Electrical stimulation of bovine carcasses cause changes in stress proteins in the longissimus dorsi muscle as revealed by proteomics

K. Hollung[1], H. Grove[1, 2], K.I. Hildrum[1] & E. Veiseth[1]
[1]Nofima Food, Matforsk AS - Food, Fisheries and Aquaculture Research, Osloveien 1, N-1430 Ås, Norway.
[2]Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432 Ås, Norway.
E-mail: kristin.hollung@matforsk.no

Abstract

Low-voltage electrical stimulation (LV-ES) of bovine carcasses is in widespread use to enhance meat tenderness. In this study, we have used proteomics as a tool to unravel the changes in protein composition in the LD muscle after LV-ES of bovine carcasses. LV-ES of carcass halves from 10 Norwegian Red bulls were performed 30-45 min after exsanguination. The other halves from each animal were kept as non-stimulated controls. The LD muscle was hot-boned and samples were collected 1 and 3 h after electrical stimulation, analyzed by 2-dimensional electrophoresis and the proteins identified. Twelve protein spots were significantly reduced in abundance by exposure to LV-ES, of which 7 proteins were identified. These were 4 variants of heat shock protein 27, 2 variants of the protein crystalline and the final protein identified was pyruvat dehydrogenase. These proteins may have a potential as markers with regard to the efficiency of LV-ES to improve tenderness in LD muscles in different carcasses.

Introduction

Electrical stimulation (ES) of beef carcasses is frequently used by industry to enhance meat tenderness. There is a general consensus that ES is beneficial in terms of quality, however reported results of the effectiveness of ES on improving meat tenderness show considerable variation (Hildrum et al., 1999, Hollung et al., 2007). It is well known that ES will increase the biochemical activity in the muscle cells, leading to ATP and creatine phosphate disappearance, accumulation of lactate and accelerated pH decline. However, the biological and physiological changes induced by ES are poorly understood. Proteomics is used in order to investigate the up- and down-regulation of metabolic muscle proteins. Understanding the molecular changes stimulated by ES may lead to development of new strategies for optimisation of the ES processing parameters.

Materials & Methods

The animals and LV-ES are described in Hollung et al. (2007). Briefly, young bulls of the Norwegian Red breed (n=8) from a performance test station (GENO) were slaughtered at a commercial abattoir at two separate days, and carcasses were halved. One half received low voltage ES (90 V, 15 Hz, 32 sec) which was applied through muscles in the neck and leg approximately 40 min (± 4 min) after stunning, while the other half served as a non-stimulated control (NES). At approximately 1h *post mortem*, the LD muscle were removed from both halves of each carcass. Samples from the hot-boned *longissimus dorsi* muscle from both halves were collected 1 and 3 h after LV-ES.

For metabolic profiling, muscle samples were separated by 2-dimensional gel electrophoresis (2-DE) according to the method described by Jia et al. (2006).

Results

Water-soluble proteins extracted from *longissimus dorsi* hot-boned from half carcasses either treated or not treated with LV-ES were separated by 2-DE. Analysis of x protein spots revealed 12 significantly changed proteins. A representative 2-DE image with indications of the significantly changed protein spots is shown in Figure 1.

