British Journal of Nutrition (2020), 123, 499–507
doi:10.1017/S000711451900309X

Arginine promotes porcine type I muscle fibres formation through improvement of mitochondrial biogenesis

Xiaoling Chen[1,2]†, Xiaoming Luo[1,2]†, Daiwen Chen[1,2], Bing Yu[1,2], Jun He[1,2] and Zhiqing Huang[1,2]*

[1]*Key Laboratory for Animal Disease-Resistance Nutrition of China Ministry of Education, Sichuan Agricultural University, Chengdu, Sichuan 611130, People's Republic of China*
[2]*Key Laboratory of Animal Disease-Resistant Nutrition, Sichuan Province, Institute of Animal Nutrition, Sichuan Agricultural University, Chengdu, Sichuan 611130, People's Republic of China*

(Submitted 18 July 2019 – Final revision received 30 October 2019 – Accepted 14 November 2019 – First published online 29 November 2019)

Abstract

The present study aimed to investigate whether arginine (Arg) promotes porcine type I muscle fibres formation via improving mitochondrial biogenesis. In the *in vivo* study, a total of sixty Duroc × Landrace × Yorkshire weaning piglets with an average body weight of 6·55 (SD 0·36) kg were randomly divided into four treatments and fed with a basal diet or a basal diet supplemented with 0·5, 1·0 and 1·5 % L-Arg, respectively, in a 4-week trial. Results showed that dietary supplementation of 1·0 % Arg significantly enhanced the activity of succinate dehydrogenase, up-regulated the protein expression of myosin heavy chain I (MyHC I) and increased the mRNA levels of *MyHC I*, troponin I1, C1 and T1 (*Tnni1*, *Tnnc1* and *Tnnt1*) in *longissimus dorsi* muscle compared with the control group. In addition, ATPase staining analysis indicated that 1·0 % Arg supplementation significantly increased the number of type I muscle fibres and significantly decreased the number of type II muscle fibres. Furthermore, 1·0 % Arg supplementation significantly up-regulated PPAR-γ coactivator-1α (PGC-1α), sirtuin 1 and cytochrome c (Cytc) protein expressions, increased *PGC-1α*, nuclear respiratory factor 1 (*NRF1*), mitochondria transcription factor B1 (*TFB1M*), *Cytc* and ATP synthase subunit C1 (*ATP5G*) mRNA levels and increased mitochondrial DNA content. In the *in vitro* study, mitochondrial complex I inhibitor rotenone (Rot) was used. We found that Rot annulled Arg-induced type I muscle fibres formation. Together, our results provide for the first time the evidence that Arg promotes porcine type I muscle fibres formation through improvement of mitochondrial biogenesis.

Key words: **Arginine: Porcine type I muscle fibres formation: Mitochondrial biogenesis: Rotenone**

Skeletal muscle is the most abundant tissue of mammals, comprising 40–50 % of the total body mass. It is a heterogeneous tissue comprised of a variety of functionally diverse muscle fibre types with contractile and metabolic properties[1]. Skeletal muscle fibres are generally classified into two major groups: type I muscle fibres expressing myosin heavy chain I (MyHC I) and type II muscle fibres expressing MyHC IIa, MyHC IIx and MyHC IIb[2]. It is well known that type I muscle fibres have more mitochondria and type IIb muscle fibres contain fewer mitochondria[3]. Mitochondrial biogenesis and function may be a new mediator of skeletal muscle fibre type[4]. Improvement of mitochondrial biogenesis and function has been reported to promote the formation of type I muscle fibres[5,6].

L-Arginine (Arg) is a nutritionally essential amino acid for animals[7]. Previous studies have suggested that Arg plays multiple physiological functions in animals, such as antioxidant activity, reproductive performance and fat deposition[8–11]. A few recent studies have shown that Arg promotes more type I muscle fibres formation in mice and MyHC I expression in porcine skeletal muscle satellite cells[12–14]. However, no information has been reported about the effect of Arg on type I muscle fibre type formation in weaning piglets. It has been reported that Arg modulates mitochondrial function[15,16]. But it remains unclear whether Arg affects type I muscle fibres formation by improving mitochondrial biogenesis and function.

It is well known that the increase of the proportion of type I muscle fibres is beneficial to the improvement of meat quality in animal husbandry[17]. Here, the aim of the present study was to investigate the role of Arg in mitochondrial biogenesis and function-related marker expression, mitochondrial DNA

Abbreviations: Arg, arginine; ATP5G, ATP synthase subunit C1; Cytc, cytochrome c; mtDNA, mitochondrial DNA; MyHC I, myosin heavy chain I; NO, nitric oxide; NOS, nitric oxide synthase; NRF1, nuclear respiratory factor 1; PGC-1α, PPAR-γ coactivator-1α; Rot, rotenone; SDH, succinic dehydrogenase; Sirt1, sirtuin 1; TFB1M, mitochondria transcription factor B1; Tnnc1, troponin C1; Tnni1, troponin I1; Tnnt1, troponin T1.

* **Corresponding author:** Zhiqing Huang, fax +86 28 8629 0976, email zqhuang@sicau.edu.cn

† These authors contributed equally to this work.

(mtDNA) content and muscle fibre type composition in weaning piglets. For further studies, mitochondrial complex I inhibitor rotenone (Rot) was used to investigate the mechanism of Arg affecting type I muscle fibres formation.

Materials and methods

Ethics statement

All animal procedures were performed according to protocols approved by the Animal Care Advisory Committee of Sichuan Agricultural University.

Animal experimental design

Sixty Duroc × Landrace × Yorkshire piglets with an average body weight of 6·55 (SD 0·36) kg were randomly assigned to four treatments. Each treatment consisted of five replicate pens of three piglets per pen. The piglets were provided a basal diet or a basal diet supplemented with 0·5, 1·0 or 1·5 % of Arg during a 28-d experimental period, while adjusting alanine to maintain the diets isonitrogenous. The basal diet was formulated according to the nutrient requirements for 7–10 kg and 11–25 kg pigs (Nutrient Requirements of Swine, 11th revised edition, 2012). The composition and nutrient levels of the diets are listed in Table 1. L-Arg (catalogue no. A8094) was obtained from Sigma. Water and feedstuff were given *ad libitum* during the experimental period. Individual body weight was measured at the beginning and end of the experimental period. Feed intake was recorded every day.

