Handbook of Meat Processing

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Part I

Technologies

Chapter 1

Chemistry and Biochemistry of Meat

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Introduction

Muscle cells are among the most highly organized cells in the animal body and perform a varied array of mechanical functions. They are required for the movement of limbs, for locomotion and other gross movements, and they must also perform finer tasks such as maintaining balance and coordination. Muscle movement and metabolism are associated with other diverse functions such as aiding in movement of blood and lymph and also in maintaining body temperature. All of these functions are dependent on cellular metabolism and the ability of the cell to maintain energy supplies. Few cells are required to generate as much force and undergo as dramatic shifts in rate of metabolism as muscle cells. The ability of living skeletal muscle to undergo relatively large intracellular changes also influences its response to the drastic alterations that occur during the first few hours following exsanguination. Thus the organization, structure, and metabolism of the muscle are key to its function and to the maintenance of its integrity both during contraction and during the early postmortem period. Ultimately, these postmortem changes will influence the suitability of meat for further processing.

Muscle Composition

The largest constituent of muscle is water (Table 1.1; U.S. Department of Agriculture 2008). In living tissue, the average water content is 75% of the weight of the muscle; however, can vary, particularly in postmortem muscle (range of 65–80%). Within the muscle, it is the primary component of extracellular fluid. Within the muscle cell, water is the primary component of sarcoplasmic (cytoplasmic) fluid. It is important in thermoregulation; as a medium for many cellular processes; and for transport of nutrients within the cell, between cells, and between the muscle and the vascular system.

The second largest component of muscle is protein (U.S. Department of Agriculture 2008). Protein makes up an average of 18.5% of the weight of the muscle, though that figure can range from 16 to 22%. Proteins serve myriad functions and are the primary solid component in muscle. The functions of proteins are quite varied. Muscle proteins are involved in maintaining the structure and organization of the muscle and muscle cells (the role of highly insoluble stromal proteins). They are also important in the contractile process. These proteins primarily are associated with the contractile organelles, the myofibril, and are thus termed myofibrillar proteins. In general, the myofibrillar proteins are not soluble at low ionic strengths found in skeletal muscle (ionic strength ≤0.15), but can be solubilized at higher ionic strengths (≥0.3). This class of proteins includes both the proteins directly involved in movement (contractile proteins) and proteins that regulate the interactions between the contractile proteins (regulatory proteins). There are also many soluble proteins (sarcoplasmic pro-

Table 1.1. Composition of Mammalian Muscle

Component	% of Muscle Weight
Water	75% (65–80%)
Protein	18.5% (16–22%)
Lipid	3% (1–13%)
Carbohydrate	1% (0.5–1.5%)
Non-Protein Nitrogenous Substances	1.7% (1–2%)
Other Non-Protein Substances (minerals, vitamins, etc.)	0.85% (0.5–1%)

Numbers in parentheses indicate the average range of that component.(U.S. Department of Agriculture, 2008)

teins) that include proteins involved in cellular signaling processes and enzymes important in metabolism and protein degradation/cellular remodeling.

The lipid content of the muscle can vary greatly due to many factors, including animal age, nutritional level of the animal, and muscle type. It is important to note that the lipid content varies inversely with the water content (Callow 1948). Some lipid is stored inside the muscle cell; however, within a muscle, the bulk of the lipid is found between muscle bundles (groupings of muscle cells). Average lipid content of skeletal muscle is about 3% of the muscle weight, but the range can be as much as 1–13% (U.S. Department of Agriculture 2008). In skeletal muscle, lipid plays roles in energy storage, membrane structure, and in various other processes in the organ, including immune responses and cellular recognition pathways.

The two major types of lipid found in skeletal muscle are triglycerides and phospholipids. Triglycerides make up the greatest proportion of lipid associated with muscle. Triglycerides (triacylglycerides) consist of a glycerol molecule in which the hydroxyl groups are esterified with three fatty acids. The melting point and the iodine number of lipid that is associated with the muscle is determined by the chain length and the degree of saturation of the fatty acids. Phospholipids (phosphoglycerides) are another type of complex lipid found in muscle. In this class of lipids, one of the hydroxyl groups of glycerol is esterified to a phosphate group, while the other constituents are fatty acids. The fatty acids associated with phospholipids are typically unsaturated. Phospholipids in skeletal muscle are commonly associated with membranes. The relative high degree of unsaturation of the fatty acids associated with the phospholipids is a contributing factor to the fluidity of the cell membranes.

Carbohydrates make up a relatively small percentage of muscle tissue, making up about 1% of the total muscle weight (range of 0.5–1.5%). The carbohydrate that makes up the largest percentage is glycogen. Other carbohydrates include glucose, intermediates of glycogen metabolism, and other mono- and disaccharides. Glycosoaminoglycans are also found in muscle and are associated with the connective tissue.

There are numerous non-protein nitrogenous compounds in skeletal muscle. They include substances such as creatine and creatine phosphate, nucleotides (ATP, ADP), free amino acids, peptides (anserine, carnosine), and other non-protein substances.

Muscle Structure

Skeletal muscle has a very complex organization, in part to allow muscle to efficiently transmit force originating in the myofibrils to the entire muscle and ultimately, to the limb or structure that is moved. A relatively thick sheath of connective tissue, the epimysium, encloses the entire muscle. In most muscles, the epimysium is continuous, with tendons that link muscles to bones. The muscle is subdivided into bundles or groupings of muscle cells. These bundles (also known as fasciculi) are surrounded by another sheath of connective tissue, the perimysium. A thin layer of connective tissue, the endomysium, surrounds the muscle cells themselves. The endomysium lies above the muscle cell membrane (sarcolemma) and consists of a base-

ment membrane that is associated with an outer layer (reticular layer) that is surrounded by a layer of fine collagen fibrils imbedded in a matrix (Bailey and Light 1989).

Skeletal muscles are highly diverse, in part because of the diversity of actions they are asked to perform. Much of this diversity occurs not only at the gross level, but also at the muscle cell (fiber) level. First, not only do muscles vary in size, they can also vary in the number of cells. For example, the muscle that is responsible for adjusting the tension of the eardrum (tensor tympani) has only a few hundred muscle cells, while the medial gastrocnemius (used in humans for walking) has over a million muscle cells (Feinstein et al. 1955). Not only does the number of cells influence muscle function and ultimately, meat quality, but also the structure of the muscle cells themselves has a profound effect on the function of living muscle and on the functionality of meat.

