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EFFECT OF HALOTHANE-SENSITIVITY AND SLAUGHTER ON MYOFILAMENT SPACING AND EXTRACELLULAR SPACE IN PIG MUSCLE

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OBJECTIVES

After slaughter, the distribution of water in muscle tissue undergoes large changes. The packing density of thick filaments per unit area increases and the cross sectional area of myofibrils decreases (Swatland & Belfry, 1985). The extracellular space is increased during the onset of rigor mortis (Heffron & Hegarty, 1974 ; Currie & Wolfe, 1980). It has been suggested that these changes lead to a movement of water from the myofibrillar compartment into the inter-myofibrillar space and then from the latter into the extracellular space (Penny, 1975; Honikel et al., 1986). The extent of these changes noticeably influences meat quality as drip loss and light scattering increase while firmness decreases with myofibrillar shrinkage (Offer & Knight, 1988). The aim of the present study was to investigate the rate and the extent of the changes affecting the myofilament spacing and the size of the extracellular space during the onset of rigor mortis in pig muscle.

MATERIAL AND METHODS

ANIMALS, SLAUGHTERING AND SAMPLING: Piétrain piglets were halothane-tested at a liveweight of about 30 kg. Six halothane-positive and 6 halothane-negative were used in the experiment. They were killed in laboratory facilities by electrical stunning and sticking at a liveweight of about 95 kg. Four samples were obtained from the Longissimus lumborum for determination of myofilament spacing and extracellular space, as follows: 1) just before slaughter, a biopsy was taken using the shot biopsy system described by Lahucky et al. (1980); a part of this sample was immediately used for analysis; 2) another part of the biopsy was incubated at 39 °C with 0.5 ml of 150 mM KCl for 1 h before use; 3) from the carcass at 1 hour after slaughter and 4) from the carcass at 24 hours after slaughter.

pH AND R-VALUE: pH was measured in the incubated biopsy and the 1 h post-mortem sample after homogenization in 0.005 M iodoacetate using a combined glass electrode. R-value (ATP/IMP) was determined according to Honikel & Fischer (1977).

MYOFILAMENT SPACING: Small muscle samples were fixed in glutaraldehyde, postfixed in osmium tetroxide then dehydrated through ethanol gradient before embedding in epoxy resin. Ultrathin sections (80-90 nm) were stained with uranyl acetate and lead citrate before observation at an accelerating voltage of 80 KV in a Philips EM electron microscope. The space between thick filaments was measured at a print magnification of x 25000 from 6 prints from different parts of the sample. Hundred measurements were made on each photograph (10 myofibrils x 10 sets of 10 filaments).

EXTRACELLULAR SPACE: Muscle strips of around 1 mm^2 in section and 5 to 10 mm in length were quickly frozen in a slush of liquid-solid nitrogen (at about -210 °C). They were transferred into aceton for 3 days at -80 °C in order to substitute water for aceton. They were then put back to room temperature and embedded in epoxy resin. Cross-sections of 1.5 µm thickness were stained with toluidine blue. The space between fibres was estimated by cutting and weighing from photographs of cross-sections at a magnification of x 300. The space was expressed as a percentage of tissue volume and was referred to as extracellular space.

RESULTS

TABLE 1. EFFECTS OF GENETIC TYPE AND SAMPLE CONDITION ON pH, R-VALUE, MYOFILAMENT SPACING AND EXTRACELLULAR SPACE.

Trait		pH	R-value	Myofilament spacing, nm	Extracellular space, %
Genetic type	Halothane-negative	5.79	1.11	26.3	8.8
	Halothane-positive	5.62	1.18	23.5	11.1
Effect [1]		NS	*	**	NS
Sample condition	Biopsy	-	-	28.1	3.2
	Incubated biopsy	5.78	1.13	26.9	4.2
	Post mortem, 1 h	5.62	1.16	22.9	15.3
	Post mortem, 24 h			21.6	17.3
Effect [1]		**	*	**	**

[1] NS: P>0.05; * P<0.05; ** P<0.01

Halothane-sensitivity affected the R-value (P<0.05) and the myofilament spacing (P<0.01) (Table1). The rate of pH fall was rather fast in both genetic types whatever the sample condition. It was higher (P<0.01) in the muscle of the slaughtered animals than in incubated biopsy. Sample condition (biopsy incubation or time post mortem) affected every trait.

