

Elevated Insulin-Like Growth Factor-I Receptors in the Avian Low Score Normal Muscle Weakness

Sandra G. Velleman, Douglas C. McFarland⁽¹⁾, Kysa K. Gilkerson⁽¹⁾, and Jane E. Pesall⁽¹⁾

Departments of Animal Science and Animal Genetics, University of Connecticut, Storrs and The Ohio State University, Ohio Agricultural and Development Center, Wooster, and (1) Department of Animal and Range Sciences, South Dakota State University, Brookings

Abstract

Insulin-like growth factor-I (IGF-I) binding to receptors was measured in pectoral muscle plasma membranes derived from birds with a genetic skeletal muscle weakness (LSN) and normal control chickens. IGF-I binding to membranes derived from day-old LSN birds was 46% greater ($P \leq 0.0001$) than controls. Although receptor dissociation constants did not differ ($P \geq 0.05$), there were 53% more ($P \leq 0.0001$) IGF receptors on membranes derived from the LSN line at one week of age. The results suggest an association between a genetic muscle weakness, LSN, and altered expression of a growth factor receptor important in muscle development.

Key words: insulin-like growth factor-I, muscle, receptor.

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Skeletal muscle development is a complex process involving the proliferation, migration, cell-cell recognition and fusion of myogenic cells to form myotubes and their eventual maturation to form functional muscle fibers. Key to the successful development of skeletal muscle is the interaction of embryonic myoblasts with the extracellular matrix. Little is known how alterations in early extracellular matrix-cell signalling events affect the structural and functional integrity of myofibrillar muscle.

In a previous study [1], the avian muscle weakness, Low Score Normal (LSN), was partially characterized. LSN birds exhibit reduced pectoral muscle function in an "exhaustion score" test (the ability of the animal to right itself from a supine position). Although the LSN birds exhibit a loss in muscle function, myosin heavy chain gene expression, muscle fiber diameter and type, myoblast cell-cell signalling, and regeneration are not altered (1; Velleman and Bandman, unpublished).

In examining the etiology of the observed muscle weakness, extracellular matrix-cell signal transduction developmental pathways must be considered. In particular, this includes the role of growth factors and their receptors in muscle development. Among these are the insulin-like growth factors (IGFs) and their cell surface receptors which play major roles in myogenesis [2].

The objective of this study was to compare the binding of IGF-I to pectoral muscle membranes derived from LSN and control White Leghorn chickens. In particular, the level of IGF-I binding and the binding affinity of membrane-bound IGF receptors was measured.

Materials and Methods

Animals and Husbandry

LSN birds used in this study were from flocks maintained by the Department of Animal Genetics at the University of Connecticut. The LSN phenotype was detected among F2 progeny in an outcross between chickens with hereditary muscular dystrophy (*am/am*) and a commercial White Leghorn stock (Pierro and Haines, unpublished). The LSN nomenclature was selected to distinguish two separate classes of birds which showed impaired ability to right themselves when repeatedly placed on their backs (exhaustion score test) at 2-3 months posthatch. Exhaustion scores of 0-3 characterized the *am* homozygotes; scores of 9-12, the LSN phenotype. The LSN phenotype has been reproduced for at least 17 generations by matings of birds with exhaustion scores between 9-12. All animal handling procedures were approved by the University of Connecticut Animal Care and Use Committee.

Membrane Preparation

One-day and one-week posthatch birds were euthanized by cervical dislocation. Pectoral muscles were removed, washed with ice-cold phosphate buffered saline (PBS) and stored at -90°C prior to processing. Membranes were prepared by a modification of a previously described procedure [3]. Briefly, tissues from 18 birds were disrupted with an IKA Ultra-Turrax T-25 tissue homogenizer (Staufen, Germany) in HEPES homogenization buffer (HHB) consisting of 0.025 M HEPES, 0.3 M sucrose, 0.005 M EDTA, 0.0001 M phenylmethyl sulfonyl fluoride (PMSF), and 0.025 M benzamidine, pH 9.0, at a ratio of 1 g of tissue to 5 ml of HHB. The homogenate was centrifuged at 2000 x g for 30 min at 4°C. The pellet was rehomogenized with HHB and centrifuged again. The resulting supernatants were pooled, filtered through a 500 µm Nitex filter and centrifuged at 20,000 x g for 30 min at 4°C. The membrane pellet was similarly washed three times with HEPES binding buffer (HBB). The final pellets were resuspended in imidazole freezing buffer (IFB) consisting of 0.24 M imidazole, 0.8 M NaCl, 75% v/v glycerol, pH 8.0. Samples were stored at -20°C.