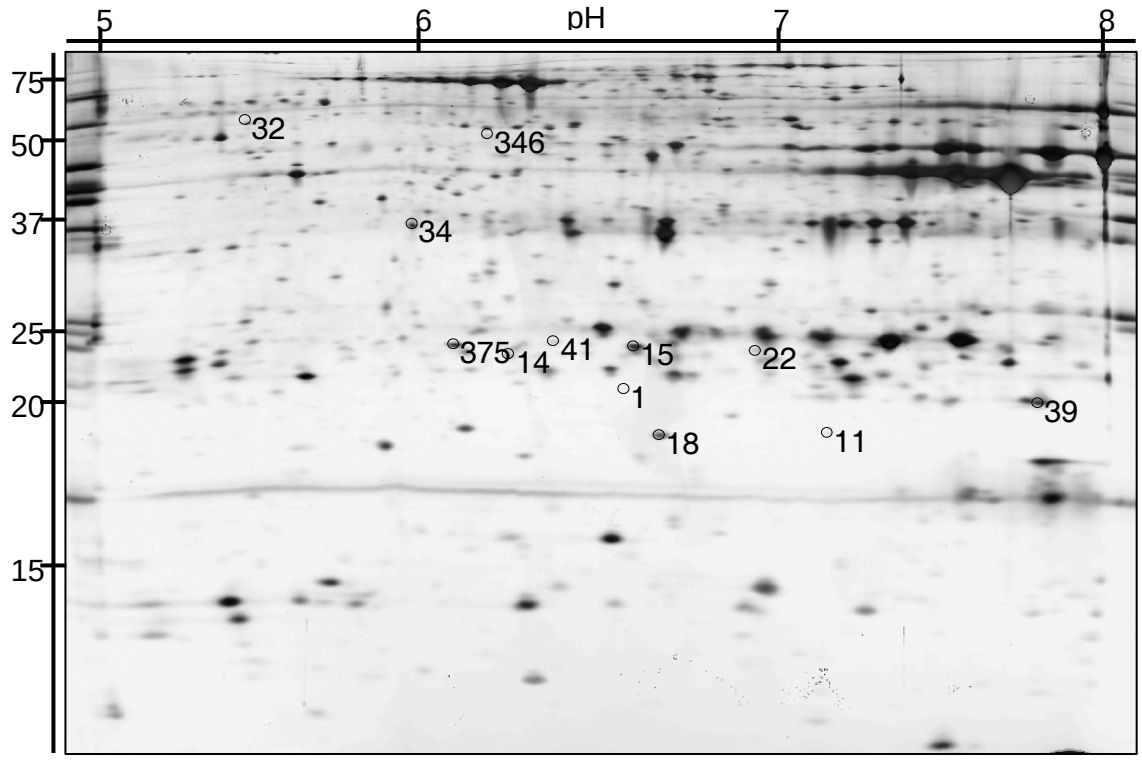

Figure 1. Representative silver stained 2-DE image of TES soluble proteins separated by IPG 5-8 and 12.5% SDS-PAGE. Significantly changed proteins according to ES treatment are marked.

Of the 12 protein spots, 7 were identified by MS. Five proteins could not be identified due to low levels of protein and difficulties in extraction of the proteins from the 2-DE gels. The identified proteins are listed in Table 1. Heat shock protein 27 and crystallin, also a small heat shock proteins, were recognized in 6 of the identified protein spots.

The relative abundance of the significant proteins in each sample is shown in the panels in Figure 2. All the changed proteins are present at lower levels in the LV-ES treated samples.

Table 1. Proteins changed in abundance by LV-ES

Spot no	**Identified proteins**	**Matched peptides/ % sequence coverage**	**NCBI accession no. (source)**
15	Heat shock protein beta-1 (HSP 27)	12/ 73	Gi|85542053 (bovine)
18	Heat shock protein, alpha-crystallin-related	13/ 98	Gi|115496724 (bovine)
22	Heat shock protein beta-1 (HSP 27)	6/ 35	Gi|85542053 (bovine)
375	Heat shock protein beta-1 (HSP 27)	10/ 61	Gi|85542053 (bovine)
11	Crystallin alpha B	8/ 31	Gi|27805849 (bovine)
39	Crystallin alpha B	13/ 66	Gi|27805849 (bovine)
34	Pyruvat dehydrogenase	14/ 43	Gi|164420789

