PE1.04 Heat shock proteins in muscle tissue of exercise stressed pigs 72.00

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Abstract—The welfare of meat producing animals as well as the quality of the meat are two very important issues in the meat sector. Some quality aspects may be linked to the stress conditions prior to slaughter and the purpose of this work was to investigate the plausibility of using specific stress induced biological markers for the indication of animal welfare and also correlation to meat quality traits. To mimic transportation pigs were exercise-stressed on a treadmill and left to rest for 0, 1 or 3 h before slaughter. The mRNA abundance in muscle tissue of heat shock proteins HSP70 and HO1 was determined and in a model system of myotube cultures the reflection of mRNA on protein expression was investigated. HO1 mRNA abundance was increased approximately 2 fold in pigs left to rest for 1 and 3 h and HSP70 mRNA was increased two fold in all the stressed pigs. The increase was observed exclusively (HSP70) or predominantly (HO1) in the biceps femoris muscle. The mRNA abundance of the HSPs did not correlate significantly to meat quality traits. However, induction of oxidative stress in myotube cultures showed that the mRNA induction was reflected in the protein expression, which merits further analysis of possible correlations between muscle HSP protein expression and meat quality. Furthermore, the stress induced increase of mRNA abundance, and possibly protein expression, of the two HSPs may be used as indicators of stress in relation to animal welfare issues.

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Index Terms—heat shock protein, meat quality, stress.

I. INTRODUCTION

ANIMAL welfare is an increasingly important issue for the consumer in the western part of the world, and stress before slaughter cinsiderably affects meat quality traits [3]. Biological markers for physical stress prior to slaughter has been investigated in several studies as variation in the levels of e.g. lactate, creatine phosphokinase or cortisol. However, several of these responses are relatively acute and exhibits great variation over a short period of time. The expression of other compounds like the heat shock proteins (HSP), are induced by a variety of stressors [1], and their presence is detectable over longer periods of time, typically several hours or days [5]. HSPs are expressed in skeletal muscle tissue [8] and are also detectable in blood [6]. The aim of this study was to identify new biomarkers related to physical stress as an indication of stress exposure partly as a marker in relation to animal welfare and partly in relation to meat quality traits. Two heat shock proteins, HSP70 and heme oxygenase 1 (HO1), were chosen as HSP70 is up-regulated at increased temperature and HO1 is regarded as more specifically induced by oxidative stress [7]. Both heat and oxidative stress are conditions expected to be triggered by physical exercise [4]. The stress induction was determined by mRNA abundance of the specific HSPs in muscle tissue, and the relation between mRNA abundance and protein expression was demonstrated in a model-system of myotube cultures where the chosen stressor was H2O2 as an inducer of oxidative stress.

II. MATERIALS AND METHODS

A. Animals and management From 10 litters of crossbreed DLY pigs (Duroc x Danish Landrace/Yorkshire) four females from each litter were allocated to four treatments: control without stress, or exercise stressed pigs slaughtered after 0, 1 or 3 h after exercise. The pigs were stressed on a treadmill with increasing speed from 0.4 km/h to an average of 5.2 km/h with increments of 0.4 km/h every 2 minutes; in total 27 minutes (SEM=0.9). Biopsies for analysis were taken at the time of sticking, snap frozen in liquid nitrogen and stored at

