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Short communication

Upregulated heat shock protein beta-1 associated with caloric restriction and high feed efficiency in *longissimus dorsi* muscle of steer

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ABSTRACT

The objective of this study was to identify myogenic proteins associated with caloric restriction and feed efficiency in bovine *longissimus dorsi* muscle. Thirty-one Korean native steers were allocated to 100% *ad libitum* (n = 16) or 80% of *ad libitum* (n = 15) groups. Regardless of nutritional level, a subset of these animals were assigned to groups with high or low feed efficiency (n = 5) at a later time point based on feed efficiency. A total of 7 differentially expressed proteins were found between groups with different nutrition levels while a total of 12 differentially expressed proteins were found between groups with different feed efficiencies. Interestingly, heat shock protein beta-1 (HSPB1) was a differentially expressed protein that showed up in both results (nutrition level and feed efficiency). It was up-regulated in both the 80% *ad libitum* group and the high feed efficiency group. In *in vitro* study, mRNA expression level of HSPB1 was increased ($P < 0.05$) after myogenic differentiation. Results of this study suggest that HSPB1 might be a myogenic protein involved in response to caloric restriction and feed efficiency in *longissimus dorsi* muscle of Korean native steer.

1. Introduction

Caloric restriction (CR) and feed efficiency (FE) could affect muscle development. It has been reported that steers exposed to low nutrition diet have enlarged muscle fibers compared to steers exposed to moderate-nutrition diet (Long et al., 2010). Restricted feeding can lead to the production of leaner carcasses (Murphy and Loerch, 1994). In general, CR has profound effect on myogenic activity of muscle stem cells such as satellite cell by altering their gene expression profile and enhancing mitochondrial energy production in mice (Cerletti et al., 2012). FE is an economically important factor in beef production. Generally, FE (g gain/kg feed) is regarded as the inverse of feed conversion ratio (FCR) or residual feed intake (RFI). Several FE studies have investigated candidate genes associated with carcass characteristics and meat quality in bovine (Baker et al., 2006; Al-Husseini et al., 2014). Lancaster et al. (2009) have reported that gains in *longissimus dorsi* muscle (LM) area are negatively correlated with FCR in growing bulls. In addition, muscle development and cytoskeletal architecture are modulated by gene expression which varies according to FE (Bottje and Kong, 2013). In this regard, we considered that CR and FE might affect muscle metabolism in LM of cattle. However, proteins involved in CR and FE during muscle development in bovine have not been identified. Therefore, it is necessary to identify a physiological marker associated with these two factors (CR and FE) in LM. Moreover, two-dimensional gel electrophoresis (2-DE) and spontaneously immortalized bovine embryonic fibroblasts (BEFS) could be used to identify genes involved in CR and FE. These genes might be used in feed development or animal selection for breeding, consequently increasing the productivity of beef cattle. Taken together, the objectives of this study were: 1) to use 2-DE to discover differentially expressed proteins common in bovine LM according to CR and FE; 2) to predict the roles of identified genes during myogenesis using BEFS.

2. Materials and methods

2.1. Animals, diets, experimental design, and sample collection

All experimental procedures involving animals were performed according to the Animal Experimental Guidelines. They were approved by the Animal Research Ethics Committee of Chungnam National University. A total of 31 Korean native steers (292.0 ± 6.95 kg) at 10 months of age were used for 4 months for this study. It has been

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Table 1
Nutrient composition (g/kg DM or as stated) of experimental diets.

Item[a]	14 months of age
DM (g/kg)	631
Crude protein	95
Ether extract	24
Neutral detergent fiber	585
Acid detergent fiber	413
Acid detergent lignin	61
NDICP	30
ADICP	17
Ash	82
Net energy for gain (KJ/kg)	2.64

[a] NDICP, neutral detergent insoluble crude protein; ADICP, acid detergent insoluble crude protein.

