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COMMENTARY

Structural plasticity of the avian pectoralis: a case for geometry and the forgotten organelle

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ABSTRACT

The avian pectoralis muscle demonstrates incredible plasticity. This muscle is the sole thermogenic organ of small passerine birds, and many temperate small passerines increase pectoralis mass in winter, potentially to increase heat production. Similarly, this organ can double in size prior to migration in migratory birds. In this Commentary, following the August Krogh principle, I argue that the avian pectoralis is the perfect tissue to reveal general features of muscle physiology. For example, in both mammals and birds, skeletal muscle fiber diameter is generally accepted to be within 10–100 µm. This size constraint is assumed to include reaction-diffusion limitations, coupled with metabolic cost savings associated with fiber geometry. However, avian muscle fiber structure has been largely ignored in this field, and the extensive remodeling of the avian pectoralis provides a system with which to investigate this. In addition, fiber diameter has been linked to whole-animal metabolic rates, although this has only been addressed in a handful of bird studies, some of which demonstrate previously unreported levels of plasticity and flexibility. Similarly, myonuclei, which are responsible for protein turnover within the fiber, have been forgotten in the avian literature. The few studies that have addressed myonuclear domain (MND) changes in avian muscle have found rates of change not previously seen in mammals. Both fiber diameter and MND have strong implications for aging rates; most aging mammals demonstrate muscular atrophy (a decrease in fiber diameter) and changes in MND. As I discuss here, these features are likely to differ in birds.

KEY WORDS: Muscle fiber diameter, Muscle ultrastructure, Myonuclear domain

Introduction

The avian pectoralis muscle is responsible for the downstroke during wing beats (Torrella et al., 1998), and this muscle complex is one of the largest organs in birds, as it accounts for up to 17–25% of their total body mass (Greenewalt, 1962; Dietz et al., 2007). In many birds, the pectoralis muscle serves as the main thermoregulatory organ, in addition to being responsible for lift and thrust (Driedzic et al., 1993). Thus it is central to survival in this animal group. Importantly, many avian species demonstrate tremendous plasticity in the mass and weight of the pectoralis muscle across seasons and during migration (Swanson, 2010; Gaunt et al., 1990). Whereas there is a large body of literature highlighting the incredible mass change in the pectoralis muscle of birds under these circumstances, there are very few studies dedicated to some important structural aspects of avian muscle physiology. In this Commentary, I argue that more detailed study of the structure of the avian pectoralis could potentially improve our understanding of muscle physiology in general.

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The diameter of skeletal muscle fibers is 10–100 µm in mammals and birds. Limits to the size of muscle fibers probably reflect a compromise between diffusion constraints and metabolic cost savings (Kinsey et al., 2011; Jimenez et al., 2013), as described by the ‘optimal fiber hypothesis’ (Johnston, 2006). Specifically, diffusion distances in small fibers are short, allowing rapid diffusion of molecules such as O_2 and ATP (Kinsey et al., 2011). However, fibers with greater diameters are less costly metabolically, because they have less membrane-associated Na^+–K^+-ATPase activity (Jimenez et al., 2013). Of course, one of the interesting properties of muscle is that fiber size changes during animal growth (Kinsey et al., 2007) and, in some birds, across seasons (Swanson, 2010). This has to do with the way muscle grows. Muscle growth in animals can follow two distinct patterns: (1) hypertrophy (see Glossary), which is an increase in muscle mass due to an increase in fiber diameter and length (Kinsey et al., 2007; Nyack et al., 2007; Jimenez et al., 2013) while fiber number remains nearly constant and (2) hyperplasia (see Glossary), which is an increase in fiber number (Sola et al., 1973; Taylor and Wilkinson, 1986; Antonio and Gonyea, 1993). Despite having low metabolic costs at rest, pectoralis muscle forms a large relative proportion of bird body mass, so that a positive correlation between basal metabolic rate (BMR; see Glossary) and the size of the pectoralis muscle has been previously shown (Chappell et al., 1999).

