Seasonal Variation of Myostatin Gene Expression in Pectoralis Muscle of House Sparrows (*Passer domesticus*) Is Consistent with a Role in Regulating Thermogenic Capacity and Cold Tolerance

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ABSTRACT

Winter acclimatization in small birds overwintering in cold climates, including house sparrows (*Passer domesticus*), is associated with improved cold tolerance, elevated summit metabolic rates (M_{sum} = maximum cold-induced metabolic rate), and increased pectoralis muscle mass compared to summer birds. Myostatin is a potent autocrine/paracrine inhibitor of skeletal muscle growth in mammals and birds and is a potential candidate for regulation of seasonal phenotypic flexibility in birds. As a first step toward examining such a role for myostatin in small birds, we measured summer and winter gene expression of myostatin and its potential metalloproteinase activators TLL-1 and TLL-2 in house sparrows from southeastern South Dakota. Gene expression of myostatin decreased significantly in winter, with summer values exceeding winter values by 1.52-fold. Moreover, gene expression of TLL-1 was also significantly reduced in winter, with summer values exceeding winter values by 1.55-fold. These data are consistent with the hypothesis that the winter increases in pectoralis muscle mass, M_{sum}, and cold tolerance in house sparrows are mediated by reduced levels of myostatin and its activator TLL-1, and they suggest the possibility that myostatin may be a common mediator of phenotypic flexibility of muscle mass in birds.

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Introduction

Small birds wintering in cold climates undergo a process of winter acclimatization that produces a winter phenotype in which cold tolerance is improved relative to summer birds (Marsh and Dawson 1989). Associated with the winter phenotype in small birds showing marked seasonal changes in cold tolerance is an increase in summit metabolic rate (M_{sum} = maximum metabolic rate in response to cold; Liknes et al. 2002; Swanson, forthcoming). Moreover, cold tolerance and M_{sum} are phenotypically correlated in small birds, both intra- and interspecifically (Swanson 2001; Swanson and Liknes 2006). Heat production in birds is accomplished primarily by shivering, with the pectoralis muscle serving as the primary site for thermogenesis (Hohtola 1982; Marsh and Dawson 1989; Hohtola et al. 1998). Thus, elevated levels of M_{sum} characteristic of the winter phenotype are associated with adjustments in the pectoralis muscle, and such adjustments could conceivably involve changes in mass-specific aerobic capacity and/or increases in muscle mass. Variation in mass-specific aerobic capacity is not a common component of winter acclimatization in small birds, but pectoralis muscle mass is generally greater in winter than in summer for small birds wintering in cold climates (Marsh and Dawson 1989; Swanson 1991, forthcoming; O'Connor 1995; Cooper 2002; Liknes 2005). Moreover, Vezina et al. (2007) documented positive intraspecific and intraindividual correlations between breast muscle size and M_{sum} for red knots *Calidris canutus*. However, the factors regulating these seasonal changes in muscle mass in birds are unknown.

One potential candidate for regulation of seasonal phenotypic flexibility in pectoralis muscle mass is myostatin, a member of the TGF-β family of growth factors. Myostatin is a potent autocrine/paracrine inhibitor of muscle growth in mammals and is a highly conserved protein, with the active peptide being identical in mice, rats, humans, pigs, dogs, chickens, and turkeys (McPherron and Lee 1997; Kocamis and Killefer 2002; Lee 2004; Matsakas and Diel 2005). Functional effects of myostatin are less well studied in birds, but available evidence suggests similar actions. Most work on birds has focused on poultry during embryogenesis and posthatch periods. Myostatin expression decreases at hatching in chicks, concurrent with rapid growth of muscle, which is consistent with an inhibitory function of myostatin on muscle hypertrophy (Mott and Ivarie 2002; Guernec et al. 2003; Velleman 2007). Furthermore, in ovo administration of antimyostatin antibodies resulted in

modest increases in muscle and body mass in chicks at 5 wk of age, also suggesting that myostatin inhibits muscle growth (Kim et al. 2006).

Inhibition of muscle growth in mammals by myostatin can occur in both developing and adult muscle (Wehling et al. 2000; Whittemore et al. 2003; Yamaguchi et al. 2006). Mutations in the myostatin gene as well as blockage of myostatin action result in dramatic increases in muscle growth (McPherron and Lee 1997; Lee 2004). This muscle growth in response to reduced myostatin levels may occur by either hyperplasia or hypertrophy, but hypertrophy is the common mechanism in adult muscle (Zhu et al. 2000; Yang et al. 2001; Nishi et al. 2002; Lee 2004; Dominique and Cabello 2006). In adult mammals, myostatin acts by signaling satellite cell quiescence in skeletal muscle (McCroskery et al. 2003), thus preventing incorporation of satellite cells into muscles and, thereby, muscle growth.

