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COLLAGEN CROSSLINKING IS ASSOCIATED WITH DECORIN PROTEOGLYCAN EXPRESSION IN MUSCLE

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INTRODUCTION

Myogenesis involves the precise regulation of a number of developmental events which includes cell adhesion and cell-cell recognition. These processes, in part, involve interaction of the cell with extracellular matrix glycosaminoglycans, proteoglycans and collagen. Decorin is a chondroitin sulfate/dermatan sulfate proteoglycan that associates with both collagen types I and II, and has been identified in a number of tissues including skeletal muscle (Carrino and Caplan, 1989). Considerable experimental data suggest that a specific functional interaction occurs between decorin and collagen in the extracellular matrix of soft tissues (Vogel and Trotter, 1987).

We examined pectoral muscle decorin levels, as well as percent collagen and collagen crosslinking concentrations, in the avian Low Score Normal (LSN) and muscular dystrophy (MD) genetic muscle disorders. The LSN and MD represent disorders of muscle development characterized by subnormal muscle development. While MD muscle exhibits abnormal cellular development, LSN myocytes are apparently normal (Velleman et al., 1993). Our objective was to determine if skeletal muscle extracellular matrix development was altered in the LSN and MD disorders.

MATERIALS AND METHODS

Control White Leghorn, LSN and MD birds 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 and a commercial White Leghorn stock. LSN birds are characterized by impaired righting ability and 40% less pectoral muscle mass than normal birds at one week posthatch.

Decorin levels were measured in muscle samples (n=6 for each treatment) using antibodies specific to the proteoglycan and immunoblot analysis (Velleman, 1995).

Collagen concentration was determined by measuring hydroxyproline (Woessner, 1961) in whole muscle (minus epimysium) hydrolyzates. Isolation and quantitation of the HP crosslink in the remaining hydrolyzate was accomplished by binary gradient reverse phase HPLC using the procedure described by Eyre et al. (1984). For *in ovo* treatments, muscle tissue from 15-20 embryos was pooled for analyses; for posthatch treatments n=6 for each treatment. Where appropriate, data was analyzed by two-way ANOVA. Differences between means were determined by Student's t-test.

RESULTS

Decorin expression, as measured by immunoblot analysis at 14 days of embryonic development, was unaffected in LSN pectoral muscle (Figure 1). However, by 20 days of embryonic development, LSN decorin levels were significantly elevated compared to control and MD birds ($P < .001$). At six weeks posthatch, LSN decorin levels did not vary significantly from the control and MD pectoral muscles ($P > .05$). However, by one year posthatch, both LSN and MD pectoral muscles exhibited a decline in decorin levels relative to control birds ($P < .001$). Collagen crosslinking and concentration at the embryonic stages did not vary from the control values, however, MD birds exhibited reduced collagen crosslinking (Figures 2 and 3). At six weeks posthatch, collagen concentration in LSN pectoral muscle was not significantly different from that in normal muscle, but covalent crosslinking of muscle collagen from LSN birds at this stage in development was markedly elevated, nearly two-fold, compared to normal animals ($P < .05$).

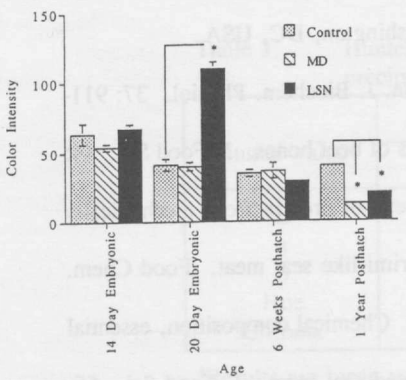

Figure 1. Quantitative analysis of decorin immunoblots for control. MD and LSN pectoral muscle protein extracts as 14 and 20 days of embryonic development and six weeks and one year posthatch. *indicates data is significantly different from control values.

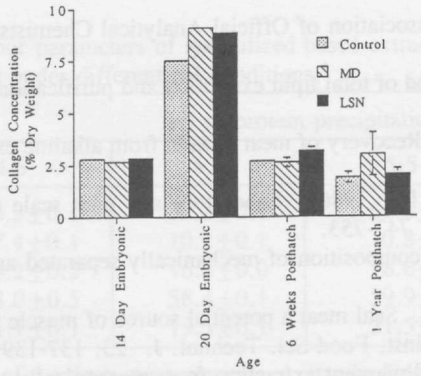

Figure 2. Collagen concentration for control, MD and LSN pectoral muscle at 14 and 20 days of embryonic development and six weeks and one year posthatch.

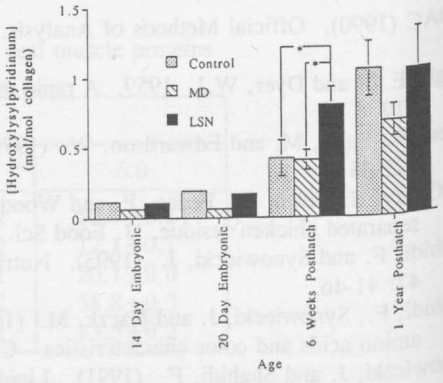

Figure 3. Hydroxylysylpyridinium concentration for control, MD and LSN pectoral muscle at 14 and 20 days of embryonic development and six weeks and one year posthatch. *Indicates data is significantly different from control values.

