THE COURSE OF RIGOR AND AGEING REGARDING TENDERNESS OF PORK FROM CARRIERS AND NON-CARRIERS OF THE RN⁻ ALLELE

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Background

The existence of the RN gene was first suggested by Naveau, (1986) and recently identified as the PRKAG3 gene (Milan *et al.*, 2000). Most work concerning the RN gene has focused on the detrimental effect on technological quality caused by the RN⁻ allele (e. g. Monin & Sellier, 1985). However, the RN gene has also been found to be of importance for the eating quality. In the greater part of the literature meat from RN⁻ carriers have been found to be more tender than meat from non-carriers of the RN⁻ allele (e. g. Jonsäll *et al.*, 2000), but no explanation has so far been suggested for the cause of this difference. The specific characteristics of the RN⁻ carriers, including low ultimate pH and high glycogen content, most likely effect rigor development and pH decline, which in turn could be of importance for the tenderness. For beef it has been shown that glycolysis and pH decline plays an important role in tenderness development (e.g. Tornberg *et al.*, 2000).

Objective

To investigate our hypothesis that the cause of the difference in tenderness between RN⁻ carriers and non-carriers lies in the course of rigor. Rigor development was followed by measurements of muscle shortening, isometric tension and pH. During ageing the myofibrillar length, as a measure of proteolytic breakdown and Warner-Bratzler shear force, reflecting tenderness, were measured in the meat.

Material and methods

The effect of the dominant RN⁻ allele on rigor development, ageing and tenderness was studied in *M. longissimus dorsi* (LD) from 11 heterozygous carriers and 5 non-carriers of the RN⁻ allele (based on glycolytic potential). The material was chosen from Hampshire x (Swedish Landrace x Yorkshire) pigs with a pH value above 6.1, measured 30 minutes post-mortem (p. m.) in LD at the last rib, in order to exclude stress-induced PSE meat. Both LDs were excised from the chosen carcasses within 45 min. p. m. Samples for all measurements were cut into pieces of 5-15 cm in length, vacuum-packed and chilled according to the same gradient (13°C at 5 hours; 8°C at 10 hours p. m.).
The ***shortening*** (percentage decrease in the initial length of the muscle strip) and ***isometric tension*** (force per unit area) of the meat was followed continuously during rigor development as described by Hertzman, *et al.* (1993), using a rigormeter (Rigotech, Reologica Instruments AB, Sweden). At 45 minutes p. m. two strips were cut parallel to the fibre axis, with a length of approximately 30 mm and a weight of about 2.0 g. The muscle strips were attached to the rigormeter and covered with paraffin oil to provide an anaerobic environment and to minimise dehydration. The measurements were carried out in a closed chamber under a controlled temperature decline (13°C at 5 hours and 8°C at 10 hours p. m. ±0.5°C). The ***pH*** was measured at 45 minutes and 5, 7, 10 and 24 hours p. m. in the LD muscle at the last rib using a Knick Portamess 911 pH meter and a Xerolyte glass combination electrode. ***Sarcomere lengths*** were measured directly on single fibres from post-rigor meat, using light microscopy (Nikon Optiphot) and an image analysis program, Image Pro Plus 3.0 (Media Cybernetics, USA) as described by Devine et al. (1999). An average of 100 sarcomeres per sample was used. ***Myofibrillar lengths*** were determined at 1, 4 and 7 days p. m. using a method developed at the Swedish Meat Research Institute (Olsson & Tornberg, 1992). Approximately 5 g of LD was homogenised with an isolation medium (I-medium: 100 mM KCl, 20 mM K phosphate, 1 mM EDTA, 1 mM NaN_3, pH 7.0) and centrifuged (2°C, 15 min, 1 000 g). I-medium was added to the sediment and one drop of the suspension was investigated under a light microscope, and analysed with an image analysis system (as above) to determine the length of the myofibrillar fragments. The average of 100 measurements was used for each sample. ***The Warner-Bratzler shear force*** was measured on LD at 1, 4 and 7 days p. m. on an Instron Universal testing machine (4301) equipped with a modified Warner-Bratzler cutting device (Bouton & Harris, 1978). The meat, that had been stored at +4°C, was cut, across the fibre direction, into 3 cm thick slices and fried to an internal temperature of 72°C on a griddle of 165 °C. The maximum shear force for 12 pieces per sample (area of 15x7mm^2 and a length of 20 mm), sheared across the fibre direction, was recorded. The results were statistically evaluated using analysis of variance and independent t-tests in SYSTAT (Wilkinson, Leland, version 7.0) and MINITAB (Release 12, Minitab Inc., USA).