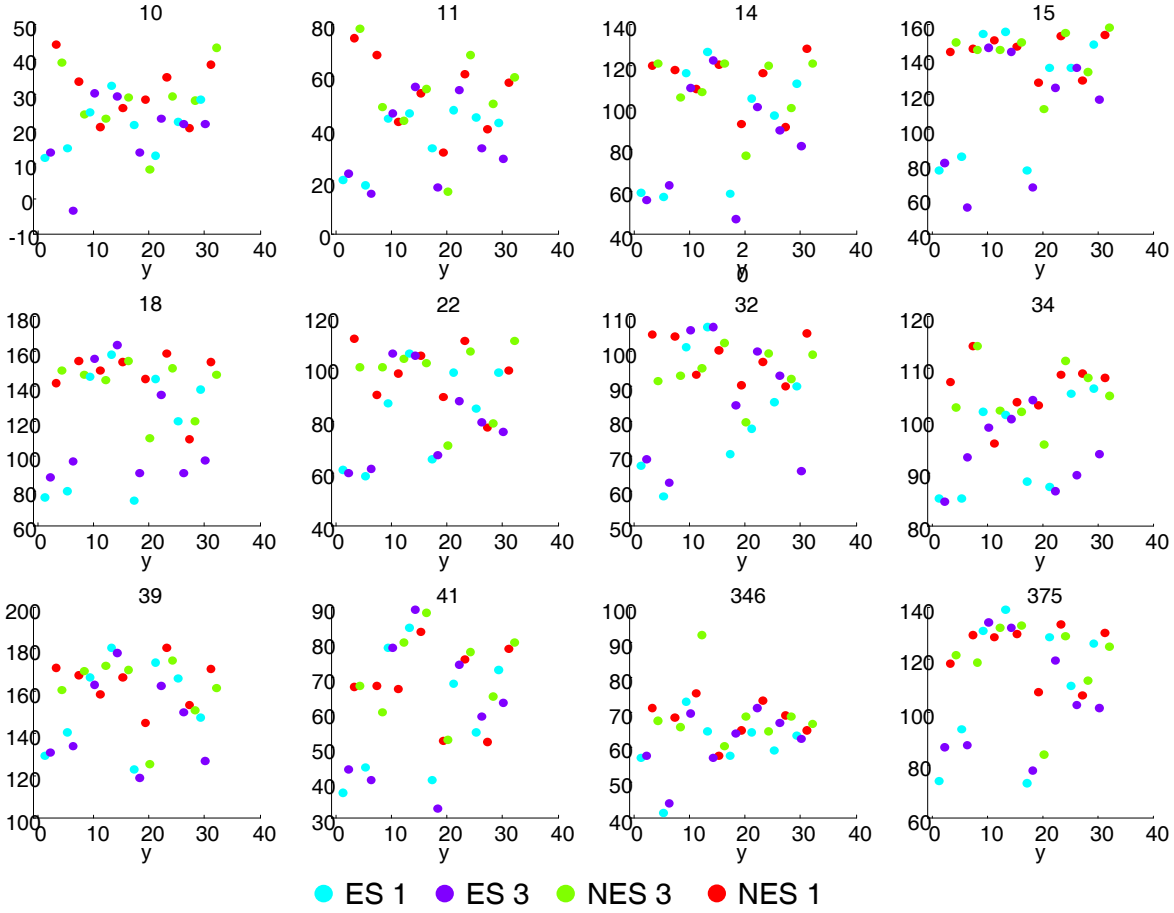

Figure 2. Relative abundance of significantly changed proteins according to ES. The different colours represent LV-ES and non-LV-ES samples collected 1 and 3 hours after stimulation.

Conclusions

The results demonstrate a shift in abundance of several small heat shock proteins in the LV-ES treated samples. How down-regulation of these muscle proteins is related to post mortem glycolysis and ageing of the muscles needs to be further studied. The observed decrease in pyruvate dehydrogenase may indicate a shift towards production of lactate which is also reflected by increased pH decline in these samples.

Acknowledgements

We thank Vibeke Høst and Barbara Grochowalska for technical assistance. This work was supported by grant NRC 167880/I10.

References

Hildrum, K.I., Solvang, M., Nilsen, B.N., Frøystein, T. and J. Berg (1999). Combined effects of chilling rate, low voltage electrical stimulation and freezing on sensory properties of bovine M. *longissimus dorsi*. Meat Sci., 52, 1-7.

Hollung K., E. Veiseth, T. Frøystein, L. Aass, Ø. Langsrud and K.I. Hildrum. Variation in the response to manipulation of *post mortem* glycolysis in beef muscles by low-voltage electrical stimulation and conditioning temperature. (2007) Meat Science 77: 372-383.

Jia, X.; Hollung, K.; Therkildsen, M.; Hildrum, K. I.; Bendixen, E (2006). Proteome analysis of early post mortem changes in two bovine muscle types: M. *longissimus dorsi* and M. *semitendinosis*. Proteomics, 6: 936-944.