Myostatin is synthesized in skeletal muscle in an inactive form that requires proteolytic removal of the N-terminal signal sequence and the propeptide to produce the active C-terminal fragment (McPherron and Lee 1997; Lee and McPherron 2001). After synthesis in skeletal muscle, myostatin is released into the circulation in the latent form bound to the propeptide and/or other proteins (follistatin, FLRG, GASP-1) that inhibit myostatin activity (Lee and McPherron 2001; Hill et al. 2002, 2003; Zimmers et al. 2002). Cleavage of the latent complexes to the active C-terminal dimer that binds to myostatin receptors is required for myostatin activity and metalloproteinases, including BMP-1/tolloid family members TLL-1 and TLL-2, can activate myostatin (Huet et al. 2001; Wolfman et al. 2003). Thus, the proteolytic processing of the latent myostatin provides another control point over myostatin regulation of muscle mass, in addition to expression of the myostatin gene itself (Lee 2004). It is worth noting here that the foregoing discussion of myostatin activation relates to mammals and that activation of the latent myostatin complex has not been studied in birds. However, in ovo injection of a polyclonal antibody binding to the myostatin propeptide (but not to mature myostatin) resulted in reduced leg muscle mass in chicks at 4–5 wk posthatch, suggesting that the myostatin propeptide inhibits myostatin activity in chicks, similar to its function in mammals (Kim et al. 2007).

As a first step in examining a potential role for myostatin in regulating seasonal phenotypic flexibility of muscle mass and summit metabolic rates in small birds, we measured summer and winter gene expression of myostatin and its potential metalloproteinase activators TLL-1 and TLL-2 in house sparrows *Passer domesticus*, which are permanent residents throughout their range in North America, including regions with cold winters (American Ornithologists' Union 1998; Tallman et al. 2002). House sparrows wintering in cold climates, including those from the population in this study, exhibit increases in thermogenic capacity, cold tolerance, and pectoralis muscle mass relative to their summer counterparts (Hart 1962; Arens and Cooper 2005a; Liknes 2005; Swanson and Liknes 2006). We hypothesize that expression of myostatin and/or its potential metalloproteinase activators TLL-1 and TLL-2 will be re-

duced in winter in a manner consistent with a role in mediating seasonal changes in M_{sum} and cold tolerance.

Material and Methods

Birds and Collection

We collected all birds by mist nets near Vermillion, Clay County, South Dakota (42°47′N). We designated birds captured from June to August as summer birds and those captured from December to February as winter birds. We captured birds under appropriate state and federal collecting permits, and all procedures were approved by the Institutional Animal Care and Use Committee at the University of South Dakota and followed the guidelines of the Ornithological Council's *Guidelines for the Use of Wild Birds in Research*.

RNA Isolation

We captured birds ($n = 8$ in summer, $n = 7$ in winter) by mist net before 0910 hours CST and transported them back to the lab, where they were provided with mixed birdseed and water at room temperature (22°C) until they were killed for dissection of pectoralis muscle. We killed birds by cervical dislocation within 1–2.5 h of capture. Next, we rapidly dissected out pectoralis muscles on ice and removed a small portion of the muscle, which was diced and immediately placed in RNAlater (Ambion, Austin, TX). The muscle stored in RNAlater was frozen at −20°C for later RT-PCR assays.

Cloning of Passer domesticus *Myostatin, TLL-1, TLL-2, and Actin*

We isolated total RNA from pancreas of house sparrow using the TRI REAGENT (Molecular Research Center, Cincinnati, OH). We used the pancreas for total RNA isolation because it was difficult to effectively disrupt muscle tissues for RNA extraction after treatment with RNAlater. Moreover, the UniGene database from the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/) shows that the expression profile of TLL-2 from *Homo sapiens* is higher in pancreas than in muscle. The RNA was reverse transcribed using Strata Script One-Tube RT-PCR System (Stratagene, La Jolla, CA) according to the manufacturer's instructions. We designed degenerate primers for conserved regions of the myostatin, TLL-1, TLL-2, and actin using primers based on the known sequences from chickens *Gallus gallus*, African clawed frogs *Xenopus laevis*, zebrafish *Danio rerio*, and mice *Mus musculus* (Table 1).