Table 1. Composition and nutrient levels of the diets

Ingredients	Contents			
	0 % Arg	0·5 % Arg	1·0 % Arg	1·5 % Arg
Maize (%)	31·52	32·14	32·49	33·20
Extruded maize (%)	37·10	37·00	36·90	36·90
Maize protein power (%)	2·85	37·00	36·90	36·90
Whey power (%)	8·00	2·85	2·85	2·85
Soyabean protein concentrate (%)	3·35	8·00	8·00	8·00
Expanded soyabean (%)	2·50	3·50	3·41	3·39
Dehulled soyabean meal (%)	2·46	2·50	2·50	2·50
Fishmeal (%)	3·60	2·46	2·46	2·46
Soyabean oil (%)	1·00	3·60	3·60	3·60
Sucrose (%)	1·00	0·90	1·10	0·90
L-Lysine·HCl (%)	0·95	1·00	1·00	1·00
DL-Methionine (%)	0·16	0·95	0·95	0·95
L-Threonine (%)	0·34	0·16	0·16	0·16
L-Tryptophan (%)	0·09	0·33	0·34	0·33
Alanine (%)	3·25	0·09	0·09	0·09
L-Arginine·HCl (%)	0	2·07	1·08	0·00
Choline chloride (%)	0·10	0·62	1·24	1·84
Limestone (%)	0·50	0·10	0·10	0·10
Dicalcium phosphate (%)	0·70	0·50	0·50	0·50
NaCl (%)	0·20	0·70	0·70	0·70
Vitamin premix* (%)	0·03	0·20	0·20	0·20
Trace mineral premix† (%)	0·30	0·03	0·03	0·03
Total (%)	100	0·30	0·30	0·30
Nutrient levels‡				
Digestible energy (Mcal/kg)§	3·50	100	100	100
Crude protein (%)	18·60	3·50	3·50	3·50
Ca (%)	0·75	18·60	18·60	18·60
Available P (%)	0·33	0·75	0·75	0·75
Digestible tryptophan (%)	0·21	0·33	0·33	0·33
Digestible lysine (%)	1·29	0·21	0·21	0·21
Digestible methionine (%)	0·38	1·29	1·29	1·29
Digestible threonine (%)	0·76	0·38	0·38	0·38
Digestible arginine (%)	0·64	0.76	0·76	0·76

* The vitamin premix supplied the following per kg diet: vitamin A, 9000 IU; vitamin D_3, 3000 IU; vitamin E, 24 IU; vitamin K, 3 mg; vitamin B_1, 3 mg; vitamin B_2, 7·5 mg; vitamin B_3, 30 mg; vitamin B_6, 3·6 mg; vitamin B_{12}, 0·036 mg; biotin, 0·15 mg and folic acid, 1·5 mg.
† The trace mineral premix supplied the following per kg diet: Fe, 100 mg; Cu, 6 mg; Mn, 4 mg; Zn, 100 mg; Se, 0·3 mg and iodine, 0·14 mg.
‡ Nutrient levels were calculated values.
§ To convert Mcal to MJ, multiply by 4·184.

Sample collection

At the end of the experiment, one piglet from each pen was slaughtered in a humane manner. Then, the *longissimus dorsi* muscle was collected, immediately frozen in liquid N_2 and stored at −80°C until further use.

Succinic dehydrogenase enzyme assay

The activity of succinic dehydrogenase (SDH) in *longissimus dorsi* muscle was determined using a commercial kit from Nanjing Jiancheng Bioengineering Institute. SDH activity was normalised to the total protein concentration.

Assay of nitric oxide synthase activity and nitric oxide content

The nitric oxide synthase (NOS) activity and nitric oxide (NO) content in *longissimus dorsi* muscle were detected by commercial biochemical kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer's instructions. The total protein concentration was measured using the Coomassie brilliant blue method (Nanjing Jiancheng Bioengineering Institute).

Analysis of mitochondrial DNA content

The relative mtDNA content was determined by real-time quantitative PCR as described above. The genomic DNA was isolated from the *longissimus dorsi* muscle of weaning piglet using a DNAiso reagent according to the manufacturer's instructions (TaKaRa). The primers for amplification of mtDNA (accession No. AF276923) were as follows: forward primer 5′-ACACCCTATAACGCCTTGCC-3′ and reverse primer 5′-AGGTGCCTGCTTTCGTAGC-3′. The primers for amplification of *β*-actin (accession No. DQ452569) were as follows: forward primer 5′-CAAAGCCAACCGTGAGAAGATG-3′ and reverse primer 5′-TGGCAAAGAGAGGCAAGAGAG-3′.

ATPase analysis

Longissimus dorsi muscle was collected and frozen in an optimal cutting temperature compound (Cat No. 4583, Sakura) before sectioning. The transverse serial sections were incubated with calcium chloride solution (pH 10·4) for 5 min and calcium chloride (pH 9·4) solution for 30 min. Then, the sections were stained with calcium chloride, cobalt nitrate and ammonium sulphide solutions. Finally, the sections were dehydrated with absolute ethyl alcohol. Sections were

Table 2. List of genes, primer sequences, GenBank accession numbers and product sizes

Genes	Primer	Sequence (5′ to 3′)	GenBank accession no.	Size (bp)
MyHC I	Forward	GGTATCGCATCCTGAACCC	NM_213855	144
	Reverse	GCCCTGCCTTGAAGAACAC		
Tnni1	Forward	CCTGCTGGGCTCTAAACACA	NM_213912	125
	Reverse	TGGCCTCGACGTTCTTTCTC		
Tnnc1	Forward	GGCACAGTGGACTTCGATGA	NM_001130243	183
	Reverse	CTCTGTGATGGTCTCGCCTG		
Tnnt1	Forward	GCAGAGAGAGCTGAGCAACA	NM_213748	75
	Reverse	CTTCTCCTCCGCCAGCTTAG		
PGC-1α	Forward	GCCCTCATTTGATGCACTG′	NM_213963	150
	Reverse	AGCTGAGTGTTGGCTGGTG		
NRF1	Forward	CCTTGTGGTGGGAGGAATGTT	XM_005657993	77
	Reverse	AGTATGCTGGCTGACCTTGTG		
TFB1M	Forward	GCAAGCAGTGAAGCAGCTA	NM_001128475	82
	Reverse	CAGACTGCCAGCTTTCCTTAC		
Cytc	Forward	TGCGGAGTGTTAAACTTTTCAGG	NM_001129970	191
	Reverse	TGCCTTAACAGGCTAGTGAACA		
ATP5G	Forward	GTGAGTCAGTCACCTTGAGC	NM_001025218	180
	Reverse	TCTGGCCTACTCAGGAAGGA		
Sirt1	Forward	ACTCTCCCTCTTTTAGACCAAGC	NM_001145750	149
	Reverse	AAACCTGGACTCTCCATCGG		
GAPDH	Forward	ACACTGAGGACCAGGTTGTG	NM_001206359	98
	Reverse	GACGAAGTGGTCGTTGAGGG		

MyHC I, myosin heavy chain I; *Tnni1*, troponin I1; *Tnnc1*, troponin C1; *Tnnt1*, troponin T1; *PGC-1α*, PPAR-γ coactivator-1α; *NRF1*, nuclear respiratory factor 1; *TFB1M*, mitochondria transcription factor B1; *Cytc*, cytochrome c; *ATP5G*, ATP synthase subunit C1; *Sirt1*, sirtuin 1; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.

photographed with a Nikon Eclipse E100 microscope equipped with a Nikon DS-U3 digital camera (Nikon Incorporation). The ratio of type I:type II fibres was calculated by using Motic Images Advanced 3.2 analysing systematical software. Light grey-stained fibres are slow type I, and dark fibres are fast type II.