Muscle cells are striated, meaning that when viewed under a polarized light microscope, distinct banding patterns or striations are observed. This appearance is due to specialized organelles, myofibrils, found in muscle cells. The myofibrils have a striated, or banded, appearance because different regions have different refractive properties. The light bands have a consistent index of refraction (isotropic). Therefore, these bands are called I-bands in reference to this isotropic property. The dark band appears dark because it is anisotropic and is thus called the A-band.

The myofibrils are abundant in skeletal muscle cells, making up nearly 80–90% of the volume of the cell. Myofibrillar proteins are relatively insoluble at physiological ionic strength, requiring an ionic strength greater than 0.3 to be extracted from muscle. For this reason, they are often referred to as "salt-soluble" proteins. Myofibrillar proteins make up approximately 50–60% of the total extractable muscle proteins. On a whole muscle basis, they make up approximately 10–12% of the total weight of fresh skeletal muscle. Therefore, they are very important in meat chemistry and in determining the functionality of meat proteins.

Myofibrils are the contractile "machinery" of the cell and, like the cells where they reside, are very highly organized. When examining a myofibril, one of the first observations that can be made is that the cylindrical organelle is made up of repeating units. These repeating units are known as sarcomeres. Contained in each sarcomere are all the structural elements needed to perform the physical act of contraction at the molecular level. Current proteomic analysis estimates that over 65 proteins make up the structure of the sarcomere (Fraterman et al. 2007). Given that the sarcomere is the most basic unit of the cell and that the number quoted in this analysis did not take into account the multiple isoforms of the proteins, this number is quite high. Many of the proteins interact with each other in a highly coordinated fashion, and some of the interactions are just now being discovered.

The structure of the sarcomere is responsible for the striated appearance of the muscle cell. The striations arise from the alternating, protein dense A-bands and less dense I-bands within the myofibril. Bisecting the I-bands are dark lines known as Z-lines. The structure between two Z-lines is the sarcomere. In a relaxed muscle cell, the distance between two Z-lines (and thus the length of the sarcomere) is approximately 2.2 µm. A single myofibril is made up of a large number of sarcomeres in series. The length of the myofibril and also the muscle cell is dependent on the number of sarcomeres. For example, the semitendinosus, a long muscle, has been estimated to have somewhere in the neighborhood of 5.8×10^4 to 6.6×10^4 sarcomeres per muscle fiber, while the soleus has been estimated to have approximately 1.4×10^4 (Wickiewicz et al. 1983). Adjacent myofibrils are attached to each other at the Z-line

by proteinacious filaments, known as intermediate filaments. Outermost myofibrils are attached to the cell membrane (sarcolemma) by intermediate filaments that interact not only with the Z-line, but also with structures at the sarcolemma known as costameres (Robson et al. 2004).

Myofibrils are made up of many myofilaments, of which there are two major types, classified as thick and thin filaments. There is also a third filament system composed primarily of the protein titin (Wang et al. 1979; Wang 1984; Wang et al. 1984; Wang and Wright 1988; Wang et al. 1991; Ma et al. 2006;). With respect to contraction and rigor development in postmortem muscle, it is the interdigitating thick and thin filaments that supply the "machinery" needed for these processes and give skeletal muscle cells their characteristic appearance (Squire 1981). Within the myofibril, the less dense I-band is made up primarily of thin filaments, while the A-band is made up of thick filaments and some overlapping thin filaments (Goll et al. 1984). The backbone of the thin filaments is made up primarily of the protein actin, while the largest component of the thick filament is the protein myosin. Together, these two proteins make up nearly 70% of the proteins in the myofibril of the skeletal muscle cell.

Myosin is the most abundant myofibrillar protein in skeletal muscle, making up approximately 50% of the total protein in this organelle. Myosin is a negatively charged protein with an isoelectric point of 5.3. Myosin is a large protein (approximately 500,000 daltons) that contains six polypeptides. Myosin consists of an alpha helical tail (or rod) region that forms the backbone of the thick filament and a globular head region that extends from the thick filament and interacts with actin in the thin filament. The head region of myosin also has ATPase activity, which is important in the regulation of contraction. Each myosin molecule contains two heavy chains (approximately 220,000 daltons each) and two sets of light chains (14,000–20,000 daltons). One of the light chains is required for enzymatic activity, and the other has regulatory functions.

Actin is the second-most abundant protein in the myofibril, accounting for approximately 20% of the total protein in the myofibril. Actin is a globular protein (G-actin) that polymerizes to form filaments (F-actin). G-actin has a molecular weight of approximately 42,000. There are approximately 400 actin molecules per thin filament. Thus the molecular weight of each thin filament is approximately 1.7×10^7 (Squire 1981). The thin filaments (F-actin polymers) are $1\,\mu m$ in length and are anchored in the Z-line.

Two other proteins that are important in muscle contraction and are associated with the thin filament are tropomyosin and troponin. Tropomyosin is the second-most abundant protein in the thin filament and makes up about 7% of the total myofibrillar protein. Tropomyosin is made up of two polypeptide chains (alpha and beta) The alpha chain has an approximate molecular weight of 34,000, and the beta chain has a molecular weight of approximately 36,000. These two chains interact with each other to form a helix. The native tropomyosin molecule interacts with the troponin molecule to regulate contraction. Native troponin is a complex that consists of three subunits. These are termed troponin I (MW 23,000), troponin C (MW 18,000), and troponin T (MW 37,000). Troponin C has the ability to bind calcium released from the sarcoplasmic reticulum, troponin I can inhibit the interaction between actin and myosin, and troponin T binds very strongly to tropomyosin. The cooperative action of troponin and tropomyosin in response to calcium increases in the sarcoplasm regulates the interaction between actin and myosin and thus is a major regulator of contraction. Calcium that is released from the sarcoplasmic reticulum is bound to the tropo-

nin complex and the resulting conformational changes within troponin cause tropomyosin to move away from sites on actin to which myosin binds and allows myosin and actin to interact.