We purified PCR fragments from agarose gels with a Zymoclean Gel DNA Recovery kit (Zymo Research, Orange, CA), cloned fragments into pCR2.1 (Invitrogen, Carlsbad, CA) according to instructions from the manufacturer, and then sequenced fragments (Iowa State University Sequencing Facility, Ames, IA). We analyzed the sequences using the nucleotide BLAST program at NCBI (http://www.ncbi.nlm.nih.gov/BLAST/).

Table 1: Degenerate PCR primers used for the isolation of *Passer domesticus* myostatin, TLL-1, TLL-2, and actin partial cDNA

Transcript	Upstream Primer	Downstream Primer
Myostatin	5′-CCTGGAAACAGCWCCDAACATYAGC-3′	5′-CACTCTCCAGAGCAGTAATTGKCCTTRTA-3′
TLL-1/TLL-2	5′-ATTCAGTCTCCCAATTATCCDGATGACTA-3′	5′-GGGCGTGAAACCCYTTCTTRYTRAT-3′
Actin	5′-GAYATGGARAAGATYTGGCAYCAYMS-3′	5′-YTTDSTRATCCACATYTGYTGRAAGGT-3′

Real-Time RT-PCR

We performed real-time RT-PCR using 200 ng total RNA per reaction. We created gene-specific primers and probes for β-actin, myostatin, TLL-1, and TLL-2 using the Primer Express Software (Applied Biosystems, Foster City, CA; Table 2). We combined RNA with primer/probe sets and TaqMan Gold RT-PCR Master Mix (Applied Biosystems). We ran real-time assays on an ABI 7000 (Applied Biosystems). The real-time PCR profile consisted of one cycle at 48°C for 30 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. We normalized data to actin mRNA levels. For quantitation of gene expression, we used the comparative C_T method ($\Delta\Delta C_T$; Livak and Schmittgen 2001; Sabirzhanov et al. 2007). We performed all reactions in duplicate and used average values from the two samples in subsequent analyses.

Statistics

Because Swanson and Liknes (2006) presented summer and winter values for M_{sum} and T_{CL} (temperature at cold limit, defined as the temperature inducing hypothermia; after Saarela et al. 1989) for this population of house sparrows without analyzing seasonal differences, we report such analyses here to establish patterns of seasonal variation for this population of sparrows. We report data as mean $\pm$ SD. We compared seasonal means for M_{sum}, T_{CL}, and mRNA expression by Student t-tests. We also compared seasonal variation in M_{sum} by generating regressions of log M_{sum} against log body mass for summer and winter birds and comparing regression lines by ANCOVA, after verifying statistical homogeneity of slopes. Statistical significance was accepted at $P < 0.05$.

Results

We found that M_{sum} was significantly greater ($t_{15} = 2.74$, $P = 0.015$) for winter sparrows (9.33 ± 0.58 mL O_2 min^{-1}, $n = 11$) than for summer sparrows (8.42 ± 0.78 mL O_2 min^{-1}, $n = 6$), a difference that amounted to a 10.8% increase in winter birds (Fig. 1). Least squares regression equations describing log M_{sum} as a function of log mass in summer and winter were

$$\text{summer}: \ \log M_{sum} = -0.69 + (1.13 \log \text{mass})$$

($n = 6$, $R^2 = 0.64$, $P = 0.055$), and

$$\text{winter}: \ \log M_{sum} = -0.09 + (0.74 \log \text{mass})$$

($n = 11$, $R^2 = 0.35$, $P = 0.055$). ANCOVA also demonstrated that winter M_{sum} was significantly greater than summer M_{sum}, after effects of body mass were controlled for ($F_{1,14} = 10.94$, $P < 0.001$). This winter increase in M_{sum} was accompanied by a significant ($t_{15} = 3.14$, $P = 0.007$) decrease in T_{CL} in helox (79% helium, 21% oxygen), from $-5.5° \pm 1.7°$C ($n = 6$) in summer to $-8.9° \pm 2.3°$C ($n = 11$) in winter, indicating improved cold tolerance in winter sparrows from the study population (Fig. 1).

