# DIFFERENCES IN MUSCLE FIBRE TYPE IN LAMB RELATE TO DIFFERENCES IN COLLAGEN SOLUBILITY AND PERI-NATAL MMP ACTIVITY.

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**Abstract – Differences in cooked meat tenderness between muscles are known to be partly due to their intramuscular connective tissue (IMCT) characteristics and may also be due to differences in muscle fibre type composition. The hypothesis that the activity of matrix metalloproteases (MMPs) responsible for IMCT degradation is related to the muscle fibre-type muscle distribution was studied in the present work. The majority of MMP expression within muscle is by the muscle fibres. Lamb *gastrocnemius* and *sternocephalicus* muscles showed differences in fibre-type distribution and the heat-solubility of IMCT collagen, and also showed differences in collagenase activity. A correlation of higher collagenase activity and a higher collagenase activity with the proportion of oxidative muscle fibres in the muscles supports the hypothesis that more oxidative muscle fibres tend to express more MMPs. This initial study on two lamb muscles will be extended to more lamb muscles and specific MMP activities to expand knowledge on this relationship.**

**Key Words –metalloproteinase, post-mortem, tenderness.**

## I. INTRODUCTION

It is well-known that different muscles in the carcasses of meat animals show different values of tenderness after cooking [1]. Such variations occur between muscles in lamb as well as in cattle [2]. Variations between muscles that contribute to variations in tenderness are thought to be partly due to variations in the properties of intramuscular connective tissue [3] and to variations in the post-mortem process of proteolysis of myofibrillar proteins, due in part to variations in enzyme activity. One factor contributing to differences in post-mortem metabolism is the muscle fibre-type composition of the muscle, which is determined during life by the physiological demands on the muscle [4;5]. Cha and Purslow [6] demonstrated that the great majority of collagenase activity (principally due to the action of matrix metalloproteinases, or MMPs) is actually produced by the muscle fibres rather than intramuscular fibroblasts. This paper tests the hypothesis that the MMP activity in a muscle is related to the muscle fibre-type distribution in this muscle, and that the heat-solubility of the intramuscular collagen within the muscle may in turn be affected by this.

## II. MATERIALS AND METHODS

*Animal and muscle samples*

Six lambs (Corriedale x Texel crossbreed) were raised at the university farm under standard conditions until 8 months old and 45 (± 8.6) kg in body weight. All were slaughtered on the same day. Within 15 min of the time each animal was killed, samples from the right-hand side of the carcass of the *M. Gastrocnemius* (GT) and *M. Sternocephalicus* (SP) muscles were taken for analysis. Muscles samples (≈5 g) were immediately frozen in carbon dioxide snow for later enzyme (MMP and SDH) detection. These samples were stored at -80 °C for determination of enzymatic activities, histochemistry and chemical analysis. The remainder was frozen at -20 °C for collagen analysis.

*Total and Soluble Collagen Content*

Muscles collagen were quantified according to the procedure of Latorre et al.[7]. Supernatant fluids and solid residues were separated and hydroxyproline quantified by HPLC-Fluorescence [8]. The percentage (%) of soluble collagen was calculated as 100 × the hydroxyproline content of the soluble phase divided by the total hydroxyproline in both the soluble phase and the solid residue.

*Enzymatic MMP activities*

Frozen tissue samples (≈ 1000 mg) were homogenized in a buffer containing 100mM Tris, 200mM NaCl and 0.1% Triton X-100 (pH 7.4) according the method of Cha and Purslow [6]. The protein concentration in the supernatant was

quantified by the Bradford method [9]. Total gelatinolytic/collagenolytic (TGC) activity in the supernatant of tissue homogenates was determined using the EnzCheck® Gelatinase/Collagenase Assay Kit (Molecular Probes, OR, USA).

*Enzyme histochemistry to determine muscle fibre- type*

Muscle specimens frozen at -80 °C were washed in ice cold PBS buffer and resuspended in dry ice before being cut in cryostat set at -20°C. The resulting sections were stained for SDH activity [10].The sections were viewed under a Leica 500 microscope and photographed (Leica, Microsystems, Switzerland,) using the Leica Application Suite (LAS) including DFC Twain software for image capture. The number of positively stained cells (positive reaction=dark colour) were analysed and quantified using Fiji software (Fiji processing package, ImageJ, GNU General Public License) and the percentage of darkly stained (Fibre-Types I & IIa) versus Intermediate and palely stained fibres (Fibre-Types IIx & IIb) was calculated.

*Statistical analysis*

Data were expressed as mean and standard errors (SE) of six animals for both muscles. Data were statistically analysed by Student's-T test. Differences were considered significant at $p< 0.05$.

## III. RESULTS AND DISCUSSION

Total collagen and percentage soluble collagen for each muscle is shown in Table 1. The GT and SP muscles in lamb present noticeable differences in total collagen, reflecting their different *in-vivo* functions. However, the percentage of heat-soluble collagen does not differ greatly between these two muscles.