Real-time quantitative PCR

The total RNA from *longissimus dorsi* muscle was extracted using the RNAiso Plus reagent (TaKaRa) according to the manufacturer's instructions. The RNA concentration and purity were determined using a Beckman DU-800 spectrophotometer (Beckman Coulter). Subsequently, reverse transcription was performed using a PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa) according to the manufacturer's instructions. Real-time quantitative PCR was performed using SYBR select Master Mix (Applied Biosystems) by a 7900HT Real-Time PCR Detection System (384-cell standard block) (Applied Biosystems). Sequences of the primers used for real-time quantitative PCR are listed in Table 2. Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) mRNA was used as an internal control, and the relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ analysis method[18].

Western blot analysis

Western blot analysis was performed as described before[12]. In brief, protein was isolated from *longissimus dorsi* muscle using radio immunoprecipitation assay lysis buffer (Pierce). Protein concentrations were determined using the bicinchoninic acid protein assay kit (Pierce). Equal amounts of protein were separated by 8 % SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore) using a wet Trans-Blot System (Bio-Rad). After blocking with 5 % fat-free milk in Tris-buffered saline-0·1 % Tween-20 for 1 h, the membrane was incubated overnight with primary antibodies at 4°C. Subsequently, the membrane was incubated with corresponding horseradish-peroxidase-conjugated secondary antibody for 1 h at 37°C. The primary antibodies used were: anti-Cytc (catalogue no. 10993-1-AP, ProteinTech Biotechnology), anti-MyHC I (catalogue no. M8421, Sigma), anti-sirtuin 1 (Sirt1) (catalogue no. 8469S, Cell Signaling), anti-PGC-1α (PPAR-γ coactivator-1α) (catalogue no. 2178S, Cell Signaling) and anti-β-actin (catalogue no. 4967S, Cell Signaling). The protein bands were visualised by the Clarity Western ECL Substrate (Bio-Rad). The densities of bands were determined using the Gel-Pro Analyzer version 4.2 (Media Cybernetics). β-Actin protein was used as a loading control.

Cell culture and treatments

The isolation and identification of porcine skeletal muscle satellite cells were performed as described in our previous study[13]. Porcine skeletal muscle satellite cells were grown in Dulbecco's modified Eagle's medium/F12 supplemented with 15 % fetal bovine serum and with 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in a 5 % CO_2 atmosphere. The isolated cells were induced to differentiate in Dulbecco's modified Eagle's medium/F12 medium supplemented with 2 % of horse serum (Hyclone), when cells were grown to about 80 % confluence. The differentiation medium was replaced with a fresh medium every 24 h. After 3 d of differentiation, the cells were treated with different concentrations (0, 50, 100 and 200 μg/ml) of Arg for 3 d. To explore the molecular mechanism, the cells were pretreated with

Table 3. Effect of arginine (Arg) on growth performance of piglets (n 15)
(Mean values with their standard errors)

	0 % Arg		0·5 % Arg		1·0 % Arg		1·5 % Arg	
Items	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial weight (kg)	6·55	0·03	6·56	0·05	6·54	0·03	6·57	0·04
Final weight (kg)	14·15	0·89	14·07	0·17	14·62	0·40	15·42	0·28
ADG (g)	271·66	31·31	268·10	4·99	288·57	13·99	316·19	10·61
ADFI (g)	456·50	41·60	443·64	15·57	495·33	26·50	517·17	6·47
F:G	1·70	0·06	1·65	0·04	1·72	0·06	1·64	0·04

ADG, average daily gain; ADFI, average daily feed intake; F:G, feed-gain ratio.

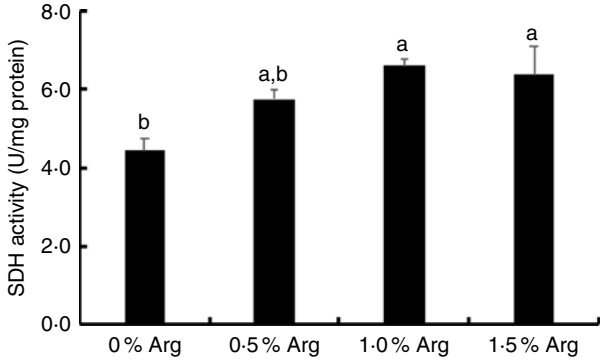

Fig. 1. Effect of arginine (Arg) on succinic dehydrogenase (SDH) activity in *longissimus dorsi* muscle of weaning piglets. Results are mean values with their standard errors from five piglets. [a,b]Values with unlike letters are significantly different ($P < 0·05$).

Rot (catalogue no. R8875, Sigma) (dissolved in dimethyl sulfoxide) for 1 h before being treated with Arg.

Statistical analysis

Data are expressed as mean values with their standard errors. Statistical analysis was performed using SPSS 22.0 statistical software. All data were analysed by one-way ANOVA followed by Tukey's test. A value $P < 0·05$ was considered to be statistically significant.

Results

Effect of arginine on growth performance of pigs

Compared with the control group, dietary Arg supplementation had no effect on growth performance of pigs, including final body weight, average daily gain, average daily feed intake and feed-gain ratio (Table 3).

Effect of arginine on succinic dehydrogenase activity of skeletal muscle of pigs

As shown in Fig. 1, 1·0 and 1·5 % Arg supplementation significantly increased ($P < 0·05$) the activity of SDH in the *longissimus dorsi* muscle and there was no significant difference between 0·5, 1·0 and 1·5 % Arg treatment groups.

