Determination of *perimysium* and *endomysium* thickness in bovine, ovine and caprine *Semimembranosus* and *Semitendinosus* muscles by video image analysis.

Gastón Torrescano†, Lluís Luján*, Armida Sánchez-Escalante†, Begoña Giménez, Pedro Roncalés and José Antonio Beltrán.

Dept. of Animal Production and Food Science. University of Zaragoza. C. Miguel Servet 177. 50013 ZARAGOZA, SPAIN. (1)
† Affiliated with Centro de Investigación en Alimentación y Desarrollo A.C., Apartado postal 1735, Hermosillo, Sonora 83000, México; presently at. (1)
* Dept. of Animal Pathology. University of Zaragoza. C. Miguel Servet 177. 50013 ZARAGOZA, SPAIN.

BACKGROUND

Tenderness of meat is the sum total of the mechanical strength of skeletal muscle tissue and its weakening during post-mortem aging of meat. The former depends on species, breed, age, sex and individual skeletal muscle tissue of animal and fowl (Takahashi, 1996). Stanton & Light (1988) indicated that subtle modifications occur in perimysial collagen during conditioning which can be correlated with catheptic action on this component of meat connective tissue and which probably led to the physical weakening of the matrix. Post-mortem changes in *endomysium* and *perimysium* during chicken aging showed that these structures disintegrated into several thin sheets within 12 h post-mortem, and that many gaps were opened in the cross-section of *endomysium* and *perimysium* (Liu *et al*, 1994). Schmitt *et al.* (1979) informed that the perimysial network is arbitrarily separated in different levels of organization, the primary is the thicker and surrounds large muscle fiber bundles which are subdivided into smaller ones by a thinner network, the so-called secondary perimysium. The thickness of perimysial sheets, as determined by microscopy, ranges from 10 μm for the secondary to 100 μm for the primary.

OBJECTIVE

In the present work the objective was to visualize and measure the distribution and the thickness of the *perimisyum* and *endomysium* in two muscles from different animal species using video image analysis.

METHODS

Samples. The species used were bovine (Swiss Brown breed), caprine (Murciano-Granadina breed) and ovine (Rasa Aragonesa breed), all of them about one year old. Whole *Semimembranosus* (SM) and *Semitendinosus* (ST) muscles were excised and a portion from the center of each muscle was cut parallel to the muscle fibers. Approximately 1 cm^3 samples of both muscles were taken 24 h post-mortem, frozen with isopentane in liquid nitrogen (-160 °C) and stored at -80 °C.

Histology. Six serial 10 μm thick muscle sections (cut perpendicularly to the muscle fiber) were obtained from each sample with a cryostat (Reichert-Jung GmbH) at -20 °C. The samples were stained using the Picro-Sirius red method of Flint and Pickering (1984).

Video image analysis (VIA). The samples were examined using an operational system consisting of an optical microscope (Leitz Laborlux 12) equipped with a high definition CCD (Charge-Coupled-Device) colour camera (Hitachi VK-C220E). The video camera was connected to a computer with specific software for digital image analysis (MIP 4 ADV, Consulting de Imagen Digital, S.L. & Microm España). A PAL standard 50Hz colour video system was used for image digitalization. The six serial sections were digitized using a X4 objective and a X10 ocular. Interactive analysis were used to measure the thichness of *endomysium* (En) and *perimysium* (Pe).

Statistical analysis. All determinations were made taking 10 replicates for every sample making a total of 60 determinations for both *endomysium* and *perimysium*. The statistical analysis was performed with SPSS (Statistical Package for the Social Sciences) for Windows version 7.5.2s (1995). Significance of differences among muscle samples were determined by analysis of variance (ANOVA) using the Least Square Difference method of the General Linear Models (GLM). Differences were considered significant at the $P<0.05$ level.

RESULTS AND DISCUSSION

Figure 1 shows representative images of interactive analysis measured by VIA using muscle tissue stained with Picro-Sirius and observed under a light microscope. The primary Pe observed in the figure shows a varying thickness among species and muscles. Meanwhile, the En presents visually only a small variation among species. Table 1 shows the results of Pe and En thickness of SM and ST muscles from of all the three species evaluated by VIA. Bovine SM muscle ($P<0.05$) presented the thickest primary Pe, secondary Pe and En. The same results were obtained in ST muscle. Bovine, caprine and ovine species had significant differences ($P<0.05$) in their primary Pe in both muscles. Secondary Pe presented no significant differences in both muscles in caprine and ovine species, while significant difference ($P<0.05$) with bovine was observed in both muscles, too. *Endomysium* showed the same results in SM muscle than those obtained in secondary Pe, but significant differences were observed ($P<0.05$) in ST muscle among species, being the ovine that presented the thinnest En in this muscle.