reported that intramuscular fat is mainly developed after 14 months of age (Cianzio et al., 1985). Therefore, experimental period of 4 months was used to investigate the effect of CR and FE on muscle development stage. Thirty-one Korean native steers were randomly distributed to 16 pens (2 animals/pen). Of these 31 steers, 16 were provided normal feed *ad libitum* while the remaining 15 were assigned to the CR group and fed *ad libitum* of 80% of normal feed intake consumed on a previous day. One steer was removed due to mechanical accident. During the 4-month feeding trial, chemical composition of the experimental diet of the normal group was 2.64 KJ/kg net energy for gain. Experimental diets were calculated to meet the requirement of the National Research Council (NRC, 2001) (Table 1). Individual daily feed intake was measured using an automated feeding machine (TMR FEEDER; Dawoon, Incheon, Korea). Body weight was recorded monthly before morning feeding. FE (g gain/kg intake) was calculated for the total experimental period (Table 2). Animals were divided into low FE (LF: n = 5) and high FE (HF: n = 5) groups. Those with median values were excluded. Difference in FE of the two groups was 20 or more.

For proteomic analysis, each LM sample was obtained at age of 14 months (normal group: n = 7, CR group: n = 5, LF group: n = 5, and HF group: n = 5) using a spring-loaded biopsy instrument (Biotech Nitra, Republic of Slovakia). Whole blood sample (10 ml) was taken *via* jugular veins after morning meal and added into a tube containing EDTA (Becton and Dickson, New Jersey, USA).

2.2. Protein extraction and two-dimensional gel electrophoresis

Proteomic analysis was performed for pooled samples (100 μg) containing equal quantities of protein from LM sample of each group to identify differentially expressed proteins. Weinkauf et al. (2006) have reported that sample pooling is efficient in 2-DE as it reduces non-specific expression background. Differentially expressed spots with at least a 2-fold change in intensity were subjected to ESI-Q-TOF/MS analysis. Details of 2-DE and ESI-Q-TOF/MS analysis have been previously described (Jin et al., 2012).

2.3. Blood variables

Whole blood (1 ml) was subjected to complete blood cell count analysis using HM2 (VetScan HM2 Hematology System, Abaxis, USA). Plasma albumin, blood urea nitrogen, glucose, total cholesterol, triglyceride, total protein, and γ-glutamyl transpeptidase levels were measured using Toshiba Accute Biochemical Analyzer-TBA-40FR (Toshiba Medical Instruments, Otawara-shi, Tochigi-ken, Japan).

2.4. Cell culture

MyoD-overexpressing BEFS cells (BEFS-MyoD) undergoing differentiation into myogenic lineages were used in this study. Details for cell culture have been described previously (Yin et al., 2010),

2.5. Total RNA extraction and real-time PCR analysis

Details of RNA isolation, cDNA synthesis, and real-time PCR procedure have been described previously (Zhang et al., 2014). Primers were designed using National Center for Biotechnology Information Primer-BLAST (Table 3). Relative fold-changes were determined using $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001). All data were normalized against β-actin as housekeeping gene.

2.6. Statistical analysis

Data (6 observations for body weight, 147 observations for daily feed intake, and 1 observation for blood composition) were presented as mean with standard error of mean (SEM). They were analyzed with independent-sample *t*-test. Real-time PCR data from BEFS cell lines were presented as mean ± SD. They were analyzed using Tukey's test. Statistical analysis was performed using SPSS software package (SPSS Inc., Chicago, IL, USA). *P*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Growth performance

Body weight of steers in the restricted group with 80% of *ad libitum* feed intake was 8.8% lower at 14 months of age compared to that of steers in the normal group with 100% *ad libitum* intake. In addition, feed intake, average daily gain, and FE at 9–14 months of age were significantly different. Although steers in the HF group consumed 15.2% less feed on average than steers in the LF group, average daily gain of steers in the HF group was greater ($P = 0.08$) than that in the LF group. Therefore, HF steers utilized nutrients more efficiently than LF steers (56.8 g/kg *vs* 31.4 g/kg for HF *vs.* LF groups, $P < 0.05$) (Table 2).

Table 2
Effects of caloric restriction and feed efficiency on growth performance.

