany et al., 2018; El et al., 2013).

5 | CONCLUSION

This study demonstrates that the hearts of Tibetan pigs are heavier and wider than those of Landrace pigs and that Tibetan pigs have a higher number of and denser arteries and arterioles than Landrace pigs. The analysis of the hearts structure revealed that the high-altitude Tibetan pigs had more developed hearts and abundant arteries to ensure their circulation in a low oxygen environment. In addition, the HIF pathway might be one of the major factors that influence the RBC count, HGB level and number of arterioles, which might be regulated through the environment. The findings also show that the regulation of the heart function of Tibetan pigs in a hypoxic environment is manifested at various levels to ensure breathing under extreme environmental conditions.

ACKNOWLEDGEMENT

The study was supported by National Natural Science Foundation of China (31760644, 32060730).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Formal analysis, investigation, methodology and writing-original draft: Yanan Yang. *Conceptualization, data curation, supervision, validation, visualization, writing-review and editing*: Caixia Gao and Tianliang Yang. *Formal analysis, investigation, methodology, project administration, writing-review and editing*: Yuzhu Sha, Yuan Cai, Xinrong Wang, Qiaoli Yang, Chengze Liu and Biao Wang. *Methodology, resources, supervision, validation, visualization, writing-review and editing*: Shengguo Zhao.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/vms3.639

ORCID

Yanan Yang https://orcid.org/0000-0001-5684-7014
Caixia Gao https://orcid.org/0000-0001-8183-9753
Tianliang Yang https://orcid.org/0000-0003-0292-2052
Yuzhu Sha https://orcid.org/0000-0003-0023-8628
Yuan Cai https://orcid.org/0000-0003-2828-2794
Xinrong Wang https://orcid.org/0000-0001-9052-2387
Qiaoli Yang https://orcid.org/0000-0003-4577-6366
Chengze Liu https://orcid.org/0000-0002-6822-4890
Biao Wang https://orcid.org/0000-0001-8845-2674
Shengguo Zhao https://orcid.org/0000-0001-8744-375X

REFERENCES

Ai, H., Yang, B., Li, J., Xie, X., Chen, H., & Ren, J. (2014). Population history and genomic signatures for high-altitude adaptation in Tibetan pigs. *BMC Genomics*, *15*, 834. https://doi.org/10.1186/1471-2164-15-834

Almohanna, A. M., & Wray, S. (2018). Hypoxic conditioning in blood vessels and smooth muscle tissues: Effects on function, mechanisms, and unknowns. *American Journal of Physiology Heart and Circulatory Physiology*, *315*, H756–H770. https://doi.org/10.1152/ajpheart.00725.2017

Amany, A. M., Mohamed, M. M. M., Samah, R. K., Gamal, A. S., & Haytham, A. A. (2018). Moringa oleifera extract attenuates the CoCl induced hypoxia of rat's brain: Expression pattern of HIF-1α, NF-kB, MAO and EPO. *Biomedicine & Pharmacotherapy*, *109*, 1688–1697. https://doi.org/10.1016/j.biopha.2018.11.019

Avivi, A., Shams, I., Joel, A., Lache, O., Levy, A P., & Nevo, E. (2005). Increased blood vessel density provides the mole rat physiological tolerance to its hypoxic subterranean habitat. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *19*, 1314–1316. https://doi.org/10.1096/fj.04-3414fje

Beall, C. M., Cavalleri, G. L., Deng, L., Elston, R. C., Gao, Y., Knight, J., Li, C., Li, J. C., Liang, Y., Mccormack, M., Montgomery, H. E., Pan, H., Robbins, P. A., Shianna, K. V., Tam, S. C., Tsering, N., Veeramah, K. R., Wang, W., Wangdui, P., … Zheng, Y. T. (2010). Natural selection on EPAS1 (HIF2alpha) associated with low hemoglobin concentration in Tibetan highlanders. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 11459–11464. https://doi.org/10.1073/pnas.1002443107

Bhattarai, D., Xu, X., & Lee, K. (2018). Hypoxia-inducible factor-1 (HIF-1) inhibitors from the last decade (2007 to 2016): A "structure-activity relationship" perspective. *Medicinal Research Reviews*, *38*, 1404–1442. https://doi.org/10.1002/med.21477

Defaria Yeh, D., Stefanescu Schmidt, A. C., Eisman, A. S., Serfas, J. D., Naqvi, M., Youniss, M. A., Ryfa, A. D., Khan, A. A., Safi, L., Tabtabai, S. R., Bhatt, A. B., & Lewis, G. D. (2018). Impaired right ventricular reserve predicts adverse cardiac outcomes in adults with congenital right heart disease. *Heart (British Cardiac Society)*, *104*, 2044–2050. https://doi.org/10.1136/heartjnl-2017-312572

