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Review

# Apoptosis or autophagy, that is the question: Two ways for muscle sacrifice towards meat

Alessandro Lana, Lello Zolla*

*Department of Ecological and Biological Sciences, University of Tuscia, Largo dell'Universita, snc, 01100 Viterbo, Italy*

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ABSTRACT

*Background:* Meat derives from muscle, but they are extremely different. The slaughtered muscle undergoes a number of biological changes during the maturation period, which is pivotal for the transformations that permit to obtain the final marketable product.
*Scope and approach:* In this review a general process driving muscle-to-meat conversion is described, despite all the factors that can affect and diversify every individual process. We focus our attention on the switch from the normal, aerobic metabolism to the post-slaughter, anaerobic one, underlining all the consequences in terms of muscle reactions driving and influencing the transformation. The massive production of ROS is the pivotal event of the muscle-to-meat conversion, and muscle cells are stimulated to react as to cope with the oxidative stress. Despite the mobilization of defensive machineries, it soon becomes overwhelming and unsustainable: muscle cells are forced to die.
*Key findings and conclusions:* ROS can induce both autophagy and apoptosis. Their role in muscle conversion is not completely clear, despite their differences have large influence on meat maturation and final product. A deeper understanding is pivotal on this argument as to better manage meat production.


## 1. Introduction

It is of fundamental importance to stress the striking differences existing between muscle and meat, which do not consist only in the muscular tissue before and after the killing of the animal: the post-slaughter period, and the time granted for meat aging, are the cradle for the intrinsic revolution undergone by the muscle at the molecular level, necessary to obtain the eatable, marketable meat. Muscles stop to be muscles, and become meat, when they lose their ability to contract as a result of ATP depletion. Every meat has its specific peculiarities, coming upstream from the genetic settings of the species, and rolling over a series of additional factors all influencing the final product: age, sex, nutritional status, pre-slaughter conditions and level of stress of the living animal, post-slaughter handling, storage modalities and so on (Van de Perre, Permentier, De Bie, Verbeke, & Geers, 2010; Sierra & Oliván, 2013). All of these points affect the molecular processes of muscle-to-meat conversion, thus giving a picture of its complexity; in the light of it, we are not far from a realistic view affirming that each individual animal presents its own meat, as to say that every meat is different from each other.

Because of the extraordinary economical importance of meat products, many efforts have been directed to a better understanding of muscle conversion to improve the quality of the final product put on the market. In the multifactorial ocean above mentioned, the research has been capable to fix some bullet points shared by each developing meat that help to clarify the underlying molecular mechanism. In this review, we want to give a picture of the state-of-the-art of our comprehension of this process. We describe the energetic gears of muscular tissue in physiological conditions to explain the traumas suffered by muscle cells after the animal sacrifice, which brutally overturn cellular environmental conditions; we then focus on the pivotal role of the inevitably increasing oxidative stress and on the subsequent muscular reactions, leading to the hamletic question: 'apoptosis or autophagy?'

## 2. The switch: from aerobiosis to anoxia

Muscle architecture probably shows one of the higher degree of tissue spatial organization that permits the generation of mechanical energy by means of the conversion of chemical energy. The

* Corresponding author.
*E-mail address:* zolla@unitus.it (L. Zolla).

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chemical energy used is supplied by ATP, coming from the phosphodiester bound between the inorganic phosphate group and the ADP. The actomyosin complex is able to dissociate only in the presence of ATP, because of the conformational change induced by ATP binding on a specific site of the myosin globular head; if available, the contraction cycle can restart.

The availability of ATP is principally dependent on three sources: muscular ATP and phosphocreatine reservoirs, oxidative phosphorylation and anaerobic glycolysis. We can categorize them in this way, but clearly in the healthy functioning muscle they are intertwined faces of the same cube: muscles are highly ATP-demanding tissues, and the ATP reservoirs alone are not able to sustain such a heavy need in the long period; the rapid consumption of this amount is buffered by the phosphocreatine shuttle, which can be considered as a medium between the core engine of the system, namely oxidative phosphorylation, and the myosin ATPase activity. In the presence of O2, during aerobic metabolism, electron chain reactions permit the massive production of ATP in mitochondria; here, the mitochondrial isoform of creatine kinase enzyme loads creatine molecules with the high-energy phosphate group supplied by the ATP tokens, and the resulting phosphocreatine is able to diffuse into the cytoplasm. The cytosolic creatine kinase replenishes the muscle fibers with ATP by reloading ADP. This mechanism works when muscular cells have an adequate oxygen supply, but in anaerobic conditions their metabolism undergoes a switch, whose central energy producer becomes glycolysis. In living animals, the anaerobic metabolism resettles for example during an intensive physical exercise; the accumulation of lactic acid is the consequence of oxidative phosphorylation and Krebs cycle stoppage, because of the upstream accumulation of pyruvate (as the last glycolytic product) that needs to be decreased to permit glycolytic progression. The reduction of pyruvate to lactate, thanks to the electrons given by glycolytic NADH (that in turn needs this alternative way to be oxidized to NAD+, given the stop of oxidative phosphorylation) is the solution. The consequent accumulation of lactate in living animals is solved by the continuous blood stream, able to transport it from peripheral tissues to the liver, where it is converted back to glucose (Cori cycle).

