

Systems Pathology: Muscle System

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About

These represent the course notes for the skeletal muscle system for VETM 2220. They are broken down into several sections based on disease process.

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Chapter 1

Introduction

1.1 The anatomy of muscle

The basic structural unit of muscle is the **myofiber**, which represents a single, long, tubular cell (Figure 1.1). Within each myofiber are many tightly packed **myofibrils**, which are composed of actin and myosin filaments, and which form the contractile machinery of the muscle. It is the arrangement of myofibrils that form the striated appearance of skeletal muscle that can be appreciated under light microscopy (Figure 1.2). The cytoplasm of a myofiber is known as the **sarcoplasm**, while the cell membrane is called the **sarcolemma**. Each myofiber is surrounded by a small amount of connective tissue called the **endomysium**. Multiple myofibers form a **fasicle** that is surrounded by another layer of connective tissue, the **perimysium**. Finally, multiple fascicles group together to form a muscle.

Skeletal muscle is characteristically multinucleated: each myofiber has 100s of nuclei scattered along its length, almost always found along the periphery of the cell. Each nucleus within a myofiber controls a specific portion of the myofiber, and each nucleus acts independently. Nuclei within muscle fibers are *terminally differentiated*, meaning they can no longer divide, thus limiting the capacity for regeneration. On the other hand, having multiple nuclei within a cell provides a distinct benefit: localized damage, affecting a small number of nuclei, will not necessarily kill the entire cell, giving the myofiber an opportunity to regenerate. We will discuss muscle regeneration in more detail in the section on Necrosis and regeneration.

Closely associated with individual myofibers are **satellite cells**. The nuclei of satellite cells are indistinguishable from myofiber nuclei under light microscopy. Satellite cells are a type of stem cell, and are important in muscle regeneration and repair.

Myofibers can be subclassified based on certain properties that reflect their function. The classification is based on three physiologic properties:

1. Rate of contraction (fast vs. slow)
2. Rate of fatigue (fast vs. slow)
3. Type of metabolism (oxidative, glycolytic, or mixed)

Taking these characteristics into consideration leads to three subtypes: Type 1, Type 2a, and Type 2b (Table 1.1). Note that muscles are rarely, if ever, composed of a single subtype: they are a mixture of the different subtypes, though one type often predominates.

1.2 The function of muscle

The contraction of muscle is a complex, orchestrated process in which myofilaments (the components of myofibrils) undergo a conformational change that results. The contraction of muscle is initiated at the