For contraction to occur, the thick and thin filaments interact via the head region of myosin. The complex formed by the interaction of myosin and actin is often referred to as actomyosin. In electron micrograph images of contracted muscle or of postrigor muscle, the actomyosin looks very much like cross-bridges between the thick and thin filaments; indeed, it is often referred to as such. In postmortem muscle, these bonds are irreversible and are also known as rigor bonds, as they are the genesis of the stiffness (rigor) that develops in postmortem muscle. The globular head of myosin also has enzymatic activity; it can hydrolyze ATP and liberate energy. In living muscle during contraction, the ATPase activity of myosin provides energy for myosin bound to actin to swivel and ultimately pull the thin filaments toward the center of the sarcomere. This produces contraction by shortening the myofibril, the muscle cell, and eventually, the muscle. The myosin and actin can disassociate when a new molecule of ATP is bound to the myosin head (Goll et al. 1984). In postrigor muscle, the supply of ATP is depleted, resulting in the actomyosin bonds becoming essentially permanent.

Muscle Metabolism

From a metabolic point of view, energy use and production in skeletal muscle is simply nothing short of amazing in its range and responsiveness. In an actively exercising animal, muscle can account for as much as 90% of the oxygen consumption in the body. This can represent an increase in the muscle's metabolic rate of as much as 200% from the resting state (Hargreaves and Thompson 1999).

Central to the existence of the muscle cell is the production of adenosine triphosphate (ATP), the energy currency of the cell. ATP consists of adenosine (an adenine ring and a ribose sugar) and three phosphate groups (triphosphate). Cleavage of the bonds between the phosphates (P_i) and the rest of the molecule provides energy for many cellular functions, including muscle contraction and the control of the concentrations of key ions (like calcium) in the muscle cell. Cleavage of P_i from ATP produces adenosine diphosphate (ADP), and cleavage of pyorphosphate (PP_i) from ATP produces adenosine monophosphate (AMP). Since the availability of ATP is central to survival of the cell, there is a highly coordinated effort by the cell to maintain its production in both living tissue and in the very early postmortem period.

Muscular activity is dependent on ample supplies of ATP within the muscle. Since it is so vital, muscle cells have developed several ways of producing/regenerating ATP. Muscle can use energy precursors stored in the muscle cell, such as glycogen, lipids, and phosphagens (phosphocreatine, ATP), and it can use energy sources recruited from the blood stream (blood glucose and circulating lipids). Which of these reserves (intracellular or circulating) the muscle cell uses depends on the activity the muscle is undergoing. When the activity is of lower intensity, the muscle will utilize a higher proportion of energy sources from the blood stream and lipid stored in the muscle cell. These will be metabolized to produce ATP using aerobic pathways. Obviously, ample oxygen is required for this process to proceed. During high intensity activity, during which ATP is used very rapidly, the muscle uses intracellular stores of phosphagens or glycogen. These two sources, however, are utilized very quickly and their depletion leads to fatigue. This is not a trivial point. Concentration of ATP in skeletal muscle is critical; available ATP must remain above

approximately 30% of the resting stores, or relaxation cannot occur. This is because relaxation of contraction is dependent on ATP, which is especially important because removal of calcium from the sarcoplasm is an ATP-dependent process (Hargreaves and Thompson 1999).

The primary fuels for muscle cells include phosphocreatine, glycogen, glucose lactate, free fatty acids, and triglycerides. Glucose and glycogen are the preferred substrates for muscle metabolism and can be utilized either aerobically (oxidative phosphorylation) or anaerobically (anaearobic glycolysis). Lipid and lactate utilization require oxygen. Lipids are a very energy-dense storage system and are very efficient with respect to the high amount of ATP that can be generated per unit of substrate. However, the rate of synthesis of ATP is much slower than when glycogen is used (1.5 mmol/kg/sec for free fatty acids compared with 3 mmol/kg/sec for glycogen utilized aerobically and 5 mmol/kg/sec when glycogen is used in anaerobic glycolysis) (Joanisse 2004).

Aerobic metabolism, the most efficient energy system, requires oxygen to operate, and that oxygen is supplied by the blood supply to the muscle and by the oxygen transporter, myoglobin. It has been estimated that in working muscle, the myoglobin is somewhere in the neighborhood of 50% saturated. Under conditions of extreme hypoxia (as found in postmortem muscle), oxygen supplies are depleted because blood flow is not sufficient (or does not exist), and myoglobin oxygen reserves are depleted if this state continues long enough. Prior to exsanguination, the oxidation of glycogen or other substrates to form water and carbon dioxide via oxidative phosphorylation is a very efficient way for the cell to regenerate ATP. However, after exsanguination, the muscle cell must turn solely to anaerobic pathways for energy production.

Phosphocreatine in living, rested muscle is available in moderate abundance compared with ATP (100 mmol/kg dry muscle weight for phosphocreatine compared with 25 mmol/kg dry muscle weight for ATP) but very low abundance compared with glycogen (500 mmol/kg dry muscle weight for glycogen). Phosphocreatine can easily transfer a phosphate group to ADP in a reaction catalyzed by creatine kinase. This reaction is easily reversible and phosphocreatine supplies can be readily restored when ATP demand is low. In living muscle, when activity is intense, this system can be advantageous, as it consumes H^+ and thus can reduce the muscle cell acidosis that is associated with anaerobic glycolysis. Another advantage of the system is that the catalyzing enzyme is located very close to the actomyosin ATPase and also at the sarcoplasmic reticulum (where calcium is actively taken up from the sarcoplasm to regulate contraction) and at the sarcolemma. However, this system is not a major contributor to postmortem metabolism, as the supplies are depleted fairly rapidly.

In general, glycogen is the preferred substrate for the generation of ATP, either through the oxidative phosphorylation or through anaerobic glycolysis (Fig. 1.1). One of the key steps in the fate of glycogen is whether or not an intermediate to the process, pyruvate, enters the mitochondria to be completely broken down to CO_2 and H_2O (yielding 38 mol of ATP per mole of oxidized glucose-1-P produced from glycogen or 36 mol if the initial substrate is glucose), or if it ends in lactate via the anaerobic glycolysis pathway. The anaerobic pathway, while comparatively less efficient (yielding 3 mol of ATP per mole of glucose-1-P produced from glycogen or 2 mol if the initial substrate is glucose), is much better at producing ATP at a higher rate. Early postmortem muscle obviously uses the anaerobic pathway, as oxygen supplies are rapidly depleted. This results in the buildup of the end product, lactate (lactic acid), resulting in pH decline.

