Kinetics of changes in gene and microRNA expression related with muscle inflammation and protein degradation following LPS-challenge in weaned piglets

Ping Kang, Xingfa Huang, Zhicheng Wan, Tianzeng Liang, Yang Wang, Xiangen Li, Jing Zhang, Huiling Zhu and Yulan Liu (ORCID)

Abstract

To test the dynamic changes of the expression of genes and microRNA in the gastrocnemius muscle after LPS challenge, 36 piglets were assigned to a control group (slaughtered 0 h after saline injection) and LPS groups (slaughtered at 1 h, 2 h, 4 h, 8 h, and 12 h after LPS treatment, respectively). After LPS treatment, the mRNA expression of IL-1β, IL-6, and TNF-α reached maximal levels at 1 h, 2 h, and 1 h, respectively ($P < 0.05$), and mRNA expression of TLR4, NODs, muscle-specific ring finger 1, and muscle atrophy F-box peaked at 12 h ($P < 0.05$). Moreover, the expression of miR-122, miR-135a, and miR-370 reduced at 1 h, 1 h, and 2 h, respectively ($P < 0.05$), and miR-34a, miR-224, miR-132, and miR-145 reached maximum expression levels at 1 h, 1 h, 2 h, and 4 h, respectively ($P < 0.05$). These results suggested that mRNA expression of pro-inflammatory cytokines was elevated in the early stage, mRNA expression of genes related to TLR4 and NODs signaling pathways and protein degradation increased in the later phase, and the expression of microRNA related to muscle inflammation and protein degradation changed in the early stage after LPS injection.

Keywords

MicroRNAs, muscle, inflammation, protein degradation, piglet, lipopolysaccharide

Date received: 29 July 2020; revised: 10 September 2020; accepted: 14 October 2020

Introduction

Skeletal muscle is not only the most widely distributed tissue in the body, it is an important site for regulating the metabolism of the whole body. However, inflammation can cause muscle wasting, which is characterized by accelerated protein degradation.[1] Many systemic diseases such as cancer are accompanied by muscle atrophy[2,3] and might result from the inflammation.[4]

TLRs play an essential role in the innate immune system and TLR4 is the central component of the sole mammalian LPS sensor.[5] LPS can stimulate TLR4 to initiate MyD88, followed by activation of IL-1 receptor associated kinase (IRAK) 1, which finally leads to NF-κB activation. Similarly, NLR 1 and 2 play an important role in the development of the innate

immune response. Receptor-interacting protein kinase (RIPK) 2 is an essential downstream adaptor protein in the NOD signaling pathway, which can result in an NF-κB-mediated pro-inflammatory response.[6] NF-κB can induce the transcription of cytokines, including IL-1β, IL-6, and TNF-α. These pro-inflammatory cytokines can directly induce muscle protein degradation,

Hubei Key Laboratory of Animal Nutrition and Feed Science, Hubei Collaborative Innovation Center for Animal Nutrition and Feed Safety, Wuhan Polytechnic University, China

Corresponding author:
Yulan Liu, Wuhan Polytechnic University, Xuefu South Road 68, Changqing Garden, Dongxihu District, Wuhan, Hubei 430023, China.
Email: yulanflower@126.com

or lead to muscle atrophy by up-regulating the mRNA expression of forkhead box O1 (FOXO1) and two muscle-specific E3 ligases, muscle atrophy F-box (MAFbx) and muscle-specific ring finger (MuRF) 1,[7,8] which is the major pathway of protein degradation in skeletal muscle.[1]

MicroRNAs (miRNAs) are a recently discovered class of small non-coding RNAs, which play a distinct role in inflammation. Many mammalian miRNAs can regulate immune and inflammatory responses.[9,10] In addition, miRNAs can be a new therapeutic frontier for muscle wasting in chronic kidney disease.[11]

We have found that the levels of pro-inflammatory cytokines in plasma and mRNA expression of genes related to inflammation and protein degradation in muscles increased in weaned pigs 4 h after LPS challenge.[12,13] Previous studies have reported that innate immune cells could be in diverse states with different degrees of pro- and anti-inflammatory phenotypes,[14,15] accompanied by varying consequences for host defense and inflammation.[16] Although LPS has been used for decades for the induction of some pro-inflammatory cytokines, the response and kinetics of inflammation varied extensively depending on the LPS timing. Therefore, this study was conducted to test the expression of selected pro-inflammatory mediators and miRNAs in TLR4, NODs, and FOXO/MAFbx-MuRF1 signaling pathways in muscle at different time points after LPS challenge in weaning piglets. Our study might reveal a dynamic change mechanism for the inflammation and protein degradation in the muscle of weaning piglets challenged by LPS.