		Nutritional level[a]		Feed efficiency[b]		SEM[c]		*P*-values[d]	
Trait	Age	Normal	Restricted	HF	LF	N	FE	N	FE
Initial BW, kg	10	294.4	289.3	287.6	307.2	9.80	19.05	ns	ns
Final BW, kg	14	370.9	338.1	359.4	356.4	12.29	28.00	*	ns
Daily feed intake (kg/d)	10–14	12.7	11.4	10.8	12.8	0.49	1.22	**	ns
Average daily gain (g/d)	10–14	642.9	410.1	603.4	413.4	45.44	78.19	**	*
Feed efficiency (g gain/kg feed)	10–14	51.0	36.6	56.8	31.4	4.39	5.70	**	**

[a] Steers were fed *ad libitum* (normal group, n = 16) or 80% of *ad libitum* (restricted group, n = 15).
[b] Steers were assigned to groups with high (HF, n = 5) and low feed efficiency (LF, n = 5) regardless of nutritional level.
[c] N, nutritional level; FE, feed efficiency.
[d] Probability values for the effect of nutritional level (N) and feed efficiency (FE); (* $P < 0.05$, ** $P < 0.01$, and ns = non-significant).

Table 3
Primers sequences, length and accession number.

Gene[a]	Accession number[b]	Sequence (5′ to 3′)	Length (bp)
HSPB1	NM_001025569	F: CCTGGACGTCAACCATTC R: GCTTGCCAGTGATCTCCAC	77
Desmin	NM_001081575	F: GGACCTGCTCAATGTCAAGA R: GGAAGTTGAGGGCAGAGAAG	109
Beta-actin	NM_173979	F: GCGTGGCTACAGCTTCACC R: TTGATGTCACGGACGATTTC	54

[a] HSPB1, heat shock protein beta-1.
[b] Database protein names and accession numbers: NCBI (http://www.ncbi.nlm.nih.gov).

3.2. Differentially expressed proteins of longissimus dorsi muscle according to nutritional level and feed efficiency

Results of 2-DE are shown in Fig. 1. Between normal and CR groups, one protein was up-regulated by CR while 6 proteins were down-regulated by CR (Table 4). Between HF and LF groups, 7 proteins were up-regulated by HF while 5 proteins were down-regulated by HF (Table 5). Interestingly, heat shock protein beta-1 (HSPB1) was found to be commonly up-regulated by both CR and HF.

3.3. mRNA expression of heat shock protein beta-1 during bovine myogenesis

In order to investigate the relationship between HSPB1 and bovine myogenesis, BEFS-MyoD cell line was used. Bovine myogenic differentiation was confirmed by an increase in the expression level of Desmin, a myogenic marker gene. The mRNA expression level of HSPB1 was increased ($P < 0.05$) after initial- and post- myogenic differentiation (Fig. 2).

3.4. Responses of complete blood cell count and metabolites to different nutritional levels and feed efficiencies

Regarding complete blood count analysis, CR had no effect (Table 6). However, the HF group had lower ($P < 0.05$) hemoglobin and hematocrit counts than the LF group. Regarding plasma metabolites, the concentration of total protein was lower ($P < 0.05$) in the CR group compared to that in the normal diet group.