Due to the stoppage of the circulation after slaughter, the critical event, from which all the following fall as more or less direct consequences, is the switch from a normal, aerobic metabolism to an anaerobic one, and this is the common event for every kind of meat considered; we can fix three bullet points from the moment of the slaughter: 1) pre-rigor mortis phase, where, despite the sudden lack of oxygen, muscles are still excitable utilizing oxygen reservoirs (hemoglobin, myoglobin; Ouali et al., 2006) and ATP and phosphocreatine reservoirs; 2) rigor mortis phase, beginning temporally from 3 to 6 hours after slaughter and physiologically when ATP levels drop. From now on, muscle can be considered meat; it will undergo a great number of physical and chemical changes; 3) the post-rigor mortis phase, also designated as tenderization phase. Skeletal muscles are among the major oxygen-consuming tissues, characterized by a high rate of mitochondrial respiration and a correspondently high risk of ROS (reactive oxygen species) production (Murphy, 2009). Nowadays, ROS are no longer seen only as harmful compounds (Barbieri & Sestili, 2011); beside their toxicity, many physiological signaling functions in muscle have been recognized: a transient, moderate increase can be the fuse for triggering a healthy process (as it is in living animals), whereas an excessive and uncontrolled production falls into irremediable cellular damages. During the process of muscle-to-meat conversion, ROS activity is inevitably persistent in time, and the progressive accumulation mobilizes all defensive responses that the muscular cell can produce; in the slaughtered muscle, the production of ROS progressively increases, while antioxidant capacities constantly decrease: our recent works shows the ongoing impairment of muscle energetic system in Piedmontese Longissimus Dorsi meat (Longo, Lana, Bottero, & Zolla, 2015), describing the inversion of GSH/GSSG ratio and the drop of ATP levels, as well as the impairment of phosphocreatine shuttle. All these conditions promote oxidative stress.

### 2.1. Sources of ROS in skeletal muscle

Like any other kind of cell, the muscular cell is subjected to the 'Oxygen paradox' (Davies, 1995), with the oxygen being the two-face central molecule of higher eukaryotes aerobic metabolism: on the one hand, oxygen is the final acceptor of the electron transport chain; on the other hand, it shows a dangerous 'dark side', assisted by the innate imperfections of the mechanism of oxidative phosphorylation.

Mitochondria are considered the major sources of ROS production, because of the heavy use of O2 in their metabolic activities (Brand, 2010) and of the intrinsic hallmarks of the oxidative phosphorylation, which is based on the oxygen as the final acceptor of electrons donated by reducing substrates: indeed, one electron at a time is used for $O_2$ reduction to $H_2O$, and between the first and the final step, the intermediate products are all ROS ($O_2 \rightarrow O_2^{-\bullet} \rightarrow H_2O_2 \rightarrow {}^{\bullet}OH \rightarrow H_2O$) (Grivennikova & Vinogradov, 2013); the superoxide anion $O_2^{-\bullet}$ is the primary species and the precursor of the other reactive species (not only oxygen-derived but also nitrogen-derived; Radi, Cassina, & Hodara, 2002), produced by NADPH oxidase, cytochrome P450-dependent oxygenases and xanthine oxidase; its enzymatic dismutation by superoxide dismutase is the responsible for the formation of H2O2. $H_2O_2$ in turn is transformed to hydroxyl radical •OH by the Fenton reaction because of the comodulation guaranteed by the presence of metal ions (Altun et al., 2007), especially iron ions contained into mitochondria and myoglobin molecules of muscles. Overall, at least ten mitochondrial enzymes contribute to ROS production (Marchi et al., 2012).

Although mitochondria are generally accepted as the major source of ROS in muscular cells, in the last years a few evidences contradicted this hypothesis, opening the debate on the argument (Brown & Borutaite, 2012).

The PLA2 (phospholipase A2), particularly the calcium-dependent isoform, has been proposed to turn on under stress conditions to trigger ROS production (Gong et al., 2006). Furthermore, it has been demonstrated that xanthine oxidase, under prolonged ischemic conditions (such as in the slaughtered muscle), is operative in both mitochondrial and cytoplasmic production of ROS (Kelley et al., 2010).

The slaughtered muscle can undergo inflammation reaction; the infiltrated polymorphoneutrophils activate NADPH oxidases by means of ROS production (respiratory burst) and secrete cytokines within the muscle, which bind specific membrane receptors and activate cyclooxygenases- and xanthine oxidase-ROS producing enzymes; a positive feedback cycle takes place thanks to the secretion of interleukins (IL-1, IL-6, IL-8) and TNFα by injured endothelial cells into the muscle, so triggering a continuous ROS production (Ji, 2007). While this inflammation-triggered ROS formation is essential under physiologic conditions to the restoration of antiseptic environment and normal homeostasis, an uncontrolled stress status contributes to the decrease of antioxidant defensive mechanisms.

Other important sources are NADPH oxidases within the sarcoplasmic reticulum and transverse tubules (Holmstrom & Finkel, 2014).