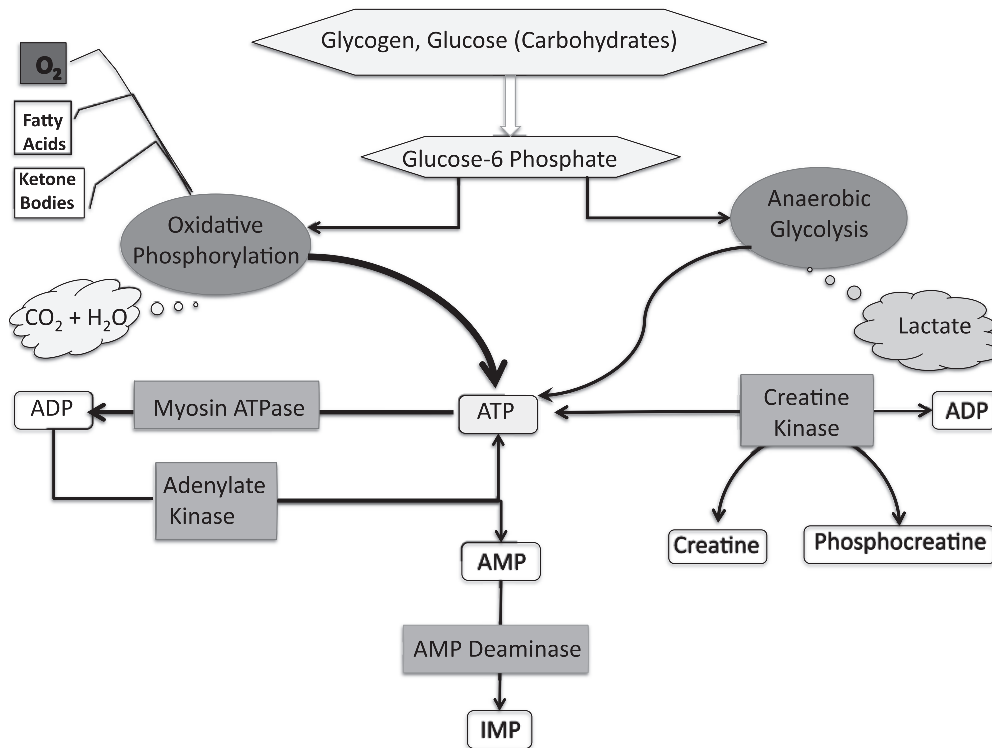

Figure 1.1. ATP production in muscle.

Major Postmortem Changes in Muscle

Tenderization

During refrigerated storage, it is well known that meat becomes more tender. It is commonly accepted that the product becomes more tender because of proteolytic changes occurring in the architecture of the myofibril and its associated proteins. There are several key proteins that are degraded during postmortem aging.

Titin

Titin (aka connectin) is a megaprotein that is approximately 3 megadaltons in size. In addition to being the largest protein found in mammalian tissues, it is also the third-most abundant. A single titin molecule is estimated to be between 2 and 2.5 µM in length. In striated muscle, titin thus spans fully half of a sarcomere, with its C-terminal end localizing in the M-line at the center of the sarcomere and the N-terminal forming an integral part of the Z-line. Titin aids in maintaining sarcomeric alignment of the myofibril during contraction. Titin integrates the Z-line and the thick filaments, maintaining the location of the thick filaments between the Z-lines. Titin is also hypothesized to play a role in generating at least a portion of the passive tension that is present in skeletal muscle cells. During development of the myofibril, titin is one of the earliest proteins expressed, and it is thought to act as a "molecular ruler" by providing a scaffolding or template for the developing myofibril (Clark et al. 2002).

Due to the aforementioned roles of titin in living cells, it is quite conceivable that

its degradation in postmortem muscle would lead to weakening of the longitudinal structure of the myofibrillar sarcomere and integrity of muscle. This weakening, in conjunction with other changes in postmortem muscle, could lead to enhanced tenderness. The degradation of titin has been observed in several studies (Lusby et al. 1983; Zeece et al. 1986; Astier et al. 1993; Huff-Lonergan et al. 1995; Melody et al. 2004; Rowe et al. 2004a, b). When titin is degraded, a major degradation product, termed $T_{2,}$ is observed that migrates only slightly faster under SDS-PAGE conditions than intact titin. This product migrates at approximately 2,400 kDa (Kurzban and Wang 1988, 1987; Huff-Lonergan et al. 1995). Another titin degradation product that has been observed by SDS-PAGE analysis migrates at approximately 1,200 kDa (Matsuura et al. 1991; Huff-Lonergan et al. 1995). This latter polypeptide has been shown to contain the portion of titin that extends from the Z-line to near the N_2 line in the I-band (Kimura et al. 1992), although the exact position that the 1200 kDa polypeptide reaches in the sarcomere is still not certain. The 1,200-kDa polypeptide has been documented to appear earlier postmortem in myofibrils from aged beef that had lower shear force (and more desirable tenderness scores) than in samples from product that had higher shear force and/or less favorable tenderness scores (Huff-Lonergan et al. 1995, 1996a, b). The T2 polypeptide can also be subsequently degraded or altered during normal postmortem aging. Studies that have used antibodies against titin have been shown to cease to recognize T2 after prolonged periods of postmortem storage or μ-calpain digestion (Ho et al. 1994; Huff-Lonergan et al. 1996a)

Nebulin

Nebulin is another mega-protein (Mr 600–900 kDa) in the sarcomere. This protein extends from the Z-line to the pointed ends of the thin filament. The C-terminal end of nebulin is embedded into the Z-line. Nebulin is highly nonextensible and has been referred to as a molecular ruler that during development may serve to define the length of the thin filaments (Kruger et al. 1991). Nebulin, via its intimate association with the thin filament (Lukoyanova et al. 2002), has been hypothesized to constitute part of a composite nebulin/thin filament (Pfuhl et al. 1994; Robson et al. 1995) and may aid in anchoring the thin filament to the Z-line (Wang and Wright 1988; Komiyama et al. 1992). Degradation of nebulin postmortem could weaken the thin filament linkages at the Z-line, and/or of the thin filaments in the nearby I-band regions (Taylor et al. 1995), and thereby weaken the structure of the muscle cell. Nebulin has also been shown to be capable of linking actin and myosin (Root and Wang 1994a, b). It has been hypothesized that nebulin may also have a regulatory function in skeletal muscle contraction (Root and Wang 1994a, b; Bang et al. 2006). Portions of nebulin that span the A-I junction have the ability to bind to actin, myosin, and calmodulin (Root and Wang 2001). More interesting, this portion of nebulin (spanning the A-I junction) has been shown to inhibit actomyosin ATPase activity (Root and Wang, 2001; Lukoyanova et al. 2002). This region of nebulin also has been suggested to inhibit the sliding velocities of actin filaments over myosin. If the latter role is confirmed, then it is also possible that nebulin's postmortem degradation may alter actin-myosin interactions in such a way that the alignment and interactions of thick and thin filaments in postmortem muscle is disrupted. This, too, could lead to an increase in postmortem tenderization. Nebulin degradation does seem to be correlated to postmortem tenderization, although the exact cause-and-effect relationship remains to be substantiated (Huff-Lonergan et al. 1995; Taylor et al. 1995;

Huff-Lonergan et al. 1996a; Melody et al. 2004).