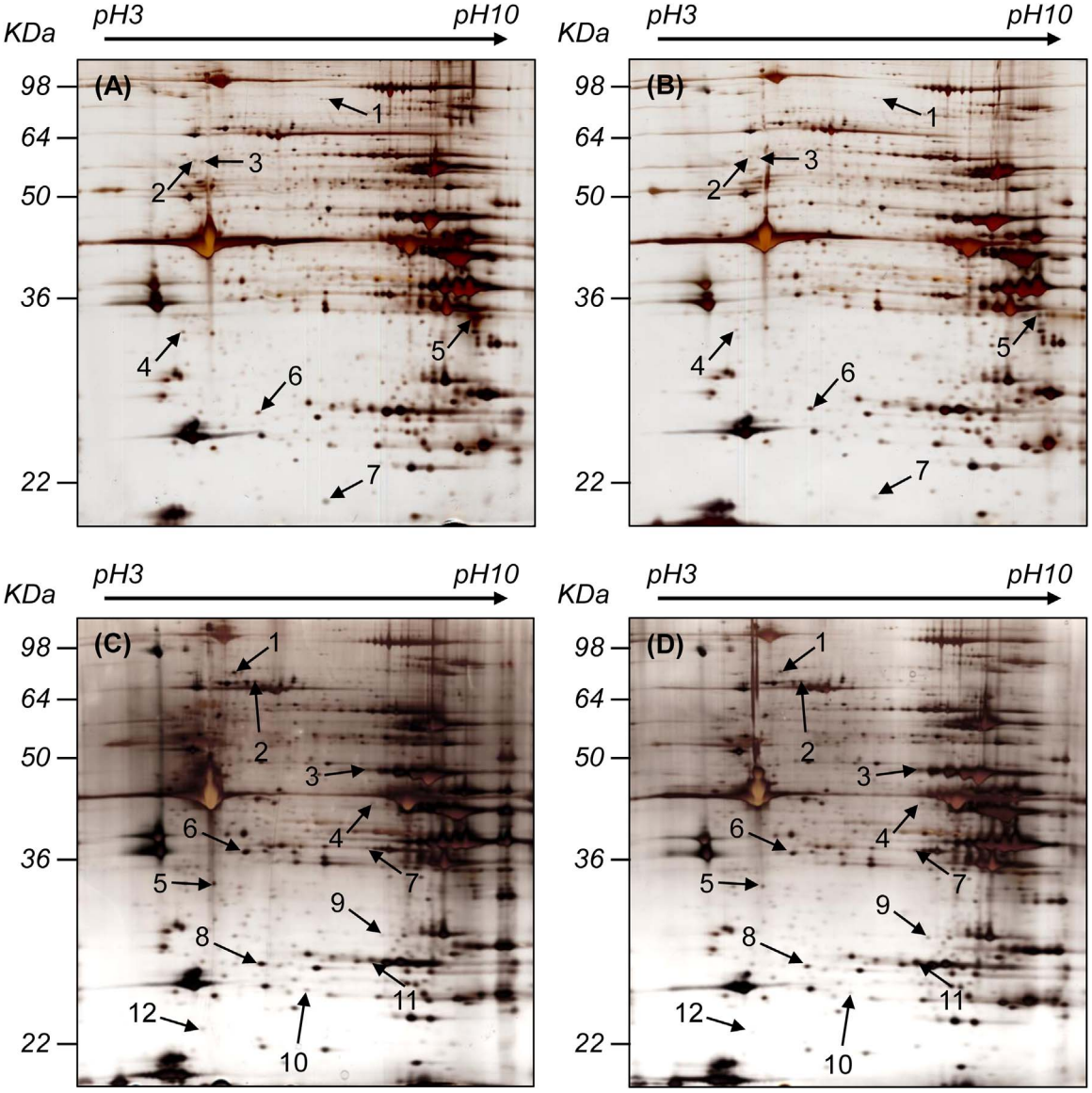

Fig. 1. 2-DE images derived from *longissimus dorsi* muscle (LM) of Korean native steer with (**A**) normal diet (N), (**B**) caloric restriction diet (CR), (**C**) high feed efficiency (HF), and (**D**) low feed efficiency (LF). Arrows indicate differentially expressed protein spots.

Table 4
Proteins differentially expressed in caloric restricted Korean.

Spot[a]	Name[b]	Accession number[b]	MW/pI[c]	Score	Sequence coverage (%)	Spot intensity		Fold change[d]
						Normal	Restricted	
1	Inner membrane protein, mitochondrial	A7E3V3	83.05/6.37	51.03	31.38	0.02	0.01	2.03
2	Alpha-1-antiproteinase precursor	P34955	46.10/6.05	14.64	16.35	0.13	0.06	2.33
3	Alpha-1 antiproteinase	P34955	46.10/6.05	4.55	4.81	0.12	0.05	2.45
4	Annexin V	P81287	36.09/4.85	47.64	50.63	0.16	0.05	3.33
5	MyoZ 1	Q8SQ24	31.67/9.17	32.44	33.99	1.08	0.39	2.73
6	Heat shock protein beta-1	Q3T149	22.40/5.98	22.18	40.80	0.29	0.62	0.47
7	Sulfotransferase, estrogen-preferring	P19217	34.62/6.67	72.35	20.00	0.47	0.22	2.20

[a] The spot number refers to Fig. 1.
[b] Database protein names and accession numbers: UniProt (www.uniprot.org).
[c] Molecular weight (MW) and isoelectric point (p*I*) of each protein were determined by 2-DE.
[d] The expression ratios of spot intensity at *ad libitum* (normal group) *versus* 80% of *ad libitum* (restricted group).

Table 5
Proteins differentially expressed in Korean native steers differing in feed efficiency.