Materials and methods

Experimental design

The Animal Care and Use Committee of Wuhan Polytechnic University approved the animal use protocol for this research. A total of 36 crossbred castrated barrows (Duroc × Large White × Landrace, 7.02 ± 0.21 kg, 28 ± 3 d) were selected and assigned randomly to six groups ($n = 6$) including control (saline injection) and LPS (Escherichia coli serotype 055: B5; Sigma Chemical, St. Louis, MO) groups in which the piglets were slaughtered at five different times after LPS challenge. Each group had six replicated pens, and each pen had one pig.[13,17] Diets were formulated to meet or exceed National Research Council (NRC) requirements for all nutrients.[18] All piglets were kept under a 12 h light/dark cycle, allowed ad libitum access to water and feed, and placed individually in pens (1.80×1.10 m) during a 14-d adaption period. The ambient temperature was maintained at 22–25°C and the living environment was in accordance with animal

welfare guidelines. Signs of diarrhea, sickness, and abnormal behavior were not observed throughout the adaption period.

Muscle sample collection

On d 15, the piglets were injected intraperitoneally with either E. coli LPS at 100 μg/kg body mass (BM) or the equivalent amount of sterile saline (0.9%) according to our previous study.[13] Then the piglets in the LPS groups were slaughtered under anesthesia with an intramuscular injection of sodium pentobarbital (80 mg/kg BM) at 1 h, 2 h, 4 h, 8 h, and 12 h after LPS injection. The piglets in the control group were slaughtered in the same way at 0 h after saline injection. Then the gastrocnemius muscle was removed under the semitendinosus and biceps flexor cruris (left hind leg), frozen in liquid nitrogen immediately, then stored at −80°C for further analysis. Previous studies found that piglets with muscle fiber atrophy had high levels of MAFbx in the gastrocnemius muscle, therefore this muscle was selected to study muscle atrophy.[19,20]

Muscle mRNA expression analysis

The gene expression was conducted by real-time PCR according to the previous study.[17] Briefly, total RNA was isolated using Trizol reagent (#108, TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China), and cDNA was synthesized by a PrimeScript® RT reagent kit (#RR047A, TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) according to the manufacturer's instructions. Quantitative results of the mRNA expression for the target genes were carried out using a SYBR® Premix Ex TaqTM (Tli RNaseH Plus) quantitative PCR kit (#RR420A, TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China). The PCR cycling conditions were 95°C × 30 s, followed by 40 cycles of 95°C × 5 s and 60°C × 34 s. The primer sequences are shown in Table 1, and the $2^{-\triangle\triangle CT}$ method was used to calculate the mRNA expression of the target genes relative to GAPDH.[21]

Muscle miRNA expression analysis

Total RNA was isolated by the Trizol reagent (#9108, TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) according to the manufacturer's protocol. Mature miRNAs were detected and quantified using primers obtained from the mirVana[TM] quantitative RT-PCR Primer Set (Gene Pharma, Shanghai, China) and SYBR Green PCR Mix (Takara, Japan). The miRNA expression was normalized to the level of U6 and analyzed by the $2^{-\triangle\triangle CT}$ method.[21]

Table 1. Primer sequences used for real-time PCR.

Gene	Forward (5′-3′)	Reverse (5′-3′)	Product length (bp)	Accession numbers
IL-1β	GCTAACTACGGTGACAACAATAATG	CTTCTCCACTGCCACGATGA	186	NM_214055.1
IL-6	AAGGTGATGCCACCTCAGAC	TCTGCCAGTACCTCCTTGCT	151	JQ839263.1
TNF-α	TCCAATGGCAGAGTGGGTATG	AGCTGGTTGTCTTTCAGCTTCAC	67	NM_214022.1
TLR4	TCAGTTCTCACCTTCCTCCTG	GTTCATTCCTCACCCAGTCTTC	166	GQ503242.1
MyD88	GATGGTAGCGGTTGTCTCTGAT	GATGCTGGGGAACTCTTTCTTC	148	AB292176.1
IRAK1	CAAGGCAGGTCAGGTTTCGT	TTCGTGGGGCGTGTAGTGT	115	XM_003135490.1
NOD1	CTGTCGTCAACACCGATCCA	CCAGTTGGTGACGCAGCTT	57	AB187219.1
NOD2	GAGCGCATCCTCTTAACTTTCG	ACGCTCGTGATCCGTGAAC	66	AB195466.1
RIPK2	CAGTGTCCAGTAAATCGCAGTTG	CAGGCTTCCGTCATCTGGTT	206	XM_003355027.1
NF-κB	AGTACCCTGAGGCTATAACTCGC	TCCGCAATGGAGGAGAAGTC	133	EU399817.1
FOXO1	TTCACCAGGCACCATCAT	GAGGAGAGTCGGAAGTAAGT	236	NM_214014.2
FOXO4	TGGAGTGTGACATGGATAAC	CTCATCTCTGAAGCAAGGAA	122	XM_003135172.3
MAFbx	TCACAGCTCACATCCCTGAG	GACTTGCCGACTCTCTGGAC	167	NM_001044588.1
MuRF1	ATGGAGAACCTGGAGAAGCA	ACGGTCCATGATCACCTCAT	219	FJ905227.1
GAPDH	CGTCCCTGAGACACGATGGT	GCCTTGACTGTGCCGTGGAAT	194	AF017079.1

RIPK2, receptor-interacting serine/threonine-protein kinase 2; FOXO1: forkhead box O 1; FOXO4: forkhead box O 4; MAFbx: muscle atrophy F-box; MuRF1: muscle-specific ring finger 1.