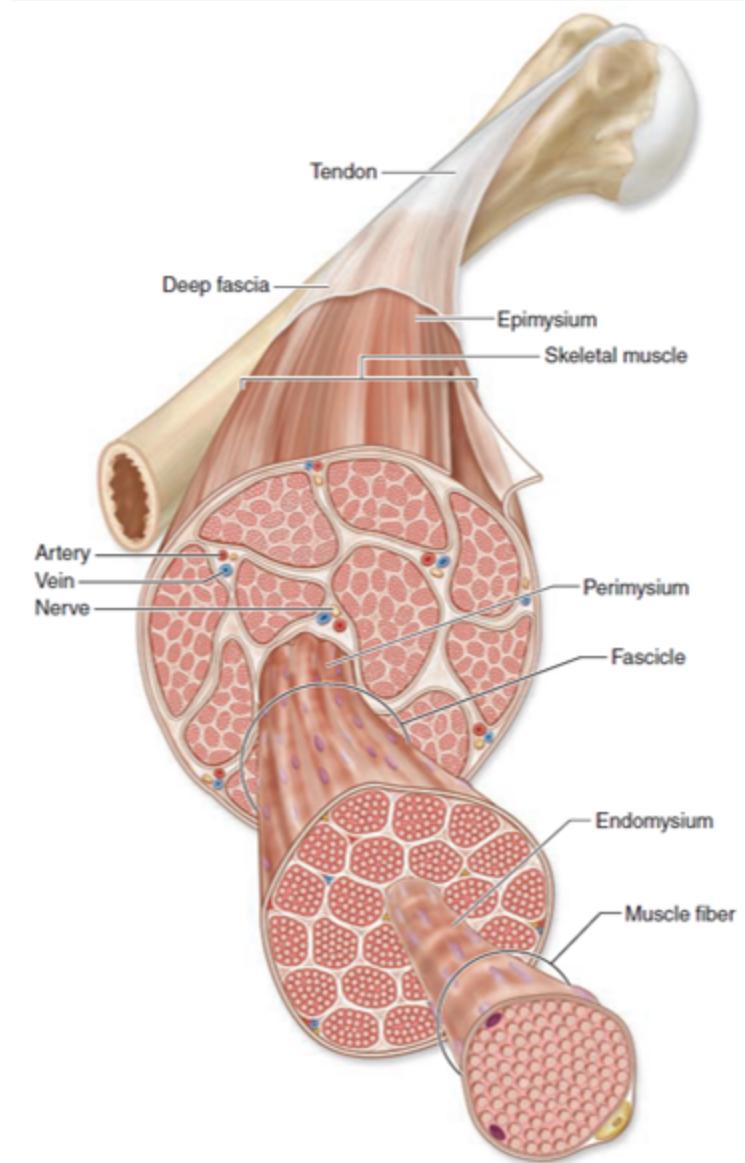


Figure 1.1: Structure and anatomy of muscle

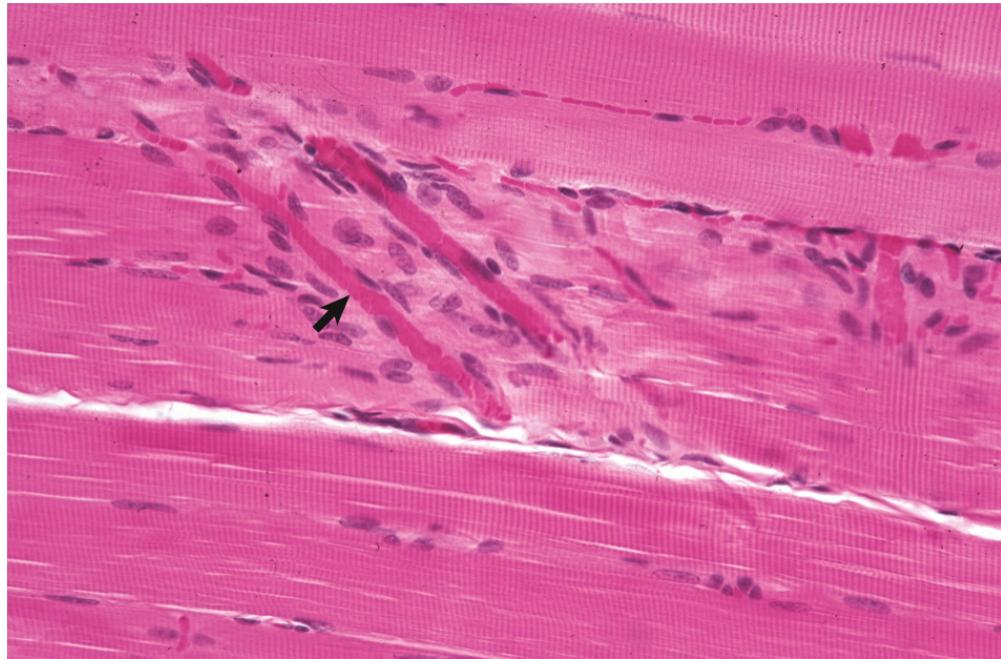


Figure 1.2: Normal skeletal muscle demonstrating striations. The arrow indicates a small capillary.

Table 1.1: Properties of different myofiber types

Fiber.type	Physiologic.characteristics	Morphologic.characteristics
Type 1	slow twitch, fatigue resistant, oxidative, aerobic, 'red'	High mitochondrial and fat content, low glycogen
Type 2a	Fast twitch, oxidative, glycolytic, fatigue resistant	Intermediate mitochondria, fat, and glycogen content
Type 2b	Fast twitch, fatigue sensitive, glycolytic, 'white'	Low mitochondrial and fat content, high glycogen

motor end plate by the release of acetylcholine from a motor neuron into the neuromuscular junction. This depolarizes the myofiber, resulting in the release of **calcium** from the sarcoplasmic reticulum. It is the binding of calcium to the myofilament troponin that results in the contraction of the sarcomere. Importantly, calcium must be pumped back into the sarcoplasmic reticulum in an ATP-dependent process. Once this occurs, the muscle enters into a relaxed, resting state.

