# THE EFFECT OF INTRAMUSCULAR WATER FLUID TRANSPORT ON *RIGOR MORTIS*

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## Background

*Rigor mortis* is one of the main macroscopic events after death in all animals, and it is of importance to understand how this event affects the quality and quantity of meat. For the meat producer it is important to understand *rigor mortis* in order to optimise slaughter and processing procedures for different meats. Our understanding of the biochemical reactions *post mortem* and how these affect the meat quantity and quality from different animals is limited (Devine *et al.*, 1999, Erikson, 2001).

Development of *rigor mortis* has been studied by several methods such as loss of extensibility (Bendall, 1973, Briskey *et al.*, 1962, Honikel *et al.*, 1983, Schmidt *et al.*, 1968) muscle shortening (Currie and Wolfe, 1979), tension development (Nuss and Wolfe, 1980-81), resistance to strain (Lepetit *et al.*, 1998) and by combination of muscle tension and shortening (Hertzman *et al.*, 1993, Olsson *et al.*, 1994, Rosenvold *et al.*, 2003).

After death, catabolic reactions predominate. ATP concentrations decline, and pH in mammalian meat are lowered to around 5.6 as a result of the formation of lactic acid. ATP is continuously used for muscle relaxation and transport of $Ca^{2+}$ across the sarcoplasmatic membrane. Partially due to the interactions between actin and myosin after death, ATP is lowered to around zero and the actomyosin bridges formed are used to explain *rigor mortis*. These bridges are also used to explain the stiffness that characterizes *rigor mortis*. The actomyosin bridges are judged as irreversible. However, it is also agreed that the stiffness is resolved without any changes in the binding between actin and myosin. Thus, another factor has to be introduced to explain the resolution of *rigor mortis*.

All the catabolic reactions after death increase the number of molecules within the muscle. Glycogen is converted to glucose that is further metabolised to lactate. Proteolysis and lipolysis give amino- and fatty acids and ions are released. Together with other enzymatic reactions the increased number of molecules changes the osmotic pressure ($\pi = cRT$) to a higher value. It has been found that the osmotic pressure increased from an at-death level of 379 to 528 mOsmole in lamb *M. longissimus dorsi,* and one-third of the increase occurred after 24 hours (Veiseth *et al.,* 2003). It was also found that the conductivity changed from 11.3 to 5.7 mS/cm during the 12 first hours.

The volume of muscle cells, however, does not change during *rigor mortis* (Kobayashi *et al.*, 2001). In addition, our morphological study of muscle cells, immediately after death and in the *rigor* state, indicates that the shape of cells changes from a circular to a more squared form, and this is calculated to be statistically significant (Slinde *et al.*, 2003). Since the volume of the whole muscle is the same at slaughter and in *rigor* this means that the intracellular volume has increased, and this space filling create stiffness.

Tenderness of meat is a very important quality parameter, but the tenderisation process is very variable in meat. Different apparatus determines the toughness of meat that characterizes the tenderness mechanically, and the Warner-Bratzler (WB) shear force is often used. Unfortunately, most experimental measurements are generally done after the animal has passed through *rigor mortis*. These texture analyses have in general a rather high standard deviation since it is measured in many individuals, but also due to the difference in muscle fibre types and variation in the amount of connective tissue. We have found that when WB shear forces are measured from slaughter and onwards in Atlantic salmon, the WB shear force decline from the first measurement onwards (Roth *et al.*, 2002, Slinde *et al.*, 2003). Partman (1963) showed that texture analysis of fish and other animals showed such high standard deviations after slaughter that the effect of *rigor mortis* was not detected.

## Objectives

It is generally agreed that after death, the main catabolic biochemical reactions and formation of the actomyosin bridges leading to *rigor mortis* are the same in muscle of animals, birds, reptiles and fish (Partman, 1963). The purpose of the present study was to strengthen the evidence that changes in osmolarity and the flow of water into the cells are the main cause of the macroscopic felt stiffness of *rigor mortis* (Roth *et al.*, 2002, Slinde *et al.*, 2003),

## Materials and methods

Atlantic salmons (*Salmo salar*) were stunned by a blow to the head, and gill arches were cut. Muscle samples were taken from the region behind the dorsal fin. A blow by a nail through the crocodile's (*Crocodylus niloticus*) head followed by decapitation killed the animal. Crocodiles, approximately 150 cm long, were reared at ambient temperature 18 – 20°C. Right and left tail muscles *M. caudal femoral, M. longissimuis caudalis* and *M. ilioishiocaudalis* were used for measurements.