Statistical analysis

The experimental data were analyzed by ANOVA with SPSS 17.0 software. Differences among treatments were determined by Duncan's multiple range tests. All data were expressed as means ± SE. The statistical significance level for all analyses was set at $P < 0.05$. A one-way multivariate ANOVA (MANOVA) was used to determine the effect size (partial eta squared) and the statistical power of the model. As for the effect of LPS challenge on the TLR4 and NOD signaling pathways, a one-way MANOVA revealed a significant multivariate main effect for region, partial eta squared was 0.884, and power to detect the effect was 1.000. For the effect of LPS challenge on the FOXO/MAFbx-MuRF1 signaling pathway, one-way MANOVA revealed a significant multivariate main effect for region, partial eta squared was 0.628, and power to detect the effect was 1.000. For the effect of LPS challenge on the miRNA expression, a one-way MANOVA revealed a significant multivariate main effect for region, partial eta squared was 0.766, and power to detect the effect was 1.000.

Results

The mRNA expression of pro-inflammatory cytokines in muscle

As shown in Figure 1, IL-1β mRNA expression reached the maximal level at 1 h ($P < 0.05$) and declined to the basal level at 2 h after LPS injection. Maximum IL-6 mRNA expression was observed at 2 h ($P < 0.05$) and declined to the basal level at 4 h after LPS challenge. TNF-α mRNA expression increased and reached the maximal level 1 h after LPS injection ($P < 0.05$).

The mRNA expression of key genes in TLR4- and NOD-signaling pathways in muscle

As shown in Figure 2, LPS treatment elevated TLR4 mRNA expression at 4 h after LPS injection and peaked at 12 h after LPS treatment ($P < 0.05$). MyD88 mRNA expression increased at 2 h after LPS injection and reached the maximum level at 12 h after LPS treatment ($P < 0.05$). mRNA level of IRAK1 increased 1 h after LPS challenge and peaked at 8–12 h after LPS treatment ($P < 0.05$). In addition, NF-κB mRNA was elevated at 1 h ($P < 0.05$) and reached the maximum level at 8 h after LPS treatment ($P < 0.05$). Moreover, NOD1 mRNA expression started to increase at 8 h after LPS challenge ($P < 0.05$), and NOD2 mRNA expression started to increase at 1 h after LPS challenge ($P < 0.05$). The maximum mRNA expression levels of both NOD1 and NOD2 were detected at 12 h after LPS injection ($P < 0.05$). RIPK2 mRNA expression reached the maximum at 1 h ($P < 0.05$) and dropped to the basal level at 8 h after LPS challenge.

The mRNA expression of key genes in FOXO/MAFbx-MuRF1 signaling pathway in muscle

As shown in Figure 3, maximum FOXO1 and FOXO4 mRNA expression was detected at 8 h after LPS challenge ($P < 0.05$). MuRF1 and MAFbx mRNA expression started to increase at 4 h, and reached the maximum levels at 12 h, respectively ($P < 0.05$).

The expression of miRNAs in muscle

As shown in Table 2, compared with the control group, the expression of miR-135a and miR-370 reduced at 2 h
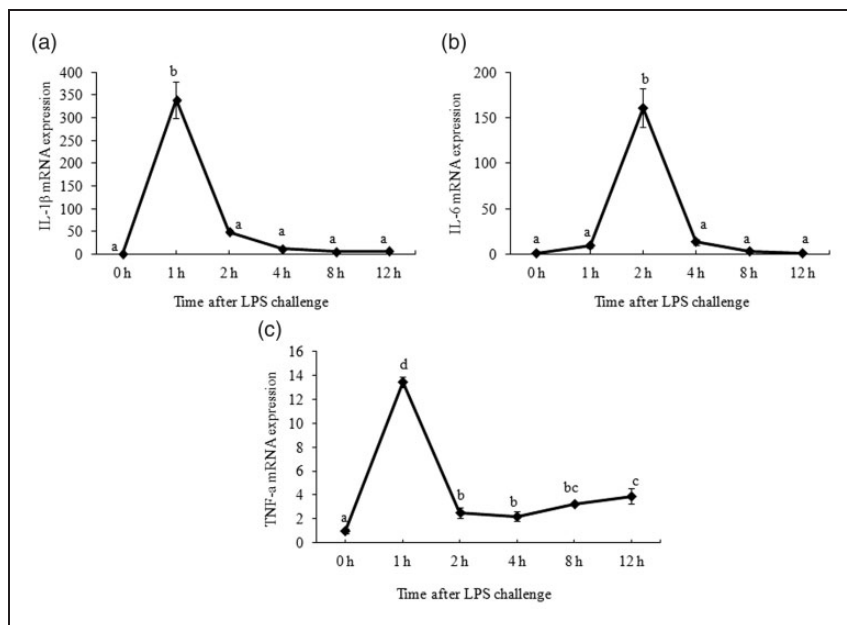
Figure 1. Effect of LPS on mRNA abundance of pro-inflammatory cytokines in muscle. Piglets were injected with 100 g/kg body mass (BM) or the equivalent amount of sterile saline. The piglets in the control group were slaughtered 0 h after saline injection. The piglets in LPS treatments were slaughtered at 1 h, 2 h, 4 h, 8 h, and 12 h after LPS injection. Values are single-point determinations and represent each of the time points listed above.

