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Genomic and lipidomic actions of nandrolone on detached rotator cuff muscle in sheep

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ABSTRACT

Reversal of fatty infiltration of pennate rotator cuff muscle after tendon release is hitherto impossible. The administration of nandrolone starting at the time of tendon release prevents the increase in fat content, but does not revert established fatty infiltration.

We hypothesised that tendon release and myotendinous retraction cause alterations in lipid related gene expression leading to fatty muscle infiltration, which can be suppressed by nandrolone through its genomic actions if applied immediately after tendon release.

The effects of infraspinatus tendon release and subsequent tendon repair at 16 weeks were studied in six Swiss Alpine sheep. In the interventional groups, 150 mg nandrolone was administered weekly after tendon release until sacrifice (N22W, n = 6) or starting at the time of repair (N6W, n = 6). Infraspinatus volume, composition, expressed transcripts, lipids, and selected proteins were analyzed at baseline, 16 and 22 weeks.

Tendon release reduced infraspinatus volume by 22% and increased fat content from 11% to 38%. These changes were not affected by repair. Fatty infiltration was associated with up-regulation of 227 lipid species, and increased levels of the adipocyte differentiation marker PPARG2 (peroxisome proliferator-activated receptor gamma 2). Nandrolone abrogated lipid accumulation, halved the loss in fiber area percentage, and up-regulated androgen receptor levels and transcript expression in the N22W but not the N6W group.

The results document that nandrolone mitigates muscle-to-fat transformation after tendon release via a general down-regulation of lipid accumulation concomitantly with up-regulated expression of its nuclear receptor and downstream transcripts in skeletal muscle. Reduced responsiveness of retracted muscle to nandrolone as observed in the N6W group is reflected by a down-regulated transcript response.

1. Introduction

1.1. Degeneration and repair of the ruptured rotator cuff muscle

Tears of rotator cuff tendons affect a considerable portion of the elderly population [1,2]. Tendon tears lead to musculotendinous retraction, which is associated with muscle transformation by a progressive increase in fat and connective tissue content at the expense of contractile material (fatty infiltration; [3–5]). Even if surgical repair is successful, this transformation of muscle structure is hitherto irreversible (reviewed in [4]) and thereby interferes with restoration of muscle function. The infiltration of muscle with fat tissue is such a risk factor for the success of tendon repair, that an increase in fat content close to, or above 50% of total

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muscle volume is considered a contra-indication for surgical repair [6]. Muscular changes may render successful reattachment of the ruptured tendon impossible (reviewed in Ref. [4]), due to shortening and stiffening of the detached and atrophic muscle. Thus the prevention of muscular transformation in the retracted muscle is a high priority to allow successful repair of the musculotendinous unit after tendon tear.

1.2. Anabolic steroids prevent fat accumulation and increase lean mass

Anabolic steroids are a proposed treatment against the accumulation of fat and associated muscle atrophy [5] because they increase lean over fat mass by promoting muscle anabolism and reducing fat content [7–9]. We recently reported that the anabolic steroid nandrolone decanoate can prevent the increase in fat content, but does not mitigate atrophy of rotator cuff muscle in sheep and rabbit models if administered at the time of tendon release [10,11]. Anabolic steroids affect a number of systemic and local aspects of lipid metabolism, which may be implicated in fat accumulation in tenotomized rotator cuff muscle. Anabolic steroids reduce lipogenic activity in the liver [12] and enhance lipolytic activity in adipose tissue [13]. Furthermore, anabolic steroids inhibit adipocyte differentiation and affect skeletal muscle directly through genomic effects on transcript expression being associated with cell growth and proliferation, connective tissue development and function as shown in rat experiments [13–17]. Tenotomy has been demonstrated to increase phospholipid and triglyceride content in rat skeletal muscle, which has been postulated to involve the infiltration and expansion of adipogenic cells, as well as de-differentiation of muscle-associated stem cells or fibroblast into adipocytes [18–22]. This raises the possibility that nandrolone prevents fat accumulation in tenotomized muscle [10,11] by multiple mechanisms in skeletal muscle including the abrogation of phospholipid and triglyceride synthesis, the enhancement of lipid oxidation [23,24], through effects on adipocyte differentiation and/or expression of adipogenic pathways in skeletal muscle.