Shear forces were measured on individual fishes and crocodiles using a Warner-Bratzler blade connected to a texture analyser. *Rigor* index was measured as "tail bending" and calculated as $IR=(L_0-L_t)/L_0 \times 100$ where L represents the vertical drop of the tail when half of the length was outside the table. $L_0$ represents the vertical drop at start and $L_t$ represents measurement during the experiment (Bito *et al.*, 1983).

## Results and discussion

We have selected fish muscle as a model to study development of *rigor mortis,* since the large swimming muscle is very homogenous with regard to fibre- and connective tissue composition. Partman (1963) states that penetration measurements show high average deviations in different fish species and an increase in rigidity in each fish during *rigor* is therefore not seen. There are also great deviations in *rigor* development within a single species. When following *rigor* development in each salmon separately as shown in Figure 1, the variation in both *rigor* index and shear force is clearly different. This is due to the biochemical status of each individual animal at slaughter. But it is very clear that the animal's shear force decreases from slaughter through *rigor mortis* while the measured *rigor* index goes through a maximum. Crocodiles are regarded as more developed than fish and have a fibre- and connective tissue structure that is more complex. Figure 2 shows that the shear force spans a much wider range in individual crocodiles reared at similar conditions when chilled at 20 and 30°C when compared to fish. The changes in shear force from slaughter and onwards decrease slightly but the standard deviations are high. We have therefore concluded that another factor besides the actomyosin binding has to contribute to the stiffness observed in *rigor mortis,* and this has to be a property that is of general nature, and must be found in all animals.

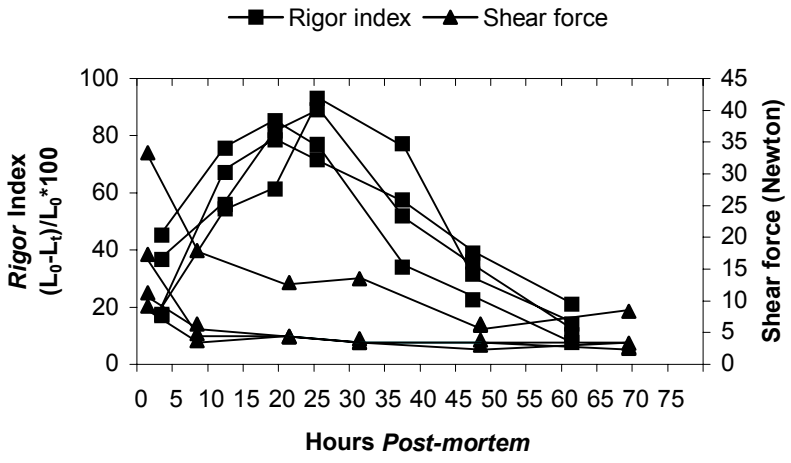


Figure 1. Measurements of Warner-Bratzler (WB) shear force and *rigor* index in four Atlantic salmon (*Salmo salar*).

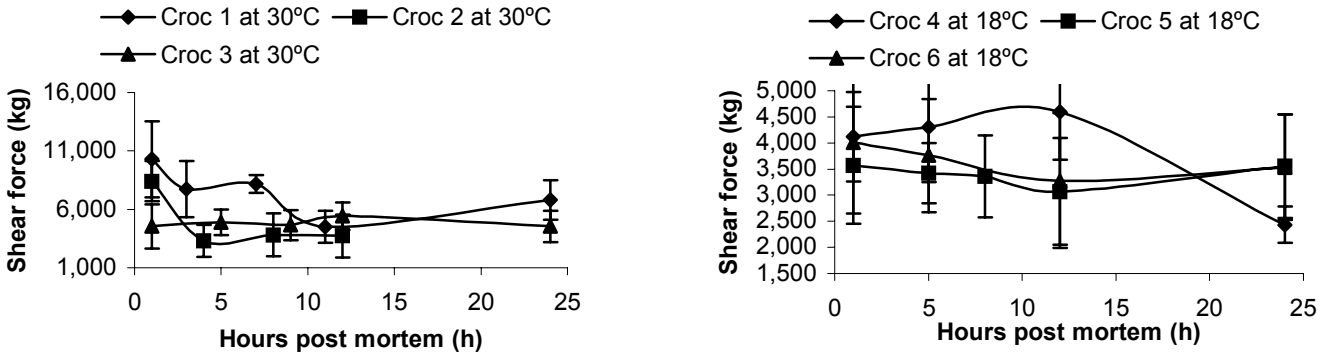


Figure 2. Change in Warner-Bratzler (WB) shear force (kg) in individual crocodile tail muscles during *rigor mortis*.