Results and discussion

The pH-time course during rigor development for RN⁻ carriers and non-carriers is shown in Figure 1. As can be seen the decrease in pH was found to be faster for RN⁻ carriers than for non-carriers between 45 minutes and 5 h p. m. ($p=0.006$), after which the rates of pH decline were the same for both groups. Due to the faster pH decline during rigor development, muscle from the RN⁻ carriers reached a significantly lower ultimate pH than that from the non-carriers ($p=0.000$). It is important to point out that the difference in pH decline did not occur in the immediate post-mortem period (i. e. < 1 h p. m.) as can be seen for stress-induced PSE meat (pH<5.9 at 1 h p. m.).

From the rigor measurements it was found that the degree of shortening (SH) did not differ significantly between the two genotypes. There was a tendency for the sarcomeres to be shorter in LD from non-carriers than from RN⁻ carriers (1.76 ± 0.11 and 1.86 ± 0.07 µm), but the degree of shortening and the course of SH during rigor development was found to be the same in both genotypes (approximately 30%).

Figure 2 shows the time courses of rigor development as followed by isometric tension (IT) using the Rigotech instrument. The RN⁻ carriers and non-carriers followed more or less the same course until 8 h p. m., after which the IT for non-carriers increased by a greater extent than the RN⁻ carriers. The maximum values of IT, of 4.0 and 5.8 kPa, were reached at 12 and 16 h p. m. in the muscles from RN⁻ carriers and non-carriers, respectively. For the RN⁻ carriers a small decline in IT was also observed between 12 h and 24 h p. m. The differences between genotypes in maximum IT and IT at 24 h p. m. were significant ($p=0.002$). Isometric tension has been suggested to be a reflection of the sum of shortening and proteolytic activity early p. m. (Devine *et al.*, 1999). The lower IT in RN⁻ carriers would then suggest a higher enzymatic activity in RN carriers than in non-carriers.

The myofibrillar fragmentation length as a function of time, which reflects the structural change caused by the proteolytic action, can be seen in Figure 4. The RN⁻ carriers were found to have significantly shorter myofibrils than non-carriers at 1 and 4 d p. m., indicating a higher proteolytic activity early post-mortem in the RN⁻ carriers. After 4 days the length of the myofibrils from RN⁻ carriers did not change, whereas the myofibrils from non-carriers shortened until 7 d p. m. The increased ageing rate in RN⁻ carriers was further seen by the results of the Warner-Bratzler shear force measurements. Meat from RN⁻ carriers exhibited a significantly lower shear force than that from the non-carriers at 1 d p. m., and the meat from non-carriers needed 7 days of ageing to reach the same tenderness attained by LD from RN⁻ carriers 4 d p. m.

(Figure 3). These results are in agreement with the results obtained by van Laack *et al.* (2001). They found LD from carriers of the RN- allele to be significantly more tender than non-carriers at 2 d p. m. but not at 7 d p. m.

Van Laack *et al.* (2001) also measured myofibrillar degradation (as 30 kDa/actin ratio) and found an increased myofibrillar degradation with decreasing pH_u, indicating a relationship between pH decline and proteolysis. Despite this no difference due to RN genotype was seen. For both beef and pork it has been shown that the rate of pH decline is of importance for the tenderness. O´Halloran *et al.* (1997a) and Tornberg, *et al*, (2000) found rapidly glycolysing LD muscles (pH 5.6 at 4-6 h p. m.) from beef to be more tender than slowly glycolysing muscles, and suggested proteolysis to be the major cause of the differences in tenderness. It should be mentioned that also a more rapid pH decline resulted in tougher meat indicating an optimum in pH decline (Tornberg *et al.*, 2000). O´Halloran *et al.*, (1997a and b) showed evidence of increased proteolysis, such as earlier appearance of the 30 kDa fragment, higher overall activity of calpains and enhanced release of cathepsins B and L in rapidly glycolysing muscles (pH_{6h}: 5.6). This is in agreement with the results of Maribo *et al.* (1999) on electrically stimulated pork. They found higher tenderness as well as increased activities of cathepsins B+L in rapidly glycolysing muscles (pH_{6h}: 5.7).

