Sports Medicine and Health Science 6 (2024) 370–377

Contents lists available at ScienceDirect

Sports Medicine and Health Science

journal homepage: www.keaipublishing.com/smhs

Research Article

Protein expression changes in Tibetan middle-to-long distance runners after the transition from high altitude to low altitude: Implications for enhancing endurance training

Di Wang, Weiping Shu *

Chengdu Sport University, Chengdu, Sichuan, China

ARTICLE INFO

Keywords:
Proteomic analysis
Tibetan
Middle-to-long distance runner
Alternate training
CFTR
CAI

ABSTRACT

The study aims to investigate the differences in protein expressions in Xizang's (Tibetan) middle-to-long distance runners after the transition from high altitude to low altitude and reveal the molecular mechanisms underlying their enhanced middle-to-long distance running performance. In the study, eleven subjects were selected from native Tibetan middle-to-long distance runners to participate in an 8-week pre-competition exercise training program consisting of a 6-week training stage in Kangding City at an altitude of 2 560 meters (m) and a subsequent 2-week training stage in Leshan City at an altitude of 360 m. Blood samples were collected twice from the runners before beginning altitude exercise training in Kangding and after going to sea level - Leshan City. Using a label-free quantitative method, peptides in the samples were analyzed by mass spectrometry. Proteomic analysis was performed to identify differentially expressed proteins and predict their biological functions. A total of 846 proteins were identified in the 21 samples, including 719 quantified proteins. In total, 49 significantly differentially expressed proteins ($p < 0.05$) were identified, including twenty-eight 0.2-fold up-regulated proteins or twenty-one 0.17-fold down-regulated proteins. The up-regulated proteins, including cystic fibrosis transmembrane conductance regulator (CFTR) and carbonic anhydrase I (CAI), were of particular interest due to their role in regulating the oxygen saturation in deep tissues. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis indicated that these proteins were mainly involved in regulating actin cytoskeleton, local adhesion, biotin absorption and metabolism, immune system, cancer, and membrane transport processes. In conclusion, Tibetan middle-to-long distance runners who resided in high-altitude areas benefited from repeated plateau-plain alternate training mode during the pre-competition period. The training mode induced positive changes in peripheral blood plasma proteins (CFTR and CAI), the biomarkers associated with aerobic capacity. Among the 11 runners, one female athlete won the gold medal in the 3 000-m running event in this competition, demonstrating that the plateau-plain alternate training mode could enhance the aerobic capacity of athletes.

1. Introduction

The Xizang's (Tibetan, China) people, after living for centuries in high-altitude areas, have developed unique physiological characteristics to tolerate a hypoxic environment. The indigenous population of the highlands have made a series of genetic, physiological, and anatomical adaptation features to cope with the low-oxygen environment due to local climatic conditions.[1] High-altitude exercise training provides an intense hypoxic body stimulation, increasing tolerance to hypoxia and lactate build-up, improving tissue oxygen transport and utilization, and ultimately enhancing athletes' aerobic capacity.[2] Proteins are the executors of genetic functions and plasma proteome refers to the collection of proteins expressed by an individual under a specific state.[3] Compared to the genome characterized by universality and homology, proteomics provides the specific, temporal, spatial, and dynamic data of the quantity and functions of all proteins expressed in a physiological or pathological state. Proteomics provide comprehensive information on dynamic processes and thereby elucidate the essence of biological phenomena and life activities.[4]

As a protein label-free quantification technology based on liquid chromatography-mass spectrometry (LC/MS), the exponentially modified protein abundance index (emPAI) method was used to analyze peptides and compare the corresponding signal intensities of peptides in different samples for relative quantification in the study.[5] Label-free protein quantification technologies do not require isotope labeling as

* Corresponding author. Chengdu Sport University, No. 2 Tiyuan Road, Chengdu, Sichuan, 610041, China.
E-mail address: shuweiping@cdsu.edu.cn (W. Shu).

https://doi.org/10.1016/j.smhs.2023.12.005
Received 15 January 2023; Received in revised form 14 December 2023; Accepted 15 December 2023
Available online 20 December 2023

List of abbreviations

m	Meters
h	Hour
min	Minute
CFTR	Cystic fibrosis transmembrane conductance regulator
CAI	Carbonic anhydrase I
COVID-19	Coronavirus Disease 2019
LC/MS	Liquid chromatography-mass spectrometry
emPAI	Exponentially modified protein abundance index
XIC	Extracted ion chromatogram
COG	Clusters of Orthologous Groups
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
PSMs	Peptide-spectrum matches
FDR	False discovery rate
FC	Fold change
HP	Haptoglobin
TF	Transferrin
TTR	Thyroxine-binding protein
BTD	Recombinant Biotinidase
CST3	Cystatin 3
NCAM	Neural cell adhesion molecule
ATP	Adenosine triphosphate
ENaC	Epithelial sodium channel
ANO1	Anoctamin-1
PDGFD	Platelet-derived growth factor D
H_2O	Water

internal standards and has the advantages of fast detection and convenience. This technology is sensitive, accurate, and suitable for high-throughput proteomic analysis.[6] In this study, the label-free method was used to quantitatively analyze the changes in the plasma proteome of indigenous Tibetan plateau (altitude phase) runners before and after training on the plain or near sea level phase. The plateau training mode is widely adopted by many athletes. Our findings provide support for molecular mechanisms underlying the enhanced aerobic capacity of athletes in high-altitude training.