1.3 Response of muscle to injury

Skeletal muscle can undergo a fairly limited range of changes in response to environmental and physiologic stimuli. Muscle can shrink (atrophy), get bigger (hypertrophy), or die (necrosis). Under certain circumstances, muscles that have been only mildly injured can regenerate. The reaction of muscle to injury tends to proceed in a fairly stereotypic fashion regardless of the inciting cause, making it difficult to determine the underlying etiology from gross or histopathological examination alone. Thus, **it is important to provide a good clinical history** when submitting a case with suspected muscle injury. Supplementary tests (special stains, culture, etc.) are also often necessary to obtain a definitive diagnosis.

1.3.1 Atrophy

Atrophy simply refers to the reducing in volume of muscle or myofiber, and is usually reversible, provided the cause can be corrected.

1.3.1.1 Denervation atrophy

Denervation atrophy is caused by the loss of a nerve that innervates a myofiber. It is rapid, severe, relatively common, and can result in the loss of more than half of the affected muscle mass in a matter of weeks. The maintenance of a normal myofiber diameter is reliant in part on an intact associated nerve, which generates trophic factors. Loss of a nerve leads to loss of the trophic factors, and results in atrophy. Interestingly, it is not a factor of contractile activity: disorders such as [Botulism] or [Tetanus] that affect the neuromuscular junction do not lead to atrophy. Denervation atrophy tends to affect both type 1 and 2 myofibers. Examples of disorders caused by denervation atrophy include [Equine laryngeal hemiplegia (“roaring”)], [“Sweeney”], [Equine motor neuron disease], [Equine protozoal myeloencephalitis], and [Radial nerve paralysis].

1.3.1.2 Disuse atrophy

Decreased contractile activity of a muscle for any reason leads to disuse atrophy. Common causes include lameness/pain or limb immobilization (e.g. cast), and it tends to occur more gradually than denervation atrophy. Disuse atrophy preferentially leads to atrophy of type 2 fibers, however, this is somewhat inconsistent, thus relying solely on type 2 atrophy to distinguish this from denervation atrophy is risky. Because the muscle fibers undergoing disuse atrophy are not being used, there is no compensatory hypertrophy.

1.3.1.3 Nutritional (malnutrition or cachexia) atrophy

Failure to supply enough dietary nutrients to maintain normal muscle mass leads to nutritional atrophy. It is a gradual form of muscle loss and tends to be generalized, though in the dog, loss of the temporal, back, and thigh muscles are often prominent. Muscle proteins undergo continuous turnover, and in states of starvation, can be used as a source of nutrients. Cachectic animals with chronic illness or neoplasia lose muscle mass due to increased circulating levels of **TNF** (also known as “cachectin”), which increases myofiber catabolism. Type 2 fibers are preferentially affected.

1.3.1.4 Atrophy of endocrine disease

This is a relatively specific category of atrophy, most commonly noted in dogs with hypothyroidism or hyperadrenocorticism. Type II fibers are preferentially affected.

1.3.2 Hypertrophy

Hypertrophy refers to grossly enlarged muscles, or to histologically enlarged myofibers. It does *not* refer to an increase in number of myofibers. It can be the result of physiologic stimulation or pathologic processes. Physiologic hypertrophy is generally the result of increased workload on the muscle. Pathologic hypertrophy occurs in response to a number of conditions. Compensatory hypertrophy of unaffected myofibers can occur in a background of neuropathic or myopathic atrophy.

1.3.3 Necrosis and regeneration

Myofiber necrosis is a non-specific finding that can accompany a variety of different diseases and conditions. Grossly, necrosis of muscle is usually only appreciated in severe cases. Necrotic muscle is typically pale to white, and may appear streaked and slightly gritty if mineralization has occurred (for example, in [White muscle disease])(Figure 1.3). Necrotic muscle can alternatively appear deeply red, if hemorrhage has occurred concurrently.

