

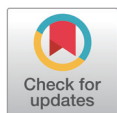
# Metabolomics reveals potential plateau adaptability by regulating inflammatory response and oxidative stress-related metabolism and energy metabolism pathways in yak

Meizhou Huang<sup>1,2#</sup>, Xin Zhang<sup>1#</sup>, Wenjun Yan<sup>3#</sup>, Jingjing Liu<sup>1</sup> and Hui Wang<sup>1\*</sup>

<sup>1</sup>Department of Toxicology, School of Public Health, Lanzhou University, Gansu 730000, China

<sup>2</sup>Academician (Expert) Workstation of Sichuan Province, The Affiliated Hospital of Southwest Medical University, Sichuan 646000, China

<sup>3</sup>Agricultural and Rural Integrated Service Center of Dachaigou Town, Tianzhu Tibetan Autonomous County, Gansu 733202, China



Received: Oct 4, 2021

Revised: Nov 14, 2021

Accepted: Nov 30, 2021

#These authors contributed equally to this work.

## \*Corresponding author

Hui Wang

Department of Toxicology, School of Public Health, Lanzhou University, Gansu 730000, China.

Tel: +86-13919330832

E-mail: [huiwang@lzu.edu.cn](mailto:huiwang@lzu.edu.cn)

Copyright © 2022 Korean Society of Animal Sciences and Technology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

## ORCID

Meizhou Huang

<https://orcid.org/0000-0003-2538-0217>

Xin Zhang

<https://orcid.org/0000-0003-0974-0139>

Wenjun Yan

<https://orcid.org/0000-0002-7636-974X>

## Abstract

Species are facing strong selection pressures to adapt to inhospitable high-altitude environments. Yaks are a valuable species and an iconic symbol of the Qinghai-Tibet Plateau. Extensive studies of high-altitude adaptation have been conducted, but few have focused on metabolism. In the present study, we determined the differences in the serum metabolomics between yaks and the closely related species of low-altitude yellow cattle and dairy cows. We generated high-quality metabolite profiling data for 36 samples derived from the three species, and a clear separation trend was obtained between yaks and the other animals from principal component analysis. In addition, we identified a total of 63 differentially expressed metabolites among the three species. Functional analysis revealed that differentially expressed metabolites were related to the innate immune activation, oxidative stress-related metabolism, and energy metabolism in yaks, which indicates the important roles of metabolites in high-altitude adaptation in yaks. The results provide new insights into the mechanism of adaptation or acclimatization to high-altitude environments in yaks and hypoxia-related diseases in humans.

**Keywords:** Yak, High-altitude adaptation, Metabolomics, Cattle, Dairy cow

## INTRODUCTION

The Qinghai-Tibetan Plateau (QTP), characterized by low temperature and hypobaric hypoxia, is the highest plateau in the world, with an average altitude > 4,000 m above sea level. Species are facing strong selection pressure to adapt to inhospitable high-altitude environments [1]. The yak (*Bos*

Jingjing Liu  
<https://orcid.org/0000-0001-9858-1867>  
Hui Wang  
<https://orcid.org/0000-0002-4136-0999>

### Competing interests

No potential conflict of interest relevant to this article was reported.

### Funding sources

National Natural Science Foundation of China (No. 31802256) and Gansu Province Science Fund for Distinguished Young Scholars (No. 20JR5RA579).

### Acknowledgements

Not applicable.

### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

### Authors' contributions

Conceptualization: Wang H.  
Data curation: Huang M, Wang H.  
Formal analysis: Huang M.  
Methodology: Zhang X, Yan W, Liu J.  
Software: Huang M.  
Validation: Wang H.  
Investigation: Yan W.  
Writing - original draft: Wang H.  
Writing - review & editing: Huang M, Zhang X, Yan W, Liu J, Wang H.

### Ethics approval and consent to participate

The animal experiment was approved and received humane care according to Ethical Committee rules of Lanzhou University, China (RIB21110301).

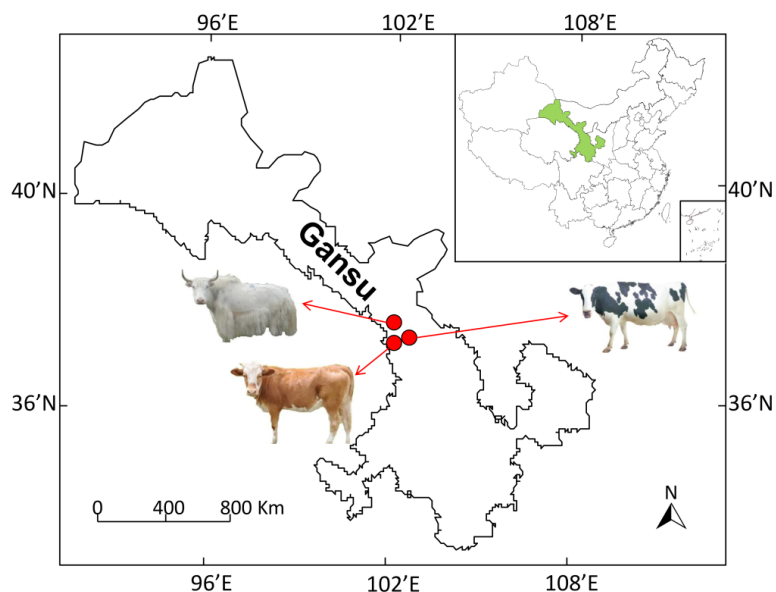
*grunniens*) is an important domesticated ruminant. It is the only large mammal inhabiting the QTP and is an iconic symbol of the QTP [2]. Yaks living at high altitudes more than 7,000 years, and must adapt to the stress of decreased oxygen availability [3]. Yaks have numerous special morphological and physiological mechanisms for life at high altitudes, e.g., blunted hypoxic pulmonary vasoconstriction [4], increased foraging ability [5], enhanced glucose uptake and aerobic respiration [6], and improved bioenergy metabolism than mammals living in the plains [7]. Genome analysis identified an expansion in yak of gene families related to sensory perception and energy metabolism compared with cattle [8], and differentially expressed miRNAs have also been found to be enriched in hypoxia-related pathways [9]. In addition, to reduce the risk of infection and disease, the activation of innate immunity was higher in yaks than in other cattle [10]. These findings partially reveal the adaptive mechanisms of yaks due to natural selection in a high-altitude and hypoxic environment, but few investigations have focused on the role of metabolites.