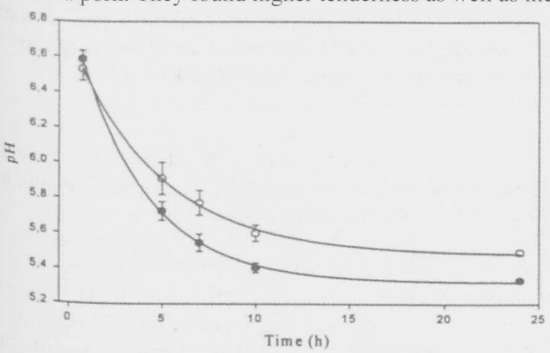

Figure 1. The pH as a function of time during rigor development in LD from RN⁻ carriers and non-carriers. Mean values are shown with standard errors.

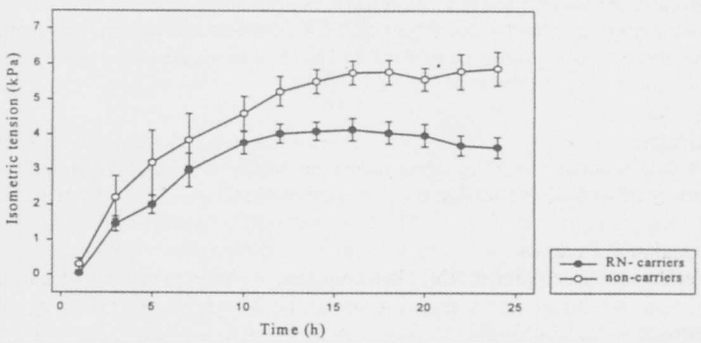

Figure 2. Isometric tension as a function of time during rigor development in LD from RN⁻ carriers and non-carriers. Mean values are shown with standard errors.

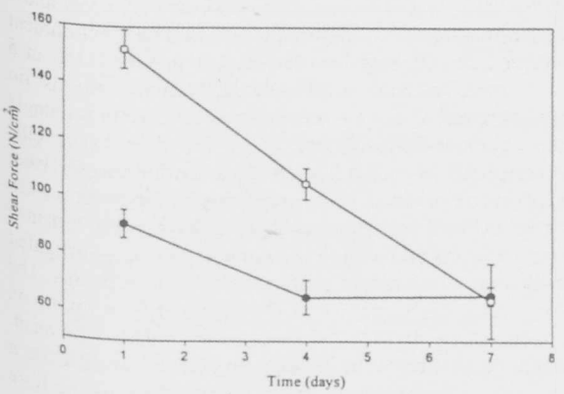

Figure 3. Shear force as a function of time during ageing of LD from RN⁻ carriers and non-carriers.

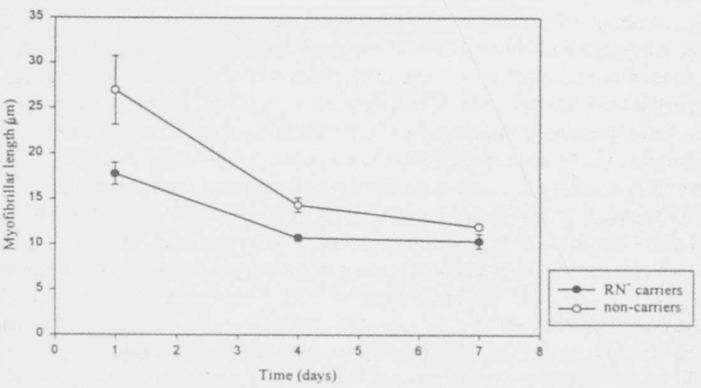

Figure 4. Myofibrillar fragmentation length as a function of time during ageing of LD from RN⁻ carriers and non-carriers.

Conclusions

In conclusion, differences observed in the course of rigor and ageing in muscle from carriers and non-carriers of the RN⁻ allele suggest that proteolytic action, as initiated by a more rapid fall in pH, is the most important factor governing the variation in tenderness of the two genotypes.

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