Micrographs of figure 1 show a large difference in thickness between the primary *perimysium* and the secondary one. It is important to take into account that the values (table 1) of the primary Pe are higher than those of the secondary Pe, in both muscles and all the species, but specially in figure 1F for Bovine ST. Bonny *et al.* (2000) reported the same results for *Gluteo Biceps* and *Pectoralis profundus* muscles; they found a large variability in primary Pe in both muscles (40 to 115 μm and 27 to 100 μm, respectively), which confirms the great variation in primary Pe thickness. If we consider thinner sheets of secondary Pe, the values obtained would modify the final result (means) and the standard deviation too.

The results of primary Pe in bovine ST muscle are in agreement with the results of Nishimura *et al.* (1995), who reported a Pe thickness of 100-200 μm. Primary Pe of ovine ST (Fig 1e) shows longitudinal gaps, suggesting the existence of two or more layers, in good agreement with the results of Nishimura *et al.* (1995) for bovine ST muscle. On the other hand, when increasing aging time, some holes (gaps) are observed (in the perimisium mainly) due probably to a weakening of the Pe structure, which after staining would give rise to a measuring error (results not shown).

CONCLUSION

Results of the measurements by VIA of intramuscular connective tissue from SM and ST muscles indicate that bovine specie have thicker primary Pe, secondary Pe and En than caprine and ovine. These results are of primary importance in order to explain the contribution of connective tissue to meat tenderness.

REFERENCES

Flint, F.O. & Pickering, K. (1984). Demonstration of collagen in meat products by an improved picro-sirius red polarization method. Analyst. 109, 1505-1506.

Bonny, J.M., Laurent,W., Labas, R., Taylor, R., Berge P. & Renau, J. P. (2000). Magnetic resonance imaging of connective tissue: a non-destructive method for characterizing muscle structure. Journal of the Science of Food and Agriculture. 81, 337-341.

Liu, A., Nishimura, T. & Takahashi, K. (1994). Structural changes in endomisyum and perimysium during postmortem aging of chicken semitendinosus muscle- Contribution of structural weakening of intra muscular connective tissue to meat tenderization. Meat Science. 38, 315-328.

Nishimura, T., Hattori. A. & Takahashi, K. (1995). Structural weakening of intramuscular connective tissue during conditioning of beef. Meat Science. 39, 127-133.

Schmitt, O, Degas, T., Perot, P., Langlois, M. R. et Dumont, B. L. (1979). Etude morpho-anatomique du périmysium (methodes de description et d'évaluation). Ann. Biol. Anim. Bioch. Biophys. 19, 1-30.

SPSS. (1995). SPSS for Windows, 7.5.2.s. SPSS Inc. Chicago, Il.

Stanton, C. & Light, N. (1988). The effects of conditioning on meat collagen: Part 2. Direct biochemical evidence for proteolytic damage in insoluble perimysial collagen after conditioning. Meat Science. 23, 179-199.

Takahashi, K. (1996). Structural weakening of skeletal muscle tissue during postmortem ageing of meat: the non-enzymatic mechanism of meat tenderization. Meat Science. 43, S67-S80.

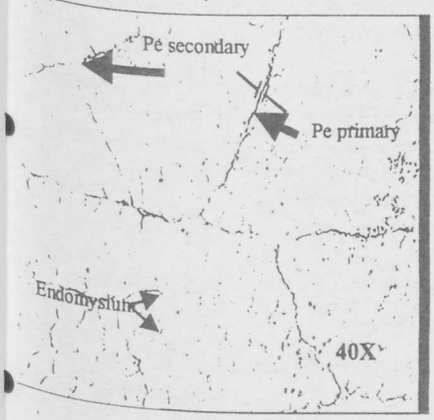
Table 1. Comparison of means (± standard errors) of *perimisyum* (Pe) and *endomysium* (En) thickness in *Semimembranosus* and *Semitendinosus* muscles in three animal species.