2. Material and methods

2.1. Research subjects

The study enrolled 11 middle-to-long distance runners (7 males and 4 females) from the Youth Sports School in Ganzi Tibetan Autonomous Prefecture. All participants were healthy and had no history of cardiovascular or cerebrovascular diseases, nor any known contraindications (Table 1,Table 2).

2.2. Ethical approval

All runners participating in the experiment were made aware of the associated experimental risks and benefits before signing an informed consent form. The study was approved by the Ethics Committee of Chengdu Sport University in 2023 (Approval No. 75).

2.3. Research methods

Eleven runners participated in an 8-week pre-competition training program, in Sichuan Province, China. Runners were trained first in Kangding City (altitude: 2 560 meters above the sea level) in the Garze Tibetan Autonomous Prefecture (hereafter referred to as Garze Prefecture) for 6-weeks and then in Leshan City (altitude: 360 m above the sea level) for 2 weeks. In the plateau phase (Kangding City), exercise training focused on basic aerobic endurance, speed endurance, and specialized speed endurance as well as additional training items such as trunk strength, explosive strength, coordination, and agility under a medium-to-high load. Additionally, multiple simulation tests and tactical exercises were performed to assist athletes in achieving their optimal exercise capacity before competition. During the 1-week training in the plain phase (Leshan City), exercise training was facilitated by adjusting and adapting to low-altitude environment and focused on basic aerobic endurance, specialized speed endurance, and explosive strength so as to optimize training while in the plain phase. Blood samples were collected twice in the training period. Each time 5 mL of fasting venous blood was taken from the median cubital vein. In the morning of the first exercise training day in Kangding (plateau phase), ten blood samples were collected (one athlete was unable to participate during the first day due to COVID-19). Eleven blood samples were collected in the morning of the fifth training day in Leshan City (plain phase). Blood samples were immediately transferred into a portable refrigeration container at 0 °C. Subsequently, plasma separation was completed within 6 hour (h) and then stored at −80 °C. Proteomic analysis was performed within 12 h after the samples were collected. Mass spectrometry analysis of proteolytic peptides was performed with the label-free quantitative method based on the extracted ion chromatogram (XIC) of peptide precursor ions. The peptides and proteins in the samples were identified and then quantified. The raw files generated from mass spectrometry were searched against corresponding databases for protein identification. The mass tolerance distribution analysis of peptides, proteins, and precursor ions was performed to evaluate the quality of the mass spectrometry data. The identified proteins were annotated with functional information from common databases, including Clusters of Orthologous Groups (COG), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG).[7–9] Quantitative analyses of the identified proteins were carried out, including the differential analysis of identified proteins in the plain and plateau exercise training phases and the clustering analysis of differentially expressed proteins. The obtained differentially expressed proteins were then subjected to functional analyses, including enrichment analysis of GO and KEGG pathways as well as network analysis, so as to reveal their interactions.

Table 1
Basic information of 11 subjects (mean ± *SD*).

Age/years	Height/cm	Weight/kg	Training Years/years	Running events
17 ± 2.23	168 ± 7.45	58.46 ± 15.27	4.17 ± 1.28	3 000 m/5 000 m

Table 2
All Athletes' blood samples number marking.

Number	Sex	Blood collection number for high-altitude training	Plain training blood collection number
1	Male	Plateau01	plain01
2	Male	Plateau02	plain02
3	Male	Plateau03	plain03
4	Male	Plateau04	plain04
5	Male	Plateau05	plain05
6	Female	Plateau06	plain06
7	Male	Plateau07	plain07
8	Male	Plateau08	plain08
9	Female	Plateau09	plain09
10	Female	Plateau10	plain10
11	Female	–	plain11

2.4. Data analysis

The raw files of mass spectrometry data in the raw format were directly imported into the Proteome Discoverer software for database search, peptide alignment, and protein quantification. To improve the quality of analysis results and reduce false positive rate, the search results were further filtered with Proteome Discoverer software. Peptide-spectrum matches (PSMs) with a confidence level above 99% were considered as credible PSMs. Proteins with at least one unique peptide segment were identified as credible ones. Only credible PSMs and proteins were retained, and a false discovery rate (FDR) validation was performed so as to remove peptides and proteins with an FDR above 1%. With Proteome Discoverer, the relative value of each PSM in each sample was first obtained based on the peak area of the original spectrum. Then, based on the quantitative information of all PSMs contained in the identified unique peptides, the relative values of unique peptides were corrected. Finally, based on the quantitative information of all unique peptides contained in each protein, the relative quantification value of each protein was corrected.

2.5. Analysis of differentially expressed proteins

Sample pairs to be compared were selected, and then the ratio of the mean values of each protein in the comparison sample pairs was calculated as the fold change (FC). A *t*-test was performed with the relative value of each protein in the two compared samples so as to determine the statistical significance. The $p \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Data quality control and identification situation

A total of 5 406 peptides and 846 proteins were identified from 21 plasma samples (Table 3 and Fig. 1).