($P < 0.05$) and miR-122 expression decreased at 1 h ($P < 0.05$). However, after LPS challenge, the expression of miR-34a and miR-145 reached maximum expression levels at 1 h and 4 h, respectively ($P < 0.05$). In addition, miR-224 maximum expression level was observed at 1 h ($P < 0.05$) and reduced at 4 h after LPS challenge ($P < 0.05$). MiR-132 expression increased from 1 h ($P < 0.05$), reached the maximum level at 2 h ($P < 0.05$), and dropped to the basal level at 4 h after LPS challenge. Moreover, miR-130a expression decreased at 4 h after LPS challenge ($P < 0.05$), and miR-223 expression increased at 1 h ($P < 0.05$). Compared with the control, miR-146a had no significant change after LPS challenge ($P > 0.05$); however, its expression reduced significantly at 8 h compared with its expression at 2 h ($P < 0.05$). No significant difference was found in miR-127, miR-30b, miR-155, and miR-199a-3p expression after LPS treatment ($P > 0.05$).

Discussion

The innate immune response is not only important for activating acquired immunity, but is the main contributor to acute inflammation induced by LPS. The inflammatory response mediated by pro-inflammatory cytokines can protect the body by removing detrimental stimuli and repairing damaged tissue.[22] However, over-production of pro-inflammatory cytokines may cause muscle atrophy evidenced by muscle protein

degradation.[1] In addition, a lot of mammalian miRNA can regulate immune function[9] and inflammatory response.[10] Previous studies reported that innate immune cells could be in diverse states with different degrees of pro- and anti-inflammatory phenotypes accompanied with varying consequences for host defense and inflammation.[14–16] Therefore, understanding the response and kinetics of changes in the expression of genes and miRNA related with inflammation and protein degradation following LPS challenge would be very important for further studies on alleviating inflammation and muscle protein loss.

As critical components of the innate immune response, TLR4 and NODs can initiate NF-κB activation through MyD88/IRAK1 signaling and RIPK2, respectively. Activation of NF-κB then triggers the expression of pro-inflammatory cytokines. In this study, we found that IL-1β, IL-6, and TNF-α mRNA expression reached maximum levels at an early stage after LPS injection; however, TLR4, MyD88, IRAK1, NOD1, and NOD2 mRNA expression reached peak levels at the later stage after LPS challenge. Basically, NF-κB mRNA expression increased at 1 h after LPS treatment, therefore these results indicated the elevated mRNA expression of pro-inflammatory cytokines could result from the increased NF-κB mRNA expression, and NF-κB could be activated through the MyD88-independent pathway at an early stage after LPS injection, which was in line with the report of
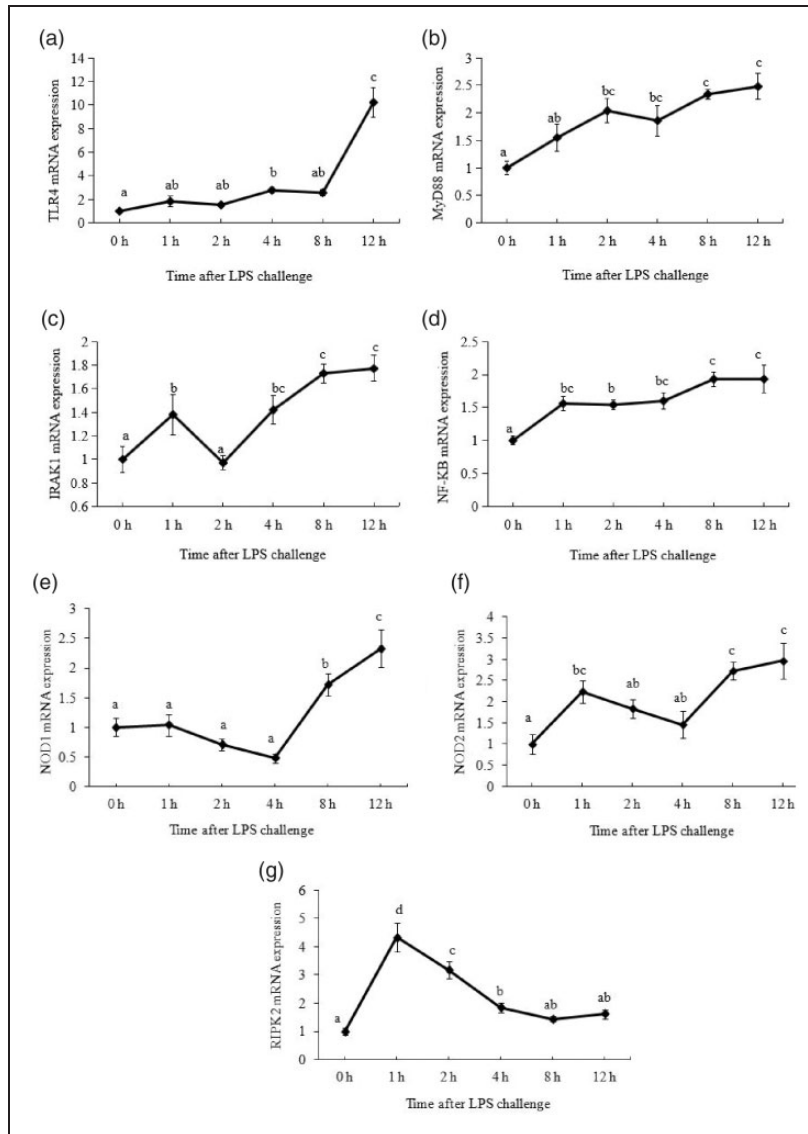
Figure 2. Effect of LPS on mRNA abundance of key genes in TLR4 and NOD signaling pathway in muscle. Piglets were injected with 100 g/kg body mass (BM) or the equivalent amount of sterile saline. The piglets in the control group were slaughtered 0 h after saline injection. The piglets in LPS treatments were slaughtered at 1 h, 2 h, 4 h, 8 h, and 12 h after LPS injection. Values are single-point determinations and represent each of the time points listed above.