Troponin-T

For many years it has been recognized that the degradation of troponin-T and the appearance of polypeptides migrating at approximately 30 kDa are strongly related to, or correlated with, the tenderness of beef (Penny et al. 1974; MacBride and Parrish 1977; Olson and Parrish 1977; Olson et al. 1977). It has been shown that purified bovine troponin-T can be degraded by μ-calpain in vitro to produce polypeptides in the 30-kDa region (Olson et al. 1977). In addition, polypeptides in the 30-kDa region found in aged bovine muscle specifically have been shown to be products of troponin-T by using Western blotting techniques (Ho et al. 1994). Often, more than one fragment of troponin-T can be identified in postmortem muscle. Increasing postmortem time has been shown to be associated with the appearance of two major bands (each is likely a closely spaced doublet of polypeptides) of approximately 30 and 28 kDa, which label with monoclonal antibodies to troponin-T (Huff-Lonergan et al. 1996a). In addition, the increasing postmortem aging time was also associated with a loss of troponin-T, as has been reported in numerous studies (Olson et al. 1977; Koohmaraie et al. 1984a, b; Ho et al. 1994). It has recently been shown that troponin-T is cleaved in its glutamic acid-rich amino-terminal region (Muroya et al. 2007). Some studies have shown labeling of two very closely spaced bands corresponding to intact troponin-T. This is likely due to isoforms of troponin-T that are known to exist in skeletal muscle (Briggs et al. 1990; Malhotra 1994; Muroya et al. 2007), including specifically bovine skeletal muscle (Muroya et al. 2007). Both the appearance of the 30- and 28-kDa bands and the disappearance of the intact troponin-T in the myofibril are very strongly related to the shear force (Penny 1976; Huff-Lonergan et al. 1996b; Huff-Lonergan and Lonergan, 1999; Lonergan et al. 2001; Rowe et al. 2003; Rowe et al. 2004a). Troponin-T is a substrate for μ-calpain, and it is hypothesized that μ-calpain is at least partly responsible for the postmortem degradation of troponin-T and the concomitant production of the 28- and 30-kDa polypeptides. Degradation of troponin-T may simply be an indicator of overall postmortem proteolysis (i.e., it occurs as meat becomes more tender). However, because troponin-T is an integral part of skeletal muscle thin filaments (Greaser and Gergely 1971), its role in postmortem tenderization may warrant more careful examination as has been suggested (Ho et al. 1994; Uytterhaegen et al. 1994; Taylor et al. 1995; Huff-Lonergan et al. 1996b). Indeed, the troponin-T subunit makes up the elongated portion of the troponin molecule and through its interaction with tropomyosin aids in regulating the thin filament during skeletal muscle contraction (Greaser and Gergely 1971; Hitchcock 1975; McKay et al. 1997; Lehman et al. 2001). It is conceivable that postmortem degradation of troponin-T and disruption of its interactions with other thin filament proteins aids in the disruption of the thin filaments in the I-band, possibly leading to fragmentation of the myofibril and overall muscle integrity. During postmortem aging, the myofibrils in postmortem bovine muscle are broken in the I-band region (Taylor et al. 1995). Because troponin-T is part of the regulatory complex that mediates actin-myosin interactions (Greaser and Gergely, 1971; Hitchcock, 1975; McKay et al. 1997; Lehman et al. 2001), it is also conceivable that its postmortem degradation may lead to changes involving thick and thin filament interactions. Regardless of whether or not troponin--T aids in disruption of the thin filament in the I-band, alters thick and thin filament interactions, or simply reflects overall protein degradation, its degradation and appearance

of polypeptides in the 30-kDa region seem to be a valuable indicator of beef tenderness (Olson et al. 1977; Olson and Parrish, 1977; Koohmaraie et al. 1984a, b; Koohmaraie 1992; Huff-Lonergan et al. 1995; Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan 1999).

Desmin

It has been suggested that desmin, an intermediate filament protein (O'Shea et al. 1979; Robson 1989) localized at the periphery of the myofibrillar Z-disk in skeletal muscle (Richardson et al. 1981), plays a role in the development of tenderness (Taylor et al. 1995; Huff-Lonergan et al. 1996a; Boehm et al. 1998; Melody et al. 2004). The desmin intermediate filaments surround the Z-lines of myofibrils. They connect adjacent myofibrils at the level of their Z-lines, and the myofibrils to other cellular structures, including the sarcolemma (Robson, 1989; Robson et al. 1995). Desmin may be important in maintaining the structural integrity of muscle cells (Robson et al. 1981, 1991). It is possible that degradation of structural elements that connect the major components (i.e., the myofibrils) of a muscle cell together, as well as the peripheral layer of myofibrils to the cell membrane, could affect the development of tenderness. Desmin is degraded during postmortem storage (Hwan and Bandman 1989; Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan, 1999; Melody et al. 2004; Rowe et al. 2004b; Zhang et al. 2006). Furthermore, it has been documented that desmin is degraded more rapidly in myofibrils from samples with low shear force and higher water-holding capacity (Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan, 1999; Melody et al. 2004; Rowe et al. 2004b; Zhang et al. 2006). A major degradation product that is often seen in beef is a polypeptide of approximately 38 kDa. This degradation product also has been shown to be present in μ-calpain-digested myofibrils (Huff-Lonergan et al. 1996a; Huff-Lonergan and Lonergan, 1999; Carlin et al. 2006). Thus, the proteolytic enzyme μ-calpain may be, at least in part, responsible for desmin degradation under normal postmortem aging conditions. Whether or not this degradation is truly directly linked to tenderization or is simply an indicator of overall postmortem proteolysis remains to be determined.