1.3. Ruptured rotator cuff muscle looses responsiveness to nandrolone

Interestingly, nandrolone does not influence fat accumulation in rotator cuff muscle in sheep and rabbit models of tendon release if administered after muscle degeneration has established [10,11]. This biological fact imposes possible limitations for full improvement of muscle function if repair of the tendon is delayed [25,26]. The main cytoplasmic receptor of anabolic steroids, the androgen receptor [17,27], is abundantly expressed in skeletal muscle. In the rat androgen receptor expression is sensitive to muscle loading and also to circulating hormone levels, i.e. being reduced with unloading and increased with nandrolone [28,29]. This suggests that the loss in responsiveness of rotator cuff muscle to anabolic steroids after tendon tear in sheep [30] involves changes in androgen receptor-mediated gene regulation.

1.4. Aim and hypotheses

The purpose of this study was to investigate 1) whether a muscle-based molecular mechanism underlies the increased fat content in sheep infraspinatus muscle 16 weeks after tendon release and 2) whether expression changes explain its mitigation when nandrolone is administered immediately after tendon release before fatty infiltration is established. As metabolism related targets of nandrolone action in skeletal muscle are not established, we first carried out a global assessment of lipid and transcript expression changes with tendon release and repair. For this purpose, we assessed to which extent alterations in transcript expression in detached infraspinatus muscle are suppressed by weekly injections of nandrolone, and whether the transcript response differs when nandrolone is administered immediately after tendon release or only after repair, and whether the response is related to androgen receptor levels. We specifically hypothesised that tendon release enhances transcript expression for gene ontologies (GOs) regulating adipocyte differentiation and lipid metabolism and associated lipid species in detached infraspinatus muscle, and that nandrolone down-regulates this expression program.

2. Materials and method

2.1. Experimental design

Eighteen female Swiss Alpine sheep (two years old; provider Staffelegg, Küttigen (AG), Switzerland) were subjected to release of the infraspinatus tendon in three interventional groups of six animals each (CONTROL: 45.3 ± 1.9 kg, N22W: 47.1 ± 0.8 kg, N6W: 48.4 ± 1.5 kg). N22W received weekly injections of 150 mg nandrolone decanoate (Deca-Durabolin®) into the gluteus maximus muscle in the post-operative phase starting immediately after tendon release. N6W received weekly intramuscular injections of 150 mg nandrolone decanoate starting at the time of tendon repair, i.e. after 16 weeks. At 16 weeks, repair was performed in all groups by a single stage repair of the tendon to the greater tuberosity. Prior to tendon release (PRE), 16 weeks after tendon release (TR) and 6 weeks after repair (END) measurements were made on the operated muscle to characterize retraction, volume and fat content of the infraspinatus muscle. The measures were compared to contralateral control muscle at the end of the experiment (END-CC). Further, biopsy samples from the infraspinatus muscle were collected. Biopsies were subjected to the characterization of muscle composition and transcript expression. Absolute values for the volumetric and cellular adjustments of infraspinatus muscle have in part been reported. They are now included as percentage change to allow the interpretation of the new measurements on molecular parameters with tendon release and repair. The experiment was approved by the local federal authorities (Veterinary Office of the Canton of Zurich, application number 72/2013).

2.2. Tendon release and reattachment

All procedures were carried out on the right shoulder essentially as described [4,30]. The tendon was released by osteotomy of the greater tuberosity ($20 \times 10 \times 10$ mm) using an oscillating saw. The tendon stump was grasped via its attached bone chip through 2 figure-of-8 stitches using Fiberwire USP No. 2 sutures (Arthrex, Inc, Naples, Florida) that passed within a 1.8-mm drill-hole in the bone chip. Subsequently, the tendon and bone chip, with the tied sutures, were wrapped in a silicon tube (Silicone Penrose drain tube, 12 mm diameter; Fortune Medical Instrument, Taipei, Taiwan) to prevent reattachment through scar tissue. Sixteen weeks later, rotator cuff repair was performed. The bone chip was reattached to its original site, or as near as possible by connecting the remaining sutures to a 3.5-mm self-tapping cortical bone screw (Synthes, Paoli, USA) with a washer. During the first three weeks of the rehabilitation period, stress to the repaired tendon was eased by reducing full weight bearing through suspension of the sheep in a loose belt and by attaching a ball to the claws. After 6 weeks of reattachment, the animals were sacrificed.

2.3. Muscle anatomy

Immediately after each surgical intervention, magnetic resonance imaging (MRI) and computed tomography of both shoulders