High-altitude hypoxia continuously affects the physical performance of people and animals [11]. Survival in high-altitude hypoxia requires a profound adaptive shift in metabolic processes [12]. In addition, hypoxia is related to homeostasis and the metabolic rate in adult tissues [13]. Organic metabolites are the reactants, intermediates or products of enzymatic reactions and represent the final products of cellular processes. The trend of contemporary scientific development is to follow systems biology. Investigation into the metabolome in response to genetic modification or physiological stimulus is a part of systems biology [14]. Identifying metabolic pathways has the potential to improve the understanding of physiological mechanisms [15]. The metabolome can reveal total metabolic profile changes in biological phenotypes and silent phenotypes [16]. In this study, the serum metabolites of yaks (*B. grunniens*), yellow cattle (*Bos taurus*) and China Holstein dairy cows (*Bos taurus*) were analyzed using a nontargeted metabolomics approach based on ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS). Comparing yaks, yellow cattle and China Holstein dairy cows may contribute to understanding evolutionary adaptation and provide meaningful data for survival at high altitudes.

## MATERIALS AND METHODS

### Sample collection

Blood samples were collected between 9:00 and 10:00 am by jugular venous puncture using vacuum tubes from 12 white yaks (Qilian Township, 37°41'6"N, 102°26'24"E, altitude: 3,600 m), 12 local yellow cattle (Tanshanling Town, 37°4'38"N, 102°24'14"E, altitude: 2,200 m), and 12 China Holstein dairy cows (Anyuan Town, 37°8'24"N, 102°37'48"E, altitude: 1,700 m) in Tianzhu County on the edge of QTP, Gansu Province, China (Fig. 1). After the blood was left to stand for 30 min, it was centrifuged at 1,000 ×g for 5 min at 4 °C. Then, the serum was extracted, immediately frozen in liquid nitrogen and stored until analysis was carried out. All animals are female, and about three years old. Yaks and yellow cattle graze the natural grassland throughout the year without supplementary feed and housing. Holstein dairy cows (milk production: 27.1 ± 0.85 kg/day, parity: 2, days in milk [DIM]: 91.6 ± 7.5 days) were fed the total mixed rations (TMR) diets *ad libitum*, the basal diet was formulated based on the Feeding Standards of Dairy Cattle in China. The three species had similar physical characteristics, and the characteristics enrolled yak, cattle, and Holstein dairy cows are shown in Table 1. The three pasture sites are traditionally used by local herders for grazing, with similar environment and climatic conditions (temperature: 19.2 ± 1.1 °C, relative humidity: 65.0 ± 2.2%), except altitude. In order to minimize the controlling variables of feeding and environmental factors among the three species, the blood was collected in August, 2021. The animal experiment was approved, and the animals received humane care according to the Ethical



**Fig. 1.** The geographic distribution of the sampling locations for white yak, yellow cattle and dairy cow on the edge of Qinghai-Tibetan Plateau, China.

**Table 1.** The characteristics of enrolled yak, cattle, and Holstein dairy cows

Group	Body condition score	Parity	Age (y)	Milk production (kg/d)	Days in milk (d)
Yak	3.5 ± 0.4	2	3	-	89.6 ± 10.5
Cattle	3.3 ± 0.5	2	3	-	87.5 ± 9.6
Holstein dairy cows	3.2 ± 0.3	2	3	27.1 ± 0.85	91.6 ± 7.5

Committee rules of Lanzhou University (RIB21110301).

### Metabolite extraction

The collected samples were thawed at 4°C, and 100 µL of sample was mixed with 400 µL of precooled methanol/acetonitrile (1:1, v/v). It was incubated at room temperature for 10 min and then centrifuged. The supernatants were collected, dried, and then resuspended in 30 µL water/acetonitrile (98:2, v/v) for MS analysis.

### Liquid chromatography conditions

First, a UPLC system (SCIEX, Framingham, MA, USA) was used for chromatographic separations. Reversed-phase separation was performed using an ACQUITY UPLC T3 column. Solvent A (ultrapure water, 0.1% formic acid) and solvent B (acetonitrile, 0.1% formic acid) comprised the mobile phase.

### Quadrupole time-of-flight mass spectrometry conditions

The metabolites were detected using a tandem mass spectrometer (TripleTOF5600plus, SCIEX). The details of the Q-TOF mass spectrometry conditions were based on our previous publication [17].

### Processing of metabolomics data

LC-MS raw data files were processed by the CAMERA and XCMS packages of R software.

Retention time (RT) and  $m/z$  data were used to identify each ion. The metabolites were annotated using the human metabolome database (HMDB) database and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis. MetaX was used to further preprocess the intensity of the peak data. The “50% rule” was applied to remove the systematic bias or technical variation by normalizing the data according to our previous publication [17], and the results showed a normal distribution after normalization processing. Outlier detection and batch effects were evaluated by principal component analysis (PCA). False discovery rate (FDR) and supervised partial least squares-discriminant analysis (PLS-DA) were conducted to adjust the  $p$  value. The important features were selected based on a variable importance in the projection (VIP) cutoff value of 1.0.

### **Determination of inflammatory cytokines and antioxidant enzymes**

The levels of interleukin-2 (IL-2), interleukin-6 (IL-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) in serum were determined using ELISA kits. The levels of malondialdehyde (MDA), total antioxidant capacity (T-AOC), and glutathione peroxidase (GSH-Px) in serum were measured by chemical colorimetry. All kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The detailed principles and methods for the detection of MDA, T-AOC and GSH-Px have been described in our previous publication [18].

### **Statistical analysis**

GraphPad Prism 9 was employed to perform statistical analyses by single factor analysis of variance (one-way ANOVA).  $P$  values less than 0.05 indicated a significant difference.