DISCUSSION

In a previous study, we demonstrated that the LSN genetic muscle weakness does not involve altered myosin heavy chain isoform switching or altered myofiber morphology (Velleman et al., 1993). The present study goes beyond an investigation of myofiber-specific development and examines alterations in two key extracellular matrix components, proteoglycan and collagen. The results reported herein support the notion that the LSN genetic muscle weakness is associated with alterations in both proteoglycan and collagen expression and organization.

Prior to 20 days of embryonic development proteoglycan expression and collagen characteristics did not differ significantly from control values. However, at 20 days of embryonic development there was a significant elevation in decorin levels, but not in the levels. At day 20 *in ovo*, collagen concentration and crosslinking in LSN muscle were similar to values from normal birds. By six weeks posthatch, there was a dramatic increase, nearly two hundred percent, in LSN collagen crosslink concentration, but collagen concentration remained constant. Considering the lack of muscle accretion which characterizes the LSN defect, it is surprising that collagen concentration was not elevated in the LSN pectoral muscle. The increase in collagen crosslinking was extremely rapid, and suggests an aberrant accumulation of mature, trivalent crosslinks. In fact, values for HP concentration in six week posthatch LSN pectoral muscle are the highest values reported for muscle tissue from any species of any age (see McCormick, 1994 for review). Phenotypically, the increase in collagen crosslinking at six weeks of age correlates with the reduction in wing flexibility and reduced ability of the birds to right themselves from a supine position in an exhaustion score test (Pierro and Haines, unpublished observation).

Molecular mechanisms responsible for rapid accumulation of crosslinks in muscle tissue are not known; we have noted that the formation of crosslinks is dependent upon molecule spatial orientation and precise stereospecific alignment of crosslinking sites on adjacent collagen molecules and fibrils (Reiser et al., 1992). The major fibrillar collagen phenotypes in muscle are types I and III; collagen fibrils may exist as either homo- or heteropolymers of the two types, with the latter probably predominating (Fleishmajer et al., 1990). In skeletal muscle type I and III collagen molecules are joined by the HP crosslink (Kuypers et al., 1994). Because HP is a trivalent crosslink and fibrillar collagen is a mixture of two discrete types, considerable flexibility exists in potential spatial arrangements of collagen molecules and in the composition of fibrils. In muscle (Kuypers et al., 1994) and non-muscle tissues containing types I and III collagen (Henkel and Glanville, 1982), both quarter dimension as well as zero dimension stagger among mixtures of types I and III collagen molecules have been demonstrated. The formation of crosslinks is dependent upon the proximity of reactive residues on adjacent collagen molecules or fibrils (Reiser et al., 1992), thus, also upon variable orientation and alignment of collagen molecules. Since decorin associates with fibrillar collagen, influences collagen formation and exhibits perturbed expression in the LSN muscle, we hypothesize that the interaction of decorin and fibrillar collagen is a key component in controlling skeletal muscle extracellular matrix development in particular, collagen crosslinking.

Considerable *in vitro* data suggest that a specific functional interaction occurs between decorin and fibrillar collagen (types I and II). Studies indicate that interactions between the core protein of decorin and collagen govern the rate and extent of collagen fibrillogenesis (Vogel and Trotter, 1987) and suggest decorin controls the maturation of fibrils into larger fibrils and fiber networks (Scott, 1988). Furthermore, the binding of decorin to type I collagen is periodic and axial along the fibril, with the decorin protein binding sites corresponding to the lateral shift or stagger of one collagen molecule to another (Pringle and Dodd, 1990). Developmental changes in decorin expression might be expected to alter the association of decorin with collagen, influence subsequent fibril formation, and thereby crosslinking patterns. Interestingly in the LSN model, an increase in decorin expression precedes a dramatic and rapid increase in collagen crosslinking, but does not alter collagen concentration. It is possible that as more decorin molecules bind to type I collagen, lateral orientation of collagen molecules is affected, thus altering location and number of crosslinks formed as well as rate of formation. Bassols and Massagué (1988) have shown that, in response to TGF-ß, decorin glycosaminoglycan chain length increases. Although the core protein of decorin interacts with collagen, it is thought that the glycosaminoglycan chains may determine the axial spacing of decorin along the collagen fibril (Hedbom and Heinegard, 1993). The mechanism by which crosslinking rates and concentrations are regulated in skeletal muscle and the potential role that decorin interaction with fibrillar collagen may play in the process awaits elucidation of collagen crosslink location and patterns of decorin binding within collagen fibrils. Nevertheless, the results described here demonstrate that appropriate expression of extracellular matrix molecules may be required for normal skeletal muscle development, structure, and function.

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