Spot[a]	Name[b]	Accession number[b]	MW/pI[c]	Score	Sequence coverage (%)	Spot intensity		Fold change[d]
						HF	LF	
1	Myosin 1	Q9BE40	22.30/5.57	102.41	14.50	0.25	0.10	2.45
2	Stress-70 protein, mitochondrial	Q3ZCH0	73.74/5.97	55.67	26.80	0.29	0.12	2.42
3	Enolase 3	Q3ZC09	47.10/7.60	47.55	28.80	0.21	0.48	0.43
4	Selectin L	P98131	41.97/7.89	11.30	27.17	0.04	0.08	0.45
5	Capping protein muscle Z-line, beta	P79136	33.74/6.01	26.51	19.93	0.23	0.08	3.11
6	Slow skeletal muscle troponin T	Q8MKH6	31.28/5.71	67.49	8.32	0.48	0.23	2.10
7	GPD1 protein	Q5EA88	37.65/6.42	34.20	29.74	0.17	0.45	0.38
8	Heat shock protein beta-1	Q3T149	22.39/5.98	22.18	40.80	0.18	0.07	2.71
9	Carbonic anhydrase II	P00921	29.11/6.41	24.75	35.89	0.07	0.22	0.37
10	Chain L, Crystal Structure Analysis of Bovine Mitochondrial Peroxiredoxin Iii	1ZYE_L	24.33/6.08	11.59	16.82	0.27	0.13	2.04
11	Triosephosphate isomerase	Q5E956	26.69/6.45	43.92	36.95	0.47	1.27	0.37
12	Desmoplakin	E1BKT9	33.24/6.47	101.97	12.63	0.25	0.09	2.84

[a] The spot number refers to Fig. 1.
[b] Database protein names and accession numbers: UniProt (www.uniprot.org).
[c] Molecular weight (MW) and isoelectric point (p*I*) of each protein were determined by 2-DE.
[d] The expression ratios of spot intensity at high feed efficiency (HF) *versus* low feed efficiency (LF) group.

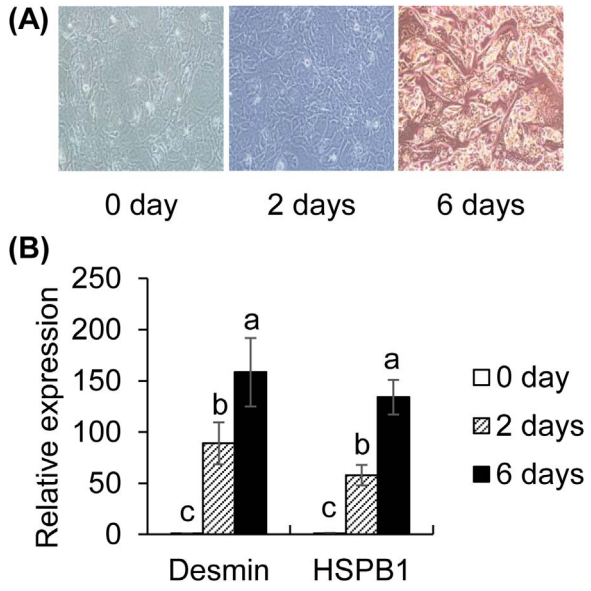

Fig. 2. mRNA expression levels of heat shock protein beta-1 (HSPB1) during bovine myogenesis or adipogenesis. (**A**) Representative photographs showing phase contrast of BEFS-MyoD. Magnification, 20X. (**B**) mRNA expression of desmin and HSPB1 in BEFS-MyoD cells at pre- (0 day), initial- (2 days), and post- differentiation (6 days). Values are mean ± SD (n = 3, a, b and c *vs.* control by Tukey's test).

4. Discussion

4.1. Blood parameters

Blood variables are useful tools to monitor animal health or animal stress. Our blood analysis results showed that CR did not have any negative impact on animal health (Table 6). Animals in the LF group had greater ($P < 0.05$) hemoglobin and hematocrit counts than those in the HF group. This might be due to the fact that LF steers are more excited and bolder than HF steers under stressful situations (Koolhaas et al., 1999). A positive relationship between RFI and hemoglobin or hematocrit has been reported because contraction of the spleen might occur after excitation (Gartner et al., 1969), consistent with our results. Plasma total protein levels in the CR and HF groups were lower ($P < 0.05$) than those of the normal diet and LF groups. Nevertheless, total protein levels of our results were within normal ranges for cattle (Kramer, 2002).

