Integrin degradation during postmortem cold storage and the level of drip loss in pork^{*)}

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Summary

Integrins are a family of transmembrane adhesion proteins. An integrin molecule is composed of two subunits called α and β , each of which has a large extracellular domain, a transmembrane fragment, and a short cytoplasmic sequence. The main function of integrin is to bind extracellular matrix proteins and the skeletal muscle cell membrane. In addition, integrin as a membrane receptor is involved in signal transduction and cell response to microenvironmental signals, by relaying information about the structure and composition of the cell environment. Postmortem integrin degradation has been the subject of several studies, mainly in pork, where the mechanisms of postmortem integrin degradation are not completely understood. Therefore, the aim of the study was to present current knowledge on the role of integrin in postmortem drip loss in pork. Research to date has shown that postmortem integrin degradation could contribute to the formation of drip channels between the cell body and cell membrane of muscle fibers, which increases the drip loss from pork.

Keywords: integrin degradation, drip loss, meat quality, pork

Meat quality is a combination of significant traits and individual properties of the raw material determining its use and nutritive value for the consumer, which develop throughout the animals' lives (33). The most important of these include tenderness, color, taste, juiciness, and aroma. After slaughter, the multistage conversion of muscle into meat is accompanied by many independent structural and biochemical changes, including the proteolysis of structural proteins that make up the muscle cytoskeleton. In recent years, the effect of structural proteins on meat quality has been the subject of many observations and analyses. Studies to date have shown that postmortem changes related to the conformation and amount of structural proteins in muscles have a direct effect on physicochemical parameters of the meat, thus contributing to its final quality (12, 17, 30, 34). Integrin is one of the major structural proteins of the cytoskeleton, which play a significant role in shaping the quality traits of meat. The effect of postmortem integrin degradation on pork quality has been the subject of several studies (3, 4, 16, 29, 32, 34, 35). Therefore, the aim of this study was to present the role of integrin in postmortem pork quality.

Location, structure and function of integrin in muscle fibers

Integrin belongs to a family of transmembrane adhesion proteins (7, 18). It is found in almost all body tissues (16). An integrin molecule is a heterodimer of non-covalently associated α and β subunits. Each subunit is a transmembrane glycoprotein and has a large extracellular domain, a transmembrane fragment, and a short cytoplasmic sequence. Integrin has a complex molecular structure, with the combination of α and β subunits underpinning the functional specificity, diversity and universality. The β chain is mainly responsible for cell membrane adhesion, although it often plays a role in extracellular matrix interaction (6, 35). To date, 18 α subunits and 8 β subunits of this protein with different specificity have been identified (9, 18).

Integrin interacts with the extracellular matrix, cell membrane and body fluid proteins (7). The main function of integrin is adhesion of the cell to the substrate and involvement in intercellular contacts (1). This protein mediates the interaction of the cytoskeleton with the extracellular matrix, thus regulating the shape, orientation and movement of the cell. As a membrane receptor, integrin is involved in signal transduction and cell response to microenvironmental

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Fig. 1. The location and structure of integrins – the $\alpha 7 \beta 1$ integrin dimer binds laminin extracellularly and actin intracellularly via the vinculin (V) and talin (T) proteins (modified after Rahimov and Kunkel (25))

signals, by relaying information about the structure and composition of the cell environment (7, 23) (Fig. 1).

Water content and drip loss of muscle fibers

Muscle contains approximately 75% water, 20% proteins, 4% lipids, 1% carbohydrates, and less than 1% vitamins and mineral salts (21). Most of the water in the muscle fibers is held within the space between actin and myosin filaments. Approximately 85% of this space is occupied by intra-myofibrillar water (8). The remaining 15% (extra-myofibrillar water) (12) can be stored in the muscle fibers within the space between myofibrils and the sarcolemma (inter-myofibrillar water), in the inter-fascicular space between muscle fibers (inter-fascicular water), and in the extra-fascicular space in between the muscle fasciculi (extra-fascicular water) (22, 23, 27) (Fig. 2).

Because water molecules are associated with structural proteins of the cytoskeleton, the level of drip loss is largely dependent on the degradation rate of proteins, such as desmin, dystrophin, troponin T, and integrin (8, 12, 15-17, 28, 31, 35).

submembrane proteins known as costameres, which include integrin. Kristensen and Purslow (15) also revealed relationships between postmortem degradation of costameric proteins and the level of drip loss from muscle. Costameres serve many important functions, such as transmitting the mechanical forces developed by the myofibrils through the sarcolemma to the extracellular matrix; coordinating folding and unfolding of the sarcolemma during contraction or relaxation; minimizing sarcolemmal damage during contraction and cell extension during relaxation; and transducing signals from the extracellular matrix to the enzyme system of the muscle (26). In this way costameres link the myofibers to the sarcolemma, providing them with integrity, cohesion and mechanical strength.

According to Offer and Cousins (20), postmortem degradation of the structural proteins of the cytoskeleton could contribute to the formation of drip channels between the cell body and sarcolemma of muscle fibers. This is when drip loss from the muscle occurs through these structures (5). Degradation of some membrane proteins (like integrin) could also contribute to the

formation of drip channels and thus might actually improve the ability of moisture to 'escape' from the muscle cell (13).

