A study on the biochemical composition of different bovine muscles

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Conclusion
In the present study the content of decorin, intramuscular fat and OH-proline in four different bovine muscles, *M. semitendinosus. M.semimembranosus. M. longissimus dorsi and M. psoas major* obtained immediately after slaughter were studied. The results showed that the amount of decorin and fat varied in the muscles. *M.semitendinosus* exhibited the highest content of decorin, whereas *M. psoas major* exhibited the highest content of fat. Minor differences were also detected in the OH-proline content. The results showed that the biochemical composition of the intramuscular connective tissue vary greatly from one muscle to another. It is premature to exclude connective tissue as a candidate for toughness before biochemical parameters such as fat and decorin have been further analyzed.

Introduction
Different muscles from the same individual vary considerably in tenderness. However, it has been difficult to establish biochemical parameters for measurement of tenderness. Intramuscular connective tissue plays an important role for meat thoughness. Electronmicroscopical studies have shown structural changes in intramuscular connective tissues between the collagen fibers during *post mortem* conditioning of beef (1), and biochemical studies have shown a decomposition of proteoglycans present in the matrix of connective tissue, such as decorin, during *post mortem* storage (2). But these results are obtained from meat stored for different time intervals after slaughter and do not provide any explanation for the intermuscular variations in tenderness at day zero. In the present study we have focused on the biochemical composition of four different bovine muscles, *M. semitendinosus. M.semimembranosus. M. longissimus dorsi and M. psoas major* from the same individual immediately after slaughter. These muscles are considered as sources for tough and tender meat. Samples were subjected to different biochemical methods to study the content of decorin, intramuscular fat and OH-prolin as a first step in order to examine whether differences in the biochemical composition of connective tissue may explain variations in meat tenderness between the different muscles.

Methods
Immediately after slaughter bovine muscles of *M. semitendinosus. M.semimembranosus. M. longissimus dorsi and M. psoas major* were cut into several pieces after removal of the epimysium. The pieces were collected randomly and powdered in liquid nitrogen. Samples were analysed for the content of OH-proline by the method of Stegeman H. and Stalder K (3) and intramuscular fat after "Fat in meat-rapid specific gravity method" (4). For fractionation of decorin powdered samples were incubated in extraction buffer consisting of 4 M guanidine-HCl, 2 % triton X-100, protease inhibitors and 0,05 M sodium acetate of pH 6 as described in (5). The extracts were then applied to ultracentrifugation in a gradient of $CsCl_2$ with a starting density of 1.37 g/ml (5), and the eluates were divided into 5 fractions called D1, D2, D3, D4 and D5, where D1 represented the bottom fraction. The fractions were finally examined by SDS-gel electrophoresis after dialysis and lyophilization, and bands were visualized by Stains all colouring. The samples after ultracentrifugation were also analysed for glycosaminoglycan (GAG) type, by treatment with c-ABC and HNO_2. C-ABC treatment degrades GAGs containing chondroitin or dermatan sulphate, and were performed by incubating 20 mg/ml sample with 0.01 unit enzyme at 37 ^{0}C over night. HNO_2 treatment which degrades GAGs containing heparan sulphate, was performed by incubation 1:1 with $HN0_2$ reagens (the supernatant of 0.5 M $BaSO_4$ mixed with 0.5 M H_2SO_4) for 10 minutes at room temperature and stopping the reaction with 1 M Tris buffer pH 8.

Results and Discussion
The results obtained from samples of freeze dried material from the different muscles after ultracentrifugation and SDS-gel electrophoresis are shown in figure 1. The figure shows the results obtained from the D4 fractions which exhibited a broad distinct band with a molecular size above 100K. This band was most strongly expressed in the sample from *M. semitendinosus*. The band was resistant to $HN0_2$ treatment but was degraded by c-ABC treatment, as illustrated in figurs 1a and b, showing that this band represented a chondroitin/dermatan sulphate proteoglycan. In a previous study a band with similar biochemical characteristics was identified by western blotting as decorin (ref 5). Decorin has previous been shown by immunohistological methods to be present in the peri-and endomysium. and to represent the major proportion of proteoglycans isolated from bovine *M. semimembranosus* (ref 5). The decorin content of *M. semitendinosus* was higher compared to the amount in other bovine muscles. Decorin is known to interact with collagen fibrils and growth factors, and influence fibrill formation and turn over(ref. 6,7). In addition decorin may play a mechanical role by linking collagen fibers and stabilizing the network of the endo- and perimysium. It therefore seems likely that decorin could be a major contributor to meat tenderness by stabilization of the mechanical network in vivo, as reflected at day zero. When decorin is degraded during post mortem storage, a destabilization of the network may occur with an increase in tenderness as result. Our results did also show variation in intramuscular fat content and OH-proline content between the different muscles. The fat content of *M. psoas major* was almost three times the levels in the other muscles (fig 2). *M. semitendinosus* showed furthermore a slightly higher content of OH-proline (fig 2). The OH-proline content has previously proven unfitted as a marker for tenderness. An explanation may be that it represents all types of collagens both fibrillar, non-fibrillar and FACIT collagens and is not specific enough as a marker. Finally, biochemical factors seems to be important in determing the quality of meat. It is well known that meat processing methods such as cooling rate influence tenderness. But differences in the biochemical composition of the muscles will most likely contribute to toughness to a much higher degree than usually believed. That no biochemical parameters have been established to relate tenderness to, does not mean that they do not exist.The present study has shown that both proteoglycans such as decorin and intramuscular fat deserve further studies.