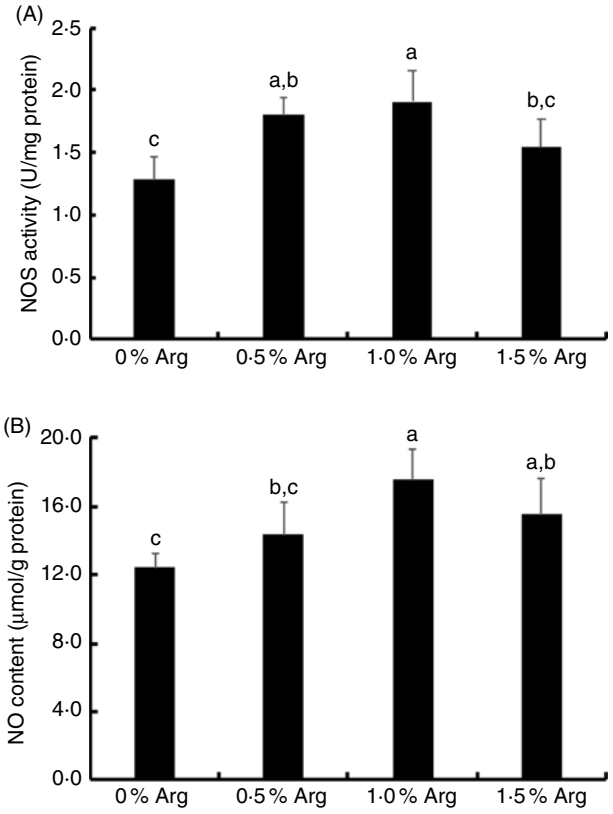

Fig. 2. Effect of arginine (Arg) on nitric oxide synthase (NOS) activity and nitric oxide (NO) content in *longissimus dorsi* muscle of weaning piglets. (A) NOS activity. (B) NO content. Results are mean values with their standard errors from five piglets. [a,b,c]Values with unlike letters are significantly different ($P < 0·05$).

Effect of arginine on nitric oxide synthase activity and nitric oxide content of skeletal muscle of pigs

The NOS activity was significantly increased ($P < 0·05$) in 0·5 and 1·0 % Arg treatment groups compared with the control group (Fig. 2(A)). As shown in Fig. 2(B), 1·0 and 1·5 % Arg treatment groups significantly increased ($P < 0·05$) the NO content compared with the control group.

Effect of arginine on muscle fibre type composition of skeletal muscle of pigs

As shown in Fig. 3, 1·0 % Arg supplementation significantly increased ($P < 0·05$) the *MyHC I*, troponin I1, C1 and T1

British Journal of Nutrition

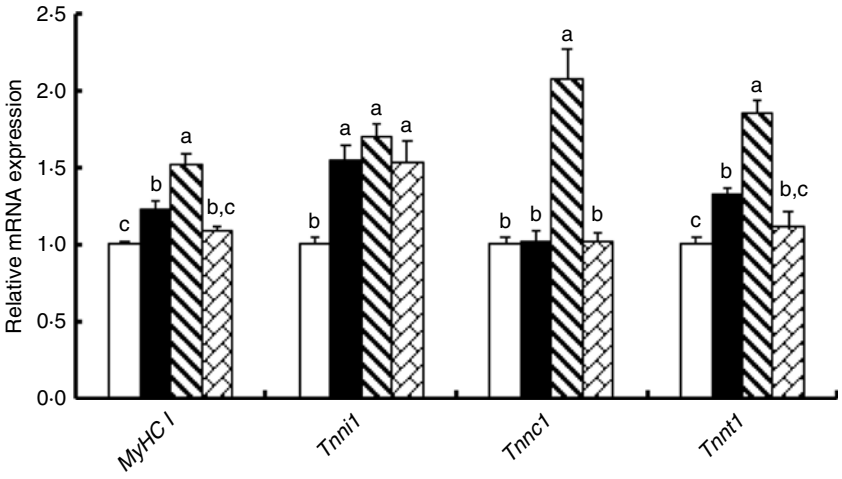

Fig. 3. Effect of arginine (Arg) on mRNA expressions of type I muscle fibres-related genes in *longissimus dorsi* muscle of weaning piglets. Results are mean values with their standard errors from five piglets. [a,b,c]Values with unlike letters are significantly different ($P < 0·05$). □, 0 % Arg; ■, 0·5 % Arg; ▧, 1·0 % Arg; and ▨, 1·5 % Arg. *MyHC I*, myosin heavy chain I; *Tnni1*, troponin I1; *Tnnc1*, troponin C1; *Tnnt1*, troponin T1.

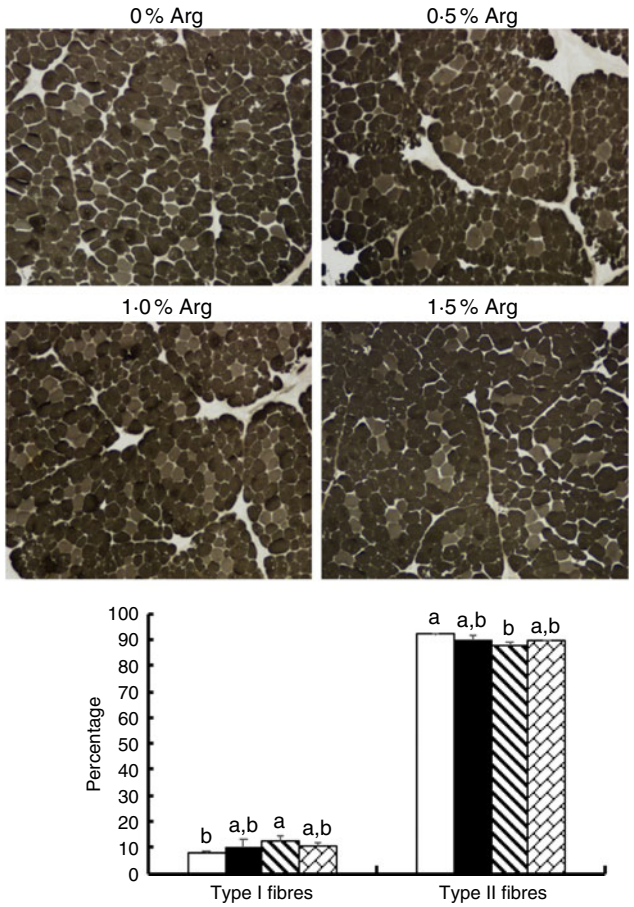

Fig. 4. Effect of arginine (Arg) on the proportion of type I and type II muscle fibres by ATPase staining analysis. Results are mean values with their standard errors from five piglets. [a,b]Values with unlike letters are significantly different ($P < 0·05$). □, 0 % Arg; ■, 0·5 % Arg; ▧, 1·0 % Arg; and ▨, 1·5 % Arg.