3.2. Protein quantification

Based on the original mass spectrometry data, the relative quantitative values of PSMs were obtained with the peak area. Then, the relative values of unique peptides were calibrated based on the quantitative information of all PSMs. Subsequently, the relative value of each protein was calibrated based on the quantitative information of all unique peptides contained in each protein. Finally, the quantitative values of proteins were obtained (Table 4).

3.3. Analysis of protein expression differences

As shown in Table 5, the ratio of the mean values of each protein in the comparison sample pairs (plain group vs. plateau group) was calculated as the fold change (FC). A *t*-test was performed with the relative values of each protein in the comparison sample pairs. The $p \leq 0.05$ was considered to be statistically significant.

3.4. Differentially expressed proteins

Table 6 shows a total of 28 up-regulated proteins in the plain group compared to the plateau group. Table 7 shows a total of 21 down-regulated proteins in the plain group compared to the plateau group.

Table 3
Overview of protein identification.

Name	Total Spectra	Matched Spectrum	Peptide	Identified Protein
ALL	1 118 136	236 466	5 406	846

Notes: Total Spectra: total number of secondary spectra; Matched spectrum: number of valid spectra; Peptide: number of identified peptides; Identified Protein: number of identified proteins.

3.5. KEGG and GO enrichment analyses and functional characterization of differentially expressed genes

3.5.1. GO enrichment analysis

GO functional enrichment analysis was performed to identify biological functions which were significantly associated with the differentially expressed proteins. First, all differentially expressed proteins were mapped to various terms in the Gene Ontology database (http://www.geneontology.org/) so as to obtain the number of proteins for each term. Then, the hypergeometric test was performed to calculate the significantly enriched GO terms in the differentially expressed proteins (Table 8).

3.5.2. KEGG enrichment analysis

KEGG enrichment analysis was performed with the hypergeometric test to identify significantly enriched pathways related to the differentially expressed proteins. Pathway enrichment analysis could identify the major biochemical and signaling pathways associated with differentially expressed proteins. The results of KEGG pathway enrichment analysis and KEGG enrichment pathway diagram are presented in Table 9 and Fig. 2, respectively.

3.6. Biological function descriptions of some differentially expressed proteins

Haptoglobin protein (HP) captures and binds free plasma hemoglobin, thus allowing degrading enzymes to access the hemoglobin, preventing iron loss through kidneys, and protecting kidneys from being damaged by free hemoglobin. Transferrin (TF) is an iron-binding transport protein that can bind two iron (III) ions and one anion (bicarbonate) and responsible for iron absorption, transport, storage, and utilization. Thyroxine-binding protein (TTR) transports thyroxine from blood to the brain and is also involved in other intracellular processes, including protein hydrolysis, nerve regeneration, autophagy, and glucose homeostasis. Biotinidase (Recombinant Biotinidase, BTD) is a protein and can transfer biotin and recycle free biotin by breaking down the normal product of carboxylase degradation, biotin-epsilon-lysine, which is bound to proteins.

Cystatin 3 (CST3), a cystatin is found in various human body fluids and secretions and has protective functions. Neural cell adhesion molecule (NCAM), a cell adhesion protein and a member of the immunoglobulin superfamily, is involved in cell-cell interactions and cell-matrix interactions during development and differentiation and plays a crucial role in regulating neurogenesis, neurite growth, and cell migration during the development of the nervous system. CFTR encodes a member of the Adenosine triphosphate (ATP) ATP-binding cassette (ABC) transporter superfamily and functions as a chloride ion channel. CFTR is a unique protein in this family and also controls the secretion and absorption of ions and water in epithelial tissues. The channel activation is mediated by a cycle of regulation domain phosphorylation, ATP binding to the nucleotide-binding domain, and ATP hydrolysis. CFTR plays an important role in regulating epithelial ion and water transport and fluid homeostasis and mediates the transport of chloride ions across cell membranes. CFTR channel activity is coupled to ATP hydrolysis. Bicarbonate (HCO_3^-) also permeates the ion channel and selectivity depends on extracellular chloride concentration. CFTR functions are realized through regulating the activity of other ion channels and transporters. CFTR also plays an important role in airway fluid balance by regulating pH and ion concentrations in the airway surface liquid and thus resists pathogens. CFTR regulates the activity of the epithelial sodium channel (ENaC) complex as well as cell surface expression of ENaC complex. CFTR inhibits the activity of ENaC channels containing genes SCNN1D, SCNN1B, and SCNN1G and not in channels containing genes SCNN1A,

