THE RELATIONSHIP BETWEEN COLLAGEN CHARACTERISTICS IN MUSCLE AND MARBLING OF BEEF

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Introduction

Marbling is a factor determining the quality grade of beef meat in North America, Asia and Australia. It refers to the appearance of white flecks or streaks of adipose tissue within connective tissue of muscles between the bundles of muscle fibres. It is thus closely linked to intramuscular fat (IMF) content. In continental Europe, IMF content in beef is lower, but it plays a major role in determining flavour. Indeed, flavour score markedly increases with increasing IMF content (Goutefongea and Valin, 1978).

The number and the diameter of intramuscular adipocytes are major factors determining IMF content and hence marbling (Cianzo *et al.*, 1985). In addition, metabolic activity of muscles was shown to differ between bovine breeds (Angus *vs* Limousin) with different ability to develop marbling (Hocquette *et al.*, 2003). The hypothesis of this work was that the characteristics of the connective tissue in which intramuscular adipocytes are included may differ between muscles of Angus and Limousin breeds as well as between muscle types. To test this hypothesis, we have determined muscle contents of type XII and XIV collagens which were previously thought to play a role in adipocyte differentiation (Ruehl *et al.*, 2005; Tahara *et al.*, 2004).

Materials and Methods

Two groups of 10 steers from two different breeds were used as previously described (Hocquette *et al.*, 2003): Angus which produces a marbled meat and Limousin which produces a weakly marbled meat. All animals had a long (6 months) finishing period with a similar cereal-rich diet [rolled wheat (47-50%), triticale (17-18%), hay (14-18%), lupins (9%), Metabolizable energy: 12 KJ/kg DM, Crude Protein: 15%], which allowed them to express their genetic potential for IMF deposition.

Animals were slaughtered at 23 months of age and samples of two muscle types were taken: *Rectus abdominis* [RA] (oxidative), and *Semitendinosus* [ST] (glycolytic). In all samples, activities of oxidative enzymes (isocitrate dehydrogenase [ICDH], cytochrome-*c* oxydase [COX]), glycolytic enzyme (phosphofructokinase [PFK]) as well as protein contents were measured as described by Hocquette *et al.* (2003). The muscle contents of two isoforms of fatty acid-binding proteins specific of muscle fibres (H-FABP) or intramuscular adipocytes (A-FABP) were determined by ELISA (Hocquette *et al.*, 2003). Total fat and triacylglycerol (TAG) contents was measured as described (Hocquette *et al.* 2003). All results were expressed per g tissue wet weight (for TAG and FABP contents) or per mg of protein in muscles (for enzyme activities).

Muscle collagen content was determined from the hydroxyproline concentration (Listrat *et al.*, 2004). The relative contents of type XII and XIV collagens within muscle were measured following western-blotting analysis. Each sample was measured in triplicate and results were expressed in arbitrary units.

Differences between genotypes and muscles were analyzed by variance analysis using SAS. Fixed effects included genotype, animal nested within genotype, muscle type and interaction between muscle and genotype. The effect of genotype was tested against animals within genotypes. A correlation analysis between the different parameters was also realized followed by a regression test to determine if the slope of the regression line was significantly different of zero.

Results and Discussion

In a previous paper (Hocquette *et al.*, 2003), we showed that the Angus muscles had a higher IMF content, as well as a more oxidative and a less glycolytic muscle metabolism that the Limousin muscles. In this paper on the same animals, we showed that the breed effect was highly significant for both total collagen and type XIV collagen contents ($P < 0.001$). For the latter, there was no muscle effect. These two parameters were indeed higher (x 1.6 and 1.7, respectively) for the Angus breed that for the Limousin breed. For type XII collagen, there was no breed effect but a significant muscle effect ($P < 0.01$), type XII collagen content being higher in RA than in ST (x 2.5). The interaction between muscle and breed effects was never significant.

High and positive correlations between protein contents of the two fatty acid binding protein (FABP) isoforms and the relative amounts of type XII and XIV collagens were observed (Table 1) when all 40 samples were included in the analysis (two muscles from two groups of ten steers). The correlations were higher for collagen XII than for collagen XIV.

	Collagen XIV		Collagen XII	
TAG	R	+0.34*		NS
A-FABP	R	+0.39*	R	+0.48**
H-FABP	R	+0.42**	R	+0.54***
COX	R	+0,28 t	R	+0.42**
ICDH	R	+0.38*	R	+0.42**
PFK	R	-0.32*		NS
LDH	R	-0.43**		NS

Table 1: Coefficients of correlation between collagen type XII and XIV relative contents and metabolic characteristics of muscles (NS: not significant; t: $P < 0.10$; *: $P < 0.05$; **: $P < 0.001$).

Some positive correlations were also observed between the contents of the two studied collagen types and the activities of oxidative enzymes.

We can thus conclude that type XII collagen is a relevant indicator of muscle type, especially associated with intramuscular fat differentiation (from its correlation with A-FABP content) and oxidative metabolism (from its correlation with H-FABP content, COX and ICDH activities), but not with IMF content. By contrast, type XIV collagen is breed specific and hence associated with muscle parameters (including IMF content) which discriminate breeds.

Recent *in vitro* studies with preadipocytes from a bovine intramuscular cell line (Tahara *et al.*, 2004) and with 3T3-L1 preadipocytes (Ruehl *et al.*, 2005) suggested that collagen types may affect adipogenesis. Our *in vivo* results support this idea since type XII and XIV collagen contents were both correlated with A-FABP content, a marker of intramuscular adipocyte differentiation. But, although both type XII and XIV collagens were associated with the metabolic activity of muscles, only type XIV collagen was associated with IMF content. This is consistent with the fact that these two collagen types could have distinct functions.

The meat of Angus is considered to be tender and tasty due to its greater ability to lay down intra-muscular marbling fat during the finishing phase. The development of adipose tissue has been proposed to disorganise the structure of the intramuscular connective tissue (Nishimura *et al.*, 1999). In addition, both type XII and XIV collagens could participate in extracellular matrix deformability, by making it more rigid or more flexible according to the constraints on the tissue (Nishiyama *et al.*, 1994). The higher proportion of type XIV collagen in the Angus muscles could favour the development of IMF or could be due to the disorganisation of the connective tissue by IMF

Conclusions

From our *in vivo* results, we can hypothesize that type XII and XIV collagen contents may be associated with metabolism of intramuscular lipids. We thus confirm previous *in vitro* studies (Tahara *et al.*, 2004; Ruehl *et al.*, 2005). Both type XII and XIV collagen may contribute to IMF accumulation, but probably by different biological mechanisms since type XII collagen content mainly differs between muscle types and type XIV collagen content mainly differs between breeds.

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