(*Tnni1*, *Tnnc1* and *Tnnt1*) mRNA levels. Furthermore, ATPase staining analysis showed that 1·0 % Arg supplementation significantly increased ($P < 0·05$) the number of type I muscle fibres and significantly decreased ($P < 0·05$) the number of type II muscle fibres compared with the control group (Fig. 4). Compared with the control group, dietary supplementation of 1·0 or 1·5 % Arg significantly increased ($P < 0·05$) the protein expression of MyHC I (Fig. 6).

Effects of arginine on mitochondrial biogenesis and function-related marker expression and mitochondrial DNA content of skeletal muscle of pigs

As shown in Fig. 5(A), 1·0 % Arg supplementation significantly increased ($P < 0·05$) the mRNA expressions of nuclear respiratory factor 1 (*NRF1*), mitochondria transcription factor B1 (*TFB1M*) and cytochrome c (*Cytc*) compared with the control group. Cytc protein expression (Fig. 6) and ATP synthase subunit C1 (*ATP5G*) mRNA expression (Fig. 5(A)) were also significantly increased ($P < 0·05$) in the 1·0 % Arg and 1·5 % Arg treatment groups. As shown in Fig. 5(B), mtDNA content of Arg-treated groups was significantly increased ($P < 0·05$) compared with the control group.

In addition, the *PGC-1α* mRNA level in *longissimus dorsi* muscle was significantly increased ($P < 0·05$) in 1·0 % Arg treatment group compared with the control group (Fig. 5(A)). As shown in Fig. 6, PGC-1α protein expression of Arg-treated groups was significantly increased ($P < 0·05$) compared with the control group. In addition, protein expressions of Sirt1 of 0·5 and 1·0 % Arg treatment groups was significantly increased ($P < 0·05$) compared with the control group (Fig. 6).

Rotenone inhibits mitochondrial biogenesis and function-related protein expression in porcine skeletal muscle satellite cells

We found that mitochondrial complex I inhibitor Rot significantly decreased the expression of mitochondrial biogenesis and function-related proteins PGC-1α and Cytc (Fig. 7). We also observed that 0·5 and 1·0 μM Rot could damage cell morphology

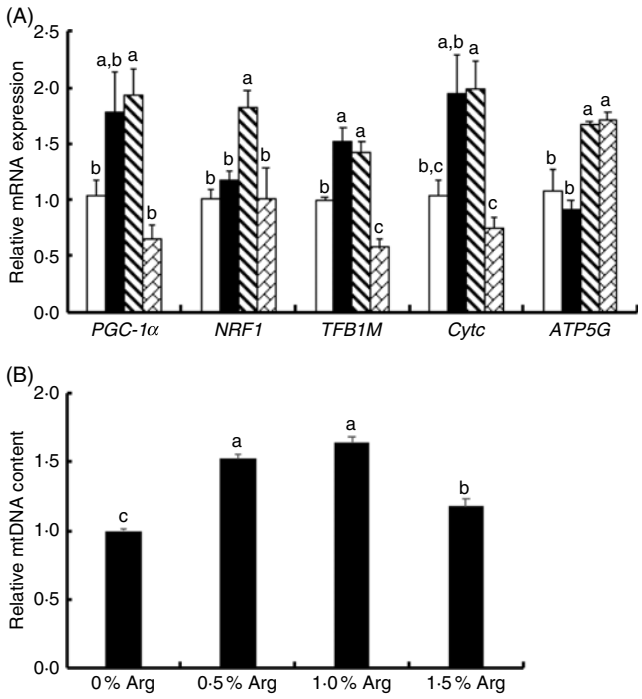

Fig. 5. Effect of arginine (Arg) on mRNA expressions of mitochondrial biogenesis and function-related genes and mitochondrial DNA (mtDNA) content in *longissimus dorsi* muscle of weaning piglets. (A) mRNA expressions of mitochondrial biogenesis and function-related genes. (B) mtDNA content. Results are mean values with their standard errors from five piglets. [a,b,c]Values with unlike letters are significantly different ($P < 0·05$). □, 0 % Arg; ■, 0·5 % Arg; ▧, 1·0 % Arg; and ▨, 1·5 % Arg. *PGC-1*α, PPAR-γ coactivator-1α; *NRF1*, nuclear respiratory factor 1; *TFB1M*, mitochondria transcription factor B1; *Cytc*, cytochrome c; *ATP5G*, ATP synthase subunit C1.

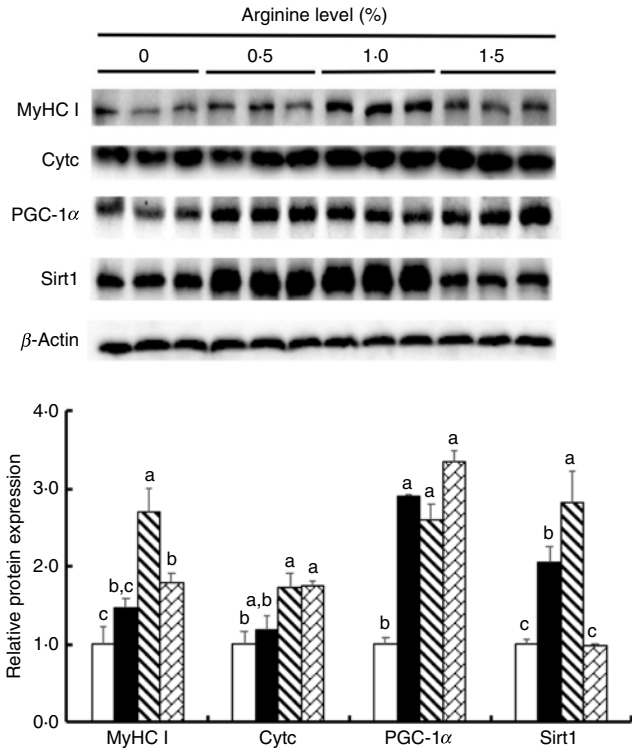

Fig. 6. Effect of arginine (Arg) on the protein expressions of myosin heavy chain I (MyHC I), cytochrome c (Cytc), PPAR-γ coactivator-1α (PGC-1α) and sirtuin 1 (Sirt1) in *longissimus dorsi* muscle of weaning piglets. Results are mean values with their standard errors from three piglets. [a,b,c]Values with unlike letters are significantly different ($P < 0·05$). □, 0 % Arg; ■, 0·5 % Arg; ▧, 1·0 % Arg; and ▨, 1·5 % Arg.

(data not shown). Therefore, Rot was supplemented with 0·1 μM in the following studies.