Filamin

Filamin is a large (*M*r = 245,000 in skeletal and cardiac muscle) actin-binding protein that exists in numerous cell types (Loo et al. 1998; Thompson et al. 2000; van der Flier et al. 2002). There are several different isoforms of filamin (Hock et al. 1990). The amount of filamin in skeletal and cardiac muscle is very low (approximately ≤0.1% of the total muscle protein). In skeletal and cardiac muscle, filamin is localized at the periphery of the myofibrillar Z-disk, and it may be associated with intermediate filaments in these regions (Loo et al. 1998; Thompson et al. 2000; van der Flier et al. 2002). Thus, postmortem degradation of filamin conceivably could disrupt key linkages that serve to help hold myofibrils in lateral register. Degradation of filamin may also alter linkages connecting the peripheral layer of myofibrils in muscle cells to the sarcolemma by weakening interactions between peripheral myofibrillar Z-disks and the sarcolemma via intermediate filament associations or costameres (Robson et al. 1995). A study using myofibrils from beef showed that some filamin was degraded to form an approximately 240-kDa degradation product that migrated as a doublet in both myofibrils from naturally aged muscle and in μ-calpain-digested myofibrils (Huff-Lonergan et al. 1996a). This same doublet formation (composed of intact and degraded filamin) has been seen in cultured embryonic skeletal muscle cells and was attributed to calpain

activity (Robson et al. 1995). Uytterhaegen et al. (1994) have shown increased degradation of filamin in muscle samples injected with $CaCl_2$, a process that has been shown to stimulate proteolysis and postmortem tenderization (Wheeler et al. 1992; Harris et al. 2001). Compared with other skeletal muscle proteins, relatively little has been done to fully characterize the role of this protein in postmortem tenderization of beef. Further studies that employ a combination of sensitive detection methods (e.g., one- and two-dimensional gels, Western blotting, immunomicroscopy) are needed to determine the role of filamin in skeletal muscle systems and postmortem tenderization.

Water-Holding Capacity/Drip Loss Evolution

Lean muscle contains approximately 75% water. The other main components include protein (approximately 18.5%), lipids or fat (approximately 3%), carbohydrates (approximately 1%), and vitamins and minerals (often analyzed as ash, approximately 1%). The majority of water in muscle is held within the structure of the muscle and muscle cells. Specifically, within the muscle cell, water is found within the myofibrils, between the myofibrils themselves and between the myofibrils and the cell membrane (sarcolemma), between muscle cells, and between muscle bundles (groups of muscle cells) (Offer and Cousins 1992).

Water is a dipolar molecule and as such is attracted to charged species like proteins. In fact, some of the water in muscle cells is very closely bound to protein. By definition, bound water is water that exists in the vicinity of nonaqueous constituents (like proteins) and has reduced mobility (i.e., does not easily move to other compartments). This water is very resistant to freezing and to being driven off by conventional heating (Fennema 1985). True bound water is a very small fraction of the total water in muscle cells; depending on the measurement system used, approximately 0.5 g of water per gram of protein is estimated to be tightly bound to proteins. Since the total concentration of protein in muscle is approximately 200 mg/g, this bound water only makes up less than a tenth of the total water in muscle. The amount of bound water changes very little if at all in postrigor muscle (Offer and Knight 1988b).

Another fraction of water that can be found in muscles and in meat is termed entrapped (also referred to as immobilized) water (Fennema 1985). The water molecules in this fraction may be held either by steric (space) effects and/or by attraction to the bound water. This water is held within the structure of the muscle but is not bound per se to protein. In early postmortem tissue, this water does not flow freely from the tissue, yet it can be removed by drying and can be easily converted to ice during freezing. Entrapped or immobilized water is most affected by the rigor process and the conversion of muscle to meat. Upon alteration of muscle cell structure and lowering of the pH, this water can also eventually escape as purge (Offer and Knight 1988b).

Free water is water whose flow from the tissue is unimpeded. Weak surface forces mainly hold this fraction of water in meat. Free water is not readily seen in pre-rigor meat, but can develop as conditions change that allow the entrapped water to move from the structures where it is found (Fennema 1985).

The majority of the water that is affected by the process of converting muscle to meat is the entrapped (immobilized) water. Maintaining as much of this water as possible in meat is the goal of many processors. Some of the factors that can influence the retention of entrapped water include manipulation of the net charge of myofibrillar proteins and the structure of the muscle cell and its components (myofibrils, cytoskeletal linkages, and membrane permeability), as well as the

amount of extracellular space within the muscle itself.

Physical/Biochemical Factors in Muscles That Affect Water-Holding Capacity

During the conversion of muscle to meat, anaerobic glycolysis is the primary source of ATP production. As a result, lactic acid builds up in the tissue, leading to a reduction in pH of the meat. Once the pH has reached the isoelectric point (pI) of the major proteins, especially myosin (pI = 5.3), the net charge of the protein is zero, meaning the numbers of positive and negative charges on the proteins are essentially equal. These positive and negative groups within the protein are attracted to each other and result in a reduction in the amount of water that can be attracted and held by that protein. Additionally, since like charges repel, as the net charge of the proteins that make up the myofibril approaches zero (diminished net negative or positive charge), repulsion of structures within the myofibril is reduced, allowing those structures to pack more closely together. The end result of this is a reduction of space within the myofibril. Partial denaturation of the myosin head at low pH (especially if the temperature is still high) is also thought to be responsible for a large part of the shrinkage in myofibrillar lattice spacing (Offer 1991).

Myofibrils make up a large proportion of the muscle cell. These organelles constitute as much as 80–90% of the volume of the muscle cell. As mentioned previously, much of the water inside living muscle cells is located within the myofibril. In fact, it is estimated that as much as 85% of the water in a muscle cell is held in the myofibrils. Much of that water is held by capillary forces arising from the arrangement of the thick and thin filaments within the myofibril. In living muscle, it has been shown that sarcomeres remain isovolumetric during contraction and relaxation (Millman et al. 1981; Millman et al. 1983). This would indicate that in living muscle the amount of water within the filamentous structure of the cell would not necessarily change. However, the location of this water can be affected by changes in volume as muscle undergoes rigor. As muscle goes into rigor, cross-bridges form between the thick and thin filaments, thus reducing available space for water to reside (Offer and Trinick 1983). It has been shown that as the pH of porcine muscle is reduced from physiological values to 5.2–5.6 (near the isoelectric point of myosin), the distance between the thick filaments declines an average of 2.5 nm (Diesbourg et al. 1988). This decline in filament spacing may force sarcoplasmic fluid from between the myofilaments to the extramyofibrillar space. Indeed, it has been hypothesized that enough fluid may be lost from the intramyofibrillar space to increase the extramyofibrillar volume by as much as 1.6 times more than its pre-rigor volume (Bendall and Swatland 1988).