Protein was analyzed using the bicinchoninic acid (BCA) assay from Pierce (Rockford, IL) using bovine serum albumin as the standard. Statistical analyses were performed using the one-way analysis of variance procedure by the Statistical Analysis System [4]. Stated differences were significant at the 0.05 level of probability.

IGF-I Binding Assay

Specific binding of [¹²⁵I]IGF-I to membranes derived from day-old chicks was determined using procedures described by McFarland et al. [3]. Due to the small amounts of pectoral muscle in these birds, competitive binding receptor assays could not be performed on membranes from this age group. One hundred micrograms of membrane protein were suspended in HBB. To this suspension were added 200,000 dpm [¹²⁵I]IGF-I (2000 Ci/mmol). Non-specific binding was determined in parallel samples containing 0.2 nmol of unlabeled IGF-I. Incubations were performed in a final volume of 333 µl in 1.5 ml microfuge tubes at 15°C. Following a 4-hr incubation period to achieve steady state binding of ligand, 1 ml of a 1:1 suspension of 31.9% polyethylene glycol (PEG) and 1.5 mg/ml bovine gamma globulin, 1.0 M Tris-HCl, pH 7.4, was added and the incubation continued in an ice-water bath for 15 min. Samples were then centrifuged in a microfuge at 12,500 x g at 4°C for 10 min. The supernatants were aspirated and the tips of the tubes containing the pellets were cut off and the radioactivity determined. Preliminary studies (not reported) using muscle membranes showed that specific binding of [¹²⁵I]IGF-I was linear between 0 and 280 µg protein per tube, and steady-state binding was reached under these incubation conditions.

To determine the affinity of IGF receptors on muscles from the 2 lines of birds, competitive binding assays were performed using membranes derived from one-week post-

hatch birds. Membranes were incubated as described above with [¹²⁵I]IGF-I in the absence or presence of increasing levels of unlabelled IGF-I. The data were analyzed with the LIGAND binding analysis computer program [5] originally written by Munson and Rodbard [6].

Results and Discussion

Membranes were prepared from the pectoral muscles of day-old and one-week posthatch chickens with a genetically-transmitted muscle weakness (LSN) and normal controls to examine the binding of IGF-I to receptors. IGF-I and -II are key growth factors involved in skeletal muscle development [2]. Avian muscles and other tissues do not possess the type II IGF receptor, and IGF-I and IGF-II binding is mediated only through the type I IGF receptor [3, 7, 8]. Therefore, only IGF-I binding was evaluated. Specific binding of IGF-I to membranes from day-old LSN birds was 46% greater than to membranes from control birds (Table 1). To determine whether the increased IGF-I binding to LSN membranes was due to increased receptor numbers or to an altered receptor dissociation constant (K_d), competitive binding assays were conducted. Figure 1a illustrates the relative differences in specific binding of IGF-I to muscle membranes derived from the 2 lines of birds at one-week posthatch. In Figure 1b, the data are expressed as a percent of maximum binding to each membrane type and illustrate the similarity of the latter curves. Scatchard analyses of the data (Table 2) show that there were 53% more IGF receptors on LSN muscle membranes compared to controls, while there were no differences in receptor affinities. In a repeated experiment using muscles from another hatch of birds (data not shown) receptor numbers in the LSN line were double those found in control membranes.