4.2. 2-DE analysis

Selsby et al. (2005) and Kim et al. (2015) have indicated that HSPB1 protein levels are increased by CR in soleus and plantaris muscle of rats compared to those in rats exposed to *ad libitum* feeding. Phosphorylated HSPB1 is also increased by serum starvation with low concentration of glucose in L6 myoblasts (Kim et al., 2014). In CR study of *Caenorhabditis elegans*, restriction of glucose metabolism can induce mitochondrial

Table 6
Analysis of blood variables in Korean native steers.

Item[a]	Nutritional level[b]		Feed efficiency[c]		SEM		*P*-value[d]	
	Normal	Restricted	HF	LF	N	FE	N	FE
Blood cell count								
White blood cell (X 103/μl)	10.5	9.8	9.3	11.3	0.86	0.90	ns	ns
Red blood cell (X 106/μl)	8.7	8.5	8.6	8.9	0.32	0.68	ns	ns
Hemoglobin (g/dl)	11.2	10.7	9.8	11.2	0.47	0.55	ns	*
Hematocrit (%)	35.8	35.1	32.8	37.5	1.42	1.75	ns	*
MCV (fl)	41.2	41.5	38.8	42.4	1.51	2.26	ns	ns
MCH (pg)	12.8	12.6	11.6	12.7	0.28	0.61	ns	ns
MCHC (g/dl)	31.1	30.4	29.9	29.9	0.37	0.31	ns	ns
Platelet (X 103/μl)	284.8	251.9	286.3	189.5	32.37	63.01	ns	ns
Metabolite								
Albumin (g/dl)	3.7	3.6	3.7	3.5	0.06	0.09	ns	ns
BUN (mg/dl)	11.6	9.9	11.7	11.8	1.01	1.76	ns	ns
Glucose (mg/dl)	83.1	83.5	84.8	81.3	2.57	4.82	ns	ns
Total cholesterol (mg/dl)	105.6	110.3	121.0	106.6	8.60	7.13	ns	ns
Triglyceride (mg/dl)	13.0	17.4	12.4	12.6	2.40	4.56	ns	ns
Total protein (g/dl)	6.3	5.9	6.2	5.8	0.15	0.19	*	*
γ-GGT (IU/l)	18.9	18.9	19.4	21.6	1.81	3.91	ns	ns

[a] MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BUN, blood urea nitrogen; γ-GGT, γ-glutamyl transpeptidase.
[b] Steers were fed *ad libitum* (normal group, n = 16) or 80% of *ad libitum* (restricted group, n = 15).
[c] Steers were assigned to groups with high (HF, n = 5) and low feed efficiency (LF, n = 5) regardless of nutritional level.
[d] Probability values for the effect of nutritional level (N) and feed efficiency (FE); (* $P < 0.05$ and ns = non-significant).

oxidative stress and ultimately strengthen its resistance of further oxidative stress (Schulz et al., 2007). Considering these points, we cautiously believe that upregulated HSPB1 might aid in physiological adaptation to stress such as nutritional deficiency and minimize oxidative damage to existing cellular components.

We also found that HSPB1 was upregulated in the HF group. Mitochondrial reactive oxygen species production is known to be higher in low RFI steers compared to that in high RFI steers (Kolath et al., 2006). A proteomic study by Vincent et al. (2015) has revealed that HSPB1 has higher abundance in the LM of low RFI pigs compared to that in the LM of high RFI pigs, consistent with our 2-DE results. It has been reported that HSPB1 expression is increased by oxidative stress in skeletal muscle cells. Over-expression of HSPB1 protein can potentially decrease reactive oxygen species generation (Mymrikov et al., 2011). Therefore, increase in HSPB1 expression of HF indicates that HF animals might have some advantages in handling increased cellular stress.