-80 °C until analysis. B. C2C12 myotube cultures and experimental setup C2C12 myoblasts (American Type Culture Collection, Manassas, VA) were seeded in 24 well plates with growth media: DMEM including 10% (v/v) fetal calf serum (FCS), 100 IU/mL penicillin, 100 µg/mL streptomycin, 3 µg/mL amphotericin B, and 20 µg/mL gentamycin, and maintained in an atmosphere of 95% air and 5% CO2 at 37 °C. Cells were grown to confluence in growth medium and left to fuse in media of only 4% (v/v) FCS. After ~4 days the differentiated myotubes were stressed by incubation in Krebs-HEPES buffer with 100 µM H2O2 for 1 h. After stress exposure myotubes were washed and incubated with fresh differentiation medium. Myotubes were harvested from duplicate wells at the time points indicated in figure 3. C. RNA extraction and real time PCR From biopsies RNA was extracted according to the trireagent extraction procedure as described by Chomczynski et al. [2] and from myotubes cultures RNA was extracted using the AllPrep RNA/protein kit (Qiagen, Albertslund, Denmark). Purified RNA adjusted to identical concentrations (88 ng/ µl from biopsies and 9 ng/ µl from cultured myotubes) was reverse transcribed with oligo-dT primers and Superscript RNase H reverse transcriptase kit (Invitrogen, Taastrup, Denmark) and then amplified with TaqMan Universal PCR Master Mix (Applied Biosystems, Stockholm, Sweden). Quantity of the mRNA from the two HSPs was detected with TaqMan gene expression assay (Applied Biosystems, Stockholm, Sweden) For PCR, 40 cycles at 95°C for 15 s and 60°C for 60 s were applied using a ABI 7900HT detection system (Applied Biosystems, Stockholm, Sweden). The relative mRNA quantity was calculated: quantity = 2ΔΔCt. D. Western blotting Protein from the myotubes were dissolved (0.125 M Tris, 4% SDS, 20% glycerol, 0.1 M DTE) and material from 2 wells were pooled and homogenized. Standardized protein quantities of the supernatant was preheated and samples (150 g) were loaded on a 10%, 18-well Criterion gel (Biorad Laboratories, CA) and run at 200 V for 1 h at RT, transferred to polyvinylidene fluoride membranes at 150 V, 1.5 mA for 1½ h at 5°C as described by Towbin, Staehelin, & Gordon [9]. Membranes were rinsed, blocked (TTBS incl. 5% nonfat dry milk washed again, and incubated with the anti-HO-1 primary antibody (rabbit anti-HO-1, Calbiochem, Darmstadt, Germany) and then the alexa flour labeled secondary antibody (Alexa Flour 488 goat-anti-rabbit, Invitrogen, Taastrup, Denmark) both diluted 1:2000 in TTBS. For visualization the membranes were scanned with an image scanner (Molecular Imager FX, Bio-Rad).

III. RESULTS AND DISCUSSION

Physical stress on a treadmill increased the mRNA abundance of both of the investigated heat shock proteins. The mRNA levels of the oxidative specific protein HO1 was not significantly different from that of the control pigs immediately after stress exposure, but was up-regulated after 1 h to twice that of the level in unstressed control pigs in BF and less up-regulated in LD. This differentiated regulation in the two muscles is to be expected from the applied type of stress exposure where the BF of the thigh is more oxidative and heavier exercised compared to the LD at the back of the animal. Already after 3 hours of rest the stimulation of HO1 expression was reduced in BF and back to baseline in LD. The mRNA of the heat sensitive protein HSP70 was significantly up-regulated in pigs slaughtered immediately after the 27 minutes of treadmill-stress, but this stimulation was only observed in BF and not in LD. BF was the more exercised muscle, and the stress-induced temperature increment in this muscle was 2 °C compared to only 1 °C increment in LD [10]. The two fold up-regulation of the HSP70 expression was maintained in all the stressed pigs irrespective of resting times of 1 and 3 hours before slaughter. No regulation of HSP70 was observed in LD in any of the stressed pigs.

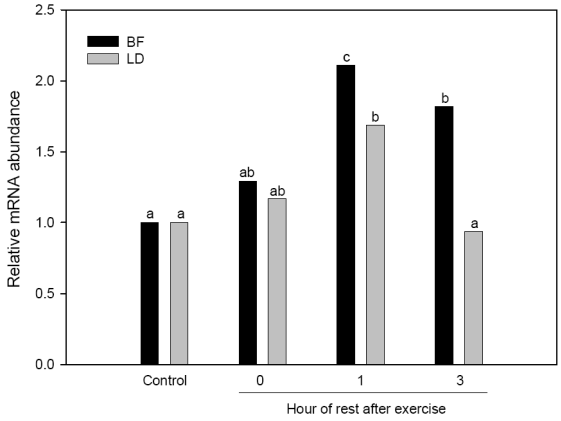

Figure 1. Relative abundance of heme oxygenase 1 (HO1) mRNA in tissue from biceps femoris (BF) and longissimus dorsi (LD) of pigs exercised and allowed to rest for 0, 1 or 3 h before slaughter. Control pigs were not stressed.