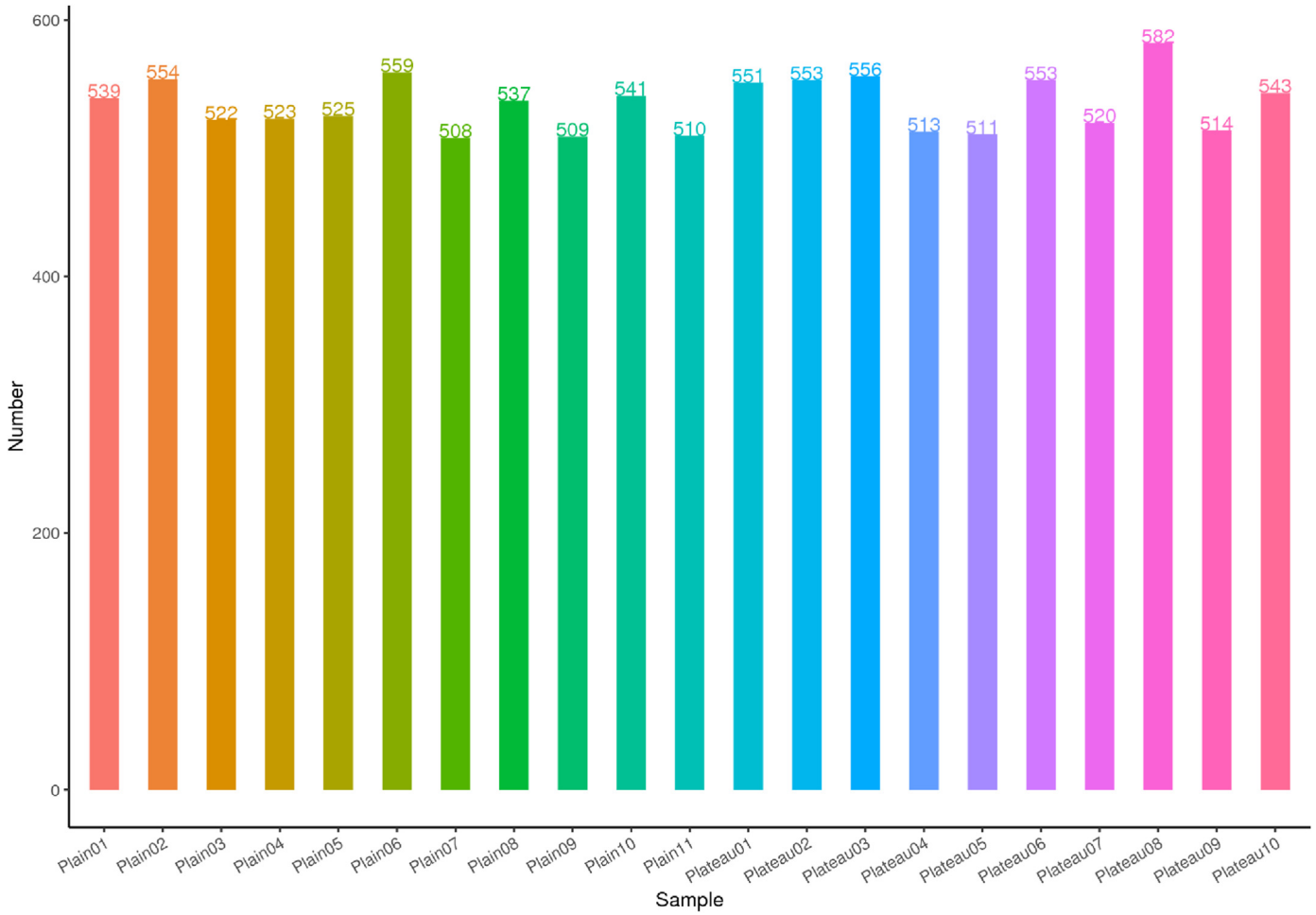

Fig. 1. Overview of Sample Protein Identification. Note: The horizontal axis represents the sample name and the vertical axis represents the number of proteins (counting only those with non-zero quantitative values). Plain during the plain phase, and Plateau is the blood sample taken from subject during the plateau phase.

Table 4
Protein quantification results.

Proteins	Descriptions	Genes	Plateau01	Plateau02	Plateau03	Plateau04	Plateau05	Plateau07
P01024	Complement C3	C3	9 442 440 943.16	7 842 282 944.19	8 726 307 061.88	9 018 293 441.941	6 859 062 184.45	10 092 009 079.87
C0JYY2	Apolipoprotein B (Including Ag(X) antigen), isoform CRA_a	APOB	1 336 312 409.86	1 509 518 557.53	1 118 050 372.35	1 363 568 818.36	921 034 342.19	1 469 786 681.60
F6KPG5	Albumin (Fragments)		855 823 180.58	5 187 623.81	9 042 753.88	10 406 002.87	4 284 956.01	8 976 014.43

Notes: (1) Protein: the database identifier of the protein based on the specimen number; (2) Description: the descriptive information of the protein in the sample database(If there is no information in the database, it will not be displayed); (3) Gene: the corresponding gene name of the protein in the database, (If there is no information in the database, it will not be displayed); (4) Other columns: the relative quantitative information of each sample.

Table 5
Analysis results of differentially expressed proteins.

Sample comparison	Num. of Total Quant	Expression differences	Fold change > 1.2	Fold change > 1.3	Fold change > 1.5	Fold change > 2.0
Plain. Vs. Plateau	631	Up-regulated	28	23	17	8
		Down-regulated	21	20	18	6

Notes: (1) Sample comparison: comparison pairs of samples; (2) Num. of Total Quant.: the number of proteins identified in both groups (proteins not identified in both groups cannot be determined to be up- or down-regulated); (3) Expression differences: type of protein expression regulation; (4) fold change: the difference in fold change.

SCNN1B, and SCNN1G. CFTR may regulate the secretion and salvage of bicarbonate in epithelial cells by affecting the transporter protein SLC4A7. Additionally, CFTR inhibits Anoctamin-1 (ANO1) chloride ion channel activity and plays a role in chloride and bicarbonate homeostasis during sperm maturation.

