Post-mortem shortening of lamb Longissimus dorsi oxidative and glycolytic fibres as affected by temperature, muscle region and skeletal restraint; its relation to meat tenderness.

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SUMMARY:

Percentages of lamb Longissimus dorsi fibre types were about 66 oxidative and 34 non-oxidative, without significant differences among muscle regions. The effects of skeletal restraint and muscle region on sarcomere shortening during rigor development were found to be highly significant; sarcomeres of caudal-ventral location were stretched by skeletal restraint while the rest were all shortened.

In excised muscles both fibre type and postmortem temperature exerted a highly significant effect on sarcomere shortening. Oxidative fibres showed a more intense shortening, already evident at temperatures well above those causing cold shortening .

Fibre shortening was found to be highly correlated to meat toughening.

INTRODUCTION:

Fibre type has been related to different factors associated with meat tenderness, such as pH (Young and Foote, 1984; Zerouala and Stickland, 1991), Z line degradation (Dutson et al., 1974), muscle zone (Totland et al., 1988), muscle shortening (Aalhus and Price, 1991) or conditioning rate (Ouali et al., 1988a; Ouali and Talmant, 1990). A recent review by Ouali (1990) dealt in detail with variability in tenderness and aging rate due to muscle type, though Seideman et al. (1988) found only small but significant correlation coefficients between tenderness and fibre type.

It is also well known that meat tenderness depends upon the sarcomere length of muscles fibres, while Smulders et al. (1990) demonstrated that this relationship is dependent on the post-mortem glycolytic rate.

In this work an attempt was made to relate post-mortem sarcomere shortening of individual fibres with their metabolic type in various regions of Longissimus dorsi muscle subject or not to skeletal restraint. The effect of temperature was also considered, since conflicting results on its influence upon sarcomere length have been reported (Honikel et al., 1986).

MATERIAL and METHODS:

1. Treatment of carcasses and muscles. Excised muscle samples consisted of 20 whole Longissimus dorsi isolated immediately post-mortem from three month-old lamb carcasses. They were maintained at different constant temperatures during rigor mortis development (0°C, 4°C, 10°C, 15°C and 20°C). Another eight muscles were not excised and completed rigor mortis within the carcass at conventional storage conditions (about 4°C). Small samples of the Longissimus dorsi cranialis, caudalis and median regions (dorsal and ventral portions for each region) were dissected after rigor onset and frozen to -160°C in liquid nitrogen. Frozen tissue blocks were stored at -20°C until analyzed. Serial sections (8-10 µm thick) were cut using a cryostat at -20°C.

2. Staining of fibres. Several sections were incubated for reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR) reaction for about 30 min. in a medium containing MTT [3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] as the electron acceptor (Gabe, 1968). The incubated sections were fixed briefly in formol saline, immersed in destilled water and mounted in glyceringelatine.

3. Fibre percentage and sarcomere length determinations. Sections were studied using a graded immersion objective (100X) on a phase contrast Nikon microscope. Type I (red, slow-twitch or oxidative), IIA (intermediate) and IIB (white, fast-twitch or glycolytic) fibres were identified according to their staining intensity. Type IIA fibres were considered together with type I fibres in this study as oxidative.

while type IIB were non-oxidative or pure glycolytic. Groups of 10 sarcomeres of 20 fibres and 10 fields for each studied region and portion were measured in longitudinal (sarcomere length) and cross (area) sections, respectively. Shortening was expressed as the percentage decrease of sarcomere length related to the mean initial value (1,86 μm). Mean shortening of muscle fibres for the study on its correlation with tenderness was calculated by averaging sarcomere lengths found for each type of fibre taking into account their relative areas (mean areas of oxidative and non-oxidative fibres were 1343 and 1723 μm^2, respectively) in muscle.

4. Tenderness evaluation. Overall tenderness was evaluated at 2 days post-mortem by a semi-trained taste panel of 10 members. Sensory scores were rated on a 9-point scale; 9 denoted extremely tender and 1 denoted extremely tough. Evaluation samples consisted of 0.7 cm thick loin steaks trimmed of visible connective tissue and fried with very little oil on a frying pan to an internal temperature of 70°C. Steaks were cut in four sections and two of them selected at random, presented to each panel member for evaluation.

5. Statistical analysis. Data were analyzed by analysis of variance and the significance of the differences between means were tested by multiple range test. Two sets of data were analyzed independently: group I (muscles exposed post-mortem to the same temperature conditions, either with or without skeletal restraint) and group II (excised muscles exposed to five different temperatures).

RESULTS and DISCUSSION:

From the results shown in Table 1 it appeared evident that no differences existed in the percentage of fibre types among the six studied regions of Longissimus dorsi . Only small deviations from average 65,9 percent oxidative and 34,1 non-oxidative glycolytic were found. Results fairly agreed with those reported by Ouali et al (1988b) (79% oxidative, 21% non-oxidative) and Solomon and Montgomery (1988) (69% and 31% respectively) for lamb Longissimus dorsi. Although no studies on the influence of Longissimus dorsi muscle region have been so far reported, Totland et al. (1988) described strong variations depending on Semitendinosus region.

Table 1.- Mean values of percentage of oxidative and glycolytic fibres in 6 different regions of lamb Longissimus dorsi.