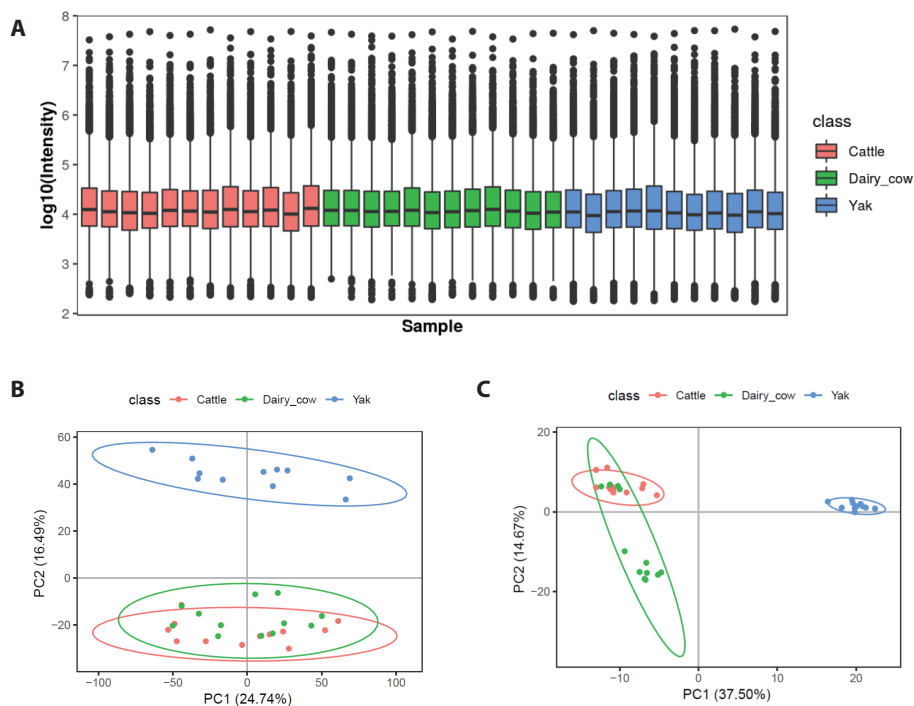
## **RESULTS**

### **Serum metabolite analysis**

Box plots were used to analyze the identified serum metabolites in yaks, yellow cattle and dairy cows. All samples showed a similar range of metabolite levels (Fig. 2A). Serum metabolomic analysis was used to determine whether the metabolic profiles of yaks are separable from those of dairy cows and yellow cattle, we used PCA for visualization. Based on the serum metabolic profiles, the score plots of the PCA model discriminating yaks, dairy cows and yellow cattle are presented in Figs. 2B and C. PCA showed that the positive mode of the total variance data was 41.23%, represented by the first two principal components (Fig. 2A), and the negative mode was 52.17% (Fig. 2B). The plot revealed that the serum metabolic profiles of yellow cattle were closely related to those of dairy cows and were not obviously changed. However, the profiles of yaks showed a clear separation trend from those of and yellow cattle and dairy cows.

### **Identification of differentially expressed serum metabolites among yaks, yellow cattle and dairy cows**

From the 1,815 detected metabolites, we investigated 63 differentially expressed metabolites (Table 2), including L-glutamine, L-glutamic acid,  $\alpha$ -linolenic acid, tauroursodeoxycholic acid, and LysoPC (ratio  $\geq 5.0$  or  $\leq 0.2$ ,  $p < 0.01$  and VIP  $\geq 1$ ). The relative concentrations of 23 metabolites were significantly higher, while 30 were significantly lower in yaks than in yellow cattle (Table 2). The relative concentrations of 11 metabolites were significantly higher and 15 were significantly lower in yaks than in dairy cows. The relative concentrations of 11 metabolites were significantly lower in yellow cattle than in dairy cows. These metabolites were carbohydrates, amino acids, lipids and their metabolites, suggesting that these metabolic pathways were different among yaks, yellow cattle, and dairy cows. Furthermore, metabolic pathways were significantly different between yaks



**Fig. 2. Comparison of distribution of serum metabolite levels among yak, dairy cow and yellow cattle.** (A) Box plots represent the distribution of metabolite peak intensity measurements from serum samples across all subjects. PCA scores plots of serum metabolomic profiles derived from UPLC-Q-TOF-MS spectra showing separation between yak and yellow cattle and dairy cow in the positive mode (B) and negative mode (C). PC, principal component; PCA, principal component analysis; UPLC-Q-TOF-MS, ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

and yellow cattle and between yaks and dairy cows, but there were similarities between yellow cattle and dairy cows based on the very few differential metabolic profiles (Table 2).

### Metabolic Kyoto encyclopedia of genes and genomes pathway analysis

KEGG analysis was used to predict metabolic pathways for all differential metabolites. Fig. 3 shows the functional enrichment of the top 5 different pathways. The most enriched functional pathways among yak, yellow cattle and dairy cow belonged to metabolic pathways: amino acid metabolism (e.g., phenylalanine, arginine, proline, glycine, valine, leucine, isoleucine and glutamine), phospholipid metabolism (lysophosphatidylcholines [LysoPCs]), and fatty acid metabolism (arachidonic acid metabolism,  $\alpha$ -linolenic acid and linolenic acid metabolism).

### Inflammatory cytokines and antioxidant levels

The levels of the inflammatory cytokines IL-2, IL-6, and TNF- $\alpha$  in yak serum were significantly higher than those in yellow cattle and dairy cows ( $p < 0.05$  or  $p < 0.01$ ) (Figs. 4A, B and C). Oxidative stress is an imbalance of reactive oxygen species (ROS) generation and elimination. High altitude-associated hypobaric hypoxia stress induces ROS production [19]. To determine if the elevated peripheral inflammatory cytokines were accompanied by reactive oxygen production or oxidative damage [20], serum levels of MDA, T-AOC and GSH-Px were measured by chemical colorimetry. The results showed that there was no significant differences in MDA, T-AOC and GSH-Px levels among yaks, yellow cattle and dairy cows ( $p > 0.05$ ) (Figs. 4D, E, and F).