Carbonic anhydrase 1 (CAI) is a zinc metalloenzyme that participates

Table 6
Up-regulated Proteins in Plain Phase Compared to Plateau Phase (Plain vs. Plateau).

Proteins	Descriptions	Genes	Plain. Vs. Plateau fold change	Plain. Vs. Plateau *p*-value	Plain. Vs. Plateau Up\Down
P06727	Apolipoprotein A-IV	APOA4	1.26	0.048	up
A0A384MEF1	Epididymis secretory sperm binding protein		1.43	0.013	up
P00738	Haptoglobin	HP	1.77	0.04	up
A0PJA6	TF protein (Fragment)	TF	1.71	0.02	up
P02766	Transthyretin	TTR	3.16	0.042	up
V9HW34	Epididymis luminalprotein213	HEL-213	1.51	0.046	up
Q8IZZ5	Coagulation factor XII-Mie		1.46	0.032	up
Q6LAM1	Heavy chain of factor I (Fragment)		2.26	0.02	up
P00742	Coagulation factor X	F10	1.26	0.01	up
P02743	Serum amyloid P-component	APCS	1.23	0.02	up
P43251	Biotinidase	BTD	1.37	< 0.01	up
P02654	Apolipoprotein C-I	APOC1	1.49	0.01	up
V9H1C1	Gelsolinexon4 (Fragment)		1.51	0.02	up
Q5NV91	V2-19protein (Fragment)	V2-19	1.31	< 0.01	up
Q7L7L0	HistoneH2Atype3	HIST3H2A	2.09	0.01	up
P01034	Cystatin-C	CST3	2.15	0.03	up
A0A2U8J995	Ig heavy chain variable region (Fragment)	Ig H	1.88	0.04	up
Q15166	Serum paraoxonase/lactonase3	PON3	1.82	0.02	up
Q16778	HistoneH2Btype2-E	HIST2H2BE	1.59	0.02	up
A0A087WWD4	Neuralcelladhesionmolecule1	NCAM1	2.00	0.01	up
Q8WTR4	Glycerophosphodiesterphosphodiesterasedomain-containingprotein5	GDPD5	1.23	0.04	up
A2N0T6	VH6Djprotein (Fragment)	VH6DJ	2.20	0.03	up
P84243	HistoneH3.3	H3F3A	3.38	0.01	up
Q63HQ4	UncharacterizedproteinDKFZp686K04147 (Fragment)	DKFZp686K04147	1.68	< 0.01	up
Q20BI4	Cystic fibrosis transmembrane conductance regulator	CFTR	1.52	< 0.01	up
Q9GZP0	Platelet-derived growth factor D	PDGFD	1.36	< 0.01	up
A0A087X097	Cadherin-23	CDH23	1.54	0.01	up
V9HWE3	Carbon icanhydrase I, isoform CRA_a	HEL-S-11	2.36	0.01	up

Table 7
Down-regulated Proteins in Plain Phase Compared to Plateau Phase (Plain vs. Plateau).

Proteins	Descriptions	Genes	Plain vs Plateau fold change	Plain vs Plateau *p*-values	Plain vs Plateau Up\Down
P21333	Filamin-A	FLNA	0.58	0.01	down
V9HWI6	EpididymissecretoryproteinLi51	HEL-S-51	0.55	< 0.01	down
B4E1C2	Kininogen1, isoform CRA_b	KNG1	0.62	< 0.01	down
A0A2Z4LCH4	Corticosteroid-binding globulin		0.79	< 0.01	down
I3L145	Sex hormone-binding globulin, isoform CRA_a	SHBG	0.44	0.01	down
V9HWB4	Epididymis secretory sperm binding protein Li89n	HEL-S-89n	0.27	0.04	down
P62328	Thymosinbeta-4	TMSB4X	0.64	0.04	down
E7EUT5	Glyceraldehyde-3-phosphatede hydrogenase	GAPDH	0.42	0.015	down
B7Z6Z4	cDNAFLJ56329, highlysimilarto Myosin lightpolypeptide6	MYL6	0.34	0.05	down
A2NYQ9	Anti-folate binding protein (Fragment)	HuVH8B	0.57	0.02	down
E9PK25	Cofilin-1	CFL1	0.45	0.03	down
A0A0G2JRQ6	Uncharacterized protein (Fragment)		0.55	0.01	down
Q9NQ79	Cartilageacidicprotein1	CRTAC1	0.38	< 0.01	down
A2N0U4	VH6Djprotein (Fragment)	VH6DJ	0.58	0.06	down
Q7Z2E8	Rearranged Vl3l gene segment (Fragment)	VL3L	0.62	0.02	down
A0A2U8J8T9	Ig heavy chain variable region (Fragment)	IgH	0.75	< 0.01	down
A0A024R1U8	Insulin-like growth factor binding protein4, isoform CRA_a	IGFBP4	0.56	0.04	down
A0A0A0MS14	Immunoglobulinheavyvariable1-45	IGHV1-45	0.64	0.04	down
Q9BWP8	Collectin-11	COLEC11	0.54	< 0.01	down
A0A2U8J9A3	Ig heavy chain variable region (Fragment)	IgH	0.57	0.03	down
A0A024R611	Coronin	CORO1A	0.69	0.013	down