As discussed above, birds show huge plasticity in the mass and weight of muscle – particularly for the pectoralis. Muscle tissue of avian species that are migrants or overwinter in colder temperate regions has a feature that is not seen in the typical mammalian study systems: a repeated increase and decrease in the mass of the tissue itself. Metabolic adjustments associated with the plasticity of the avian pectoralis muscle have been explored elsewhere (Guglielmo, 2010; Swanson, 2010), and are not a part of this Commentary. Additionally, birds provide an interesting study model with respect to aging, as they have higher mass-specific metabolic rates but live longer lives compared with similar-sized mammals (Jimenez et al., 2019a). These differences between birds and mammals imply that studies including birds represent an interesting and under-utilized avenue to unveil generalizable principles in muscle structure and function.

Here, I emphasize some of the missing pieces in the avian muscle literature. I first give examples of conditions under which the pectoralis muscle demonstrates plasticity in birds, then I go on to discuss physiological implications of changing muscle fiber size. Next, I address the lack of myonuclear domain (MND; see Glossary) studies in avian muscle, followed by a consideration of potential ploidy changes in avian muscle. Finally, I end with a discussion of potential molecular mechanisms dictating these muscle changes. Using the (sometimes few) available studies, I describe the interesting physiological implications of these traits, especially when compared with the mammalian-dominated literature in these fields.

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Glossary

Basal metabolic rate
The rate of metabolism in a post-absorptive, non-reproductive, non-thermally stressed animal.

Endopolyploidy
Ploidy level is defined as the number of copies of DNA per nucleus. When more than two copies of each chromosome are present in a nucleus, it is termed polyploidy, or endopolyploidy.

Fast glycolytic muscle fiber
These rely primarily on anaerobic metabolic pathways to produce ATP, and their morphology reflects this: they lack the high number of mitochondria seen in oxidative fibers, and have less vascularization. Fast-twitch fibers can contract quickly, but also fatigue quickly.

Hyperplasia
An increase in muscle mass due to an increase in fiber number.

Hypertrophy
An increase in muscle mass due to an increase in fiber diameter and length.

Maximum cold-induced metabolism
A measure of maximal shivering heat production seen as an index of avian cold endurance.

Metabolic scope
The difference between basal metabolic rate and peak metabolic rate.

Myonuclear domain
The amount of cytoplasm (volume) serviced by each myonucleus.

Peak metabolic rate
Maximal metabolic rate as measured by cold exposure or exercise.

Sarcolemma
Lipid bilayer plasma membrane enveloping skeletal muscle fibers.

Sarcopenia
Loss of skeletal mass and function (sometimes associated with age in mammals).

Slow oxidative muscle fiber
These rely primarily on aerobic metabolic pathways to make ATP and have several structural features that support this type of metabolism, including more mitochondria and capillaries compared with fast-twitch glycolytic fibers. Because they use aerobic means to fuel ATP production, slow oxidative fibers fatigue slowly. Generally, oxidative fibers have a smaller diameter, and less force production compared with glycolytic fibers.

Plasticity of the avian pectoralis

Avian pectoralis muscle and thermogenesis

In many birds, heat is generated by shivering, and the pectoralis muscle is considered to be the main thermogenic organ (Swanson, 2010; Swanson and Vézina, 2015). Thus, it follows that the pectoralis (as well as the flight muscles and supracoracoideus muscles) have all been studied with respect to avian shivering thermogenesis (Swanson, 2010, and references therein). Maximum metabolic rates (MMR) during cold exposure in birds generally range from three to eight times BMR, although most studies measuring MMR do so well below temperatures naturally experienced by birds (Swanson, 2010). Metabolic scope (see Glossary) during cold exposure is usually less than that measured during flight or locomotion (Brackenbury, 1984) – some species see a 5.5-fold increase over BMR during steady flight (Klaassen et al., 2000) – pointing to the fact that not all aerobic capacity in bird muscle is available for thermogenesis (Swanson, 2010). Seasonal alterations of the morphology and physiology of the pectoralis muscle are common in birds that live in temperate areas, and these changes allow birds to better match energy demands across seasons (Swanson, 2010). Many bird species have been used as model systems in this regard, and seem to demonstrate larger pectoralis muscle mass during winter compared with summer months (O'Connor, 1995; Swanson, 2010; Swanson and Vézina, 2015; Swanson et al., 2013).