Depoix, C. L., De Selliers, I., Hubinont, C., & Debieve, F. (2017). HIF1A and EPAS1 potentiate hypoxia-induced upregulation of inhibin alpha chain expression in human term cytotrophoblasts in vitro. *Molecular Human Reproduction*, *23*, 199–209. https://doi.org/10.1093/molehr/gax002

El, H. R., Marchant, D., Pichon, A., Escoubet, B., Pezet, M., Hilfiker-Kleiner, D., Hoch, M., Pham, I., Quidu, P., Voituron, N., Journé, C., Richalet, J.-P., & Favret, F. (2013). Epo deficiency alters cardiac adaptation to chronic hypoxia. *Respiratory Physiology & Neurobiology*, *186*, 146–154. https://doi.org/10.1016/j.resp.2013.01.003

Faulhaber, M., Gatterer, H., Haider, T., Linser, T., Netzer, N., & Burtscher, M. (2015). Heart rate and blood pressure responses during hypoxic cycles of a 3-week intermittent hypoxia breathing program in patients at risk for or with mild COPD. *International Journal of Chronic Obstructive Pulmonary Disease*, *10*, 339–345. https://doi.org/10.2147/COPD.S75749

He, Y. Y. (2007). *Studies on the microvasculature of infant and adult yak's heart* [Unpublished dissertation, Gansu Agricultural University].

Janaszak-Jasiecka, A., Siekierzycka, A., Bartoszewska, S., Serocki, M., Dobrucki, L. W., Collawn, J. F., Kalinowski, L., & Bartoszewski, R. (2018). eNOS expression and NO release during hypoxia is inhibited by miR-200b in human endothelial cells. *Angiogenesis*, *21*, 711–724. https://doi.org/10.1007/s10456-018-9620-y

Kim, H. J., Yoo, H. Y., Lin, H. Y., Oh, G. T., Zhang, Y. H., & Kim, S. J. (2016). Role of muscular eNOS in skeletal arteries: Endothelium-independent hypoxic vasoconstriction of the femoral artery is impaired in eNOS-deficient mice. *American Journal of Physiology Cell Physiology*, *311*, C508–C517. https://doi.org/10.1152/ajpcell.00061.2016

Kong, X. Y., Gou, X., Ma, T., Leng, J., Mao, H. Y., & Yang, S. L. (2014). Study on blood physiological indicators of adaptation to hypoxia in Tibetan Pig. *Journal of Yunnan Agricultural University*, *29*, 297–300. https://doi.org/10.3969/j.issn.1004-390X(n).2014.02.026

Kong, X., Dong, X., Yang, S., Qian, J., Yang, J., Jiang, Q., Li, X., Wang, B., Yan, D., Lu, S., Zhu, L., Li, G., Li, M., Yi, S., Deng, M., Sun, L., Zhou, X., Mao, H., & Gou, X. (2019). Natural selection on TMPRSS6 associated with the blunted erythropoiesis and improved blood viscosity in Tibetan pigs. *Comparative Biochemistry and Physiology Part B, Biochemistry & Molecular Biology*, *233*, 11–22. https://doi.org/10.1016/j.cbpb.2019.03.003

Lin, C.-J., Lan, Y.-M., Ou, M.-Q., Ji, L.-Q., & Lin, S.-D. (2019). Expression of miR-217 and HIF-1α/VEGF pathway in patients with diabetic foot ulcer and its effect on angiogenesis of diabetic foot ulcer rats. *Journal of Endocrinological Investigation*, *42*, 1307–1317. https://doi.org/10.1007/s40618-019-01053-2

Liu, H., Tang, F., Su, J., Ma, J., Qin, Y., Ji, L., Geng, H., Wang, S., Zhang, P., Liu, J., Cui, S., Ge, R.-L., & Li, Z. (2020). EPAS1 regulates proliferation of erythroblasts in chronic mountain sickness. *Blood Cells, Molecules & Diseases*, *84*, 102446. https://doi.org/10.1016/j.bcmd.2020.102446

Ma, Y.-F., Han, X.-M, Huang, C.-P., Zhong, Li, Adeola, A. C., Irwin, D. M., Xie, H.-B., & Zhang, Y.-P. (2019). Population genomics analysis revealed origin and high-altitude adaptation of Tibetan pigs. *Scientific Reports*, *9*, 11463. https://doi.org/10.1038/s41598-019-47711-6