Table 2. List of serum differential metabolites among yak, yellow cattle and dairy cow (n = 12)

NO.	Metabolites	Yak vs cattle			Yak vs dairy cow			Cattle vs dairy cow		
		Ratio	p-value	VIP	Ratio	p-value	VIP	Ratio	p-value	VIP
1	L-Acetopine	9.287	1.17E-03	2.237	5.183	4.55E-03	2.082			
2	LysoPC (18:0)	7.211	7.94E-06	2.558	7.621	3.30E-06	2.906			
3	7-Ketodeoxycholic acid	6.780	2.93E-03	2.358	0.161	5.11E-04	3.567			
4	LysoPC (16:0)	6.379	1.09E-04	2.311	0.131	6.89E-06	2.572			
5	LysoPC (18:1)	6.219	3.65E-05	2.401	5.979	3.69E-05	2.539			
6	Ganoderol A	6.042	1.81E-05	2.564	5.110	4.73E-05	2.264			
7	$\alpha$ -Linolenic acid	0.179	1.93E-05	2.342	0.181	1.73E-07	2.816			
8	Stearoylcarnitine	0.141	4.00E-13	2.782	0.103	7.26E-14	3.428			
9	Taurocholic acid	0.099	5.33E-06	3.151	0.186	7.83E-05	2.477			
10	TG (14:0)	0.098	4.37E-08	2.979	0.081	8.98E-10	1.936			
11	Heme O	0.089	3.83E-07	3.345	0.081	1.98E-07	3.623			
12	Tauroursodeoxycholic acid	0.084	3.71E-06	3.393	0.133	2.61E-05	3.119			
13	Ganodermic acid TQ	0.063	3.96E-07	3.500	0.147	2.16E-05	2.721			
14	L-Glutamine	41.331	2.93E-10	2.094	50.738	3.27E-09	2.243			
15	L-Glutamic acid	15.615	1.84E-07	1.764	16.328	3.30E-06	2.096			
16	Daucic acid	8.296	2.66E-05	1.410	6.906	5.44E-05	1.351			
17	L-Malic acid	5.635	2.69E-09	1.320	8.920	1.45E-08	1.798			
18	Pterodin N	0.095	1.69E-06	1.474	0.073	5.82E-17	1.882			
19	Linoleic acid	0.078	2.03E-06	1.744	0.060	2.18E-06	1.826			
20	Pyrocatechol sulfate	0.031	9.83E-11	2.130	0.106	1.62E-04	1.167			
21	Methyl levulinate	0.007	2.59E-17	2.578	0.019	3.04E-05	1.582			
22	LysoPC (22:6)	8.040	1.68E-09	3.084						
23	Lactapiperanol D	7.664	1.46E-08	3.178						
24	Octadecyl fumarate	6.929	1.33E-07	2.797						
25	27-Norcholestanehexol	6.225	1.89E-07	3.014						
26	Lysyl-Threonine	5.756	4.46E-07	2.940						
27	5-Hydroxy-tryptophol	5.682	1.08E-05	2.663						
28	LysoPC (22:4)	5.665	4.70E-08	2.611						
29	beta-Elemonic acid	5.555	1.21E-07	2.704						
30	L-Carnitine	5.539	7.49E-08	2.715						
31	Malonyl-Carnitin	5.461	1.78E-07	2.920						
32	Cortisol	5.210	2.99E-05	2.455						
33	Polyporusterone B	5.058	1.78E-05	2.896						
34	Acetaminophen glucuronide	0.199	5.80E-03	2.147						
35	Eicosatetraenoic acid	0.195	4.34E-06	2.348						
36	12-Ketodeoxycholic acid	0.191	1.81E-04	2.932						
37	Pterodin O	0.149	4.72E-06	2.921						
38	Phenylalanyl-Tryptophan	0.144	9.62E-08	2.968						
39	N-Heptanoylglycine	0.137	1.79E-09	2.834						
40	Hexaethylene glycol	0.125	4.52E-04	2.333						
41	Glycyrrhizin	0.107	1.10E-06	3.200						
42	Campesteryl linoleate	0.103	1.07E-04	3.643						
43	Psychosine sulfate	0.091	1.75E-09	3.191						
44	D-Xylose	0.084	3.42E-03	2.262						
45	Cellulose triacetate	0.076	2.61E-06	3.463						

Table 2. Continued

NO.	Metabolites	Yak vs cattle			Yak vs dairy cow			Cattle vs dairy cow		
		Ratio	p-value	VIP	Ratio	p-value	VIP	Ratio	p-value	VIP
46	6a-Hydroxy-paclitaxel	0.070	6.90E-13	3.368						
47	TG (18:1)	0.063	7.34E-10	3.311						
48	Paradol	0.053	2.32E-15	3.789						
49	Torvonin A	5.959	1.10E-10	1.534						
50	Allantoin	0.168	2.66E-04	1.632						
51	Ethyl pyruvate	0.154	8.04E-05	1.661						
52	2-Hydroxybutyric acid	0.148	5.76E-07	1.539						
53	Galactitol	0.078	8.58E-04	1.423						
54	Cystathionine ketimine				9.393	2.68E-04	1.256			
55	L-Phenylalanine				5.220	1.86E-08	1.467			
56	PC (16:0)				7.816	8.68E-03	2.112			
57	LysoPC (20:3)				0.196	4.88E-05	2.219			
58	TG (15:0)				0.014	4.52E-18	4.611	0.139	9.69E-05	2.035
59	Heliantriol A1							0.183	1.00E-03	1.682
60	Corbisterol							0.183	4.19E-06	5.027
61	Diacetoxypropyl stearate							0.176	9.65E-04	4.455
62	Famprofazone							0.164	4.15E-07	5.073
63	Leontogenin							0.147	2.01E-06	5.420

The ratio is > 5.0 or < 0.2.

LysoPC, lysophosphatidylcholine; TG, triglycerides.