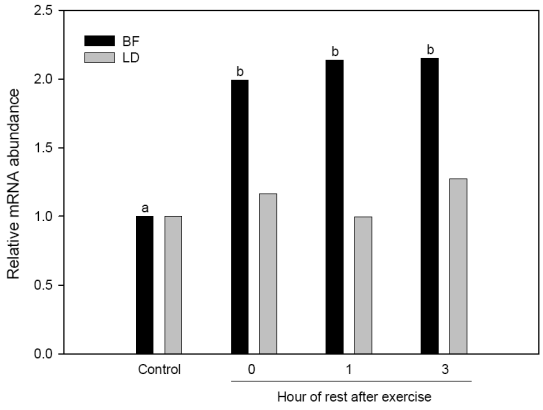

Figure 2. Relative abundance of heat shock protein 70 (HSP70) mRNA in tissue from biceps femoris (BF) and longissimus dorsi (LD) of pigs exercised and allowed to rest for 0, 1 or 3 h before slaughter. Control pigs were not stressed. An up or down-regulation of the mRNA abundance for a specific gene is expected to be reflected in the protein expression. We compared the relative abundances of HO1 mRNA with HO1 protein expression in a model system of myotube cultures (Figure 3). The increase in HO1 mRNA abundance peaked at 4 hours after stress exposure and was back to baseline after 24 h. This pattern was reflected in the protein expression with a 1-2 h delay as the protein expression peaked at 6-8 hours after stress exposure and decreasing towards baseline levels without reaching baseline within the timeframe of the investigated 36 h after stress exposure.

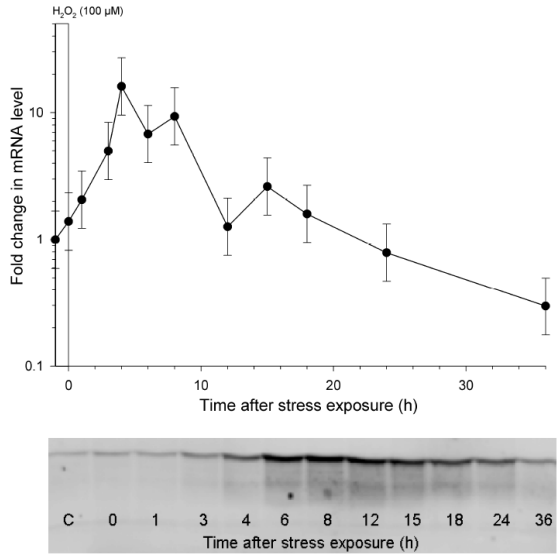

Figure 3. Relative abundance of heme oxygenase 1 (HO1) mRNA and intensity of the HO1 protein in myotubes after exposure to H2O2 for 1 h. Note different time scales. This reflection of mRNA on the HO1 protein expression observed in the model system of cultured myotubes may indicate that the observed increase in the mRNA levels of HO1 and HSP70 in muscle tissue of pigs may be followed by similar increases in protein expression. The protein expression of muscle tissue is soon to be determined, by western blotting but the extent of a subsequent protein expression will depend partly on the time lag from mRNA regulation to protein expression in muscle tissue and also the extent of protein regulation over time. One of the aims of this study was to identify possible biological markers of stress which at the same time reflects one or more meat quality traits. Correlations were calculated from meat quality traits such as pH, drip loss and texture, but no direct correlation was found. These correlations will be reanalyzed once the protein expression data is available. Furthermore, these HSPs may have potential as markers of stress exposure prior to slaughter when focusing on animal welfare issues. The mRNA is up-regulated for at least 1-3 hours after stress exposure, and the protein expression may be stimulated even longer if it reflects the results from the myotube model system.

IV. CONCLUSION

Physical stress on a treadmill increased the mRNA abundance of both heat shock proteins, HSP70 and HO1, in skeletal muscle from pigs. Correlation analysis revealed no significant correlation between the HSPs and various meat quality traits, but the fact that the mRNA abundance and possibly the proteins are up-regulated as a consequence of physical stress may be used as indicators of stress conditions as an animal welfare issue.

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