Catabolic reactions are a general property in all animals after death. Veiseth *et al*. (2004) have performed measurements of osmolar changes. Figure 3 shows the osmolarity within lamb muscle together with the change in pH. We can see that these two properties are mirror images of each other. The rather large change in osmolarity causes water to flow from the extracellular space into the cell. The volume of the cells expands and the cell walls follow the surrounding connective tissue more closely (Slinde *et al*., 2002). In Figure 4, a model of this water transport between intra- and extracellular space within a muscle has been drawn. The increase in cell volume strengthens the structure of the connective tissue and give rise to stiffness. A better understanding of the osmotic behaviour in the muscle after slaughter might increase our understanding of meat texture, as well as its water binding properties. Preliminary studies indicate that the conductivity in cells are low after slaughter, but a sudden raise is observed after some time indicating cellular membrane rupture. The osmotic changes found (Veiseth *et al*., 2004) and shown in Figure 3 may also be studied using NIR (Near Infrared Reflectance), since this method has the property of measuring water in different environments *i.e*. the intra- and extra cellular compartments.

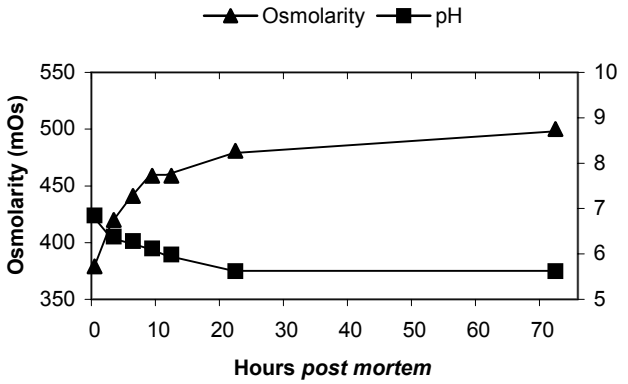


Figure 3. Data from lamb *M. longissimus dorsi* (Veiseth *et al.*, 2004) shows how the osmotic pressure increases after death, Note that the pH is almost a mirror image of the change in osmolarity.

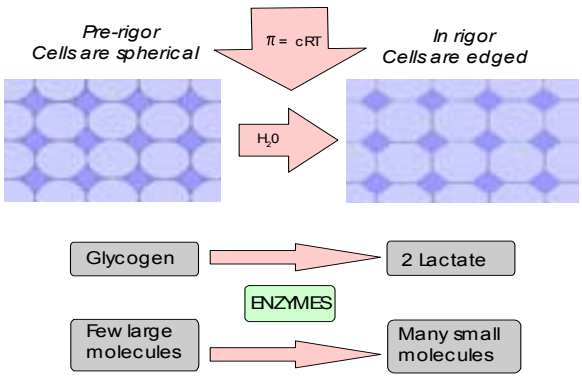


Figure 4. The osmotic change in muscle cells during *rigor mortis*. The enzymatic breakdown increases the number of molecules within the muscle cells. The contour of the cells changes from a spherical to a more edged form due to osmotic influx of water ($\pi = cRT$).

## Conclusions

Figure 1 and 2 shows the same tendency, that in individual animals, the water being intra- or extracellular does not affect the WB shear force. The WB shear force is not affected by *rigor mortis* as opposed to the *rigor* index (Figure 1 and 2). The *post mortem* reactions increase the number of molecules within the muscle cells and causes an alternation in the osmotic potential (Veiseth *et al.*, 2004). Since the volume of the muscle is constant (Kobayashi *et al.*, 2001) the increased cell volume makes the muscle stiff. Further investigation of the transport of muscle juice will increase our understanding of *rigor mortis*, the DFD and PSE syndrome as well as the water holding capacity of meat. It will also increase our understanding of tenderisation.

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