Mean threshold cycle (C_T) values (Livak and Schmittgen 2001) for actin mRNA were 14.44 ± 0.06 in summer ($n = 8$) and 14.46 ± 0.06 in winter ($n = 7$). Because C_T values are exponential rather than linear terms, we transformed C_T values to linear terms according to 2^{-C_T} (Livak and Schmittgen 2001). Transformed values did not differ significantly ($t_{13} = 0.393$, $P = 0.701$), indicating that normalizing seasonal values for myostatin, TLL-1, and TLL-2 expression to actin expression is valid for this study.

Real-time RT-PCR revealed that myostatin mRNA levels were 1.52-fold greater in summer birds than in winter birds (Fig. 2). This seasonal difference in myostatin gene expression was significant ($t_{13} = 3.97$, $P = 0.002$; Fig. 3). Reduced myostatin gene expression in winter was accompanied by a significant winter decrease in mRNA levels for TLL-1 ($t_{13} = 4.22$, $P = 0.001$), with summer levels exceeding winter levels by 1.55-fold (Fig. 3). Gene expression of TLL-2 was also significantly reduced in winter relative to summer ($t_{13} = 4.16$, $P = 0.001$), but for TLL-2, summer mRNA levels increased only to 0.5% above winter mRNA levels (Fig. 3).

Because M_{sum} and myostatin gene expression data were collected during different years, and recent weather conditions can influence metabolic rates in small birds, at least in winter (Swanson and Olmstead 1999), it is important to take recent weather conditions into account when comparing seasonal differences in M_{sum} and myostatin gene expression in this study. Summer and winter periods of measurement for M_{sum} were similar in terms of temperature to the corresponding periods for measurement of myostatin gene expression, with mean temperatures for the months in which M_{sum} was measured being 19.4°C in summer and $-3.2°$C in winter and mean temperatures for the months in which myostatin gene expression was measured being 24.2°C in summer and $-2.4°$C in winter (South Dakota Office of Climatology). Because the thermal neutral zone in summer-acclimatized house sparrows extends to 16°C (Arens and Cooper 2005b), it is unlikely that the summer temperature difference between years greatly influenced seasonal variation in M_{sum} or myostatin gene expression in this study.

Table 2: Primers for real-time RT-PCR

Target	Forward Primer	Reverse Primer	Minor Groove Binder Probe
TLL-1	TGGAGCTTTTTGATGGTCATGA	TGGCCCAGATCCACAAAATC	ACAGCAGTGAGACTTG
TLL-2	CGCGCCGAGGTGAAGA	CCCCCGGGTAGTTGTTGTC	ACGCACAATTTGG
Myostatin	TGAACCCAGGCACCGGTAT	CCAATTTTGCAGCACTGTCTTC	TGGCAGAGCATTGAT
Actin	AGGGAAATYGTKCGYGACAT	GCRGCAGTRGCCATYTC	AAGYTGTGCTATGTTGCTCTRGACTTY

Discussion

House sparrows in this population from southeastern South Dakota exhibited an 11% increase in M_{sum} in winter relative to summer, which accompanied a significant improvement in cold tolerance. Such seasonal variation in M_{sum} is consistent with the seasonal pattern for other small birds that show marked winter improvement in cold tolerance (Marsh and Dawson 1989; Swanson, forthcoming). Other house sparrow populations from regions with cold winter climates also show similar patterns of seasonal variation in M_{sum} and cold tolerance (Hart 1962; Arens and Cooper 2005a). However, the magnitude of the winter increment in M_{sum} for South Dakota sparrows was lower than those for other populations (Table 3): sparrows from Ontario and Wisconsin exhibited winter increments of 43% and 31%, respectively (Hart 1962; Arens and Cooper 2005a; Table 3). Reasons for the differences among populations are not immediately obvious, although differences in winter M_{sum} might be partially explained by differences in winter conditions among studies (Swanson and Olmstead 1999).