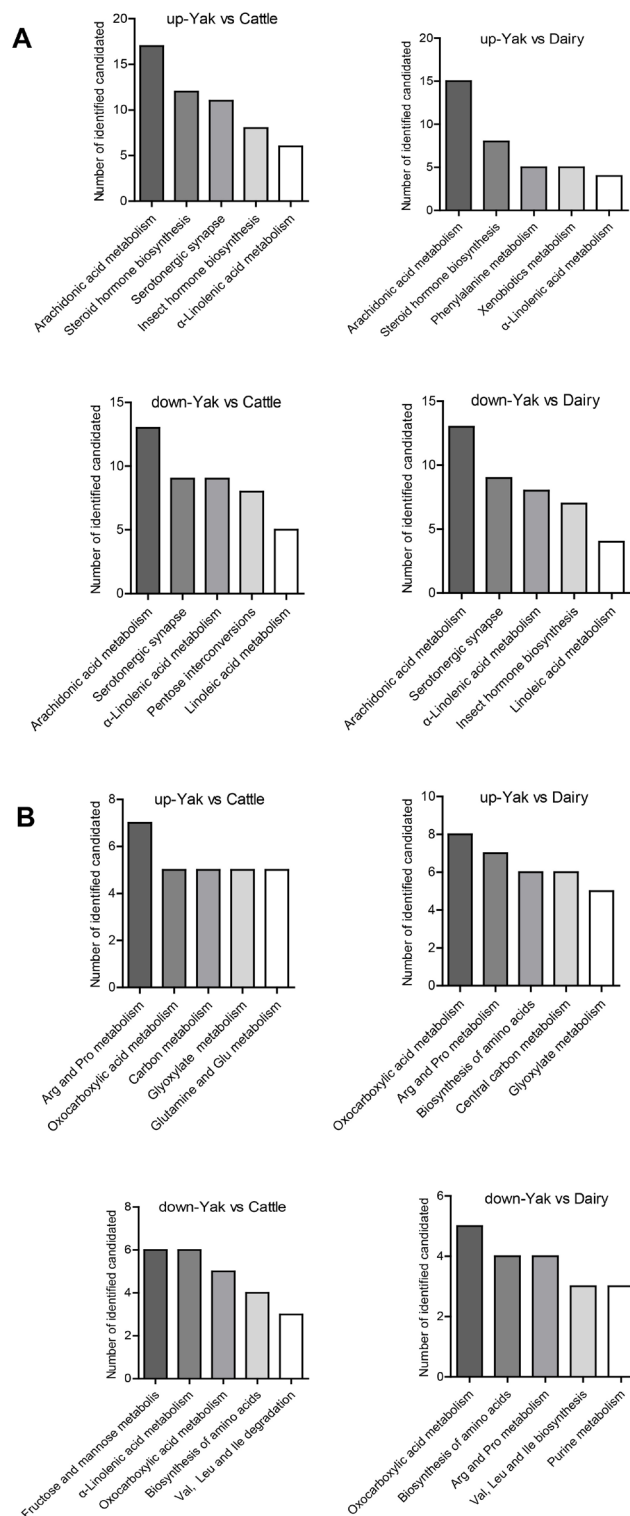
## DISCUSSION

Yaks are an iconic symbol of QTP and can be used as a model to elucidate the mechanisms of hypoxia adaptation. The three species of yak, yellow cattle and Holstein dairy cow belong to subtribe Bovina [21]. Holstein and yellow cattle should be probably separated from yak about 4.4 to 5.3 million years ago [10,22]. Systems biology is the trend of contemporary scientific development [23]. Comparative transcriptome sequencing revealed that the innate immunity were more activated in yak lung than low-altitude cattle (Sanjiang and Holstein cattle) [10,24]. Proteomics of skeletal muscle mitochondria showed that the significantly affected pathway in yaks and cattle was oxidative phosphorylation [7]. Identification of metabolic pathways using metabolomics comparisons between closely related species has the potential to provide insights into the basis of mammalian divergence and adaptation. To understand differences in the global metabolic profiles and relevant metabolic pathways of yaks, yellow cattle and dairy cows during acclimatization to high altitude, we utilized UPLC-Q-TOF-MS to determine the serum metabolite profiles of the three breeds.

We detected a clear separation trend between yaks and yellow cattle and dairy cows. A total of 63 different metabolites were obtained in serum. An integrative view plot of the metabolic changes among white yaks, yellow cattle and dairy cows was prepared (Fig. 3). The major perturbed metabolic patterns and plausible pathways are involved in amino acid metabolism, phospholipid metabolism, and fatty acid metabolism, which are associated with hypobaric hypoxia.

### Amino acid metabolism

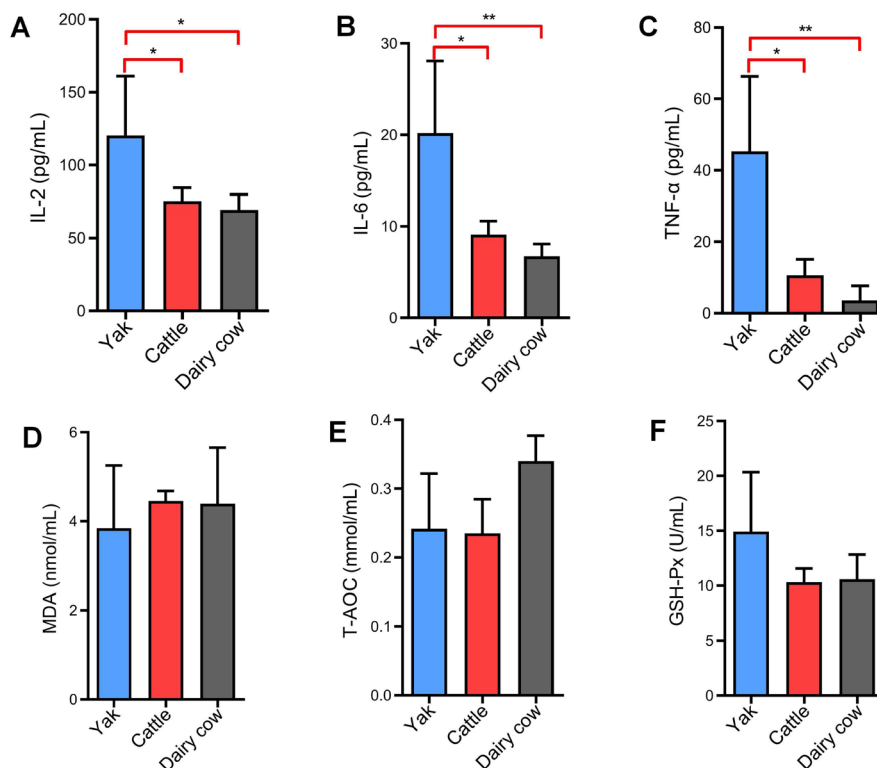
Glutamine is a key metabolite in the alanine, aspartate and glutamate metabolism pathways [25]. A previous study reported that high-altitude exposure leads to lower glutamate levels due to decreased



**Fig. 3.** The top 5 different pathways for differential metabolites (up and down) among yak, yellow cattle, and dairy cow in positive (A) and negative (B) ion modes.