Arginine affects the formation of type I muscle fibres by improving mitochondrial biogenesis in porcine skeletal muscle satellite cells

To investigate the effect of Arg on mitochondrial biogenesis and function-related protein expression and type I muscle fibres formation, porcine skeletal muscle satellite cells were treated with different concentrations of Arg (0, 50, 100 and 200 μg/ml). Western blot result showed that the maximal up-regulation of MyHC I, Cytc, PGC-1α and Sirt1 protein expression was observed in 100 μg/ml Arg treatment group (Fig. 8). In the following studies, Arg was supplemented with 100 μg/ml.

To determine whether mitochondrial biogenesis contributes to Arg-induced type I muscle fibres formation, mitochondrial complex I inhibitor Rot was used. As shown in Fig. 9, Arg increased MyHC I protein level, whereas Rot annulled the positive effect of Arg on MyHC I protein expression.

Discussion

Arg is classified as a semi-essential or conditionally essential amino acid in mammals. Arg is the unique natural precursor in the biosynthesis of NO by NOS[19]. A large number of studies showed that NO or NOS plays an important role in regulating muscle fibre type composition[20–22]. In the present study, we found that dietary supplementation of 1·0 % Arg increased NO content, NOS activity, protein expression of MyHC I and mRNA expressions of type I muscle fibres-related genes (*MyHC I*, *Tnni1*, *Tnnc1* and *Tnnt1*) in *longissimus dorsi* muscle of weaning piglets. Some studies had indicated that type I and type II muscle fibres can be identified by myosin ATPase staining[23,24]. Our data showed that 1·0 % Arg supplementation significantly increased the number of type I muscle fibres and significantly decreased the number of type II muscle fibres by ATPase staining analysis. Previous reports showed that the type I muscle fibres had higher SDH enzyme activity[25]. In the present study, the SDH activity was significantly increased by Arg in *longissimus dorsi* muscle. Taken together, these findings showed that dietary supplementation of Arg promoted type I muscle fibres formation in weaning piglets.

Mitochondria are well-known organelles that provide energy for eukaryotic cells[26]. PGC-1α, a major regulator of mitochondrial biogenesis, is expressed in skeletal muscle[27]. Sirt1 modulates PGC-1α expression and activity[28] and plays an important role in regulating mitochondrial function[29,30]. PGC-1α and Sirt1 control mitochondrial biogenesis and function via the induction and activation of several downstream nuclear transcription factors, such as NRF1, mitochondria transcription factor B1

British Journal of Nutrition

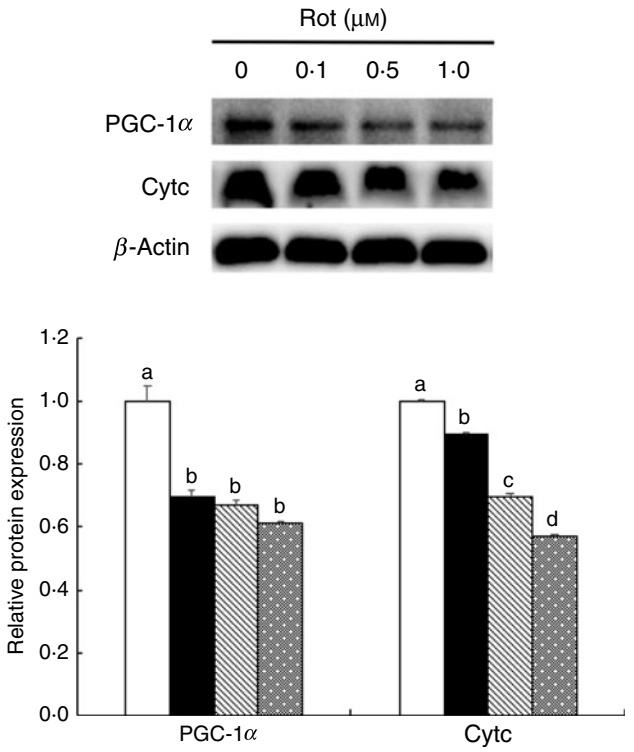

Fig. 7. Effect of rotenone (Rot) on PPAR-γ coactivator-1α (PGC-1α) and cytochrome c (Cytc) protein expression in porcine skeletal muscle satellite cells. About 80 % confluent porcine skeletal muscle satellite cells were cultured in the differentiation medium (Dulbecco's modified Eagle's medium/F12, 2 % horse serum) for 3 d and then treated with different concentrations of Rot (0, 0·1, 0·5 and 1·0 μM) for 24 h. PGC-1α and Cytc protein levels were determined by Western blot analysis. The amount of PGC-1α and Cytc was normalised to the amount of β-actin. The mean values with their standard errors of the densitometry results from three independent experiments are shown in the lower panel. [a,b,c,d]Values with unlike letters are significantly different ($P < 0·05$). □, 0 μM Rot; ■, 0·1 μM Rot; ▨, 0·5 μM Rot and ▩, 1·0 μM Rot.

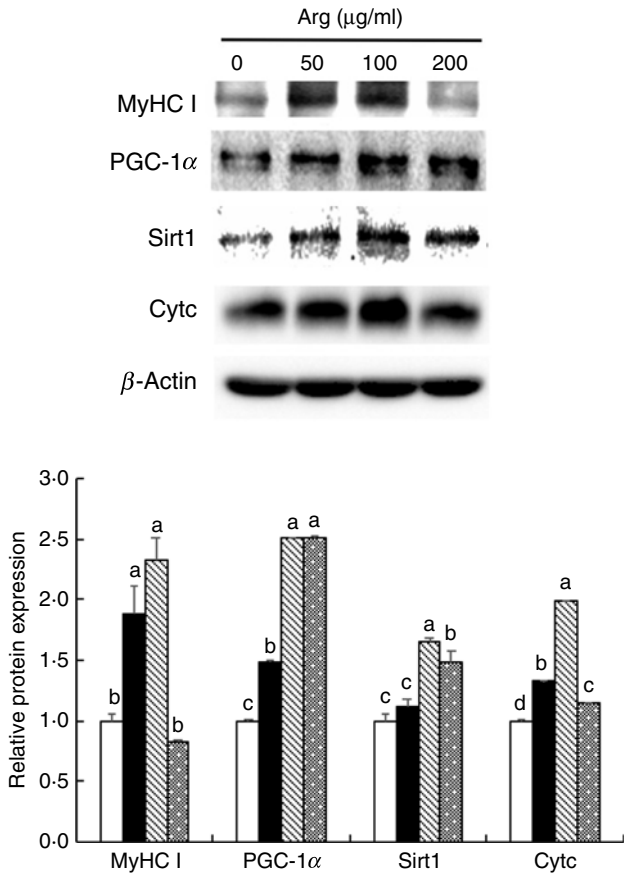

Fig. 8. Effect of arginine (Arg) on myosin heavy chain I (MyHC I), PPAR-γ coactivator-1α (PGC-1α), sirtuin 1 (Sirt1) and cytochrome c (Cytc) protein expression in porcine skeletal muscle satellite cells. About 80 % confluent porcine skeletal muscle satellite cells were cultured in the differentiation medium (Dulbecco's modified Eagle's medium/F12, 2 % horse serum) for 3 d and then treated with different concentrations of Arg (0, 50, 100 and 200 μg/ml) for 3 d. MyHC I, PGC-1α, Sirt1 and Cytc protein levels were determined by Western blot analysis. The amount of MyHC I, PGC-1α, Sirt1 and Cytc was normalised to the amount of β-actin. The mean values with their standard errors of the densitometry results from three independent experiments are shown in the lower panel. [a,b,c]Values with unlike letters are significantly different ($P < 0·05$). □, 0 μg/ml Arg; ■, 50 μg/ml Arg; ▨, 100 μg/ml Arg and ▩, 200 μg/ml Arg.