The winter increase in M_{sum} and cold tolerance in house sparrows in this study and others is consistent with the variable maximum model of winter acclimatization proposed by Liknes et al. (2002), which contends that the improved cold tolerance and enhanced shivering endurance in winter birds are associated with physiological adjustments that elevate thermogenic capacity in winter relative to summer. Two non–mutually exclusive mechanisms exist for increasing M_{sum}: enhanced mass-specific cellular aerobic capacity and increases in size of muscles involved in shivering thermogenesis (Swanson, forthcoming). Liknes (2005) found that catabolic enzyme activities associated with endurance capacity in the pectoralis of seasonally acclimatized house sparrows did not change significantly between summer and winter, suggesting that elevated mass-specific aerobic capacity does not contribute to higher winter M_{sum}. Pectoralis muscle mass, however, increased significantly in winter compared to summer for this population of house sparrows, on both wet-mass (10% increase) and dry-mass (15% increase; Liknes 2005) bases. This elevation of pectoralis muscle mass and M_{sum} in South Dakota house sparrows is consistent with winter elevation of pectoralis mass and M_{sum} in several species of small passerines (Swanson 1991; O'Connor 1995; Cooper 2002; Liknes 2005), with intraspecific correlations of M_{sum} and pectoralis mass in red knots (Vezina et al. 2007) and with positive intraspecific correlations of pectoralis mass with aerobic exercise capacity in house sparrows (Chappell et al. 1999).

Myostatin expression in pectoralis muscle was reduced by 34% in winter sparrows relative to their summer counterparts.

This is consistent with a scenario whereby reduced myostatin levels in winter birds promote pectoralis hypertrophy, which in turn produces elevated thermogenic capacity and cold tolerance. For house sparrows, lower critical temperatures in both summer and winter are usually above 16°C (Hart 1962; Arens and Cooper 2005b), and ambient temperatures in southeastern South Dakota very rarely reach these levels during winter, so prolonged shivering is necessary during much of the winter period, especially at night (Swanson and Thomas 2007). The prolonged elevation of shivering thermogenesis increases the thermogenic workload on shivering muscles. While isometric shivering is qualitatively different from muscle loading, both increase muscle workload, and they might result in similar remodeling of muscle. Muscle loading in adult mammals stimulates reductions in myostatin gene expression and myostatin levels, along with muscle hypertrophy in mice, rats, and humans (Wehling et al. 2000; Kim et al. 2005; Yamaguchi et al. 2006). Few studies have actually measured the impact of muscle hypertrophy resulting from changing myostatin levels on muscle performance. In one such study, Whittemore et al. (2003) blocked myostatin function with an inhibitory antibody in mice, and this resulted in increased muscle mass that was accompanied by enhanced grip strength, a measure of muscle
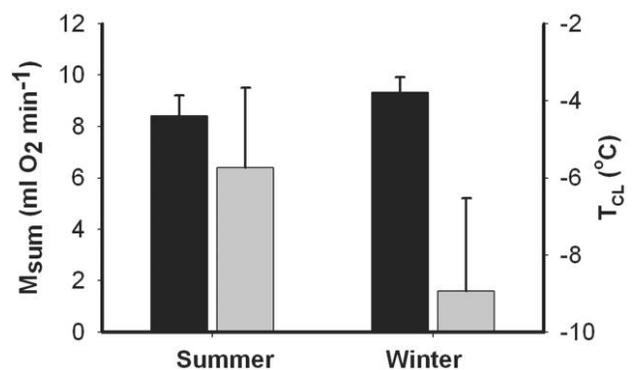
Figure 1. Seasonal variation in mean ($\pm$SD) M_{sum} (maximum cold-induced oxygen consumption; *black bars*) and T_{CL} (temperature eliciting hypothermia in an atmosphere of 21% oxygen/79% helium; *gray bars*) in house sparrows, *Passer domesticus*, from southeastern South Dakota, USA. Winter elevation of M_{sum} is correlated with reduced T_{CL} and improved cold tolerance. M_{sum} and T_{CL} data are from Swanson and Liknes (2006), so these birds are from different years than those sampled for gene expression data. Thus, the degree of variation in myostatin, TLL-1, and TLL-2 gene expression is not directly comparable to the degree of variation in M_{sum} and T_{CL}, although both represent seasonal differences between summer and winter birds. Sample sizes for M_{sum} and T_{CL} were 6 in summer and 11 in winter.
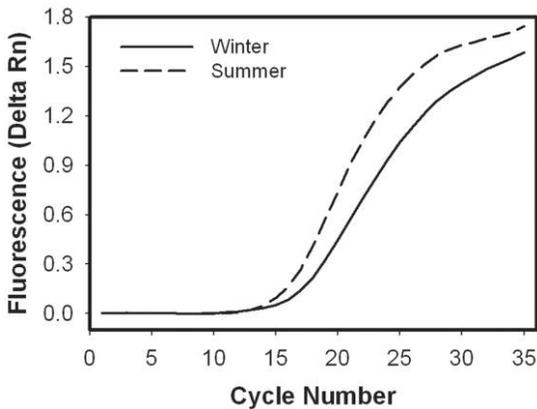
Figure 2. Representative RT-PCR traces for myostatin gene expression in pectoralis muscle of summer and winter house sparrows, *Passer domesticus*, from southeastern South Dakota, USA. Data are expressed as relative fluorescent intensity (Delta Rn). Higher fluorescence at a given cycle number signifies higher levels of myostatin mRNA. Values are from individual sparrows from December 15, 2004 (winter), and July 26, 2006 (summer).