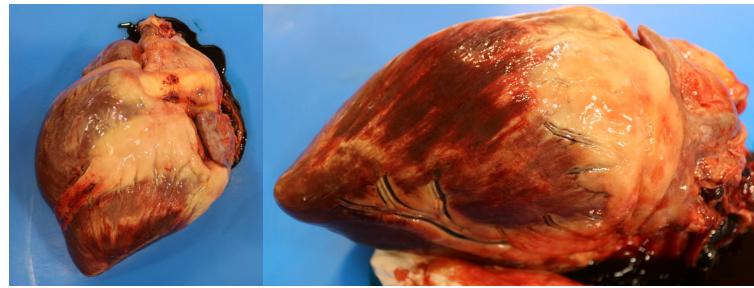


Figure 1.3: Necrosis of the myocardium. Pale white streaks bordered by hemorrhage are visible throughout the ventricle. Photo: C. Martin

The outcome of muscle necrosis is dependent on its severity. If the basal lamina remains intact, and the damage only affects a small portion of the myofiber, then muscle can regenerate. The basal lamina is a thin layer of extracellular matrix that keeps satellite cells closely associated to the myofiber, and just importantly, keeps fibroblasts out. The steps in muscle regeneration are fairly straightforward:

1. Segmental necrosis
2. Invasion of the sarcoplasm by circulating monocytes, which differentiate into macrophages.
 - i) Macrophages phagocytose cellular debris, “cleaning” up the sarcoplasm.
3. Satellite cells enter the sarcoplasm and migrate towards the center of the myofiber.
4. Satellite cells divide and form a tube (“myotube”) which produces sarcoplasm.
 - i) The myotube extends to the edges of the damaged myofiber.
 - ii) The myotube expands, though is still narrower than the unaffected myofiber.
 - iii) A row of nuclei appear in the center of the regenerating myofiber, and sarcomeres begin to form.

Note that if the basal lamina has been destroyed, or if the damage affects a large area, then healing occurs via fibrosis (scarring). Although myofiber nuclei themselves cannot divide, recall that each myofiber is accompanied by **satellite cells**, which can divide and differentiate into myofibers.

Segmental necrosis and regeneration occur following a variety of insults, most commonly metabolic, nutritional (e.g. [White muscle disease]), toxic (e.g. [Ionophore toxicity])). Determining the etiology of the damage can therefore be quite difficult. It is helpful to observe the temporal pattern of the damage: are all muscle fibers at the same stage of necrosis or regeneration, suggesting a single, massive insult? Or is there a range of changes, with some fibers showing early stages of necrosis, and others at the end of regeneration, which suggests an on-going injury? These temporal changes are known as **monophasic** (occurring at one point) or **polyphasic** (an on-going process). These can be further classified by the commonly used distribution modifiers: focal, locally extensive, multifocal, or diffuse, to help narrow down the etiology. For example, a focal, monophasic injury is more likely to be traumatic in origin than metabolic, while a multifocal, polyphasic injury could be due to the on-going lack of a nutritional requirement, as seen in [White muscle disease].

1.4 Gross evaluation of muscle

Although important, the gross examination of muscles during a necropsy can be underwhelming, and if muscular disease is suspected, samples of muscle should *always* be submitted for histopathology. During a gross examination of a carcass, muscles should be evaluated for changes in size, texture, and colour. Muscles can be bigger (hypertrophied) or, more commonly, smaller (atrophied), or normal. Difficulty in assessing the normal size of muscles for different species and breeds can be aided by comparing with normal animals, or, if unilateral disease is present, with the contralateral side.

Changes in the colour of muscle are common, are often artifactual, and are dependent on blood perfusion, age, and species. Possible colour changes, along with potential causes, are listed below.

- Pale muscles:
 - Normal in young animals
 - Common in anemic animals
 - Can be due to necrosis (ischemic)
 - Denervation
 - If streaking observed, usually due to necrosis and mineralization.
- Dark red:
 - Congestion or hemorrhage
 - Hemorrhagic necrosis
 - Inflammation
 - Hypostatic congestion (i.e. blood pooling due to gravity, and artifact found in postmortem examinations)
- Green:
 - Putrefaction (rot)
 - Eosinophilic inflammation (uncommon)

The texture of diseased muscle can range from soft to hard (mineralized). Soft muscle can be indicative of necrosis, or potentially fat infiltration.