(TFB1M), ATP5G and cytochrome c (Cytc)[6,29,31]. Furthermore, there is some evidence that improvement of mitochondria biogenesis and function may play a key role in promotion of type I muscle fibres formation and may be a new mediator of skeletal muscle fibre type[4–6]. Type I (slow-twitch) muscle fibres are rich in the mitochondria[3,32]. It has been reported that some nutrients (such as leucine and resveratrol) can regulate type I (slow-twitch) muscle fibres expression by improving the mitochondrial biogenesis and function[29,30,33]. In the present study, our data found that dietary supplementation of 1·0 % Arg significantly increased the PGC-1α, Sirt1 and Cytc protein expressions, the *PGC-1α*, *NRF1*, *TFB1M*, *Cytc* and *ATP5G* mRNA expressions and the mtDNA content, suggesting that Arg plays an important role in regulating mitochondrial biogenesis and function.

It should be noted that there might be an optimal dietary supplementation of Arg in weaning piglets. In the present study, we showed that for many of the outcome measures, 1·5 % Arg was statistically lower than 1·0 % Arg, suggesting that the effectiveness of Arg might be optimal at 1·0 %. The reason might be that dietary supplementation with 1·0 % Arg could meet the needs of pigs, but excessive supplementation was disadvantageous. The exact reasons need to be further studied in the future.

Roteone (Rot) is a common pesticide and has been reported as a specific inhibitor of mitochondrial electron transport chain complex I, the first step in the electron transport chain[34]. There is evidence that the inhibition of mitochondrial electron transport chain can lead to mitochondrial dysfunction[33]. Rot has been proven to induce mitochondrial dysfunction[35]. Here, we observed that the cellular morphology changed gradually following the increase in Rot concentration, and no obvious damage effects were observed in porcine skeletal muscle satellite cells with 0·1 μM Rot treatment, which was consistent with the result of the previous study in PC12 cells[36]. Our data indicated that 0·1 μM Rot could effectively inhibit mitochondrial electron transport chain. Interestingly, inhibition of mitochondrial electron transport chain by Rot down-regulated the expression of PGC-1α (a major regulator of mitochondrial biogenesis) and annulled Arg-induced type I muscle fibres formation.

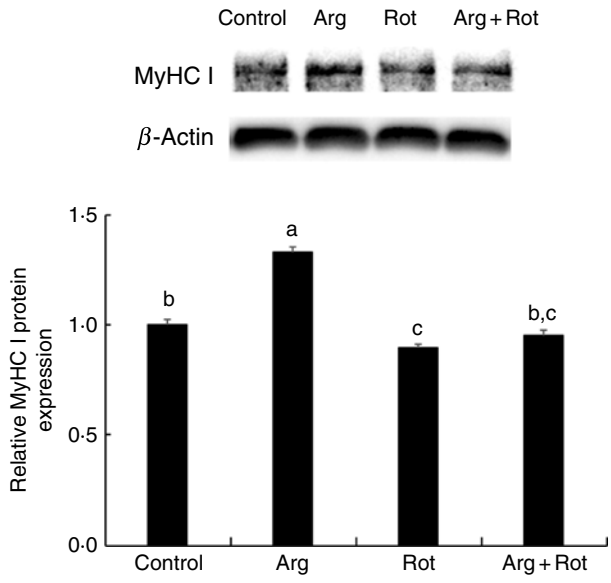

Fig. 9. Mitochondrial complex I inhibitor rotenone (Rot) annuls the effect of arginine (Arg) on protein expression of myosin heavy chain I (MyHC I) in porcine skeletal muscle satellite cells. About 80 % confluent porcine skeletal muscle satellite cells were cultured in the differentiation medium (Dulbecco's modified Eagle's medium/F12, 2 % horse serum) for 3 d and then pretreated with 0·1 μM Rot for 1 h followed by Arg (100 μg/ml) treatment for 3 d. MyHC I protein level was determined by Western blot analysis. The amount of MyHC I was normalised to the amount of β-actin. The mean values with their standard errors of the densitometry results from three independent experiments are shown in the lower panel. [a,b,c]Values with unlike letters are significantly different ($P < 0·05$).

In conclusion, we reported that Arg promotes the formation of porcine type I muscle fibres, which is dependent on the improvement of mitochondrial biogenesis. Our study not only brings new information on the nutritional function of Arg but also deeply understands the mechanism of Arg regulating the formation of type I muscle fibres.

Acknowledgements

This work was supported by the National Key R&D Program of China (no. 2018YFD0500403) and the Sichuan Youth Science and Technology Foundation (no. 2017JQ0008).

X. C. and Z. H. conceived the study and designed the experiments. X. L. carried out the experiments and analysed the data. X. C. wrote the manuscript. X. C., D. C., B. Y., J. H. and Z. H. contributed reagents/materials/analysis tools. Z. H. revised the manuscript. All authors read and approved the final manuscript.

The authors declare that there are no conflicts of interest.