Lu et al.[23] In addition, previous studies reported that TNF-α could directly activate NF-κB,[24,25] therefore increased NF-κB mRNA expression at an early stage after LPS treatment might partially result from elevated TNF-α mRNA expression, which was directly induced by LPS challenge.

LPS challenge can induce muscle atrophy, which is characterized by the elevated mRNA expression of FOXO1, MAFbx, and MuRF1.[7,8] Crossland et al. reported that pro-inflammatory cytokines could directly induce muscle protein degradation, or lead to muscle atrophy through activating these genes.[1] However, in our study, we found that FOXO1, FOXO4, MAFbx, and MuRF1 mRNA expression were elevated relatively

late compared with IL-1β, IL-6, and TNF-α mRNA expression. The results suggested these increased pro-inflammatory cytokines at an early stage could result in the muscle wasting at a later stage, indicating the muscle-wasting response could happen later than the inflammatory response after LPS treatment.

Increasing evidence has indicated miRNA could stimulate or inhibit an inflammatory response. According to previous studies, a lack of miR-145 can induce pro-inflammatory signals in the innate immune system.[26–28] Pekow et al. supposed that a loss of miR-145 pre-disposes patients to chronic inflammation in inflammatory bowel disease.[29] In line with these findings, we found that miR-145 expression reached the
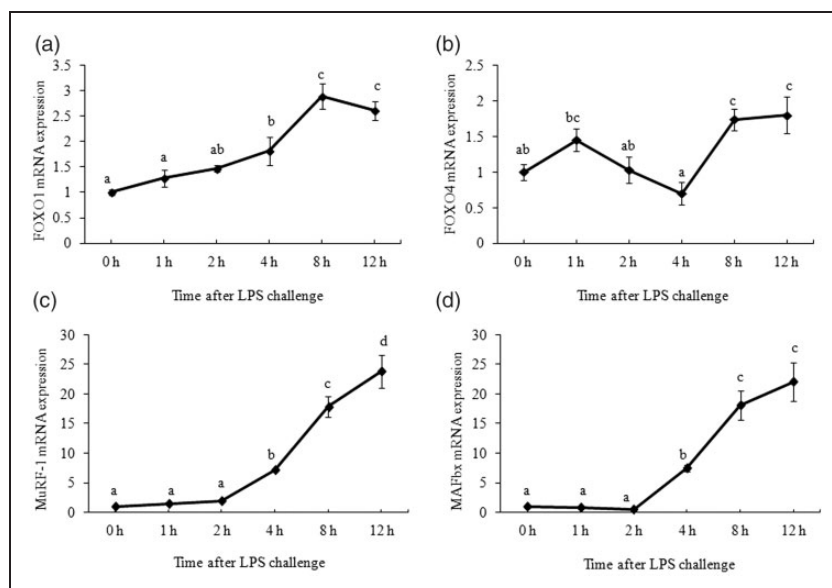
Figure 3. Effect of LPS on mRNA abundance of key genes in forkhead box O1 (FOXO)/ muscle atrophy F-box (MAFbx)-muscle-specific ring finger 1 (MuRF1) signaling pathway in muscle. Piglets were injected with 100 g/kg body mass (BM) or the equivalent amount of sterile saline. The piglets in the control group were slaughtered 0 h after saline injection. The piglets in LPS treatments were slaughtered at 1 h, 2 h, 4 h, 8 h, and 12 h after LPS injection. Values are single-point determinations and represent each of the time points listed above.