	Muscle region					
	Cranial dorsal	Cranial ventral	Median dorsal	Median ventral	Caudal dorsal	Caudal ventral
Oxidative	65,0 ± 13,14	66,3 ±13,09	65,6 ± 9,53	68,0 ± 7,64	64,8 ± 8,68	65,4 ±10,00
Glycolytic	35,0 ± 13,47	33,7 ±13,71	34,3 ± 9,04	32,0 ± 7,55	35,2 ± 8,26	34,6 ± 8,29

Table 2 (I) shows an analysis of variance of the effect of skeletal restraint, muscle region and fibre type on sarcomere shortening of muscle fibres subject or not to restraint during rigor mortis development at 4°C. As expected, skeletal restraint had a highly significant effect, in agreement with Bouton et al. (1973). Sarcomere lengths averaged 1,26 μm for excised muscles and 1,79 μm for those with skeletal attachment.

Muscle region had also a very significant effect; sarcomeres of restrained fibres shortened to a mean value of 1,63 μm in cranial and medial regions, while caudal-dorsal located averaged 1,79 μm and caudal-ventral 2,46 μm. The latter were thus even stretched at rigor mortis. The interaction between both treatments was found to be accordingly significant.

Fibre type did not exert any significant effect on the tightly limited sarcomere shortening of restrained muscles. However, results greatly differed when only data from excised muscles freed to shorten were treated by analysis of variance (Table 2, II). While muscle region had no significant influence, fibre type showed in this case a highly significant effect, with an average sarcomere length after rigor of 1,17 μm in oxidative fibres and 1,30 μm in non-oxidative considering altogether the five studied temperatures. This result agreed with the report of Aalhus and Price (1991), who demonstrated that endurance-exercised sheep increased their proportion of oxidative fibres leading up to an increased sarcomere shortening of muscles.

Table 2.- Influence of several factors on sarcomere shortening. Analysis of variance of the sarcomere length was carried out and F-ratio and significance (*** P< 0,001, N.S. not significant) given. I. Muscles held post-mortem at 4°C, either with or without skeletal restraint. II. Muscles excised from carcasses immediately after slaughter and held throughout rigor development at either 0, 4, 10, 15 or 20°C).

I.	Skeletal restraint (A)	Muscle region (B)	Type of fibre (C)	Interaction (A x B)
F-ratio	71,48	9,45	0,20	14,61
Significance	***	***	N.S.	***
II.	**Muscle region (A)**	**Type of fibre (B)**	**Temperature (C)**	**Interaction (B x C)**
F-ratio	0,45	18,20	34,16	0,87
Significance	N.S.	***	***	N.S.

As shown, oxidative fibres were found to contract more strongly than non-oxidative or pure glycolytic in response to post-mortem conditions. Furthermore, the intensity of shortening depended upon the temperature of muscle conditioning, which exerted a highly significant effect. This effect of decreasing temperature on sarcomere length is well known and was already demonstrated by Honikel et al. (1983,1986) and other authors.

Although no significant interactive effect of fibre type and temperature on shortening could be found, Fig. 1 clearly depicts that oxidative and non-oxidative fibres did not react to equal temperature conditions with the same degree of shortening. Oxidative fibres reached maximum contraction at 15°C and lower temperatures did not cause any further shortening, while non-oxidative fibres shortened with gradual intensity as temperature decreased from 20 to 0°C.

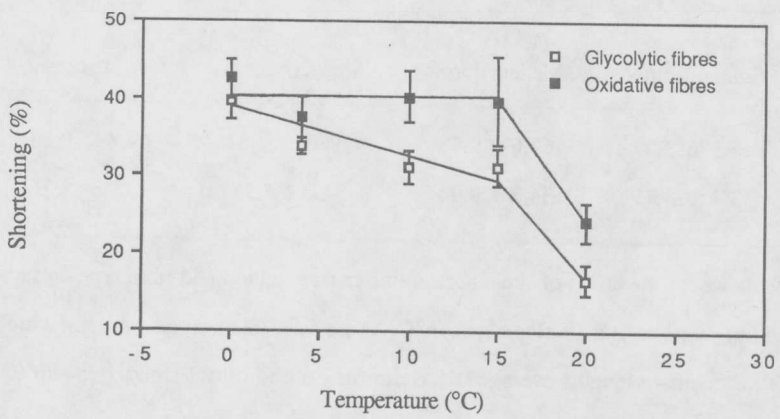

Figure 1.- Influence of temperature on sarcomere shortening for oxidative and glycolytic fibres.

Thus a close relationship seemed to exist between metabolic type of fibres and the shortening response to post-mortem temperature. As expected, Young and Foote (1984) and Aalhus and Price (1991) found that oxidative fibres showed a slower rate of post-mortem pH decrease; this slow-glycolysing behaviour of oxidative fibres would then result, according to Smulders et al. (1990), in an enhanced ability to shorten, even by effect of temperatures within the range 5 -15°C. The different response of oxidative and non-oxidative fibres to those temperatures would satisfactorily explain heterogeneities in sarcomere lengths described by Honikel et al. (1986).

Finally, although not shown, a correlation coefficient of r = -0,60 was found between sarcomere shortening of muscles and sensory tenderness (for details see Material and Methods), thus indicating a highly significant effect of shortening on meat toughening. This expected

result, at least in slow-glycolysing muscles (Smulders et al., 1990), would therefore suggest that the higher the muscle content of oxidative fibres the higher the toughness of resulting meat, and vice versa. This would essentially agree with results reported by Totland et al. (1988), who demonstrated a direct relationship between IIB fibres (non-oxidative) and tenderness, while Seideman et al. (1988) found only small but significant correlation coefficients between tenderness and fibre type.

CONCLUSIONS:

Skeletal restraint and muscle region had a highly significant effect on sarcomere shortening of muscles fibres. On the contrary, in excised muscles region did not affect sarcomere shortening while both fibre type and post-mortem temperature exerted a highly significant effect on it. Oxidative fibres showed always a more intense shortening. Fibre shortening was found to be highly correlated to meat toughening.

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