activity of glutamine synthetase [26]. In contrast, we found significantly elevated levels of glutamic acid and glucogenic amino acids that produce pyruvic acid,  $\alpha$ -ketoglutaric acid, and oxaloacetic





**Fig. 4.** The inflammatory cytokines of IL-2 (A), IL-6 (B), and TNF- $\alpha$  (C) were determined using ELISA kits, and MDA level (D), T-AOC (E) and GSH-Px activity (F) related to antioxidant defense system in serum were measured by chemical colorimetry. \* $p < 0.05$ , \*\* $p < 0.01$ . IL, interleukin; TNF, tumor necrosis factor; MDA, malondialdehyde; T-AOC, total antioxidant capacity; GSH-Px, glutathione peroxidase.

acid in the serum of yaks in comparison with yellow cattle and dairy cows [27] (Table 2). The results of this study indicate that yaks have the highest levels of protein catabolism and amino acid mobilization. Therefore, the mobilization of yak muscle protein may be a metabolic adaptation to hypobaric hypoxia. Pathway analysis also showed improved energy metabolism and promoted acclimatization to high altitude by increasing the metabolism of phenylalanine, arginine, proline and glutamine to meet the energy requirements in yaks (Fig. 3). This result is consistent with the previous finding that hypobaric hypoxia exposure can enhance glucose and amino acid metabolism [28].

### Phospholipid metabolism

Phospholipids play a role as a cellular bilayer with membrane proteins, and they are involved in the maintenance of hepatic lipid metabolism [29]. Our results show that almost all LysoPCs, including LysoPC (18:0), LysoPC (16:0), LysoPC (18:1), LysoPC (22:6) and LysoPC (22:4), were markedly increased in yaks compared with yellow cattle and dairy cows (Table 2). LysoPCs participate in the inflammatory response by mediating cell signaling pathways in monocytes and macrophages [30,31]. To verify the increased LysoPCs, the serum levels of cytokines IL-2, IL-6, and TNF- $\alpha$  were detected. The results showed that the levels of IL-2, IL-6, and TNF- $\alpha$  were significantly higher in yaks than in cattle and dairy cows (Figs. 4A, B, and C), which is consistent with the increased LysoPCs. Environmental factors such as hypobaric hypoxia, cold and UV exposure at high altitude can suppress the immune system [32]. Tumor necrosis factors and interleukins can mediate innate immunity signaling. Xin et al. [10] reported that the immune system was more

activated and the genes related to immune were up-regulated in yak compared with Sanjiang and Holstein cattle [10]. A significant elevation of LysoPCs and cytokines (IL-2, IL-6, and TNF- $\alpha$ ) might be responsible for yaks being more tolerant to hypoxia at high altitudes than yellow cattle and dairy cows by activating innate immunity system.

### Fatty acids metabolism

Hypoxia is associated with an increase in the generation of reactive oxygen species (ROS), and an excessive load of ROS generated may result in cell injury and dysfunction [33]. Excessive ROS can lead to lipid peroxidation, MDA can reflect the level of lipid peroxidation. ROS are balanced by natural antioxidant compounds such as GSH-Px, superoxide dismutase (SOD) and catalase (CAT) [34]. We found that serum MDA levels, T-AOC and GSH-Px activity were not significantly changed in yak in comparison with yellow cattle and Holstein dairy cows (Figs. 4D, E, and F), based on they had similar physical characteristics (Table 1). The results demonstrate that yaks adapt to hypoxia-induced oxidative stress at high altitudes do not by increasing antioxidant enzyme levels. Free fatty acids (FFAs) are risk factors for cardiovascular diseases and are closely related to metabolic syndromes [35]. FFAs are significant sources of ROS [36], mainly through the activation of NADPH oxidase [37]. There was a dose-dependent increase in ROS in monocytes exposed to FFAs [38]. Polyunsaturated fatty acids (PUFAs) are a favorable target for ROS [39]. Oxidative breakdown of PUFAs may affect lipid metabolism and the expression of genes and proteins related to cell differentiation [40].  $\alpha$ -Linolenic acid and linoleic acid are PUFAs. Linoleic acid contains unsaturated double bonds that are highly vulnerable to ROS [41], and has been linked to red blood cell damage by promoting redox reactions [42]. The ROS production was greater in bovine mammary epithelia cells treated with linoleic acid and  $\alpha$ -linolenic acid [43]. Arachidonic acid has been demonstrated to promote inflammatory responses by activating the mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinases (JNK) pathways by increasing TNF- $\alpha$  levels [44,45]. Arachidonic acid-derived metabolites also can propagate inflammation and oxidative stress [46]. Arachidonic acid suppressed the cell growth of hepatic cells by dose-dependently inducing the production of ROS [47]. In the present work, FFAs ( $\alpha$ -linolenic acid, linoleic acid and arachidonic acid) were significantly decreased in yaks compared with yellow cattle and Holstein dairy cows (Table 2). We speculate that yaks can decrease the level of FFAs ( $\alpha$ -linolenic acid, linoleic acid and arachidonic acid) in serum induced by hypoxia and that the decreased FFAs can attenuate cell injury and hypoxia dysfunction by inhibiting oxidative stress.

## CONCLUSION

A clear separation trend between the serum metabolic profiles of yaks and yellow cattle and dairy cows was demonstrated by PCA. In addition, a total of 63 differentially expressed metabolites were identified among the three species. Functional analysis revealed that differentially expressed metabolites were related to the innate immune activation (elevation of LysoPCs and cytokines), oxidative stress-related metabolism (arachidonic acid metabolism,  $\alpha$ -linolenic acid metabolism, and linoleic acid metabolism) and energy metabolism (fatty acid metabolism and amino acid metabolism) in yaks, which indicates the important roles of metabolites in high-altitude adaptation in yaks.

## REFERENCES

1. Tang Q, Gu Y, Zhou X, Jin L, Guan J, Liu R, et al. Comparative transcriptomics of 5 high-altitude vertebrates and their low-altitude relatives. *Gigascience*. 2017;6:gix105. <https://doi.org/10.1093/gigascience/gix105>.