Lawson (16), when analyzing the rate of integrin degradation in the *m. longissimus* of pigs during 24-h cold storage of meat postmortem, demonstrated a positive relationship between the opening of drip channels and the level of drip loss. The author concluded that for a drip loss of 2.2%, drip channels appear 9 h postmortem, whereas higher drip loss of 11.8% caused drip channels to open 3 h postmortem. Higher integrin degradation postmortem is associated with higher drip

Fig. 2. The structure of skeletal muscle with regard to different water locations: intra-myofibrillar water – between actin and myosin filaments; inter-myofibrillar water – between myofibrils and the sarcolemma; inter-fascicular water – between muscle fibers and in the inter-fascicular space; extra-fascicular water – in the extrafascicular space in between the muscle fasciculi (modified after Baechle and Earle (2))

loss (16, 35). In turn, inhibition of integrin degradation decreases drip channel formation in porcine *m. longissimus* (16). Lawson (16) also concludes that the formation and size of drip channels strictly correlates with the degradation of the β_1 -chain of integrin. It is reported that higher integrin degradation increases the formation of drip channels, which increases the level of drip loss from pork (4, 16, 35).

Zhang (34) clearly showed that increased calpain activity reduces the level of drip loss from muscle fibers. Calpains are intracellular, proteolytic enzymes that include μ -calpain, m-calpain and calpain 3. The calpain system is activated when the concentration of calcium ions reaches a proper level. Several other types of calpain are believed to exist, but to date they have never been isolated from myofibers (19). In muscle fibers, calpains directly associate with myofibrils at the Z line (14). These enzymes play a fundamental role in tenderization of meat as it ages, degrading structural proteins of the cytoskeleton and thus determining the final quality of the meat (12, 19, 34). Pfaff et al. (24) found that all β -integrin subunits can be cleaved by m-calpains. In turn, Lawson (16) and Zhang et al. (35) showed that also μ -calpain is involved in integrin degradation. According to Geesink et al. (11), μ -calpain may start the degradation of integrin and other structural proteins of the cytoskeleton, whereas in the later stages of meat aging, its functions come to a halt and are taken over by the other types of calpain (11). If calpains at the surface of the muscle fiber have been activated and integrins have been degraded, the cell membrane lifts off of the fiber and drip channels are formed (16).

An important regulator of calpain activity is calpastatin, which at the same time is a calpain inhibitor. Similar to calpains, calpastatin is found in muscle fibers mainly at the Z-line region of myofibrils (14). Geesink and Koohmaraie (10) showed that increased calpastatin levels inhibit the activity and degradation rate of cytoskeletal structural proteins of the muscle fibers. These results indicate that the calpain system is largely responsible for degradation of structural proteins, thus determining final meat quality (11).

Straadt et al. (29), who studied integrin degradation in *m. longissimus* of PSE (pale-soft-exudative) and DFD (dark-firm-dry) pigs during 7-day cold storage of meat, observed a positive correlation between integrin content and the mobility of the myofibrillar water on day 7 postmortem. The same authors noted no correlation between integrin content on day 1 postmortem and drip loss on day 7. This means that integrin degradation on day 1 postmortem reduces the mobility of the myofibrillar water. This situation may be due to the increased distance between muscle fibers. Straadt et al. (29) suggest that a strong link between integrin degradation and drip loss in pork is questionable, whereas integrin degradation seems to have an impact on the succeeding development in the mobility of the myofibrillar water. In turn, Yin et al. (32), who

investigated integrin degradation and μ -calpain activity in *m. longissimus* of PSE and DFD pigs during 5-day cold storage of meat, found more integrin degradation products in the *m. longissimus* of PSE pigs compared to RFN (red-firm-nonexudative) pigs on day 1 postmortem. According to the same authors, RFN pork is characterized by lower content of undegraded 80 kDa μ -calpain and greater intensity of autolyzed 76 kDa product compared to PSE pork. The results obtained suggest that the degree of μ -calpain activity and integrin degradation in PSE meat can contribute to the high drip loss during the storage of meat postmortem.

Bérard et al. (4), when analyzing the effect of birth weight (BtW) and litter size (S – small, ≤ 10 piglets/litter; L – large, ≥ 14 piglets/litter) on integrin degradation in porcine *m. longissimus* and *m. semitendinosus* at 30 min, 24 h and 72 h of cold storage of meat, showed no correlation between integrin degradation and the level of drip loss in both muscles. Integrin degradation was more rapid in the *m. longissimus* of animals from L compared to S litters. What is more, integrin degradation in *m. longissimus* at 24 h postmortem and in *m. semitendinosus* at 72 h postmortem was unrelated to the level of drip loss. It was observed, however, that the increase in integrin degradation was paralleled by a decrease in drip loss from *m. longissimus* and *m. semitendinosus*. In addition, integrin degradation in *m. semitendinosus* at 72 h postmortem was 3 times as rapid as in *m. longissimus*, which could help to partly explain the differences seen in drip loss between the two muscles studied. At 72 h postmortem, integrin degradation in *m. longissimus* was lower in pigs from S litters than L litters (4). The investigated factor, such as BtW and litter size, had little effect on integrin degradation in the *m. longissimus* and *m. semitendinosus*. Regardless of the BtW group, the level of undegraded integrin in *m. longissimus* at 72 h postmortem was 47% greater in pigs from S litters compared to L litters. No differences in integrin degradation were observed for the *m. semitendinosus*.

Conclusions

To sum up, degradation of integrin and of other structural proteins postmortem is probably started by μ -calpain. In the later stages, its functions come to a halt and are taken over by the other calpain types. Furthermore, research to date shows that postmortem degradation of integrin, which is found in costameres, can contribute to the level of drip loss from muscle fibers in pork. In addition, higher degradation of the β_1 -chain of integrin leads to the formation of drip channels between the cell body and cell membrane of myofibers, thus increasing the level of drip loss. The processes that shape physicochemical characteristics of meat, such as drip loss, are still complex and involve many factors, because they result not only from the specific microstructure of muscle tissue, but also from many interactions taking place in the muscles postmortem.

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