Meilhac, S. M., & Buckingham, M. E. (2018). The deployment of cell lineages that form the mammalian heart. *Nature Reviews Cardiology*, *15*, 705–724. https://doi.org/10.1038/s41569-018-0086-9

Padget, R. L., Mohite, S. S., Hoog, T. G., Justis, B. S., Green, B. E., & Udan, R. S. (2019). Hemodynamic force is required for vascular smooth muscle cell recruitment to blood vessels during mouse embryonic development. *Mechanisms of Development*, *156*, 8–19. https://doi.org/10.1016/j.mod.2019.02.002

Peng, Yi, Cui, C., He, Y., Ouzhuluobu, Zhang, H., Yang, D., Zhang, Q., Bianbazhuoma, Yang, L., He, Y., Xiang, K., Zhang, X., Bhandari, S., Shi, P., Yangla, Dejiquzong, Baimakangzhuo, Duojizhuoma, Pan, Y., … Su, B. (2017). Down-regulation of EPAS1 transcription and genetic adaptation of Tibetans to high-altitude hypoxia. *Molecular Biology and Evolution*, *34*, 818–830. https://doi.org/10.1093/molbev/msw280

Qi, X., Zhang, Qu, He, Y., Yang, L., Zhang, X., Shi, P., Yang, L., Liu, Z., Zhang, F., Liu, F., Liu, S., Wu, T., Cui, C., Ouzhuluobu, Bai, C., Baimakangzhuo, Han, J., Zhao, S., Liang, C., & Su, B. (2019). The transcriptomic landscape of yaks reveals molecular pathways for high altitude adaptation. *Genome Biology and Evolution*, *11*, 72–85. https://doi.org/10.1093/gbe/evy264

Shakhsheer, B. A., Lec, B., Zaborin, A., Guyton, K., Defnet, A. M., Bagrodia, N., Kandel, J. J., Zaborina, O., Hernandez, S. L., & Alverdy, J. (2017). Lack of evidence for tissue hypoxia as a contributing factor in anastomotic leak following colon anastomosis and segmental devascularization in rats. *International Journal of Colorectal Disease*, *32*, 539–547. https://doi.org/10.1007/s00384-016-2737-9

Srinivasan, S., Chitalia, V., Meyer, R. D., Hartsough, E., Mehta, M., Harrold, I., Anderson, N., Feng, H., Smith, L. E. H., Jiang, Y., Costello, C. E., & Rahimi, N. (2015). Hypoxia-induced expression of phosducin-like 3 regulates expression of VEGFR-2 and promotes angiogenesis. *Angiogenesis*, *18*, 449–462. https://doi.org/10.1007/s10456-015-9468-3

Tetsuo, S., Kyoko, F., Kanami, O., Maiko, E. S., Hirohisa, S., Kenji, M., & Akio, M. (2016). Recent advances in understanding the roles of vascular endothelial cells in allergic inflammation. *Allergology International*, *65*, 21–29. https://doi.org/10.1016/j.alit.2015.08.001 https://doi.org/10.19707/j.cnki.jpa.2019.04.011

Vozdek, R., Long, Y., & Ma, D. K. (2018). The receptor tyrosine kinase HIR-1 coordinates HIF-independent responses to hypoxia and extracellular matrix injury. *Science Signaling*, *11*, eaat0138. https://doi.org/10.1126/scisignal.aat0138

Wang, X., Yang, Y., & Li, M. (2020). Vascular morphology and expression of angiogenic factors in sheep ovaries and their relation with the lambing rate. *Reproduction in Domestic Animals*, *11*, 1132–1138. https://doi.org/10.1111/rda.13751

Williams, A. M., Ainslie, P. N., Anholm, J. D., Gasho, C., Subedi, P., & Stembridge, M. (2019). Left ventricular twist is augmented in hypoxia by β-adrenergic-dependent and β-adrenergic-independent factors, without evidence of endocardial dysfunction. *Circulation Cardiovascular Imaging*, *12*, e008455. https://doi.org/10.1161/CIRCIMAGING.118.008455

Xin, J., Zhang, H., He, Y., Duren, Z., Bai, C., Chen, L., Luo, X., Yan, D.-S., Zhang, C., Zhu, X., Yuan, Q., Feng, Z., Cui, C., Qi, X., Ouzhuluobu, Wong, W. H., Wang, Y., & Su, B. (2020). Chromatin accessibility landscape and regulatory network of high-altitude hypoxia adaptation. *Nature*, *11*, 4928. https://doi.org/10.1038/s41467-020-18638-8