- org/10.1093/gigascience/gix105
2. Cui GX, Yuan F, Degen AA, Liu SM, Zhou JW, Shang ZH, et al. Composition of the milk of yaks raised at different altitudes on the Qinghai-Tibetan Plateau. *Int Dairy J.* 2016;59:29-35. <https://doi.org/10.1016/j.idairyj.2016.02.046>
  3. Qiu Q, Wang L, Wang K, Yang Y, Ma T, Wang Z, et al. Yak whole-genome resequencing reveals domestication signatures and prehistoric population expansions. *Nat Commun.* 2015;6:10283. <https://doi.org/10.1038/ncomms10283>
  4. Dolt KS, Mishra MK, Karar J, Baig MA, Ahmed Z, Pasha MAQ. cDNA cloning, gene organization and variant specific expression of HIF-1 $\alpha$  in high altitude yak (*Bos grunniens*). *Gene.* 2007;386:73-80. <https://doi.org/10.1016/j.gene.2006.08.004>
  5. Shao B, Long R, Ding Y, Wang J, Ding L, Wang H. Morphological adaptations of yak (*Bos grunniens*) tongue to the foraging environment of the Qinghai-Tibetan Plateau. *J Anim Sci.* 2010;88:2594-603. <https://doi.org/10.2527/jas.2009-2398>
  6. Xin JW, Chai ZX, Zhang CF, Zhang Q, Zhu Y, Cao HW, et al. Signature of high altitude adaptation in the gluteus proteome of the yak. *J Exp Zool B Mol Dev Evol.* 2020;334:362-72. <https://doi.org/10.1002/jez.b.22995>
  7. Long L, Zhu Y, Li Z, Zhang H, Liu L, Bai J. Differential expression of skeletal muscle mitochondrial proteins in yak, dzo, and cattle: a proteomics-based study. *J Vet Med Sci.* 2020;82:1178-86. <https://doi.org/10.1292/jvms.19-0218>
  8. Qiu Q, Zhang G, Ma T, Qian W, Wang J, Ye Z, et al. The yak genome and adaptation to life at high altitude. *Nat Genet.* 2012;44:946-9. <https://doi.org/10.1038/ng.2343>
  9. Guan J, Long K, Ma J, Zhang J, He D, Jin L, et al. Comparative analysis of the microRNA transcriptome between yak and cattle provides insight into high-altitude adaptation. *PeerJ.* 2017;5:e3959. <https://doi.org/10.7717/peerj.3959>
  10. Xin JW, Chai ZX, Zhang CF, Zhang Q, Zhu Y, Cao HW, et al. Transcriptome profiles revealed the mechanisms underlying the adaptation of yak to high-altitude environments. *Sci Rep.* 2019;9:7558. <https://doi.org/10.1038/s41598-019-43773-8>
  11. Julian CG, Wilson MJ, Moore LG. Evolutionary adaptation to high altitude: a view from in utero. *Am J Hum Biol.* 2009;21:614-22. <https://doi.org/10.1002/ajhb.20900>
  12. O'Brien KA, Griffin JL, Murray AJ, Edwards LM. Mitochondrial responses to extreme environments: insights from metabolomics. *Extrem Physiol Med.* 2015;4:7. <https://doi.org/10.1186/s13728-015-0026-9>
  13. Araldi E, Schipani E. Hypoxia, HIFs and bone development. *Bone.* 2010;47:190-6. <https://doi.org/10.1016/j.bone.2010.04.606>
  14. Sauer U, Heinemann M, Zamboni N. Getting closer to the whole picture. *Science.* 2007;316:550-1. <https://doi.org/10.1126/science.1142502>
  15. Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, et al. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. *Science.* 2012;336:1040-4. <https://doi.org/10.1126/science.1218595>
  16. Dunn WB, Broadhurst DI, Atherton HJ, Goodacre R, Griffin JL. Systems level studies of mammalian metabolomes: the roles of mass spectrometry and nuclear magnetic resonance spectroscopy. *Chem Soc Rev.* 2011;40:387-426. <https://doi.org/10.1039/B906712B>
  17. Wang H, Liu Z, Wang S, Cui D, Zhang X, Liu Y, et al. UHPLC-Q-TOF/MS based plasma metabolomics reveals the metabolic perturbations by manganese exposure in rat models. *Metallomics.* 2017;9:192-203. <https://doi.org/10.1039/C7MT00007C>
  18. Wang H, Liu Z, Huang M, Wang S, Cui D, Dong S, et al. Effects of long-term mineral block supplementation on antioxidants, immunity, and health of Tibetan sheep. *Biol Trace Elem Res.*