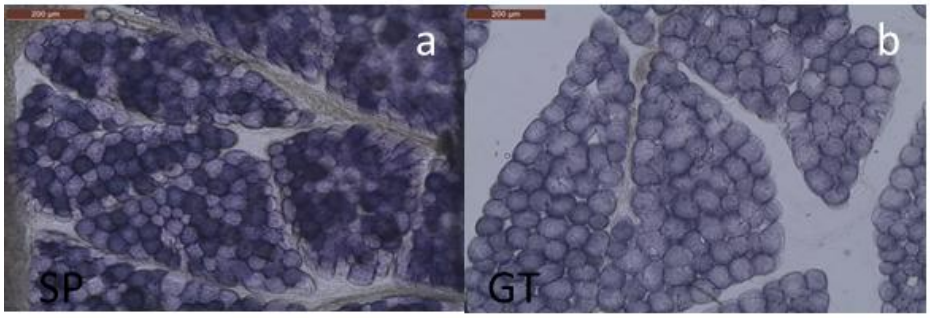
Values of enzyme activity in each muscle (pooled across all animals, Table 1) showed that the total gelatinase/collagenase activity was lower in the GT than the SP muscle. However, the SP muscle samples presented two clear "groups" of animals. One set of three animals with higher activities (229 U/mg protein) and the other with lower activity (19.8 U/mg protein). Despite these differences, the SP/GT activity ratio for all animals was >1 (1.3-9.2). Sylvestre et al. [12] showed that the MMP-2 activities in lamb muscle and intramuscular connective tissue solubility varied with growth rate.

Table 1 Total collagen, soluble collagen content and peri-mortal gelatinase/collagenase activities in *Gastrocnemius* (GT) and *Sternocephalicus* (SP) muscles from lamb. N=6 in all cases.

| | GT | ST |
|---|---|---|
| Total Collagen (g/100g wet tissue) | 10.7 ± 1.2 (0.59)* | 4.42 ± 0.80 (0.40)** |
| % Soluble collagen | 1.55 ± 0.38 (0.15) | 2.09 ± 0.48 (0.24) |
| U/mg protein | 26.1 ± 6.25 (3.13) | 125 ± 158 (79) |
| U/g wet tissue. | 974 (80) | 5341 (3333) |

All the results are average, standard deviation and numbers in parentheses represent SE.
Asterisks (* ;**) indicated significantly different between pair muscles, by t-test ($p< 0.05$).

The transverse histological sections of muscles stained with the SDH technique showed normal muscle structure without pathological damage. Figure 1a shows micrographs from cryosections of both muscles stained for SDH. There are clear differences in muscle fibre-type between the two muscles. As expected, the SP muscle contains mainly "oxidative" fibres (Type I and IIa). In contrast, the GT muscle principally contains fibres with intermediate-pale staining for SDH (type IIx and IIb).




Figures 1- Frozen section of **a)** *Sternocephalicus* (SP) and **b)** *Gastrocnemius* (GT) muscle from lamb, stained for succinate dehydrogenase activity.

Figure 2 shows the percentage of the deep and intermediate-pale staining groups for SP and GT lamb muscle. There is an association between the higher proportion of oxidative fibres in the SP muscle and the higher collagenase/gelatinae activity, in comparison with the GT muscle. This result confirms the hypothesis put forward by Cha and Purslow [6] that MMP activity muscles may be related to the muscle fibre-type composition of each muscle. We also note that % of heat-soluble collagen was higher in the SP muscle, suggesting that the higher collagenase/gelatinase activity present at the time of slaughter in the muscle may affect the thermal stability of is intramuscular collagen.

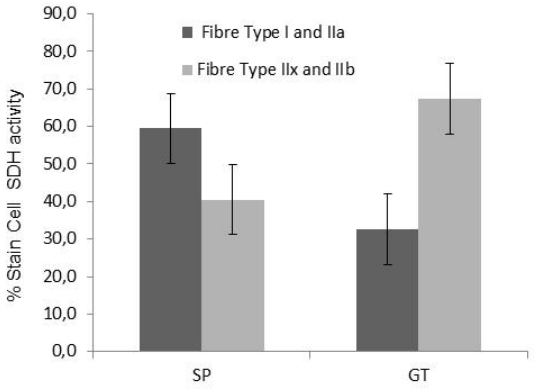



Figure 2- Muscle fibre-type composition as percentage (%) of cells stained dark (oxidative, corresponding to Types I & IIa) and Intermediate/Pale (Types IIx & IIb). Error bars correspond to one standard deviation (n=4).

## IV. CONCLUSION

This study shows that muscle fibre-type differences between two muscles in lambs are also related to the activity of enzymes responsible for degrading intramuscular tissue and the heat-solubility of intramuscular collagen. Results indicated that it could be possible a relation between proportion of highly-oxidative fibres with higher collagenase activity and a higher percentage of heat-soluble collagen. This study on two muscles from lambs warrants extension into a study on a wider range of functional muscles.

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