Lametsch et al. (2006) have suggested that HSPB1 might have an important role in hypertrophic muscle growth during compensatory growth in pigs. For this reason, we speculated that upregulated HSPB1 protein expression in CR and HF groups might improve nutrient utilization for myogenic differentiation by reducing mitochondrial oxidative stress in LM of steers. Thus, an *in vitro* experiment was conducted to predict the association between HSPB1 and bovine myogenesis using BEFS-MyoD cells. Results of the *in vitro* experiment revealed that the mRNA expression level of HSPB1 was increased after myogenic differentiation. Mymrikov et al. (2011) have indicated that HSPB1 might be able to regulate all elements of the cytoskeleton and remodel it. Ito et al. (2001) have reported that HSPB1 is expressed highly before differentiation of C2C12 myoblast and continuously accumulated after myogenic differentiation of C2C12 cells into myotubes. Moreover, HSPB1 and phosphorylation of HSPB1 play important roles in organizing myofibril structure and regulation of muscle mass. They are correlated with muscle hypertrophy (Sugiyama et al., 2000; Kawano et al., 2007; Hamelin et al., 2006).

5. Conclusion

Our results revealed that increased HSPB1 protein expression in LM was associated with CR and HF. HSPB1 mRNA expression was also increased after myogenic differentiation of BEFS-MyoD. Taken together, HSPB1 might be a key protein associated with CR and FE. It might play an important role in skeletal muscle mechanism related to energy utilization. It might be useful for improving the productivity of steers.

Statement on conflict of interest

The authors have no conflict of interest to declare.

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References

Al-Husseini, W., Gondro, C., Quinn, K., Herd, R.M., Gibson, J.P., Chen, Y., 2014. Expression of candidate genes for residual feed intake in Angus cattle. Anim. Genet. 45, 12–19.
Baker, S.D., Szasz, J.I., Klein, T.A., Kuber, P.S., Hunt, C.W., Glaze Jr., J.B., Falk, D., Richard, R., Miller, J.C., Battaglia, R.A., Hill, R.A., 2006. Residual feed intake of purebred Angus steers: effects on meat quality and palatability. J. Anim. Sci. 84, 938–945.
Bottje, W., Kong, B.W., 2013. Cell biology Symposium: feed efficiency: mitochondrial function to global gene expression. J. Anim. Sci. 91, 1582–1593.
Cerletti, M., Jang, Y.C., Finley, L.W., Haigis, M.C., Wagers, A.J., 2012. Short-term calorie restriction enhances skeletal muscle stem cell function. Cell Stem Cell 10, 515–519.
Cianzio, D.S., Topel, D.G., Whitehurst, G.B., Beitz, D.C., Self, H.L., 1985. Adipose tissue growth and cellularity: changes in bovine adipocyte size and number. J. Anim. Sci. 60, 970–976.
Gartner, R.J., Callow, L.L., Granzien, C.K., Pepper, P.M., 1969. Variations in the concentration of blood constituents in relation to the handling of cattle. Res. Vet. Sci. 10, 7–12.
Hamelin, M., Sayd, T., Chambon, C., Bouix, J., Bibe, B., Milenkovic, D., Leveziel, H., Georges, M., Clop, A., Marinova, P., Laville, E., 2006. Proteomic analysis of ovine muscle hypertrophy. J. Anim. Sci. 84, 3266–3276.
Ito, H., Kamei, K., Iwamoto, I., Inaguma, Y., Kato, K., 2001. Regulation of the levels of small heat-shock proteins during differentiation of C2C12 cells. Exp. Cell Res. 266, 213–221.
Jin, Y.C., Li, Z.H., Hong, Z.S., Xu, C.X., Han, J.A., Choi, S.H., Yin, J.L., Zhang, Q.K., Lee, K.B., Kang, S.K., Song, M.K., Kim, Y.J., Kang, H.S., Choi, Y.J., Lee, H.G., 2012. Conjugated linoleic acid synthesis-related protein proteasome subunit alpha 5 (PSMA5) is increased by vaccenic acid treatment in goat mammary tissue. J. Dairy Sci. 95, 4286–4297.
Kawano, F., Matsuoka, Y., Oke, Y., Higo, Y., Terada, M., Wang, X.D., Nakai, N., Fukuda, H., Imajoh-Ohmi, S., Ohira, Y., 2007. Role(s) of nucleoli and phosphorylation of