The myofilament spacing was higher in biopsy (P<0.05), incubated biopsy (P<0.05) and 1 h-post mortem muscle (P<0.01) from halothane-negative animals than in those from halothane-positive animals (Figure 1). It decreased very fast during the first hour post-mortem (P<0.01 in both genetic types). It tended to decrease in biopsy during incubation but not significantly (P>0.05). The extracellular space increased sharply during the first hour after slaughter (P<0.01 in both genetic types). It tended to increase in incubated biopsy but not significantly (P>0.05).

The changes in pH, myofilament spacing and extracellular space were parallel in post mortem muscle. All these characteristics had almost reached their ultimate values at 1 h after slaughter (an ultimate pH of 5.4-5.5 can be expected in Piétrain pigs killed after minimum stress, as it was the case here). By contrast, myofilament spacing and extracellular space had undergone little change although pH was much decreased in biopsy after 1 h incubation .

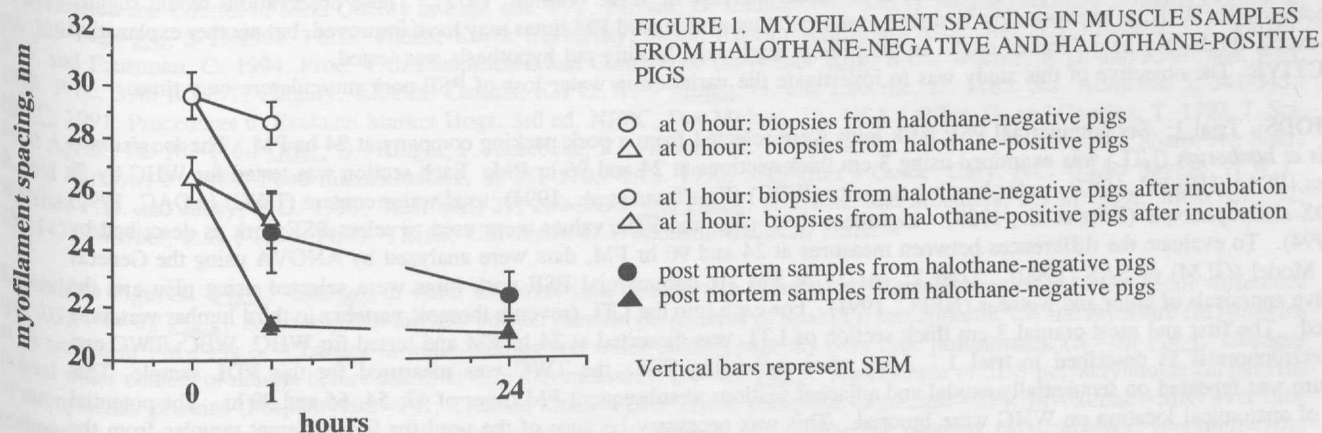

FIGURE 1. MYOFILAMENT SPACING IN MUSCLE SAMPLES FROM HALOTHANE-NEGATIVE AND HALOTHANE-POSITIVE PIGS

CONCLUSIONS.

Changes in myofilament spacing and extracellular space follow pH changes in the Longissimus of slaughtered pigs, but not in muscle taken by biopsy then incubated. In both incubated biopsies and 1 h-post mortem muscle, the pH value was low and close to its ultimate value. However the extracellular space undergone little change in incubated biopsies. This suggests that 1) the pH fall might be not the primary factor responsible for the changes in extracellular space in anoxic muscle, and 2) that stress associated with slaughter could play a prominent role in the post mortem changes of muscle extracellular space in slaughtered animals.

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