Table 2. Effect of LPS treatment on miRNAs abundance in muscle.[e]

Item	Post-LPS					
	0 h	1 h	2 h	4 h	8 h	12 h
miR-135a	1.00 ± 0.32^b	0.89 ± 0.43^b	0.55 ± 0.14^a	0.50 ± 0.19^a	0.39 ± 0.12^a	0.46 ± 0.19^a
miR-370	1.00 ± 0.39^c	0.82 ± 0.33^{bc}	0.65 ± 0.08^{ab}	0.75 ± 0.19^{bc}	0.43 ± 0.16^a	0.37 ± 0.14^a
miR-122	1.00 ± 0.45^c	0.38 ± 0.11^a	0.39 ± 0.10^a	0.57 ± 0.24^{ab}	0.35 ± 0.04^a	0.71 ± 0.22^{bc}
miR-34a	1.00 ± 0.25^a	1.67 ± 0.31^c	1.25 ± 0.28^{ab}	1.06 ± 0.23^a	1.59 ± 0.58^{bc}	0.94 ± 0.22^a
miR-145	1.00 ± 0.36^{ab}	1.06 ± 0.50^{ab}	1.52 ± 0.57^{bc}	1.66 ± 0.44^c	1.08 ± 0.35^{ab}	0.88 ± 0.24^a
miR-127	1.00 ± 0.46	1.15 ± 0.49	1.55 ± 0.52	1.30 ± 0.21	1.14 ± 0.39	1.03 ± 0.50
miR-30b	1.00 ± 0.15	1.02 ± 0.60	0.90 ± 0.30	0.65 ± 0.10	0.97 ± 0.21	0.65 ± 0.30
miR-155	1.00 ± 0.20	0.78 ± 0.14	1.32 ± 0.58	0.70 ± 0.10	0.69 ± 0.20	1.22 ± 0.97
miR-199a-3p	1.00 ± 0.28	0.98 ± 0.14	0.86 ± 0.24	0.73 ± 0.38	1.01 ± 0.21	0.93 ± 0.17
miR-224	1.00 ± 0.22^c	1.63 ± 0.42^d	0.88 ± 0.38^{bc}	0.58 ± 0.26^{ab}	0.46 ± 0.10^a	0.63 ± 0.21^{ab}
miR-132	1.00 ± 0.18^a	1.60 ± 0.48^b	2.29 ± 0.23^c	1.22 ± 0.27^{ab}	1.18 ± 0.27^a	1.23 ± 0.49^{ab}
miR-130a	1.00 ± 0.23^b	1.16 ± 0.11^b	1.10 ± 0.31^b	0.77 ± 0.17^a	0.56 ± 0.13^a	0.60 ± 0.11^a
miR-223	1.00 ± 0.32^a	1.58 ± 0.47^b	1.70 ± 0.61^b	1.71 ± 0.63^b	1.44 ± 0.25^{ab}	1.66 ± 0.39^b
miR-146a	1.00 ± 0.27^{abc}	1.31 ± 0.72^{bc}	1.41 ± 0.23^c	1.19 ± 0.34^{bc}	0.87 ± 0.35^{ab}	0.65 ± 0.12^a

[abcd]Labeled means in a row without a common letter differ, $P < 0.05$.
[e]Values are mean $\pm$ SE, $n = 6$ (one pig per pen).
miRNA: micro RNA.

maximum level in the present study; however, the mRNA expression of IL-1β and TNF-α dropped to the basal level from the maximum 2 h after LPS challenge, indicating miR-145 had an anti-inflammatory response in a later stage of inflammation. In addition, miR-370 can reduce the inflammatory reaction by targeting TLR4.[30] In line with this study, in our experiment the minimum mRNA expression of miR-370 was observed at 12 h after LPS injection; on the contrary, the maximum mRNA expression of TLR4 and its downstream transcription factors MyD88 and IRAK1 was reached 12 h after LPS treatment.

MiR-127 can be significantly induced in an inflammation-related lung injury.[31,32] Increased expression of miR-127 in macrophages augments pro-inflammatory cytokine production.[33] Similarly, miR-

155 is a major regulator of TLR signaling, and plays an important role in regulating immune cell development, function, and disease.[34,35] Tili et al. found a high expression of miR-155 could produce more TNF-α in mice challenged with LPS.[36] However, in this study, LPS injection had no effect on the expression of miR-155 and miR-127. The differences might result from the various LPS doses, tissues, animals, and sample collection times after LPS challenge. This could also explain our miR-30b expression results. In this study, we found LPS challenge had no effect on miR-30b expression over the experimental time points; however, Fordham et al. reported that enhanced miR-30b expression could inhibit the release of pro-inflammatory cytokines such as TNF-α and IL-6 induced by LPS.[37] miR-224 is a transcriptional target of NF-κB, and its up-regulation can activate the LPS- and NF-κB-dependent TNF-α inflammatory pathway.[38] In agreement with these results, in the present study we also found the maximum expression of miR-224 was reached at 1 h, as well as the mRNA expression of IL-1β and TNF-α after LPS injection.