During the development of rigor, the diameter of muscle cells decreases (Hegarty 1970; Swatland and Belfry 1985) and is likely the result of transmittal of the lateral shrinkage of the myofibrils to the entire cell (Diesbourg et al. 1988). Additionally, during rigor development, sarcomeres can shorten; this also reduces the space available for water within the myofibril. In fact, it has been shown that drip loss can increase linearly with a decrease in the length of the sarcomeres in muscle cells (Honikel et al. 1986). More recently, highly sensitive low-field nuclear magnetic resonance (NMR) studies have been used to gain a more complete understanding of the relationship between muscle cell structure and water distribution (Bertram et al. 2002). These studies have suggested that within the myofibril, a higher proportion of water is held in the I-band than in the more protein-dense A-band. This observation may help explain why shorter sarcomeres (especially in cold-shortened

muscle) are often associated with increased drip losses. As the myofibril shortens and rigor sets in, the shortening of the sarcomere would lead to shortening and subsequent lowering of the volume of the I-band region in myofibril. Loss of volume in this myofibrillar region (where much water may reside), combined with the pH-induced lateral shrinkage of the myofibril, could lead to expulsion of water from the myofibrillar structure into the extramyofibrillar spaces within the muscle cell (Bendall and Swatland 1988). In fact, recent NMR studies support this hypothesis (Bertram et al. 2002). It is thus likely that the gradual mobilization of water from the intramyofibrillar spaces to the extramyofibrillar spaces may be key in providing a source of drip.

All the previously mentioned processes influence the amount of water in the myofibril. It is important to note that shrinkage of the myofibrillar lattice alone could not be responsible for the movement of fluid to the extracellular space and ultimately out of the muscle. The myofibrils are linked to each other and to the cell membrane via proteinacious connections (Wang and Ramirez-Mitchell 1983). These connections, if they are maintained intact in postmortem muscle, would transfer the reduction in diameter of the myofibrils to the muscle cell (Diesbourg et al. 1988; Morrison et al. 1998; Kristensen and Purslow 2001; Melody et al. 2004). Myofibril shrinkage can be translated into constriction of the entire muscle cell, thus creating channels between cells and between bundles of cells that can funnel drip out of the product (Offer and Knight 1988). Extracellular space around muscle fibers continually increases up to 24 hours postmortem, but gaps between muscle fiber bundles decrease slightly between nine and 24 hours postmortem, perhaps due to fluid outflow from these major channels (Schafer et al. 2002). These linkages between adjacent myofibrils and myofibrils and the cell membrane are made up of several proteins that are associated with intermediate filament structures and structures known as costameres. Costameres provide the structural framework responsible for attaching the myofibrils to the sarcolemma. Proteins that make up or are associated with the intermediate filaments and costameres include (among others) desmin, filamin, synemin, dystrophin, talin, and vinculin (Greaser 1991). If costameric linkages remain intact during the conversion of muscle to meat, shrinkage of the myofibrils as the muscle goes into rigor would be transmitted to the entire cell via these proteinacious linkages and would ultimately reduce volume of the muscle cell itself (Offer and Knight 1988b; Kristensen and Purslow 2001; Melody et al. 2004). Thus, the rigor process could result in mobilization of water not only out of the myofibril, but also out of the extramyofibril spaces as the overall volume of the cell is constricted. In fact, reduction in the diameter of muscle cells has been observed in postmortem muscle (Offer and Cousins 1992). This water that is expelled from the myofibril and ultimately the muscle cell eventually collects in the extracellular space. Several studies have shown that gaps develop between muscle cells and between muscle bundles during the postrigor period (Offer et al. 1989; Offer and Cousins 1992). These gaps between muscle bundles are the primary channels by which purge is allowed to flow from the meat; some investigators have actually termed them “drip channels.”

Postmortem Changes in Muscle That Influence Quality

As muscle is converted to meat, many changes occur, including: (1) a gradual depletion of available energy; (2) a shift from aerobic to anaerobic metabolism favoring the production of lactic acid, resulting in the pH of the tissue declining from near neutrality to 5.4–5.8; (3) a rise in ionic strength, in part, because of the inability of ATP-dependent

calcium, sodium, and potassium pumps to function; and (4) an increasing inability of the cell to maintain reducing conditions. All these changes can have a profound effect on numerous proteins in the muscle cell. The role of energy depletion and pH change have been covered in this chapter and in other reviews (Offer and Trinick 1983; Offer and Knight 1988a). What has not been as thoroughly considered is the impact of other changes on muscle proteins, such as oxidation and nitration.

Protein Oxidation

Another change that occurs in postmortem muscle during aging of whole muscle products is increased oxidation of myofibrillar and sarcoplasmic proteins (Martinaud et al. 1997; Rowe et al. 2004a, b). This results in the conversion of some amino acid residues, including histidine, to carbonyl derivatives (Levine et al. 1994; Martinaud et al. 1997) and can cause the formation of intra- and/or inter-protein disulfide cross-links (Stadtman 1990; Martinaud et al. 1997). In general, both these changes reduce the functionality of proteins in postmortem muscle (Xiong and Decker 1995). In living muscle, the redox state of muscle can influence carbohydrate metabolism by directly affecting enzymes in the glycolytic pathway. Oxidizing agents can also influence glucose transport. Hydrogen peroxide (H_2O_2) can mimic insulin and stimulate glucose transport in exercising muscle. H_2O_2 is increased after exercise, and thus oxidation systems may play a role in signaling in skeletal muscle (Balon and Yerneni 2001). Alterations in glucose metabolism in the ante- and perimortem time period do have the potential to cause changes in postmortem muscle metabolism and thus represent an important avenue of future research.