Although many have linked the size of the pectoralis muscle in passerines with maximum cold-induced metabolism (M_{sum}; see Glossary) (Vézina et al., 2006, 2007, 2011; Swanson, 2010; Swanson et al., 2013; Petit and Vézina, 2014), others have found that increases in muscle mass do not correlate with M_{sum} (Noakes et al., 2020) or no correlation was found between pectoralis size and M_{sum} (Barceló et al., 2017; Milbergue et al., 2018). Thus, heat production may not be a mere function of pectoralis muscle mass, but also of cellular aerobic capacity. Conversely, species that demonstrate no seasonal variation in pectoralis muscle mass, such as house finches in Colorado, also see no changes in M_{sum} (Dawson et al., 1983; Carey et al., 1989). This implies that increases in pectoralis muscle mass (and heart mass) may partially be the drivers of seasonal flexibility in temperate small passerines and may allow these species to survive potentially harsh winter temperatures (Swanson and Vézina, 2015). However, it may be informative to consider the underlying ultrastructural changes in pectoralis in this case (see below).

Avian pectoralis muscle and migration

Bird migration is a physiologically demanding period of time. The actual migration time is sandwiched between two (or more) periods of hyperphagia, where birds deposit mass rapidly (Guglielmo and Williams, 2003). During migration itself, birds perform endurance flights at high metabolic rates for up to 100 h (Guglielmo and Williams, 2003), and migratory birds generally have well-developed exercise organs, such as their pectoralis muscle, heart and lungs (Swanson, 2010; Vágási et al., 2016). Rapid pectoralis muscle changes are known to occur in response to increases in workload (Petit and Vézina, 2014; Zhang et al., 2015) or in preparation for migration (Swanson, 2010; Price et al., 2011). Prior to migration, many bird species increase their body mass and, concomitantly, their pectoralis muscle size, an adaptation that is often described as an enhancement for power output, as in the case of semipalmated sandpipers (*Calidris pusilla*) (Driedzic et al., 1993). Body mass is positively correlated with muscle mass in birds that undergo migration (Lindstrom et al., 2000; Bauchinger and Biebach, 2005). However, migration distance does not correlate with flight muscle sizes (Vágási et al., 2016). After migration, many birds exhibit up to a 25% decrease in muscle mass (Swanson, 2010).

It should be noted that many studies looking at muscle mass changes during migration refer to the increase in bird muscle mass as hypertrophy without considering the actual structural changes underlying this change in organ size. If these changes occur via increases in fiber number, they would more accurately be termed hyperplasia. Historically, there have only been three studies that quantified the ultrastructural change in pectoralis muscle; these studies determined that the increase in mass was most likely to be the result of hypertrophy, although hyperplasia could not be ruled out (Marsh, 1984; Gaunt et al., 1990; Evans et al., 1992). Dunlin and sanderlings increase their muscle mass pre-migration due to a significant increase in fiber area (i.e. hypertrophy; Evans et al., 1992). Fiber area changes are greater than body mass changes in these birds (Evans et al., 1992). The authors of this study noted that hypertrophy may occur to different extents in different parts of the pectoralis muscle (Evans et al., 1992). It is noteworthy to mention here that muscle fiber sizes can be diffusion limited (as discussed above); fibers that are too large may be affected by decreases in the rates of diffusion of important metabolites (Kinsey et al., 2007). One way around this challenge within muscle tissue is to recruit muscle fibers via hyperplasia, as seen in the black seabass (Priester et al., 2011). Thus, this could be a mechanism by which birds increase the mass of their musculature while preventing diffusion limitations. As an

extreme example of the plasticity of the pectoralis, when eared grebes (*Podiceps nigricollis*) molt all of their feathers, they are flightless and their pectoralis muscle mass is greatly reduced in size. However, within two weeks prior to migration departure, the grebes double the size of their muscle mass (Gaunt et al., 1990). The mechanism via which muscular proteins are upregulated remains unexplored, as does the activity of each nucleus within avian skeletal muscle.