- 2016;172:326-35. <https://doi.org/10.1007/s12011-015-0593-z>
19. Bakonyi T, Radak Z. High altitude and free radicals. *J Sports Sci Med.* 2004;3:64-9.
  20. Chen H, Cao G, Chen DQ, Wang M, Vaziri ND, Zhang ZH, et al. Metabolomics insights into activated redox signaling and lipid metabolism dysfunction in chronic kidney disease progression. *Redox Biol.* 2016;10:168-78. <https://doi.org/10.1016/j.redox.2016.09.014>
  21. Zhang K, Lenstra JA, Zhang S, Liu W, Liu J. Evolution and domestication of the Bovini species. *Anim Genet.* 2020;51:637-57. <https://doi.org/10.1111/age.12974>
  22. Gu Z, Zhao X, Li N, Wu C. Complete sequence of the yak (*Bos grunniens*) mitochondrial genome and its evolutionary relationship with other ruminants. *Mol Phylogenet Evol.* 2007;42:248-55. <https://doi.org/10.1016/j.ympev.2006.06.021>
  23. Liao WT, Liu B, Chen J, Cui JH, Gao YX, Liu FY, et al. Metabolite modulation in human plasma in the early phase of acclimatization to hypobaric hypoxia. *Sci Rep.* 2016;6:22589. <https://doi.org/10.1038/srep22589>
  24. Lan D, Xiong X, Ji W, Li J, Mipam TD, Ai Y, et al. Transcriptome profile and unique genetic evolution of positively selected genes in yak lungs. *Genetica.* 2018;146:151-60. <https://doi.org/10.1007/s10709-017-0005-8>
  25. Guo L, Tan G, Liu P, Li H, Tang L, Huang L, et al. Three plasma metabolite signatures for diagnosing high altitude pulmonary edema. *Sci Rep.* 2015;5:15126. <https://doi.org/10.1038/srep15126>
  26. Radák Z, Asano K, Fu Y, Nakamura A, Nakamoto H, Ohno H, et al. The effect of high altitude and caloric restriction on reactive carbonyl derivatives and activity of glutamine synthetase in rat brain. *Life Sci.* 1998;62:1317-22. [https://doi.org/10.1016/S0024-3205\(98\)00065-4](https://doi.org/10.1016/S0024-3205(98)00065-4)
  27. Lapierre H, Lobley GE, Doepel L, Raggio G, Rulquin H, Lemosquet S. Triennial Lactation Symposium: mammary metabolism of amino acids in dairy cows. *J Anim Sci.* 2012;90:1708-21. <https://doi.org/10.2527/jas.2011-4645>
  28. Horscroft JA, Murray AJ. Skeletal muscle energy metabolism in environmental hypoxia: climbing towards consensus. *Extrem Physiol Med.* 2014;3:19. <https://doi.org/10.1186/2046-7648-3-19>
  29. Oh HA, Lee H, Park S, Lim Y, Kwon O, Kim JY, et al. Analysis of plasma metabolic profiling and evaluation of the effect of the intake of *Angelica keiskei* using metabolomics and lipidomics. *J Ethnopharmacol.* 2019;243:112058. <https://doi.org/10.1016/j.jep.2019.112058>
  30. Duong CQ, Bared SM, Abu-Khader A, Buechler C, Schmitz A, Schmitz G. Expression of the lysophospholipid receptor family and investigation of lysophospholipid-mediated responses in human macrophages. *Biochim Biophys Acta Mol Cell Biol Lipids.* 2004;1682:112-9. <https://doi.org/10.1016/j.bbalip.2004.03.002>
  31. Kabarowski JH. G2A and LPC: regulatory functions in immunity. *Prostaglandins Other Lipid Mediat.* 2009;89:73-81. <https://doi.org/10.1016/j.prostaglandins.2009.04.007>
  32. Mishra KP, Ganju L. Influence of high altitude exposure on the immune system: a review. *Immunol Invest.* 2010;39:219-34. <https://doi.org/10.3109/08820131003681144>
  33. Clanton TL. Hypoxia-induced reactive oxygen species formation in skeletal muscle. *J Appl Physiol.* 2007;102:2379-88. <https://doi.org/10.1152/jappphysiol.01298.2006>
  34. Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense systems, and schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry.* 2013;46:200-6. <https://doi.org/10.1016/j.pnpbp.2013.02.015>
  35. Boden G. Obesity and free fatty acids. *Endocrinol Metab Clin North Am.* 2008;37:635-46. <https://doi.org/10.1016/j.ecl.2008.06.007>
  36. Ghosh A, Gao L, Thakur A, Siu PM, Lai CWK. Role of free fatty acids in endothelial

- dysfunction. *J Biomed Sci.* 2017;24:50. <https://doi.org/10.1186/s12929-017-0357-5>
37. Cury-Boaventura MF, Curi R. Regulation of reactive oxygen species (ROS) production by C18 fatty acids in Jurkat and Raji cells. *Clin Sci.* 2005;108:245-53. <https://doi.org/10.1042/CS20040281>
  38. Zhang WY, Schwartz E, Wang Y, Attrep J, Li Z, Reaven P. Elevated concentrations of nonesterified fatty acids increase monocyte expression of CD11b and adhesion to endothelial cells. *Arterioscler Thromb Vasc Biol.* 2006;26:514-9. <https://doi.org/10.1161/01.ATV.0000200226.53994.09>
  39. Behn C, Araneda OF, Llanos AJ, Celedón G, González G. Hypoxia-related lipid peroxidation: evidences, implications and approaches. *Respir Physiol Neurobiol.* 2007;158:143-50. <https://doi.org/10.1016/j.resp.2007.06.001>
  40. Bork CS, Baker EJ, Lundbye-Christensen S, Miles EA, Calder PC. Lowering the linoleic acid to alpha-linoleic acid ratio decreases the production of inflammatory mediators by cultured human endothelial cells. *Prostaglandins Leukot Essent Fatty Acids.* 2019;141:1-8. <https://doi.org/10.1016/j.plefa.2018.12.001>
  41. Fedor DM, Adkins Y, Mackey BE, Kelley DS. Docosahexaenoic acid prevents trans-10, cis-12-conjugated linoleic acid-induced nonalcoholic fatty liver disease in mice by altering expression of hepatic genes regulating fatty acid synthesis and oxidation. *Metab Syndr Relat Disord.* 2012;10:175-80. <https://doi.org/10.1089/met.2011.0113>
  42. Yuan T, Fan WB, Cong Y, Xu HD, Li CJ, Meng J, et al. Linoleic acid induces red blood cells and hemoglobin damage via oxidative mechanism. *Int J Clin Exp Pathol.* 2015;8:5044-52.
  43. Basiricò L, Morera P, Dipasquale D, Tröschler A, Bernabucci U. Comparison between conjugated linoleic acid and essential fatty acids in preventing oxidative stress in bovine mammary epithelial cells. *J Dairy Sci.* 2017;100:2299-309. <https://doi.org/10.3168/jds.2016-11729>
  44. Balsinde J, Winstead MV, Dennis EA. Phospholipase A2 regulation of arachidonic acid mobilization. *FEBS Lett.* 2002;531:2-6. [https://doi.org/10.1016/S0014-5793\(02\)03413-0](https://doi.org/10.1016/S0014-5793(02)03413-0)
  45. Saito Y, Watanabe K, Fujioka D, Nakamura T, Obata J, Kawabata K, et al. Disruption of group IVA cytosolic phospholipase A2 attenuates myocardial ischemia-reperfusion injury partly through inhibition of TNF- $\alpha$ -mediated pathway. *Am J Physiol Heart Circ Physiol.* 2012;302:H2018-30. <https://doi.org/10.1152/ajpheart.00955.2011>
  46. Rink C, Khanna S. Significance of brain tissue oxygenation and the arachidonic acid cascade in stroke. *Antioxid Redox Signal.* 2011;14:1889-903. <https://doi.org/10.1089/ars.2010.3474>
  47. Qin XY, Lu J, Cai M, Kojima S. Arachidonic acid suppresses hepatic cell growth through ROS-mediated activation of transglutaminase. *FEBS Open Bio.* 2018;8:1703-10. <https://doi.org/10.1002/2211-5463.12511>