Table 8
Results of gene ontology (GO) **enrichment.**

GO_ID	GO_Term	GO_Class	*p*	Adjusted *p*	*x*	*y*	*n*	*N*	Golevl	ProtID
GO:0003677	DNA binding	MF	< 0.01	0.10	3	3	27	220	5	Q7L7L0 Q16778 P84243
GO:0016788	hydrolase activity, acting on ester bonds	MF	0.07	0.10	2	4	27	220	4	Q15166 Q8WTR4
GO:0016020	membrane	CC	0.08	0.10	4	14	27	220	2	Q6LAM1 Q20BI4 Q9GZP0 A0A087X097
GO:0003779	actin binding	MF	0.11	0.10	2	5	27	220	5	P62328 E9PK25
GO:0003785	actin monomer binding	MF	0.12	0.10	1	1	27	220	6	P62328

Notes: (1) Map ID: ID of enriched KEGG pathways; (2) Map Title: name of enriched KEGG pathways; (3) *p*: *p*-value of enrichment analysis (4) Adjusted *p*: adjusted *p*-value; (5) *x*: number of differentially expressed proteins associated with the pathway; 6); *y*: number of background proteins associated with the pathway; (7) *n*: the number of differentially expressed proteins annotated by KEGG; (8) *N*: the number of background proteins annotated by KEGG; (9) ProtID: list of enriched proteins.

Table 9
Results of kyoto encyclopedia of genes and genomes (KEGG) **enrichment.**

Map ID	Map Title	*p*	Adjusted *p*	*x*	*y*	*n*	*N*	Prot ID
map05034	Alcoholism	0.01	0.3	3	5	32	298	Q7L7L0 Q16778 P84243
map04810	Regulation of actin cytoskeleton	0.02	0.3	5	16	32	298	A0A384MEF1 V9H1C1 P62328 E9PK25 Q9GZP0
map04977	Vitamin digestion and absorption	0.061	0.3	2	4	32	298	P06727 P43251
map00780	Biotin metabolism	0.11	0.3	1	1	32	298	P43251
map02010	ABC transporters	0.11	0.3	1	1	32	298	Q20BI4

Notes: (1) Map ID: ID of enriched KEGG pathways; (2) Map Title: name of enriched KEGG pathways; (3) *p*: *p*-value of enrichment analysis (4) Adjusted *p*: adjusted *p*-value; (5) *x*: number of differentially expressed proteins associated with the pathway; 6); *y*: number of background proteins associated with the pathway; (7) *n*: the number of differentially expressed proteins annotated by KEGG; (8) *N*: the number of background proteins annotated by KEGG; (9) ProtID: list of enriched proteins.

in various biological processes, including respiration, calcification, and acid-base balance. CAI is widely distributed and can reduce interstitial carbon dioxide (CO_2) concentration and increase oxygen saturation. Platelet-derived growth factor D (PDGFD) protein is a member of platelet-derived growth factor family with an unusual N-terminal domain or a CUB domain. PDGFD is a mesenchymal cell mitogen and a growth factor involved in embryonic development, cell proliferation, migration, survival, and chemotaxis.

3.7. Alterations in athletic performance and plasma protein markers of athletes in "the alternate training mode during pre-competition period

As shown in Table 10, after the high-altitude phase to low-altitude alternate training phase for two times before the competition, significant differences ($p < 0.05$) were observed in the timed running distances of athletes reported by the 12-min running tests completed and reported as Plateau A and Plain P. These results indicate that the athletes were able to maintain a high level of aerobic capacity through the training mode and enhance their athletic performance. In the high-altitude training phase and the plain training phase, male middle-to-long distance runners showed significant differences ($p < 0.05$) in both CFTR and CAI. However, female runners showed significant differences ($p < 0.05$) in CFTR and not CAI.

4. Discussion

Insufficient oxygen supply during exercise can cause a decrease in cerebral and skeletal muscle oxygenation potentially leading to central and peripheral fatigue. Decreased brain oxygenation during intensive exercise can decrease cortical neural activity and lead to central fatigue. Increasing exercise intensity can decrease oxygenation of the working muscles, alter muscle cell metabolism, impair the ability to control muscle contraction, and ultimately lead to fatigue. Improving muscle tissue oxygen saturation is crucial for enhancing endurance performance.[7] Therefore, increasing muscle tissue oxygen saturation is essential for improving endurance performance. After the high-altitude to low-altitude alternate training, the skeletal muscle oxygen-carrying hemoglobin concentration and oxygen saturation of Tibetan athletes were significantly improved, so that the sports ability of athletes was enhanced to provide effective aerobic exercise reserves for plain competition.[8]

CAI is one of the main proteins in red blood cells and ranks second after hemoglobin. CAI catalyzes the reaction $CO_2 + H_2O$ (water) $\rightarrow H_2CO_3$ and accelerates the hydration of carbon dioxide so as to eliminate CO_2 from tissues and increase oxygen saturation. CAI plays an important regulatory role in red blood cell differentiation, especially in the later differentiation stage.