M. *Semimembranosus*			
	Connective Tissue		
A. Species	primary Pe	secondary Pe	Endomysium
CAPRINE	$20.39^{3a} \pm 1.94$	$11.06^{2a} \pm 1.21$	$10.07^{1a} \pm 1.64$
OVINE	$28.17^{3b} \pm 4.55$	$12.12^{2a} \pm 1.38$	$10.37^{1a} \pm 1.54$
BOVINE	$111.29^{3c} \pm 9.37$	$33.13^{2b} \pm 4.13$	$14.81^{1b} \pm 2.83$

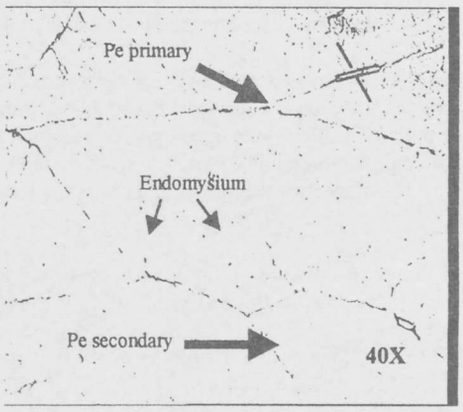
M. *Semitendinosus*			
	Connective Tissue		
A. Species	primary Pe	secondary Pe	Endomysium
CAPRINE	$22.10^{3a} \pm 1.89$	$12.61^{2a} \pm 1.11$	$11.99^{1b} \pm 0.88$
OVINE	$29.73^{3b} \pm 4.24$	$12.65^{2a} \pm 1.31$	$10.41^{1a} \pm 1.81$
BOVINE	$121.19^{3c} \pm 6.29$	$35.38^{2b} \pm 2.84$	$14.13^{1c} \pm 2.87$

a-c Means in the same column with different superscript differ significantly ($P<0.05$).
1-3 Means in the same row with different superscript differ significantly ($P<0.05$).
Thickness value in micron

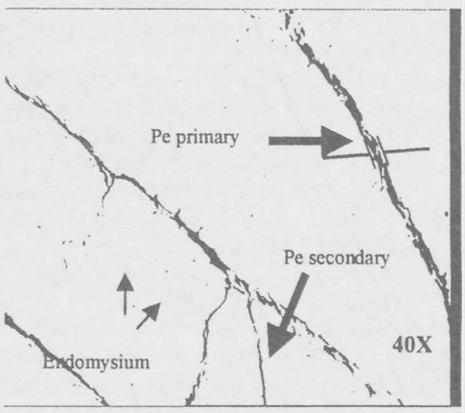
gure 1. Measures by VIA of cross-sections of intramuscular connective tissue from SM and ST muscles in three animal species



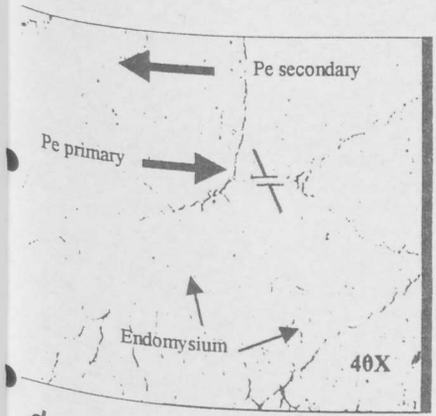
a. Caprine *Semimembranosus* muscle



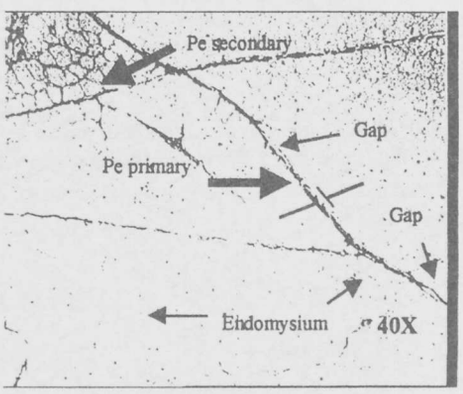
b. Ovine *Semimembranosus* muscle



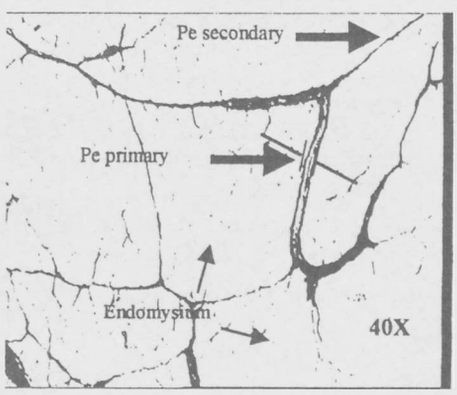
c. Bovine *Semimembranosus* muscle



d. Caprine *Semitendinosus* muscle



e. Ovine *Semitendinosus* muscle



f. Bovine *Semitendinosus* muscle