performance. Thus, these data suggest that reduced myostatin gene expression leads to muscle hypertrophy in adult mammals and that the muscle hypertrophy increases muscular performance (but see Amthor et al. 2007).

The increased demand for sustained thermogenesis in small birds overwintering in regions with cold climates also results in muscle remodeling and leads to muscle hypertrophy (Liknes 2005; Swanson, forthcoming) and elevated M_{sum}, which is an integrated measure of overall muscle performance during shivering. Reduced pectoralis levels of myostatin mRNA in winter relative to summer sparrows in this study suggest that downregulation of myostatin gene expression may promote winter hypertrophy of the pectoralis muscle and the subsequent increases in M_{sum} and cold tolerance. Given that pectoralis muscle hypertrophy is a common component of the winter phenotype in small birds (Swanson 1991; O'Connor 1995; Cooper 2002; Liknes 2005), it seems likely that myostatin downregulation could function as a prominent mediator of seasonal phenotypic flexibility in these birds.

Seasonal phenotypic flexibility of body and flight-muscle masses is also associated with other portions of the annual cycle of birds, such as migration and molt. Changes in body mass are usually tracked by similar changes in flight-muscle mass, so that increased wing loading generally is accompanied by increased flight-muscle mass. These changes occur both during molt, where wing loading increases as a result of decreased wing area (Gaunt et al. 1990; Jehl 1997; Lind and Jakobsson 2001), and during migration and winter, where muscle workload increases (Marsh and Storer 1981; Marsh 1984; Lindström et al. 2000; Swanson, forthcoming). These data suggest that flight muscles in birds generally respond to increased workload with hypertrophy, a response similar to that of mammals. However, birds can modify flight-muscle size independent of workload (Swaddle and Biewener 2000), and changes in flight-

muscle mass before migration can occur without training, in response to endogenous rhythms (Dietz et al. 1999). This suggests that the regulation of phenotypic flexibility of flight-muscle mass in birds is more complicated than simply a use-disuse phenomenon, but because alteration of myostatin levels can produce both increases and decreases in muscle size, myostatin appears to be a candidate for such regulation.

Because myostatin is secreted in an inactive form that requires cleavage for activation, adjustments in myostatin-activating proteins could also lead to changes in muscle remodeling. Metalloproteinases appear to be required to cleave myostatin to produce the active form in mammals (McPherron and Lee 1997; Lee and McPherron 2001; Lee 2004). The BMP-1/tolloid family metalloproteinases are capable of cleaving the myostatin propeptide to produce the active form (Huet et al. 2001; Wolfman et al. 2003). The BMP-1/tolloid family member TLL-2 is expressed specifically during skeletal muscle development in mammals (Scott et al. 1999), suggesting that it may be active in cleaving myostatin during embryogenesis (Lee 2004). In this study, TLL-1 mRNA showed a 35% reduction in winter sparrows relative to their summer counterparts, and TLL-2 mRNA levels were also reduced in winter, but only by 0.5%. These data suggest that TLL-1 and TLL-2 metalloproteinases may be responsible for cleaving myostatin to produce the active form in skeletal muscle in adult birds. The greater winter reduction in TLL-1 expression relative to that of TLL-2 suggests that TLL-1 may be the primary metalloproteinase responsible for mediating seasonal myostatin-induced changes in muscle mass and suggests the interesting possibility that TLL-
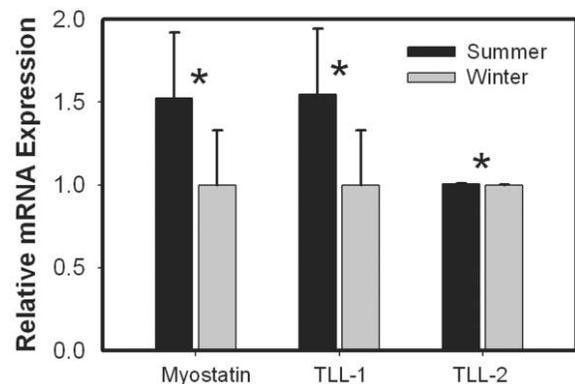
Figure 3. Seasonal variation in mRNA levels (mean ± SD, normalized to winter values) for myostatin and its metalloproteinase activators, TLL-1 and TLL-2, in house sparrows, *Passer domesticus*, from southeastern South Dakota, USA. Levels of mRNA were significantly reduced (denoted by asterisks) in winter ($n = 7$) compared to summer ($n = 8$) for myostatin and both metalloproteinase activators. Relative increases in gene expression in summer compared to winter were 1.52-fold for myostatin and 1.55-fold for TLL-1, suggesting that winter reductions in myostatin and TLL-1 expression contribute to winter hypertrophy of pectoralis muscle. TLL-2 expression was also significantly higher in summer than in winter, but the seasonal difference was only 0.5%, suggesting little physiological role for seasonal changes in TLL-2 expression in regulating seasonal changes in pectoralis muscle mass.

