AGE-RELATED CHANGES IN COLLAGEN CHARACTERISTICS OF PORCINE LOIN AND HAM MUSCLES

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BACKGROUND

Collagen, the main component of muscle connective tissue, greatly influences beef toughness. Properties rather than concentration of collagen play an important role in determining meat quality (Bailey and Light, review, 1989). Concerning pig meat, gelatin resulting from collagen thermal solubilization is involved in the cohesion of cooked products, for example ham slices. It can be expected that, as observed in the bovine species (Hill, 1966; Bailey and Light, 1989), the properties of muscle collagen can change with age in the pig.

OBJECTIVES

The purpose of this study was to describe the age-related changes in intramuscular collagen characteristics of pig muscles from birth up to usual slaughter stage. Two muscles differing in their anatomical location (loin *vs* ham) and amount of collagen (Baland and Monin, 1987) were used : *Longissimus dorsi* (LD) and *Biceps femoris* (BF). Collagen content and heat-solubility were determined, and immunohistolocation of type I, III and IV collagens was investigated on both muscles taken at four different stages of growth.

METHODS

A total of 32 Large White x (Large White x Landrace) pigs were used. They were slaughtered at 4 stages : birth (1.5 kg body weight (BW) / one day) (1), beginning of the fattening period (30 kg BW / 79 days) (30), middle of the fattening period (70 kg BW / 128 days) (70), and usual slaughter stage (105 kg BW / 164 days) (105). Each group contained 8 pigs (4 castrated males and 4 females) from 8 different litters. Pigs were fed *ad libitum* with standard diets all along the experiment.
Muscle samples were taken on the day after slaughter. For stage 1, the entire LD and BF muscles were taken. At the other stages, a 3-cm thick transverse slice was taken from the middle part of LD (at the last rib level) and BF muscles, trimmed of epimysium, minced, freeze-dried, powderized and stored under vacuum at -20°C before biochemical analyses. From one pig per group, a small piece of LD and BF muscles was taken in the middle part of the slice, frozen in isopentane cooled by liquid nitrogen and stored at -80°C until histological analyses. Hydroxyproline content was determined according to Bergman and Loxley (1963). Data were multiplied by 7.14 (Etherington and Sims, 1981) and collagen content were expressed as mg per g of dried muscle. Heat-solubility was determined according to Hill (1966) and expressed as percentage of collagen content. All determinations were performed in triplicate. Muscle location of type I, III and IV collagens was investigated on transverse serial sections using rabbit anti-bovine type I, anti-bovine type III and anti-human type IV collagen antiserums (Institut Pasteur, Lyon, France), and fluorescein (FITC)-conjugated mouse anti-rabbit antibody (Jackson, West Grove, PA, USA) as second antibody. Biochemical data were submitted to an analysis of variance using the SAS GLM prodecure (SAS, 1989). Stage of growth and sex were considered as fixed effects in the model.

RESULTS AND DISCUSSION

Content and heat-solubility of collagen. In LD muscle, collagen content markedly decreased from birth up to stage 105 ($p<.001$), particularly between birth and stage 30 (50%) (Table 1). The percentage of heat-soluble collagen decreased from birth up to stage 105, rapidly at the beginning of growth and more and more slowly thereafter. No influence of sex was found on collagen content, whereas castrated males tended to show a higher collagen heat-solubility than females (average across stages of 30.9 and 27.5%, respectively, $p=.06$). Collagen content of BF muscle was higher at birth than at any other stages of growth (Table 1). It strongly decreased between birth and stage 30 (50%, $p<.001$), as observed in LD muscle, but remained quite constant thereafter. Heat-solubility of BF collagen followed the same general trend than in LD, with a decrease all along the growth, particularly during the first months of life. Sex had no influence on amount or heat-solubility of collagen in BF. In both muscles, the decrease in heat-solubility during growth was due to a large decrease in soluble collagen content all along this period, specially between birth and stage 30 (60%, $p<.001$), whereas amount of insoluble collagen decreased between birth and stage 30 (40%, $p<.001$) and remained constant thereafter. For each stage of growth, collagen content was higher in BF than in LD (30%, $p<.001$) in accordance with results obtained in 90 to 100 kg live weight pigs by Baland and Monin (1987) and Pertersen et al. (1994). Collagen heat-solubility did not differ between LD and BF whatever the growth stage, as reported by Boccard et al. (1979) in the bovine species. The higher collagen content observed at birth as well as the general trend of muscle collagen content during growth are consistent with results obtained in bovine muscle from neonatal period up to adult stage (Boccard et al., 1979; Nishimura et al., 1996), and in pig muscle betwen 29 and 64 kg BW (Andersen et al., 1992). Even though a large increase in myofiber diameter was observed during postnatal growth up to stage 105 (figure 1), leading to a "dilution" of intramuscular collagen, the variations in LD and BF collagen content between stages 30 and 105 were rather small, suggesting that some collagen is still synthesized in muscle at stage 105. The decrease in collagen heat-solubility during growth observed in this study is in agreement with earlier findings of Hill (1966), Boccard et al. (1979) and Nishimura et al. (1996) in beef muscles. The enduring decrease in collagen solubility at stage 105 means that collagen crosslink maturation still takes place at this stage of growth.
Immunohistolocation of collagen types. Specificity and control of antibodies were tested in the pig as described by Listrat et al. (1998). Since no difference was observed between LD and BF muscles, results are presented only for LD (Figure 1). From birth up to stage 105, type I and III collagens are located in perimysium and endomysium, while type IV is located exclusively in endomysium. Whatever the stage of growth, immunolabelling with anti-type I collagen antibody is very strong and much weaker with anti-type III, particularly in perimysium. In endomysium, labelling with anti-type IV is higher than with anti-type I and III collagen antibodies. Our

observations are in accordance with previous results obtained on bovine muscles from foetuses (Listrat et al., 1998) and adults (Bailey and Light, 1989).

CONCLUSION

The present results show large age-related changes in amount and heat-solubility of porcine muscle collagen, whereas location of type I, III and IV collagens was not modified from birth up to usual slaughter stage. Major decrease in collagen content occured during the first months of growth, while collagen heat-solubility was still decreasing up to 105 kg BW (5.5 months old). This suggests that slaughter age of pigs could therefore be an important factor in the determination of the textural properties of meat and meat products.

LITERATURE

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Table 1. Influence of growth stage on amount and heat-solubility of collagen in *L. dorsi* and *B. femoris* muscles.

	L. dorsi					*B. femoris*				
	1	30	70	105	Sign[a]	1	30	70	105	Sign[a]
Collagen (mg/g)	42.6 a	21.7 b	19.9 bc	17.0 c	***	59.7 a	28.7 b	27.9 b	26.4 b	***
Heat solubility (%)	43.8 a	33.2 b	22.5 c	17.4 d	***	45.8 a	32.6 b	25.0 c	18.2 d	***

[a] *** = P <.001. Means within row affected different letters differ significantly (P <.05).

Figure 1. Immunohistochemical analyses with antibodies directed against type I, III and IV collagens on tranverse serial sections of *L. dorsi* muscle taken at different stages of growth. P : perimysium; E : endomysium (X 20).