In postmortem muscle, these redox systems may also play a role in influencing meat quality. The proteolytic enzymes, the calpains, are implicated in the proteolysis that is involved in increasing the tenderness of fresh meat and in influencing fresh meat water-holding capacity (Huff-Lonergan and Lonergan 2005). Because μ-calpain and m-calpain enzymes contain both histidine and SH-containing cysteine residues at their active sites, they are particularly susceptible to inactivation by oxidation (Lametsch et al. 2008). Therefore, oxidizing conditions in postmortem muscle lead to inactivation or modification of calpain activity (Harris et al. 2001; Rowe et al. 2004a, b; Maddock et al. 2006). In fact, evidence suggests oxidizing conditions inhibit proteolysis by μ-calpain, but might not completely inhibit autolysis (Guttmann et al. 1997; Guttmann and Johnson 1998; Maddock et al. 2006). In postmortem muscle, there are differences between muscles in the rate that postmortem oxidation processes occur (Martinaud et al. 1997). It has been noted that differences in the rate of oxidation in muscle tissue are seen when comparing the same muscles between animals and/or carcasses that have been handled differently (Juncher et al. 2001). These differences may arise because of differences in diet, breed, antemortem stress, postmortem handling of carcasses, etc. In fact, there have been reports of differences between animals and between muscles in the activity of some enzymes involved in the oxidative defense system of muscle (Daun et al. 2001). Therefore, there may be genetic differences in susceptibility to oxidation that could be capitalized on to improve meat quality. It is reasonable to hypothesize that differences in the antioxidant defense system between animals and/or muscles would influence calpain activity, proteolysis, and thus tenderization.

Exposure to oxidizing conditions (H_2O_2) under postmortem-like conditions inhibits calpain activity (Carlin et al. 2006). In a series of in vitro assays using either a fluorescent peptide or purified myofibrils as the substrate it was shown that the presence of oxidizing species does significantly impede

the ability of calpains to degrade their substrates. Oxidation with H_2O_2 significantly limits proteolytic activity of μ- and m-calpain against the fluorescent peptide Suc-Leu-Leu-Val-Tyr-AMC, regardless of the pH or ionic strength. Similar results were seen when using purified myofibrils as the substrate. This inhibition was reversible, as addition of reducing agent (DTT) to the oxidized samples restored activity. Oxidation also has been shown to slow the rate of μ-calpain autolysis and could be part of the mechanism underlying some of the retardation of activity (Guttmann et al. 1997; Carlin et al. 2006).

Oxidation does occur early in postmortem meat, and it does influence proteolysis (Harris et al. 2001; Rowe et al. 2004b). Rowe et al. (2004) showed that there was a significant increase in proteolysis of troponin-T in steaks from alpha-tocopherol-fed steers after 2 days of postmortem aging compared with steers fed a conventional feedlot diet. This indicates that very low levels of oxidation can influence proteolysis and that increasing the level of antioxidants in meat may have merit in improving tenderness in future studies. In fact, low levels of oxidation may be the cause of some heretofore-unexplained variations in proteolysis and tenderness that have been observed in meat.

Nitric Oxide and S-Nitrosylation

Nitric oxide (NO) is often used as a general term that includes NO and reactive nitrogen species (RNS), like S-nitrosothyols, peroxynitrate, and metal NO complexes. In living tissue, NO is involved in arteriole dilation that increases blood flow to muscles, resulting in increased delivery of nutrients and oxygen to the muscle (Kobzik et al. 1994; Stamler et al. 2001). NO species are also implicated in glucose homeostasis and excitation-contraction coupling. The gas NO is produced in biological systems by a family of enzymes known as nitric oxide synthases (NOS). There are three major isoforms of NOS: neural, inducible, and endothelial. Skeletal muscle expresses all three isoforms; however, the neural form, nNOS, is thought to be the predominant isoform (Kaminski and Andrade 2001). These enzymes utilize arginine as a substrate and catalyze the following reaction: L-arginine+NADPH+O_2 forming L-citrulline+$^{\bullet}$NO+$NADPH^+$. NO is important in biological systems, particularly because of its role as a second messenger. However, while NO rapidly diffuses through tissues, NO itself is a relatively short-lived species. It does have the ability to combine with other biomolecules that also have physiological importance.

One example of this is its ability to combine with superoxide to form the highly oxidizing molecule peroxynitrite. Proteins are important biological targets of peroxynitrite, particularly proteins containing cysteine, motioning, and/or tryptophan (Radi et al. 2000). Several enzymes are known to be inactivated by peroxynitrite. Among these is the sarcoplasmic reticulum Ca^{2+}-ATPase (Klebl et al. 1998). One indirect effect of NO is S-nitrosylation. In most cases, S-nitrosylation events involve amines and thiols. Nitric oxide can interact with cysteines to form nitrosothiols that can alter the activity of the protein. Because of this, it has been suggested that S-nitrosylation may function as a post-translational modification much like phosphorylation (Jaffrey et al. 2001). Some proteins, such as the ryanodine receptor and the cysteine protease caspase-3, have been shown to be endogenously nitrosylated, further supporting the suggestion that formation of nitrosothiols may be an important regulatory step (Hess et al. 2001; Hess et al. 2005). μ-Calpain is also a cysteine protease that could be influenced by S-nitrosylation. Small thiol peptides like glutathione can be impacted by nitrosative stress to form compounds like S-nitrosoglutathione (GSNO). These compounds can, in turn, influence other proteins

by transnitrosating other reduced thiols (Miranda et al. 2000).

Aspects of skeletal muscle function that can be affected by increased NO production include inhibition of excitation-contraction coupling, increased glucose uptake, decreased mitochondrial respiration, and decreased force production. The decrease in force is apparently because of an inhibitory effect that NO has on actomyosin ATPase activity, which leads to less cross-bridge cycling. S-nitroslyation of the ryanodine receptor (calcium release channel in the sarcoplasmic reticulum) may also play a role on modulating contraction. This protein is responsible for releasing calcium from the sarcoplasmic reticulum into the sarcoplasm. S-nitrosylation of a cysteine in the ryanodine receptor will increase its activity. This effect is reversible (Kobzik et al. 1994). Because muscle contains all the compounds needed to form these intermediates, it stands to reason that they could be important in the conversion of muscle to meat.

It is clear that the composition, structure, and metabolic properties of skeletal muscle have enormous impacts on the quality of fresh meat and, in turn, its suitability as a raw material for further processed meat. Continued attention to factors that regulate changes in early postmortem muscle will improve the quality and consistency of fresh meat. This, in turn, will improve the consistency of the quality of further processed products.

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