Xu, X., Xu, H., Qimuge, A., Liu, S., Wang, H., Hu, M., & Song, L. (2019). MAPK/AP-1 pathway activation mediates AT1R upregulation and vascular endothelial cells dysfunction under PM2.5 exposure. *Ecotoxicology and Environmental Safety*, *170*, 188–194. https://doi.org/10.1016/j.ecoenv.2018.11.124

Xu, X.-H., Bao, Y., Wang, X., Yan, F., Guo, S., Ma, Y., Xu, D., Jin, Li, Xu, J., & Wang, J. (2018). Hypoxic-stabilized EPAS1 proteins transactivate DNMT1 and cause promoter hypermethylation and transcription inhibition of EPAS1 in non-small cell lung cancer. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *32*, 6694–6705. https://doi.org/10.1096/fj.201700715

Xu, Y. J., Gong, X. L., Duan, M. Q., Wu, L. C., BianBa, Q. D., Guo, X. Y., Qi, Y. T., Qiangba, Y. Z., & Shang, P. (2019). Determination of blood physiological parameters in Tibet pig and Yorkshire pig at high altitude. *Journal of Plateau Agriculture*, *3*, 421-3+49.

Xu, Z., & Rothstein, S. J. (2018). ROS-Induced anthocyanin production provides feedback protection by scavenging ROS and maintaining photosynthetic capacity in Arabidopsis. *Plant signaling & Behavior*, *13*, e1451708. https://doi.org/10.1080/15592324.2018.1451708

Yang, Y., Gao, C., Yang, T., Sha, Y., Cai, Y., Wang, X., Yang, Q., Liu, C., Wang, B., & Zhao, S. (2021). Characteristics of Tibetan pig lung tissue in response to a hypoxic environment on the Qinghai–Tibet Plateau. *Archives Animal Breeding*, *64*, 283–292. https://doi.org/10.5194/aab-64-283-2021

Yu, J., Liang, F., Huang, H., Pirttiniemi, P., & Yu, D. (2018). Effects of loading on chondrocyte hypoxia, HIF-1α and VEGF in the mandibular condylar cartilage of young rats. *Orthodontics & Craniofacial Research*, *21*, 41–47. https://doi.org/10.1111/ocr.12212

Zhang, B., Ban, D., Gou, X., Zhang, Y., Yang, L., Chamba, Y., & Zhang, H. (2019). Genome-wide DNA methylation profiles in Tibetan and Yorkshire pigs under high-altitude hypoxia. *Journal of Animal Science and Biotechnology*, *10*, 25. https://doi.org/10.1186/s40104-019-0316-y

Zhang, B., Chamba, Y., Shang, P., Wang, Z., Ma, J., Wang, L., & Zhang, H. (2017). Comparative transcriptomic and proteomic analyses provide insights into the key genes involved in high-altitude adaptation in the Tibetan pig. *Scientific Reports*, *7*, 3654. https://doi.org/10.1038/s41598-017-03976-3

Zhang, P., & Zuo, Z. L. (1997). Scanning electron microscope observation of microvasculature in the right ventricular wall of pigs. *Heilongjiang Animal Husbandry and Veterinary*, *1986*, 25–27.

Zhang, W., Fan, Z., Han, E., Hou, R., Zhang, L., Galaverni, M., Huang, J., Liu, H., Silva, P., Li, P., Pollinger, J. P., Du, L., Zhang, X., Yue, B., Wayne, R. K., & Zhang, Z. (2014). Hypoxia adaptations in the grey wolf (*Canis lupus chanco*) from Qinghai-Tibet Plateau. *PLoS Genetics*, *10*, e1004466. https://doi.org/10.1371/journal.pgen.1004466

Zhang, Y. F., Xu, X. P., & Yu, H. (2015). A preliminary study on the physiological and biochemical indexes of Tibetan pig blood in Shi Hezi. *Gansu Animal Husbandry and Veterinary*, *45*, 51-2. https://doi.org/10.15979/j.cnki.cn62-1064/s.2015.05.020

Zhou, J. X., Yu, S. J., He, J. F., & Cui, Y. (2015). Comparative structural organization and morphological analyses of intrapulmonary artery of adult yak and cattle. *Chinese Journal of Veterinary Medicine*, *35*:1840–1844. https://doi.org/10.16303/j.cnki.1005-4545.2015.11.22

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Yang, Y., Gao, C., Yang, T., Sha, Y., Cai, Y., Wang, X., Yang, Q., Liu, C., Wang, B., & Zhao, S. (2022). Vascular characteristics and expression of hypoxia genes in Tibetan pigs' hearts. *Veterinary Medicine and Science*, *8*, 177–186. https://doi.org/10.1002/vms3.639