Muscle fiber diameter in birds

At rest, the tissue-specific metabolism of skeletal muscle is relatively low. However, because skeletal muscle is the tissue that makes up the largest fraction of body mass, it has a strong contribution to whole-animal metabolic rate (Martin and Fuhrman, 1955; Rolfe and Brown, 1997). Thus, in small mammals, skeletal muscle accounts for up to 30% of the metabolic rate at rest (Martin and Fuhrman, 1955; Rolfe and Brown, 1997); this figure rises to 90% for peak metabolic rate (PMR; see Glossary) as elicited by locomotion (Weibel and Hoppeler, 2005). In birds, the pectoralis muscle contributes up to 25% of the total body mass (Greenewalt, 1962; Dietz et al., 2007); thus, it may be a tissue where considerable basal metabolic savings could be accrued. As discussed above, there is a strong link between the size of a muscle fiber and the cost of ion pumping. The surface area to volume ratio of a muscle fiber and the rate of cell metabolism are positively correlated with the rates of Na^+–K^+-ATPase activity, and larger muscle fibers are therefore not as metabolically expensive to maintain (Johnston et al., 2003; Jimenez et al., 2011, 2013; Kielhorn et al., 2013). Na^+–K^+-ATPase activity in the muscle sarcolemma (see Glossary) is responsible for 19–40% of the resting metabolic rate in muscles (Gregg and Milligan, 1982; Milligan and McBride, 1985; Rolfe and Brown, 1997), and muscle can represent up to 25% of the total body mass (Greenewalt, 1962; Dietz et al., 2007); therefore any change in the surface area to volume ratio of muscle fibers resulting from seasonal plasticity or migration would have implications for the cost of maintaining the muscle mass and, in turn, for the BMR of the animal. Thus, the geometry of the muscle fiber can have whole-animal metabolic implications. For example, fiber diameters are closely related to the body mass of quail, where larger, faster-growing quail have significantly larger fiber diameters than smaller quail with lower growth rates (Jimenez et al., 2018). In addition, following migration in eared grebes, a decrease in pectoralis muscle size is accompanied by a 60% decrease in fiber diameter during atrophy (Gaunt et al., 1990). In fact, fiber diameters in birds differ with life-history traits. In addition, fiber diameters in avian muscle are more plastic and responsive to environmental stress than originally thought, and the pattern of muscle fiber diameter as birds age seems to differ from the commonly accepted mammalian paradigm. These issues are discussed in more detail below.

Life history

Tropical birds have an 18% lower BMR and a 30% lower PMR as elicited by cold exposure or exercise in a flight wheel compared with temperate bird species (Wiersma et al., 2007a,b). In a study published in 2014, we used 16 phylogenetically paired species of tropical and temperate birds and assessed fiber diameters in the pectoralis muscle as well as maximal Na^+–K^+-ATPase activity, with the aim of testing the idea that differences in life history can be related to differences in muscle structure (Jimenez and Williams, 2014a). In general, we found that temperate birds have larger muscle fiber diameters and, concomitantly, lower maximal activity of Na^+–K^+-ATPase than tropical birds. These results were surprising, considering that we anticipated that tropical birds (which have lower whole-animal metabolic rates than temperate birds) would have larger diameter fibers that would be metabolically cheaper. Based on our findings, we suggested that the larger diameter fibers found in temperate species would allow the birds to have increased force production and a greater muscle mass (especially in winter months) relative to tropical birds (Wiersma et al., 2012), while still minimizing basal metabolic costs. Tropical birds perform shorter flights and have a reduced muscle mass; we therefore proposed that the smaller muscle fibers are the result of reduced selection for muscle performance in these birds (Jimenez and Williams, 2014a).

