Absence of Myostatin (GDF-8) Decreases Toughness of the Knee Ligaments in Mice, and is Associated with Decreased Expression of Tenascin C and Type 1 Collagen

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INTRODUCTION

Congenital absence of myostatin (GDF-8) is known to significantly increase muscle mass, and myostatin inhibitors can improve muscle mass and strength in laboratory animals[1]; however, it has also been shown that absence of myostatin decreases the size and strength of the leg tendons in mice, suggesting that myostatin-deficiency may alter the integrated growth, development, and adaptation of the myotendon complex.[2] We tested the hypothesis that myostatin deficiency is in fact associated with patellar ligament (tendon) weakness in laboratory mice, and we also sought to determine if myostatin deficiency altered strength of the anterior cruciate ligament (ACL). We examined tenascin expression in these tissues using real-time PCR, in order to explore the molecular mechanisms underlying the effects of myostatin deficiency on tendon and ligament mechanical properties. Tenascins are a family of extracellular matrix (ECM) glycoproteins that contribute to matrix structure, and tenascin expression is upregulated with mechanical loading. Furthermore, tenascins are thought to play a direct role in the pathophysiology of tendon injury, as sequence variants of tenascin C (TNC) are associated with tendinopathies and Achilles tendon ruptures.

METHODOLOGY

Adult mice, 4-6 months of age, were included for study. The sample utilized in this study includes 12 wild-type (+/+) mice and 12 myostatin-deficient (-/-) mice, six males and six females per genotype. Animals were sacrificed according to IACUC-approved procedures, weighed, and the left gastrocnemius weighed. The left quadriceps muscle was dissected free superior to the patella and weighed. The collateral ligaments were dissected free as was the posterior cruciate ligament, preserving the intact ACL and patellar ligament (PL). The ACL was first tested in tension, followed by tensile testing of the patellar ligament. Biomechanical testing was conducted using a Vitrodyne materials testing system. The cross-sectional area of the ACL and patellar ligament was calculated by multiplying the width of the tendon by its thickness. Stiffness, toughness (area under the curve), and peak load were measured directly from force-displacement curves.

Quantitative Real-time PCR (RT-PCR) was performed using the Cepheid SmartCycler. ACL and patellar ligament tissue was harvested from the right knee of mice, tissue was homogenized, and the resultant RNA isolated. The RNA was reverse transcribed into cDNA, and this cDNA library was used in real-time PCR. A total of three genes were used in RT-PCR analysis: GAPDH (housekeeping gene), Tenascin C, and Type I Collagen. Each gene was amplified for 45 cycles. A melt curve was used to assess purity of gene products and the data was normalized by GAPDH using the cycle threshold (CT) comparative method[3].

RESULTS

ANOVAs demonstrate that mice lacking myostatin showed significantly (P<.001) greater body weight (+25%), quadriceps mass (+70%), and gastrocnemius mass (+85%) compared to wild-type mice. Mice lacking myostatin did, however, also show significantly lower peak force and toughness of the patellar ligament during tensile testing (Fig. 1a), and significantly lower toughness of the ACL (Fig. 1b) compared to wild-type mice. Stiffness, ultimate stress, and ultimate strain of the ACL and patellar ligament did not differ significantly between genotypes. ACL cross-sectional area was similar between genotypes whereas tendon area was slightly (~10%), but not significantly, lower in myostatin-deficient mice. Real-time PCR data showed marked differences between genotypes in relative gene expression. Mice lacking myostatin showed significantly lower expression levels of tenascin C and type 1 collagen in both the patellar ligament (Fig. 2a) and the ACL (Fig. 2b).

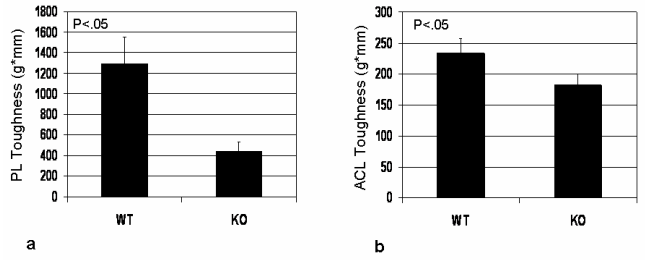

Fig. 1. Toughness of the patellar ligament (PL; a) and anterior cruciate ligament (ACL; b) in normal (wt) and myostatin-deficient (ko) mice. Means and standard errors are shown.

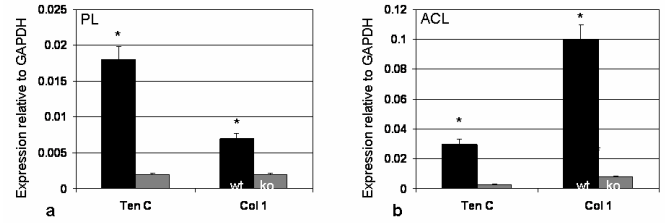

Fig. 2. Expression of tenascin C (Ten C) and type 1 collagen (Col 1) relative to the housekeeping gene GAPDH in the patellar ligament (PL; a) and anterior cruciate ligament (ACL; b) in normal (wt, solid bars) and myostatin-deficient (ko, shaded bars) mice. Means and standard errors are shown. * P<.05.

DISCUSSION

It was previously shown that myostatin and the myostatin receptor (ActRIIB) are expressed in tendon, and that myostatin directly stimulates collagen synthesis in cultured tendon fibroblasts.[1] Consistent with these previous findings, collagen expression was decreased in both the patellar ligament and ACL of myostatin-deficient, suggesting myostatin signaling plays a significant role in connective tissue metabolism. We also found lower toughness of the patellar ligament and ACL, as well as lower peak strength of the patellar ligament, in myostatin-deficient mice, suggesting that decreased collagen and tenascin expression is associated with diminished mechanical properties of these tissues.

Myostatin expression in injured skeletal muscle is known to contribute to increased fibrosis, whereas myostatin inhibitors such as follistatin decrease fibrosis in regenerating muscle and improve muscle repair.[4,5] While myostatin deficiency may increase muscle mass and muscle regeneration, as well as increase bone formation,[6] our results indicate that loss of myostatin function may inhibit collagen synthesis in connective tissues and perhaps attenuate the mechanical and structural adaptation of tendons and ligaments.

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