CFTR is closely related to cell energy metabolism. When cells are activated, increased ATP metabolism results in elevated cellular cyclic adenosine monophosphate (cAMP) levels, which can activate protein kinase A to phosphorylate the R regulatory region of CFTR protein and open the CFTR channel. In contrast, when a cell is in a resting state or experiencing insufficient energy, the activity of the CFTR ion channel is inhibited so as to allow the cell to conserve energy.[9] The increased expression of CFTR protein is positively correlated with improved sperm motility, suggesting that CFTR protein may participate in regulating sperm and its corresponding energy metabolism.[10] Additionally, CFTR has a positive effect on the recovery process of body nervous excitability of athletes, a process closely related to cell charging and discharging. Slow charging may impair the ability of recruited muscles to perform work. Results indicate that changes in altitude or altitude-related factors affected the neural excitability of athletes are due to the upregulation of CFTR expression.

The upregulation of CFTR and CAI will likely have a synergistic effect on the clearance of CO_2 and energy metabolism, leading to an increase in tissue oxygen saturation and an improved athletic ability. Therefore, athletes may achieve better competition results in the plain (near sea level) competition after training at altitude.

5. Conclusion

After plateau exercise training, Tibetan middle-to-long distance runners competed in the plains at or near sea level where oxygen is more abundant. When athletes arrived at the plains, their bodies had undergone beneficial changes for improving athletic performance. Proteomic analysis revealed that the plasma concentrations of CAI and CFTR proteins significantly increased after arrival at the plain. CAI protein catalyzes the synthesis of carbonic acid, reduces CO_2 content in the body, increases tissue oxygen saturation, which ultimately lead to enhanced sports performance. CFTR is an important regulatory factor in energy metabolism and provides enhanced kinetic energy for athletes. Therefore, the increase in plasma CAI and CFTR proteins after arrival from higher altitudes to lower altitudes provide an explanation for the improved performance of middle-to-long distance runners who train at high altitudes.

In the future, through omics study, the training and competition of Tibetan middle-to-long distance runners at different altitudes will be further investigated so as to identify additional proteomics-related genes that positively impact sports performance and improve the training of middle-to-long distance runners at high altitudes.

Submission statement

This manuscript is original and has not been published and will not be submitted elsewhere for publication while being considered. And the study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time.