Seasonality and thermal stress

As temperature is one of the major modifiers of metabolic rates in endotherms (Swanson, 2010), it makes sense that birds would have adaptive responses to mediate its effects. These responses have been investigated across seasons in Central New York, using both small black-capped chickadees and larger rock pigeons. In the former, spring-phenotype birds have significantly larger fiber diameters than summer-phenotype birds. However, there is no seasonal difference in fiber diameter in rock pigeons (Jimenez et al., 2019b). Chickadees and rock pigeons both have larger pectoralis muscles in the winter (Saarela and Hohtola, 2003; Petit et al., 2014; Vézina et al., 2017). Thus, it seems that black-capped chickadees and rock pigeons are both phenotypically flexible across seasons, but their strategies differ. The pectoralis of black-capped chickadees shows an increase in muscle mass (which is mediated by hypertrophy) in spring relative to summer. This might allow them to save energy during the colder months, as a larger body mass is associated with lower thermogenic costs (Stager et al., 2015; Milbergue et al., 2018). The larger muscle fiber diameters of spring-phenotype chickadees may contribute to reductions in basal metabolic costs associated with the activity of Na^+–K^+-ATPase, while simultaneously providing increases in force production to improve shivering. For comparison, rock pigeons also have greater muscle mass at lower acclimation temperatures (Saarela and Hohtola, 2003). However, as rock pigeons are larger than chickadees they are likely to face a lower selection pressure for metabolically cheaper fibers – this is because they do not require the same increases in thermogenic capacity or force production (for shivering) in colder months as the smaller black-capped chickadee. Additionally, fiber diameters in black-capped chickadees seem to be homogenous and are significantly smaller (32–38 µm) compared with those of rock pigeons that demonstrate a heterogeneous population of small and large muscle fibers (small fibers 30–40 µm; large fibers 60–80 µm). Arguably, there is already a cost saving associated with these large fibers in rock pigeons compared with black-capped chickadees. Thus, pigeons may be able to grow their muscle mass via hyperplasia (Jimenez et al., 2019b). In terms of heat stress, we have also previously found that heat-shocked house sparrows (*Passer domesticus*) decrease muscle fiber diameter within 24 h of a heat-shock treatment, and that fiber diameter can return to control conditions after recovering at room temperature for 24 h after the 24 h heat-shock treatment. Na^+–K^+-ATPase activity increases significantly when fiber diameters decrease due to heat shock (Jimenez and Williams, 2014b). Thus, muscle fibers in small passerine birds may be quickly adaptable and plastic.

Aging

In humans, muscle fiber diameters tend to decrease with age, in a process termed sarcopenia (see Glossary; Young et al., 1984; Lexell et al., 1988; Frontera et al., 2000). However, it is not known whether these age-related changes affect all (or most) animals or whether they are specific to mammals (Young et al., 1984; Lexell et al.,

1988; Frontera et al., 2000). The pectoralis muscle of aging black-legged kittiwakes and thick-billed murres does not show signs of atrophy (Brown et al., 2019; Jimenez et al., 2019c), in opposition to the mammalian aging paradigm, which includes a decrease in fiber diameter as part of the muscular atrophy process. These papers are the first to note a lack of muscle atrophy with age in wild birds.

Are myonuclei the forgotten organelle in avian muscle studies?

Muscle is a post-mitotic, multinucleated tissue (i.e. a syncytium). Therefore, to grow by hypertrophy, new nuclei may need to draw into the muscle fiber from a population of satellite cells. These are stem-like cells located under the basement membrane and the sarcolemma of each muscle fiber, and it is thought that the number of satellite cells is fixed throughout an animal's life (Bruusgaard et al., 2010; Van der Meer et al., 2011; Jimenez and Kinsey, 2012). Each myonucleus in a muscle fiber controls a certain volume of cytoplasm known as a myonuclear domain (MND) (Qaisar and Larsson, 2014; Box 1). The cytoplasm of a muscle fiber must be highly organized in order to contain the necessary metabolic and contractile machinery. One could therefore think of the regulation of muscle fiber size and/or cross-sectional area in terms of the balance between production and degradation of cytoplasmic components (Hughes and Schiaffino, 1999; Van der Meer et al., 2011). In chickens, post-natal development of muscle fibers involves both an increase in fiber size and an increase in the number of nuclei (derived from satellite cells), demonstrating that fiber size may not maintain a constant MND (Hughes and Schiaffino, 1999; Brack et al., 2005). In adult birds, satellite cells may proliferate following stretching (Winchester and Gonyea, 1992). The majority of the work on MND has been performed using mammalian muscle (Box 1), and the role of this organelle in avian muscle seems to have been forgotten. Below, I discuss the few studies on birds that have measured MND changes. In the Brown et al. (2019) study on aging birds, we highlight all the avian studies that have quantified muscular fiber size and capillary density, but we also point out that Brown et al. (2019) was the first study we could find that measured MND in birds. Thus, changes associated with the myonuclei have been mostly ignored in this field.