1.5 Biopsy techniques

As noted above, a biopsy of muscle should always accompany any suspected muscular disease. Muscle is highly susceptible to artifact, but proper preparation of the sample can help in ensuring a diagnostic sample. Muscle retains the ability to contract after biopsy/death, and this contraction can result in artifactual histological changes that mimic true pathological changes. These changes, noted as *contraction band artifact*, can be prevented relatively easily: shortly after removal, secure the muscle to a rigid surface prior to fixation. A simple and manageable technique whether in the field or clinic is to place a strip of muscle along a tongue depressor, and anchor it into place using two needles (FIGURE XXX GET PICTURE), prior to placing the sample in formalin. As a general rule, a muscle biopsy should not exceed ~ 1 cm in diameter (with myofibers running lengthwise).

Chapter 2

Congenital and inherited myopathies

Chapter 3

Circulatory disturbances

We describe our methods in this chapter.

Chapter 4

Physical injuries

Chapter 5

Toxic myopathies

We have finished a nice book.

Chapter 6

Degenerative/necrotizing myopathies

We have finished a nice book.

Chapter 7

Myopathies associated with endocrine disorders

We have finished a nice book.

Chapter 8

Myopathies associated with serum electrolyte imbalance

We have finished a nice book.

Chapter 9

Immune-mediated myopathies

9.1 Masticatory myositis

9.2 Acquired myasthenia gravis

We have finished a nice book.

Chapter 10

Infectious myositis

10.1 Clostridial myositis

10.2

Chapter 11

Parasitic myositis

11.1 Sarcocystosis

11.2 *Neospora caninum* and *Toxoplasma gondii*

11.3

Chapter 12

Neoplasms of muscle

Primary neoplasms of the striated muscle are rare in veterinary species. Although usually found in muscle, they can arise in unexpected locations devoid of striated muscle, such as the bladder.

12.1 Rhabdomyoma

As its name suggests, a rhabdomyoma is a benign tumour of striated muscle. It is seen most frequently in the hearts of pigs, particularly the red wattle breed and are typically incidental findings. They often present grossly as circumscribed, smooth-surfaced, nodular masses embedded in the myocardium. A similar neoplasm occasionally arises on the larynx of dogs. Laryngeal rhabdomyomas may cause respiratory difficulty or altered bark. They are typically minimally invasive and tend not to metastasize.

12.2 Rhabdomyosarcoma

These are the malignant counterparts to rhabdomyomas. They are most common in the dog. Counterintuitively, they occur more frequently at sites that normally lack skeletal muscle versus those that don't. There are a variety of subtypes, however, whether there is any prognostic significance to differentiating them is uncertain. They all tend to be locally invasive and metastatic. Prognosis is poor for all subtypes. Grossly, the tumours appear as pale, white to tan, firm masses, often with areas of necrosis. The botryoid ("cluster of grapes") subtype occurs in the bladder as a polypoid mass. The histologic appearance of these tumours is quite variable, though occasionally elongated, variably striated, myotube-like cells known as "strap cells" are present, which is suggestive of rhabdomyosarcoma. Immunohistochemistry is usually required to definitively diagnose these tumours.

12.3 Tumours of the s

The supporting mesenchyme of muscle can occasionally produce a (usually) malignant neoplasm. Granular cell tumours occur in the tongue of dogs and cats. They are composed of densely packed round cells with PAS positive granules. Hemangiosarcomas can arise in the muscles of dogs and horses, and aspiration of these large, intramuscular masses typically only reveals hemorrhage. Like their splenic cousins, they metastasize frequently.

12.4 Secondary tumours

Muscles are occasionally infiltrated by local neoplasms. Infiltrative lipomas are characterized by relatively well differentiated adipocytes crawling and invading through myofibers. They are highly invasive and require excision. Other neoplasms, such as subcutaneous mast cell tumours, lymphoma, hemangiosarcomas, and soft tissue sarcomas can invade into muscle. Metastasis to muscle is uncommon but does occur.