ribosomal protein S6 and/or HSP27 in the regulation of muscle mass. Am. J. Physiol. Cell Physiol. 293, C35–44.
Kim, J.H., Lee, Y., Kwak, H.B., Lawler, J.M., 2015. Lifelong wheel running exercise and mild caloric restriction attenuate nuclear EndoG in the aging plantaris muscle. Exp. Gerontol. 69, 122–128.
Kim, M.Y., Lee, J.U., Kim, J.H., Lee, L.K., Park, B.S., Yang, S.M., Jeon, H.J., Lee, W.D., Noh, J.W., Kwak, T.Y., Jang, S.H., Lee, T.H., Kim, J.Y., Kim, B., Kim, J., 2014. Phosphorylation of Heat Shock Protein 27 is increased by cast immobilization and by serum-free starvation in skeletal muscles. J. Phys. Ther. Sci. 26, 1975–1977.
Kolath, W.H., Kerley, M.S., Golden, J.W., Keisler, D.H., 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. J. Anim. Sci. 84, 861–865.
Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925–935.
Kramer, J., 2002. Normal Hematology of Cattle, Sheep, and Goats. Williams & Wilkins, Philadelphia.
Lametsch, R., Kristensen, L., Larsen, M.R., Therkildsen, M., Oksbjerg, N., Ertbjerg, P., 2006. Changes in the muscle proteome after compensatory growth in pigs. J. Anim. Sci. 84, 918–924.
Lancaster, P.A., Carstens, G.E., Ribeiro, F.R., Tedeschi, L.O., Crews Jr., D.H., 2009. Characterization of feed efficiency traits and relationships with feeding behavior and ultrasound carcass traits in growing bulls. J. Anim. Sci. 87, 1528–1539.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25, 402–408.
Long, N.M., Prado-Cooper, M.J., Krehbiel, C.R., DeSilva, U., Wettemann, R.P., 2010. Effects of nutrient restriction of bovine dams during early gestation on postnatal growth, carcass and organ characteristics, and gene expression in adipose tissue and muscle. J. Anim. Sci. 88, 3251–3261.
Murphy, T.A., Loerch, S.C., 1994. Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. J. Anim. Sci. 72, 2497–2507.
Mymrikov, E.V., Seit-Nebi, A.S., Gusev, N.B., 2011. Large potentials of small heat shock proteins. Physiol. Rev. 91, 1123–1159.
NRC, 2001. National Academy Press, Washington, DC, USA.
Schulz, T.J., Zarse, K., Voigt, A., Urban, N., Birringer, M., Ristow, M., 2007. Glucose restriction extends Caenorhabditis elegans life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab. 6, 280–293.
Selsby, J.T., Judge, A.R., Yimlamai, T., Leeuwenburgh, C., Dodd, S.L., 2005. Life long calorie restriction increases heat shock proteins and proteasome activity in soleus muscles of Fisher 344 rats. Exp. Gerontol. 40, 37–42.
Sugiyama, Y., Suzuki, A., Kishikawa, M., Akutsu, R., Hirose, T., Waye, M.M., Tsui, S.K., Yoshida, S., Ohno, S., 2000. Muscle develops a specific form of small heat shock protein complex composed of MKBP/HSPB2 and HSPB3 during myogenic differentiation. J. Biol. Chem. 275, 1095–1104.
Vincent, A., Louveau, I., Gondret, F., Trefeu, C., Gilbert, H., Lefaucheur, L., 2015. Divergent selection for residual feed intake affects the transcriptomic and proteomic profiles of pig skeletal muscle. J. Anim. Sci. 93, 2745–2758.
Weinkauf, M., Hiddemann, W., Dreyling, M., 2006. Sample pooling in 2-D gel electrophoresis: a new approach to reduce nonspecific expression background. Electrophoresis 27, 4555–4558.
Yin, J., Jin, X., Beck, S., Kang, D.-H., Hong, Z., Li, Z., Jin, Y., Zhang, Q., Choi, Y.-J., Kim, S.-C., Kim, H., 2010. In vitro myogenic and adipogenic differentiation model of genetically engineered bovine embryonic fibroblast cell lines. Biotechnol. Lett. 32, 195–202.
Zhang, Q., Lee, H.G., Kang, S.K., Baik, M., Choi, Y.J., 2014. Heat-shock protein beta 1 regulates androgen-mediated bovine myogenesis. Biotechnol. Lett. 36, 1225–1231.