Seasonality and thermal stress

In black-capped chickadees, when fiber diameter increases across seasons by hypertrophy, MND also increases (Jimenez et al., 2019a). This indicates that each myonucleus must maintain a proper balance between synthesis and degradation for a greater area of the muscle fiber, (Van der Meer et al., 2011). In addition, each myonucleus may need to respond to an increased demand for protein turnover. It is possible that MND increases prior to satellite cells being incorporated into the myofiber, as stated in Box 1.

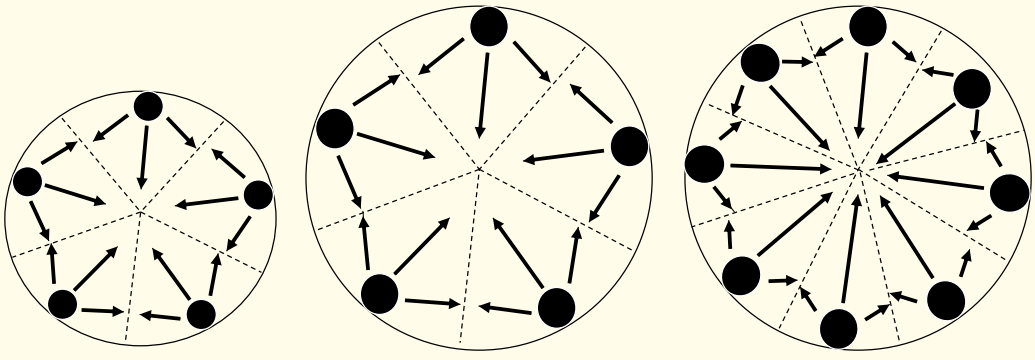
Cold-acclimated chickadees exposed to a sudden 15°C drop in temperature are able to modify their pectoralis ultrastructure within

Box 1. Myonuclear domain in mammals

In mammals, myonuclear domain (MND) size differs across fiber types; slow oxidative fibers have a smaller MND than glycolytic fibers (Van der Meer et al., 2011). The effects of changing muscle fiber size on MND are not clear: some studies report no changes to MND size with hypertrophic muscle growth, whereas others report an increase in MND (Hikida et al., 1997; McCarthy and Esser, 2007; O'Connor and Pavlath, 2007; Brooks et al., 2009).

Whether a change in MND is imperative for hypertrophic muscle growth is controversial (Van der Meer et al., 2011). Currently, we do not know the sequence of events and time course that is required for muscle fiber architectural changes (Van der Meer et al., 2011). For example, one study demonstrated that an increase in fiber size during hypertrophy is preceded by the recruitment of more nuclei (Bruusgaard et al., 2010). However, others have stated that changes in MND and fiber size take up to 4 weeks, and some have seen changes within 1–2 weeks (Van der Meer et al., 2011). These conflicting results have led to the notion that MND size may not be as carefully regulated as once thought (Gundersen and Bruusgaard, 2008).

A positive relationship between fiber size/cross-sectional area and MND would be potentially disadvantageous, as this would result in insufficient nuclei per fiber to accommodate increasing diffusion distances. A positive relationship between fiber size and MND implies that growing muscle cells may not recruit satellite cells (there may not be any left to recruit). This would increase the protein turnover load per nucleus (Kinsey et al., 2011). In mammals, decreased innervation appears to cause sarcopenia, with associated decreases in MND (Hughes and Schiaffino, 1999). Studies have shown conflicting results when examining age-related changes in myonuclear number for humans, mice and rats – some studies show no change in the number of myonuclei, whereas others report that there are more myonuclei with age (Van der Meer et al., 2011). Myonuclei can be eliminated from the muscle fiber via caspases without the destruction of the cell, a pathway that is probably triggered by atrophic conditions (Van der Meer et al., 2011), although this notion has been challenged (Gundersen and Bruusgaard, 2008). In contrast, it seems that myonuclei that are newly acquired during hypertrophy in mouse muscle are not lost, even after 3 months of denervation (Bruusgaard et al., 2010; Van der Meer et al., 2011). In fact, during atrophy, the number of nuclei has been shown to be conserved for up to 28 days in both oxidative and glycolytic fibers (Bruusgaard and Gundersen, 2008). The Box figure shows depictions of a single muscle fiber in cross-section.