Table 3: Summit metabolism (M_{sum}) measures for house sparrows *Passer domesticus*

Method and Location	Summer		Winter		
	M_{sum} (mL O_2 min^{-1})	Mass (g)	M_{sum} (mL O_2 min^{-1})	Mass (g)	Reference
Air:					
Ontario	7.0 (8)	27.1	10.0 (8)	29.4	Hart 1962
Helox:					
Poland	6.7 ± .4 (17)	25.6	…	…	Koteja 1986
Wisconsin	8.4 ± 1.1 (15)	26.4 ± 1.3	10.9 ± 1.4 (16)	27.6 ± 1.6	Arens and Cooper 2005a
South Dakota	8.4 ± .8 (6)	26.8 ± 1.7	9.3 ± .6 (11)	27.1 ± 1.4	Swanson and Liknes 2006

Note. Values are presented ±SD. Sample sizes are included in parentheses. "Method" refers to M_{sum} measurement at very cold temperatures in air or at more moderate temperatures in helox (79% helium/21% oxygen).

1 may be a primary activator of myostatin in adults but that TLL-2 fulfills this role during embryogenesis. The very minor seasonal difference in TLL-2 expression in this study suggests that changes in TLL-2 expression are unlikely to be physiologically relevant contributors to seasonal differences in pectoralis muscle mass in house sparrows.

The winter reductions in myostatin and TLL-1 mRNA levels in house sparrow pectoralis muscle in this study suggest that myostatin is downregulated in winter and that this downregulation is accomplished both by reduced gene expression of myostatin and by reduced processing of latent myostatin by its metalloproteinase activators. Inasmuch as gene expression does not always reflect active protein levels, however, future studies should document seasonal variation in active myostatin and TLL-1 protein levels to elucidate a possible role for posttranslational processing in seasonal changes in muscle mass. The data in this study are consistent with the hypothesis that a downregulation of active myostatin protein in winter contributes to winter hypertrophy of pectoralis muscle, which in turn mediates organism-level changes in M_{sum} and cold tolerance that contribute to the winter phenotype. Because winter increases in pectoralis muscle mass are a common element of winter acclimatization in small birds, these data suggest that myostatin might play a prominent role in skeletal muscle remodeling associated with the phenotypic flexibility that produces seasonal phenotypes in small birds.

Myostatin is one of several growth factors and extracellular matrix proteoglycans that are involved in the regulation of skeletal muscle growth and remodeling in birds (Velleman 2007). Given that numerous regulatory factors and signal transduction pathways are involved in regulating muscle growth, it is likely that myostatin expression is upregulated or downregulated seasonally along with other muscle growth regulators and in concert with signaling pathways relating to exercise and cellular energy status (e.g., Guernec et al. 2003; Nader 2007). Detailing such interactions would be a fruitful avenue for future research. Future studies should also address functional responses in small birds (e.g., muscle hypertrophy, thermogenic capacity) to myostatin inhibition. Functional studies using inhibitory myostatin antibodies (Bogdanovich et al. 2002; Whittemore et al. 2003; Kim et al. 2006) would be a reasonable next step. These data also suggest that myostatin could be a can-didate for mediation of other portions of the avian annual cycle where flight muscle masses are known to vary, such as migration (e.g., Marsh 1984; Lindström et al. 2000) and molt (Gaunt et al. 1990; Jehl 1997).

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