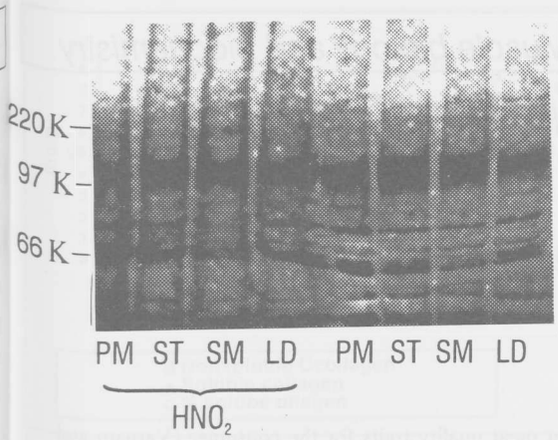

Fig. 1a) SDS-gel electrophoresis.
Lyophilized samples from D4-fractions were treated with HNO_2 and subjected to SDS-gel electrophoresis on tris-glycine gradient gels (4-20 %). The bands were visualized by Stains all colouring. Lanes 1-4 from left represent HNO_2 treated samples, while lanes 5-8 represent non-treated samples.
PM (*psosas major*), ST (*semitendinosus*), LD (*longissimus dorsi*), SM (*semimembranosus*).

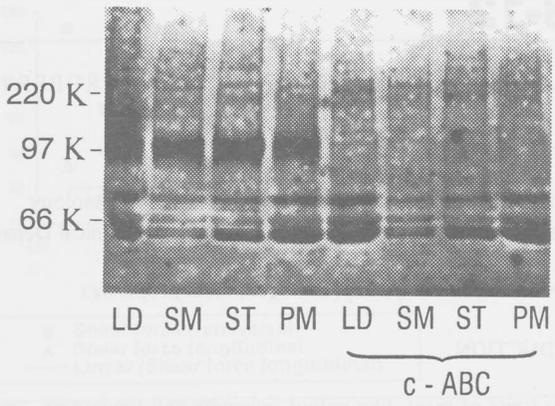

Fig. 1b) SDS-gel electrophoresis.
Lyophilized samples from D4-fractions were treated with c-ABC and subjected to SDS-gel electrophoresis on tris-glycine gradient gels (4-20 %). The bands were visualized by Stains all colouring. Lanes 1-4 from left represent non treated samples, while lanes 5-8 represent the same samples after c-ABC digestion.
PM (*psosas major*), ST (*semitendinosus*), LD (*longissimus dorsi*), SM (*semimembranosus*).

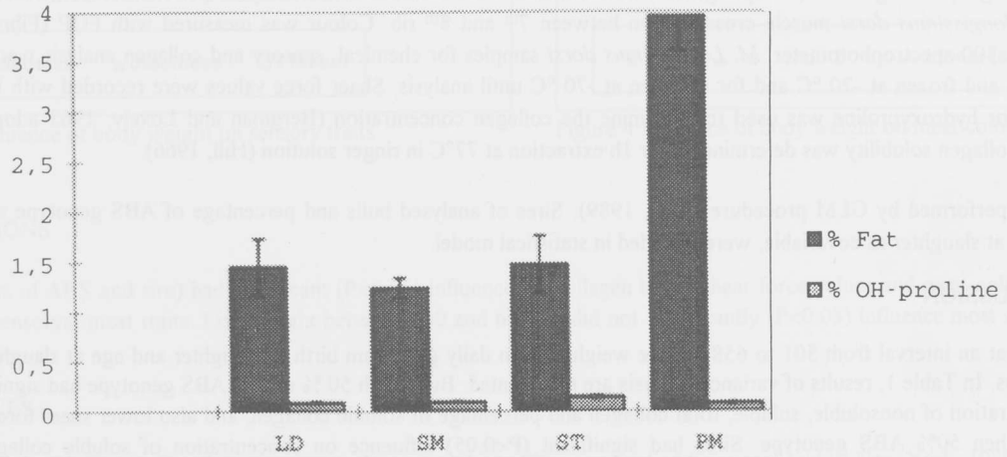

Fig.2
The figure shows the percent fat and OH-proline content in bovine muscles, *M. longissimus dorsi, M. semimembranosus. M. semitendinosus and M. psosas major.*

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