3 h of the temperature change (Vézina et al., 2020). Within 3 h, these birds are able to increase the number of nuclei per millimeter of fiber by 15% (considering the raw data), and MND decreases by the same amount. This suggests that the addition of satellite cells into existing myofibers can be rapid (Vézina et al., 2020). However, it is not clear where these extra nuclei originate from; are they derived from endoreduplication or myogenic progenitor cells? It is also not known whether they undergo division or if they become depleted. Similarly, there are questions relating to the mitotic profiles with respect to the potential nuclear division. Increases in total protein synthesis due to a hypertrophic stimulus are evident within hours of exposure to the stimulus (Bruusgaard et al., 2010). There are several implications of decreased MND during an acute thermal challenge. If all nuclei show a similar rate of production, a decreased MND may represent a decrease in protein turnover load per nucleus, reducing the nuclear workload. Alternatively, the implication may be that nuclei are not producing products at a sufficient rate, such that it is necessary to recruit more nuclei in order to increase protein turnover rates (Brooks et al., 2009). Additionally, the very quick incorporation of new nuclei into muscle fibers in response to cold temperatures might precede a further increase in fiber diameter (which could allow greater force production in order to improve thermogenic capacity; Bruusgaard et al., 2010).

Aging

In aging black-legged kittiwakes, we found that the MND increased with fiber diameter, indicating that the number of nuclei does not increase in proportion to fiber size (Brown et al., 2019). Similar results have been reported in the white muscle of fishes, which also shows an increase in MND following muscle fiber hypertrophy (Jimenez and Kinsey, 2012). However, in aging thick-billed murres, we found a negative correlation between MND and age (Brown et al., 2019). It has been reported that slow oxidative muscle fibers (see Glossary) in rats display a decrease in MND with age, whereas MND does not change with age in fast glycolytic muscle fibers (see Glossary) in rats and humans (Brooks et al., 2009; Cristea et al., 2010). Other studies in humans show a significant decrease in MND with age (Manta et al., 1987; Cristea et al., 2010). In our study on thick-billed murres (Brown et al., 2019), although MND decreased with age, fiber diameter did not change, as predicted by mammalian work. Thus, it may be that chronic exercise, as performed by these foraging birds, may limit the sarcopenia and loss of satellite cell function seen in aging mammals. It is thought that 'filling up' muscle fibers with nuclei by exercising throughout the lifetime may prevent muscle weakness during old age (Bruusgaard et al., 2010); a high level of exercise may also allow thick-billed murres to maintain proper muscular function into old age.

Migration

There are currently no data on what happens to MND during avian migration or exercise when the size of the pectoralis muscle increases and decreases rapidly, as in the dramatic case of eared grebes (Gaunt et al., 1990). Applying the August Krogh principle, a muscle mass that undergoes cyclical yearly increases and decreases could be very informative in providing generalizable principles regarding the flexibility and plasticity of MND. To further elucidate these patterns, work that could be done in this area would include time-point experiments following structural changes in muscle mass in birds with different migratory strategies. These experiments could provide answers regarding phenotypic plasticity in muscle mass and could determine whether exercise is associated with myonuclei filling up muscle fibers, which seems to be unresolved in mammalian studies (Box 1).

Are all myonuclei equal?

In animals, cell size is highly correlated with DNA content (Conlon and Raff, 1999; Gregory, 2001; Comai, 2005), with increases in ploidy presumably leading to larger cell volumes (Vinogradov et al., 2001). Thus, another option for compensating for increases in MND is an increase in the ploidy of each nucleus. An increase in ploidy is often associated with an alteration in cellular morphology, physiology and behavior (Galitski et al., 1999; Comai, 2005). Animals that grow their anaerobic muscle strictly through hypertrophic means, such as fish and crustaceans, have demonstrated that this tissue is also endopolyploid (see Glossary; Jimenez et al., 2010; Jimenez and Kinsey, 2012). Others have found variation in DNA content in myotubes of *Xenopus* (Daczewska and Saczko, 2003). A potential area of future work, then, would be to quantify ploidy in avian musculature, as this concept has not been tested in a system that undergoes rapid cycles of hypertrophy and atrophy. This may also begin to address the question of whether the amount of DNA correlates with cell size (Kozłowski et al., 2020).