In addition, we found the expression of miR-132, IL-1β, and NF-κB increased 1 h after LPS injection and the expression levels of miR-132 and IL-6 reached the maximum at 2 h. These results are inconsistent with previous studies, which reported that miR-132 played an anti-inflammatory role by suppressing IL-1β, IL-6, and NF-κB.[39,40] Li et al. thought there might be a highly negative correlation between miR-130a and TNF-α;[41] however, we did not find this relationship between them in our study. Moreover, Wang et al. reported that miR-223 inhibited inflammation by suppressing TLR4 signaling in macrophages.[42] However, in this study, we did not find this inhibitory response in muscle for miR-223 after LPS challenge.

Apart from stimulating or inhibiting inflammatory response, miRNA could be a new therapeutic frontier for muscle wasting in chronic kidney disease.[11] In this study, the mRNA expression of FOXO1, MuRF1, and MAFbx had a negative relationship with the expression of miR-34a, miR-224, miR-132, and miR-130a. These results indicated that these miRNAs might play an important role in inhibiting protein degradation by reducing the mRNA expression of FOXO1, MuRF1, and MAFbx in the late phase after LPS treatment. On the contrary, the expression of miR-145 was elevated 4 h after LPS injection, which was in line with the mRNA expression of FOXO1, MuRF1, and MAFbx, indicating that miR-145 could stimulate protein degradation after LPS injection.

In conclusion, the mRNA expression of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α elevated in the early stages, mRNA expression of genes related to the TLR4 and NOD signaling pathway and protein degradation increased in the later phase after LPS injection, and miRNA related to muscle inflammation and protein degradation changed in the early stage after LPS treatment.

ORCID iD

Yulan Liu https://orcid.org/0000-0001-9617-9305

References

1. Crossland H, Constantin-Teodosiu D, Gardiner SM, et al. A potential role for Akt/FOXO signaling in both protein loss and the impairment of muscle carbohydrate oxidation during sepsis in rodent skeletal muscle. *J Physiol* 2008; 586: 5589–5600.
2. Gomes MD, Lecker SH, Jagoe RT, et al. Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy. *P Natl Acad Sci USA* 2001; 98: 14,440–14,445.
3. Kandarian SC and Jackman RW. Intracellular signaling during skeletal muscle atrophy. *Muscle Nerve* 2006; 33: 155–165.
4. Grivennikov SI, Greten FR and Karin M. Immunity, inflammation, and cancer. *Cell* 2010; 140: 883–899.
5. Beutler B. TLR4: Central component of the sole mammalian LPS sensor. *Curr Opin Immunol* 2000; 12: 20–26.
6. Park JH, Kim YG, McDonald C, et al. RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. *J Immunol* 2007; 178: 2380–2386.
7. Cheung WW, Rosengren S, Boyle DL, et al. Modulation of melanocortin signaling ameliorates uremic cachexia. *Kidney Int* 2008; 74: 180–186.
8. Workeneh BT and Mitch WE. Review of muscle wasting associated with chronic kidney disease. *Am J Clin Nutr* 2010; 91: 1128S–1132S.
9. Qiu C, Ma J, Wang ML, et al. MicroRNA-155 deficiency in CD8+ T cells inhibits its anti-glioma immunity by regulating FOXO3a. *Eur Rev Med Pharmaco* 2019; 23: 2486–2496.
10. O'Connell RM, Rao DS and Baltimore D. microRNA regulation of inflammatory responses. *Annu Rev Immunol* 2012; 30: 295–312.

11. Mak RH, Cheung WW. MicroRNAs: A new therapeutic frontier for muscle wasting in chronic kidney disease. *Kidney Int* 2012; 82: 373–374.

12. Kang P, Wang Y, Li XG, et al. Effect of flaxseed oil on muscle protein loss and carbohydrate oxidation impairment in a pig model after lipopolysaccharide challenge. *Brit Nutr* 2020; 123: 859–869.

13. Liu Y, Chen F, Odle J, et al. Fish oil increases muscle protein mass and modulates Akt/FOXO, TLR4, and NOD signaling in weaning piglets after lipopolysaccharide challenge. *J Nutr* 2013; 143: 1331–1339.

14. Hanke ML, Heim CE, Angle A, et al. Targeting macrophage activation for the prevention and treatment of Staphylococcus aureus Biofilm infections. *J Immunol* 2013; 190: 2159–2168.

15. Bunt SK, Clements VK, Hanson EM, et al. Inflammation enhances myeloid-derived suppressor cell cross-talk by signaling through Toll-like receptor 4. *J Leukocyte Biol* 2009; 85: 996–1004.

16. Marrotte EJ, Chen DD, Hakim JS, et al. Manganese superoxide dismutase expression in endothelial progenitor cells accelerates wound healing in diabetic mice. *J Clin Invest* 2010; 120: 4207–4219.

17. Zhu H, Wang H, Wang S, et al. Flaxseed oil attenuates intestinal damage and inflammation by regulating necroptosis and TLR4/NOD signaling pathways following lipopolysaccharide challenge in a piglet model. *Mol Nutr Food Res* 2018; 62: e1700814.