References

1. Choi Y & Kim B (2009) Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. *Livest Sci* **122**, 105–118.
2. Zhang M, Liu Y, Fu C, *et al.* (2014) Expression of MyHC genes, composition of muscle fiber type and their association with intramuscular fat, tenderness in skeletal muscle of Simmental hybrids. *Mol Biol Rep* **41**, 833–840.
3. Schiaffino S & Reggiani C (2011) Fiber types in mammalian skeletal muscles. *Physiol Rev* **91**, 1447–1531.
4. Venhoff N, Lebrecht D, Pfeifer D, *et al.* (2012) Muscle-fiber transdifferentiation in an experimental model of respiratory chain myopathy. *Arthritis Res Ther* **14**, R233.
5. Lin J, Wu H, Tarr RT, *et al.* (2002) Transcriptional co-activator PGC-1α drives the formation of slow-twitch muscle fibres. *Nature* **418**, 797–801.
6. Zhang L, Zhou Y, Wu WJ, *et al.* (2017) Skeletal muscle-specific overexpression of PGC-1α induces fiber-type conversion through enhanced mitochondrial respiration and fatty acid oxidation in mice and pigs. *Int J Biol Sci* **13**, 1152–1162.
7. Jobgen WS, Fried SK, Fu WJ, *et al.* (2006) Regulatory role for the arginine-nitric oxide pathway in metabolism of energy substrates. *J Nutr Biochem* **17**, 571–588.
8. Mateo RD, Wu G, Bazer FW, *et al.* (2007) Dietary L-arginine supplementation enhances the reproductive performance of gilts. *J Nutr* **137**, 652–656.
9. Ma X, Lin Y, Jiang Z, *et al.* (2010) Dietary arginine supplementation enhances antioxidative capacity and improves meat quality of finishing pigs. *Amino Acids* **38**, 95–102.
10. Fouad AM, El-Senousey HK, Yang XJ, *et al.* (2013) Dietary L-arginine supplementation reduces abdominal fat content by modulating lipid metabolism in broiler chickens. *Animal* **7**, 1239–1245.
11. Zheng P, Yu B, He J, *et al.* (2013) Protective effects of dietary arginine supplementation against oxidative stress in weaned piglets. *Br J Nutr* **109**, 2253–2260.
12. Chen XL, Guo YF, Jia G, *et al.* (2018) Arginine promotes skeletal muscle fiber type transformation from fast-twitch to slow-twitch via Sirt1/AMPK pathway. *J Nutr Biochem* **61**, 155–162.
13. Chen XL, Guo YF, Jia G, *et al.* (2018) Arginine promotes slow myosin heavy chain expression via Akirin2 and the AMP-activated protein kinase signaling pathway in porcine skeletal muscle satellite cells. *J Agric Food Chem* **66**, 4734–4740.
14. Chen XL, Jia G, Zhao H, *et al.* (2019) Arginine induces skeletal muscle fiber type conversion by upregulating Akirin2 and AMPK/PGC-1α in mice. *Biologia* **74**, 709–715.
15. Mabalirajan U, Ahmad T, Leishangthem GD, *et al.* (2010) L-Arginine reduces mitochondrial dysfunction and airway injury in murine allergic airway inflammation. *Int Immunopharmacol* **10**, 1514–1519.
16. Kurhalyuk N & Tkachenko H (2007) L-Arginine modulates mitochondrial function in rat liver during physical training. *Bull Vet Inst Pulawy* **51**, 641–647.
17. Chang KC, da Costa N, Blackley R, *et al.* (2003) Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. *Meat Sci* **64**, 93–103.
18. Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta Ct}$ method. *Methods* **25**, 402–408.
19. Palmer RMJ, Ashton DS & Moncada S (1988) Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* **333**, 664–666.
20. Martins KJB, St-Louis M, Murdoch GK, *et al.* (2012) Nitric oxide synthase inhibition prevents activity-induced calcineurin–NFATc1 signalling and fast-to-slow skeletal muscle fibre type conversions. *J Physiol* **590**, 1427–1442.
21. Suwa M, Nakano H, Radak Z, *et al.* (2015) Effects of nitric oxide synthase inhibition on fiber-type composition, mitochondrial biogenesis, and SIRT1 expression in rat skeletal muscle. *J Sports Sci Med* **14**, 548–555.

22. Moon Y, Balke JE, Madorma D, *et al.* (2017) Nitric oxide regulates skeletal muscle fatigue, fiber type, microtubule organization, and mitochondrial ATP synthesis efficiency through cGMP-dependent mechanisms. *Antioxid Redox Signal* **26**, 966–985.
23. Zhang D, Wang X, Li Y, *et al.* (2014) Thyroid hormone regulates muscle fiber type conversion via miR-133a1. *J Cell Biol* **207**, 753–766.
24. Reyes NL, Banks GB, Tsang M, *et al.* (2015) Fnip1 regulates skeletal muscle fiber type specification, fatigue resistance, and susceptibility to muscular dystrophy. Proc Natl Acad Sci U S A 112, 424–429.
25. Tang K, Wagner PD & Breen EC (2010) TNF-alpha-mediated reduction in PGC-1alpha may impair skeletal muscle function after cigarette smoke exposure. *J Cell Physiol* **2010**, 222, 320–327.
26. Nunnari J & Suomalainen A (2012) Mitochondria: in sickness and in health. *Cell* **148**, 1145–1159.
27. Lehman JJ, Barger PM, Kovacs A, *et al.* (2000) Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J Clin Invest* **106**, 847–856.
28. Reznick RM & Shulman GI (2006) The role of AMP-activated protein kinase in mitochondrial biogenesis. *J Physiol* **574**, 33–39.
29. Price NL, Gomes AP, Ling AJ, *et al.* (2012) SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab* **15**, 675–690.
30. Chen XL, Xiang L, Jia G, *et al.* (2019) Leucine regulates slow-twitch muscle fibers expression and mitochondrial function by Sirt1/AMPK signaling in porcine skeletal muscle satellite cells. *Anim Sci J* **90**, 255–263.
31. Vazquez F, Lim JH, Chim H, *et al.* (2013) PGC1α expression defines a subset of human melanoma tumors with increased mitochondrial capacity and resistance to oxidative stress. *Cancer Cell* **23**, 287–301.
32. Zierath JR & Hawley JA (2004) Skeletal muscle fiber type: influence on contractile and metabolic properties. *PLoS Biol* **2**, e348.
33. Peng K, Tao Y, Zhang J, *et al.* (2016) Resveratrol regulates mitochondrial biogenesis and fission/fusion to attenuate rotenone-induced neurotoxicity. *Oxid Med Cell Longev* **2016**, 1–12.
34. Yuhki K, Miyauchi T, Kakinuma Y, *et al.* (2001) Endothelin-1 production is enhanced by rotenone, a mitochondrial complex I inhibitor, in cultured rat cardiomyocytes. *J Cardiovasc Pharm* **38**, 850–858.
35. Yee AG, Freestone PS, Bai JZ, *et al.* (2017) Paradoxical lower sensitivity of locus coeruleus than substantia nigra pars compacta neurons to acute actions of rotenone. *Exp Neurol* **287**, 34–43.
36. Wang H, Dong XG, Liu ZX, *et al.* (2018) Resveratrol suppresses rotenone-induced neurotoxicity through activation of Sirt1/Akt1 signaling pathway. *Anat Rec* **301**, 1115–1125.