What are the potential molecular mechanisms?

Myostatin is an inhibitor of muscle growth, and it may regulate hypertrophy and hyperplasia (Lee and McPherron, 2001; Price et al., 2011). Myostatin is downregulated at low temperatures, which might permit an increase in size and mass of the pectoralis muscle in house sparrows (Swanson et al., 2009). Conversely, at higher temperatures, increased levels of myostatin could prevent muscle growth, leading to atrophy. However, some work has reported that the expression of the myostatin gene remains unchanged across seasons in house sparrows (Swanson et al., 2009; Swanson and Merkord, 2013) or across temperature treatment groups in juncos (Zhang et al., 2018), although other work does show differential expression of myostatin across seasons in chickadees (Cheviron and Swanson, 2017). Others have found reductions in myostatin protein levels with cold or exercise training in house sparrows (Zhang et al., 2015). In exercise-trained European starlings (*Sturnus vulgaris*) that increased muscle mass there is no change in the expression of myostatin mRNA, but there is a significant increase in the expression of insulin growth factor-1 (IGF-1) (Price et al., 2011). The cellular production of IGF-1 increases in response to growth hormone produced by the pituitary (Dantzer and Swanson, 2012). In vertebrates, the receptor for IGF-1 (IGF-1R) promotes growth (Holzenberger et al., 2003). IGF-1 is also associated with the activation of hypertrophic muscle growth (Stitt et al., 2004), protein synthesis and satellite cell proliferation (Rennie et al., 2004). Thus, IGF-1 signaling seems to be a determinant for muscle growth, and may also be temperature sensitive. White-throated sparrows (*Zonotrichia albicollis*) show increases in muscle size that can be induced by changes in photoperiod, although this is associated with simultaneous upregulation of both myostatin and IGF-1. The fact that these seemingly antagonistic proteins are upregulated in tandem may allude to a role in the regulation of cell size (Price et al., 2011) as muscle mass increases. Of course, much remains to be done in order to determine the ultimate and proximate mechanisms underlying seasonal changes in muscle fiber diameter and those that occur in response to temperature and migration. It will also be necessary to investigate alternative molecular mechanisms (e.g. BMR signaling; Cheviron and Swanson, 2017).

As noted above, Bruusgaard et al. (2010) found that it may be beneficial for muscle fibers to 'fill up' with myonuclei by increasing workload or exercise prior to senescence; this may provide resistance to atrophy. Satellite cells are recruited into the muscle fiber upon their release from myostatin inhibition (Amthor et al., 2009). Work on myostatin-null mice has demonstrated that post-natal muscle

hypertrophy does not involve satellite cell recruitment, and myostatin-null mice have fewer satellite cells compared with wild-type mice (Amthor et al., 2009), thus challenging the tenet that myostatin-driven hypertrophic growth involves satellite cell recruitment, and demonstrating clear increases in MND. It may be that birds such as the eared grebe, which shows tremendous and rapid pectoralis muscle changes, employ this type of mechanism to be able to upregulate and downregulate their muscle mass so efficiently (Gaunt et al., 1990).

Conclusions

Here, I have highlighted the fact that birds have an extremely plastic pectoralis muscle which, in some species, changes in mass cyclically across the year. This plasticity is unlike that noted in any mammalian species [although Yacoe (1983) describes flexibility in pectoralis muscle in bats], and yet the muscle structure literature contains more data on mammals than birds. Structural studies of avian muscle are lacking, but using this tissue to address structural questions could allow us to clarify generalizable principles in muscle physiology. Some important aspects of structure in muscle that could be better understood by using the avian pectoralis as a study system include muscle fiber diameter, which has been linked to whole-animal metabolic rate, and MND, which could determine the rate of protein turnover in muscle tissue. Additionally, the underlying molecular mechanisms that may dictate these structural changes are of great interest, and these mechanisms could also potentially be investigated through studies of the avian pectoralis.

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Competing interests

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