18. National Research Council (NRC). *Nutrient requirements of swine*. 10th ed. Washington, DC: National Academic Press; 1998.

19. Ooi PT, da Costa N, Edgar J, et al. Porcine congenital splay leg is characterised by muscle fibre atrophy associated with relative rise in MAFbx and fall in P311 expression. *BMC Vet Res* 2006; 2.

20. Drew B, Phaneuf S, Dirks A, et al. Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol-Reg I* 2003; 284: R474–R480.

21. Livak KJ and Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and $2^{-\Delta\Delta CT}$ method. *Methods* 2001; 25: 402–408.

22. Medzhitov R. Origin and physiological roles of inflammation. *Nature* 2008; 454: 428–435.

23. Lu YC, Yeh WC and Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine* 2008; 42: 145–151.

24. Wu Y and Zhou BP. TNF-alpha/NF-kappaB/Snail pathway in cancer cell migration and invasion. *Brit J Cancer* 2010; 102: 639–644.

25. Bouwmeester T, Bauch A, Ruffner H, et al. A physical and functional map of the human TNF-alpha/NF-kappa B signal transduction pathway. *Nat Cell Biol* 2004; 6: 97–105.

26. Chen X, Guo X, Zhang H, et al. Role of miR-143 targeting KRAS in colorectal tumorigenesis. *Oncogene* 2009; 28: 1385–1392.

27. Akao Y, Nakagawa Y and Naoe T. MicroRNAs 143 and 145 are possible common onco-microRNAs in human cancers. *Oncol Rep* 2006; 16: 845–850.

28. Starczynowski DT, Kuchenbauer F, Argiropoulos B, et al. Identification of miR-145 and miR-146a as mediators of the 5q- syndrome phenotype. *Nat Med* 2010; 16: 49–58.

29. Pekow JR, Dougherty U, Mustafi R, et al. miR-143 and miR-145 are downregulated in ulcerative colitis: Putative regulators of inflammation and protooncogenes. *Inflamm Bowel Dis* 2012; 18: 94–100.

30. Tian D, Sha Y, Lu JM, et al. MiR-370 inhibits vascular inflammation and oxidative stress triggered by oxidized low-density lipoprotein through targeting TLR4. *J Cell Biochem* 2018; 119: 6231–6237.

31. Milosevic J, Pandit K, Magister M, et al. Profibrotic role of miR-154 in pulmonary fibrosis. *Am J Resp Cell Mol* 2012; 47: 879–887.

32. Xie T, Liang J, Liu N, et al. MicroRNA-127 inhibits lung inflammation by targeting IgG Fcɣ receptor I. *J Immunol* 2012; 188: 2437–2444.

33. Ying H, Kang Y, Zhang H, et al. MiR-127 modulates macrophage polarization and promotes lung inflammation and injury by activating the JNK pathway. *J Immunol* 2015; 194: 1239–1251.

34. Quinn SR and O'Neill LA. A trio of microRNAs that control Toll-like receptor signaling. *Int Immunol* 2011; 23: 421–425.

35. O'Connell RM, Chaudhuri AA, Rao DS, et al. Inositol phosphatase SHIP1 is a primary target of miR-155. *P Natl Acad Sci USA* 2009; 106: 7113–7118.

36. Tili E, Michaille JJ, Cimino A, et al. Modulation of miR-155 and miR-125b levels following lipopolysaccharide/ TNF-alpha stimulation and their possible roles in regulating the response to endotoxin shock. *J Immunol* 2007; 179: 5082–5089.

37. Fordham JB, Naqvi AR and Nares S. Regulation of miR-24, miR-30b, and miR-142-3p during macrophage and dendritic cell differentiation potentiates innate immunity. *J Leukocyte Biol* 2015; 98: 195–207.

38. Rudnicki A, Shivatzki S, Beyer LA, et al. microRNA-224 regulates Pentraxin 3, a component of the humoral arm of innate immunity, in inner ear inflammation. *Hum Mol Genet* 2014; 23: 3138–3146.

39. Kong H, Yin F, He F, et al. The Effect of miR-132, miR-146a, and miR-155 on MRP8/TLR4-induced astrocyte-related inflammation. *J Mol Neurosci* 2015; 57: 28–37.

40. Liu F, Li Y, Jiang R, et al. miR-132 inhibits lipopolysaccharide-induced inflammation in alveolar macrophages by the cholinergic anti-inflammatory pathway. *Exp Lung Res* 2015; 41: 261–269.

41. Li ZC, Han N, Li X, et al. Decreased expression of microRNA-130a correlates with TNF-α in the development of osteoarthritis. *Int J Clin Exp Patho* 2015; 8: 2555–2564.

42. Wang J, Bai X, Song Q, et al. miR-223 inhibits lipid deposition and inflammation by suppressing toll-like receptor 4 signaling in macrophages. *Int J Mol Sci* 2015; 16: 24,965–24,982.