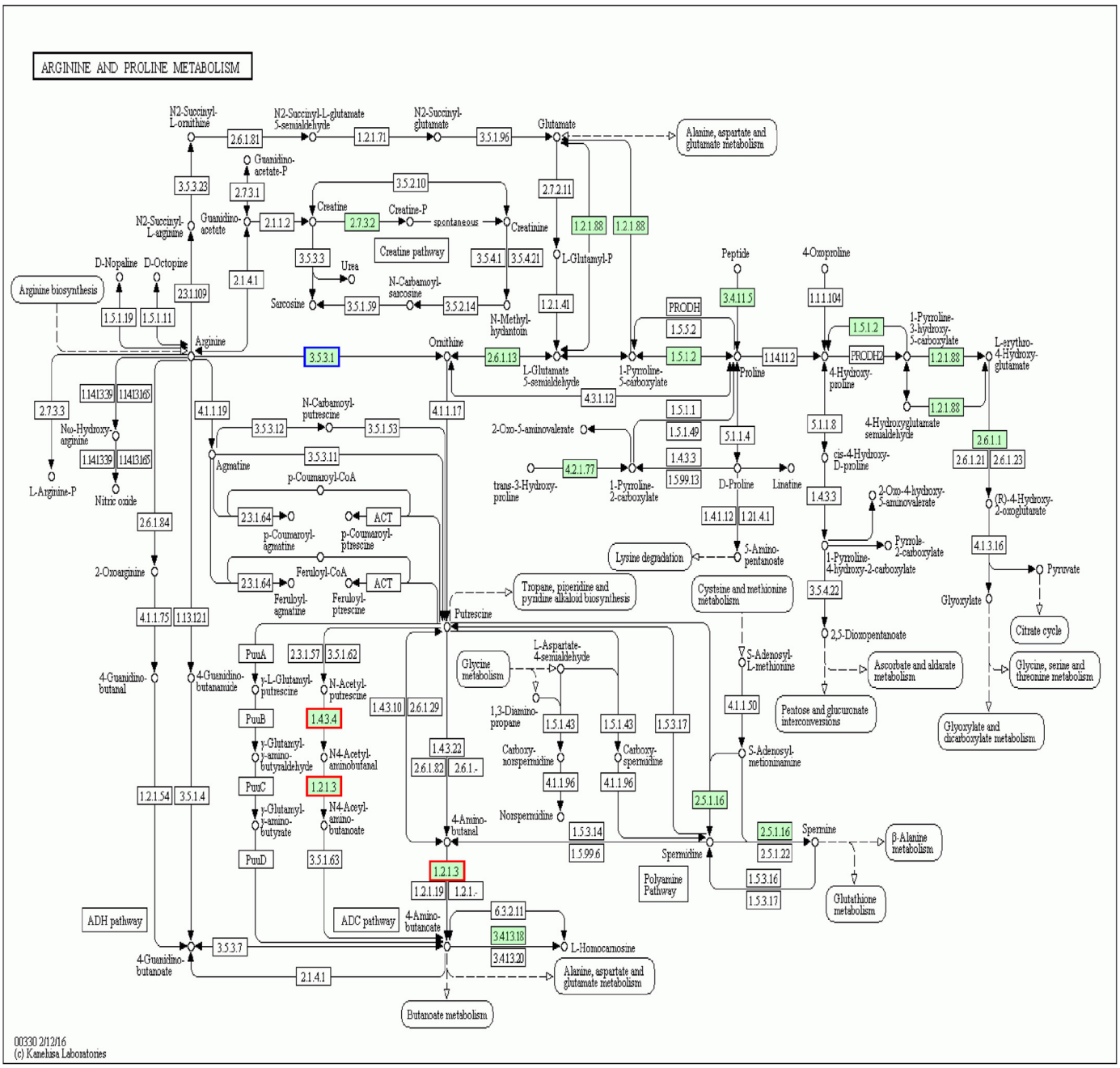

Fig. 2. Diagram of Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment pathways.

Table 10
Alterations in Athlete's Exercise Performance and Plasma Protein Markers in the Alternate Training Mode. Cystic fibrosis transmembrane conductance regulator (CFTR), carbonic anhydrase I (CAI).

sex	age/ years	height/ cm	weight/ kg	Plateau a	Plain *p*	Plateau plasma	Plain plasma	*p*	Plateau plasma	Plain plasma	*p*
				12-min run (m)	12-min run (m)	CFTR Measure-ments	CFTR Measureme-nts		CAI measurements	CAI measurements	
Male *n* = 7	16.84 ± 0.83	172.00 ± 1.91	58.07 ± 1.66	3 016.23 ± 60.62	3 595.00 ± 146.72*	9 327 283.50 ± 5 213 962.91	14 849 768.88 ± 2 746 141.04	0.03*	30 724 286.70 ± 14 107 644.06	96 617 581.95 ± 34 361 850.30	0.014*
Female *n* = 4	15.70 ± 1.00	162.25 ± 2.50	49.07 ± 4.35	2 615.00 ± 327.67	3 005.01 ± 170.00*	10 010 430.33 ± 1 427 866.671	17 301 870.63 ± 2 695 347.64	0.04*	76 225 560.75 ± 16 260 742.59	118 696 683.70 ± 36 987 035.66	0.458

Note: Data are represented by mean ± *SD*. * in the table indicates $p < 0.05$.

No data have been fabricated or manipulated (including images) to support your conclusions. No data, text, or theories by others are presented as if they were our own. The submission has been received explicitly from all co-authors. And authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

Ethical approval statement

All runners participating in the experiment were made aware of the associated experimental risks and benefits before signing an informed consent form. The study was approved by the Ethics Committee of Chengdu Sport University in 2023 (Approval No. 75).

Di Wang collected data and wrote the article. Weiping Shu designed studies and edited the article.

Authors' contributions

Di Wang: Data curation, Writing – original draft. **Weiping Shu:** Conceptualization, Methodology, Writing – review & editing.

Conflict of interest

All authors declare that they have no conflict of interest.

References

1. Wu YA, Niu WZ, Li B, et al. Comparison of physical capacity between high altitude natives and migrants at 3680m altitude. *J Prev Med Chin People's Liberation Army*. 1993;6:416–420.
2. Yu XY. Current situation and development prospect of plateau training for native plateau athletes. *Track and Field*. 2013;9:31–35.
3. Aebersold R. Mann M. Mass-spectrometric exploration of proteome structure and function. *Nature*. 2016;537:347–355. https://doi.org/10.1038/nature19949.
4. Zaro BW, Noh JJ, Mascetti VL, et al. Proteomic analysis of young and old mouse hematopoietic stem cells and their progenitors reveals post-transcriptional regulation in stem cells. *eLife Sciences*. 2020;9:e62210. https://doi.org/10.7554/eLife.62210.
5. Goeminne LJE, Gevaert K, Clement L. Experimental design and data-analysis in label-free quantitative LC/MS proteomics: a tutorial with MSqRob. *J Proteonomics*. 2018; 171:23–36. https://doi.org/10.1016/j.jprot.2017.04.004.
6. Bai XY, Yuan ML. Study on high altitude training model of native plateau race walk athletes. *J Capital Univ Phys Educ Sports*. 2018;30(1):55–59. https://doi.org/10.14036/j.cnki.cn11-4513.2018.01.011.
7. Amann M, Kayser AB. Nervous system function during exercise in hypoxia. *High Alt Med Biol*. 2009;10(2):149. https://doi.org/10.1089/ham.2008.1105.
8. Laughlin MH, Roseguini B. Mechanisms for exercise training-induced increases in skeletal muscle blood flow capacity: differences with interval sprint training versus aerobic endurance training. *J Physiol Pharmacol*. 2009;59(Suppl 7):71–88.
9. Sharma S, Hanukoglu A, Hanukoglu I. Localization of epithelial sodium channel (ENaC) and CFTR in the germinal epithelium of the testis, Sertoli cells, and spermatozoa. *J Mol Histol*. 2018;49(2):195–208. https://doi.org/10.1007/s10735-018-9759-2.
10. Li B, Shen YZ, Deng JC, et al. The expression of CFTR protein in human sperms and its correlation with sperm motility. *Chinese Journal of Andrology*. 2021;35(4):17–20.