The results establish an association between a genetic muscle weakness and the level of an important growth factor receptor in the pectoral muscle of the LSN avian model. When compared with control birds at one week posthatch, LSN body weights were lower (38.84 ± 1.28 g vs. 72.65 ± 1.25 g) and the pectoral muscle weights were lower (0.74 ± 0.04 g vs. 2.336 ± 0.10 g). The defective development of pectoral muscles in LSN birds is illustrated

Table 1. [¹²⁵I]IGF-I binding to muscle membranes derived from day-old LSN and control birds.

Line	DPMs [¹²⁵ I]IGF-I specifically bound/100 micrograms of protein ¹
LSN	16,499 ± 165 ²
Control	11,330 ± 450

¹Values represent the mean ± SE of 4 replicate samples

²Means are significantly different (P ≤ 0.0001)

IGF-I receptors in LSN birds

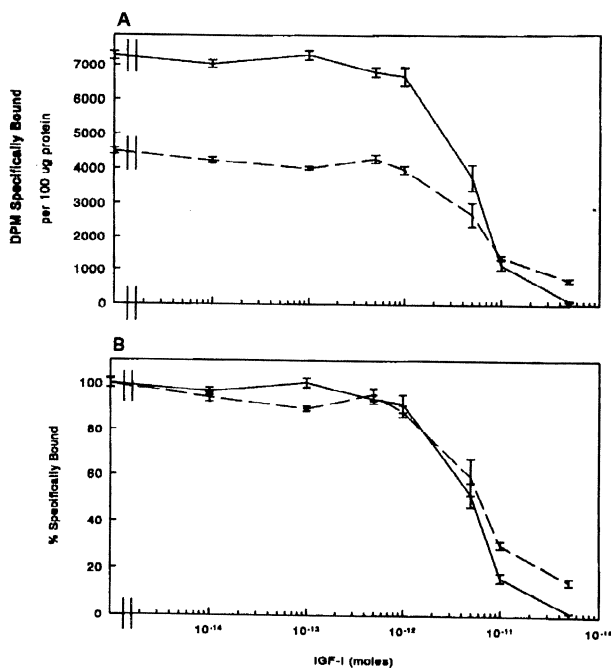


Figure 1. Displacement curves of $[^{125}\text{I}]$ binding to LSN (—) or Control (---) muscle membranes. Membranes were incubated 4 hr at 15°C with $[^{125}\text{I}]\text{IGF-I}$ and increasing concentrations of unlabelled IGF-I. (a) Data are expressed based on DPMs specifically bound, (b) Data are expressed based on the percent of maximally bound DPMs. Each point represents the mean $\pm$ SE of 4 observations.

Table 2. IGF-I receptor dissociation constants (Kds) and receptor numbers on membranes derived from LSN and control birds.

	LSN	Control
Kd	$1.56 \times 10^{-8} \text{ M}^a$	$1.73 \times 10^{-8} \text{ M}^a$
Receptors per µg protein	$9.90 \times 10^6,^a$	$6.46 \times 10^6,^b$

^{a,b} Means within a comparison with no common superscripts differ ($P \leq 0.0001$)

by comparing the percentage of body weight this muscle represents in the 2 lines. The pectoral muscles of control birds were 3.2% of the total body weight, while those from the LSN birds were 1.9%. While the specific genetic defect in the LSN line is currently unknown, it is tempting to speculate that an altered regulation of IGF receptor expression is involved. A prerequisite to skeletal muscle hypertrophy is the differentiation and fusion of myogenic

satellite cells to adjacent muscle fibers [9, 10]. Since IGFs stimulate satellite cells to leave the cell cycle and differentiate [2], muscle cells with a greater number of IGF receptors may fuse to form myotubes prematurely during early muscle development. This would result in a smaller pool of satellite cells for continued development of the affected muscles. The results have established that birds with the LSN genetic muscle weakness have altered expression of IGF receptors in the pectoral muscles. Further examination of the LSN defect will focus on determining if and how changes in IGF receptor numbers impact skeletal muscle extracellular matrix organization and cell signal transduction pathways.

Address correspondence to:

Dr. Sandra G. Velleman, The Ohio State University/OARDC, Department of Animal Science, 1680 Madison Ave, Wooster, Ohio 44691 USA, phone 216 263 3905, fax 216 263 3949.

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