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Undernutrition regulates the expression of a novel splice variant of myostatin and insulin-like growth factor 1 in ovine skeletal muscle

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ABSTRACT

Undernutrition suppresses the growth of skeletal muscles and alters the expression of insulin-like growth factor 1 (IGF1), a key mitogen, and myostatin, a potent inhibitor of myogenesis. These changes can explain, at least in part, the reduced growth of skeletal muscles in underfed lambs. We have recently identified a myostatin splice variant (MSV) that binds to and antagonizes the canonical signaling of myostatin. In the present study, we hypothesized that the expression of MSV would be reduced in conjunction with myostatin and IGF1 in response to underfeeding in skeletal muscles of sheep. Young growing ewes were fed either ad libitum or an energy-restricted diet (30% of maintenance requirements) for 28 d. This regime of underfeeding resulted in a 24% reduction in body mass ($P < 0.001$) and a 36% reduction in the mass of the semitendinosus muscles relative to controls ($P < 0.001$) by day 28. The concentrations of MSV and IGF1 messenger RNA (mRNA) were reduced (both $P < 0.001$), but myostatin mRNA was not altered in semitendinosus muscles. Unlike the reduced expression of mRNA, the abundance of MSV protein was increased ($P < 0.05$) and there was no change in the abundance of myostatin protein. Our results suggest that undernutrition for 28 d decreases the signaling of myostatin by increasing the abundance of MSV protein. Although this action may reduce the growth inhibitory activity of myostatin, it cannot prevent the loss of growth of skeletal muscles during undernutrition.

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1. Introduction

Animals are often subjected to a reduced supply of food as a consequence of prolonged and unfavorable changes in climate and weather. Identification of downstream molecular targets of undernutrition and their expression patterns are essential to better understand how skeletal muscles adapt to match the restricted substrate availability. Two growth factors, myostatin and insulin-like growth factor 1 (IGF1), have been shown to mediate the effects of nutritional perturbations in skeletal muscles. In addition, a novel myostatin splice variant (MSV) has recently been identified, which regulates the bioavailability of canonical myostatin. However, the role of MSV and its interplay with myostatin and IGF1 in this adaptive response of skeletal muscle to undernutrition is unknown.

Myostatin, a key inhibitor of myogenesis, acts as a protein ligand of a homodimer (25 kDa) [1–4]. Binding of the myostatin ligand to its canonical receptor results in the inhibition of the proliferation of myoblasts and protein synthesis [5–8]. Nutrition has a variable impact on the

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expression of myostatin. Short-term fasting is associated with decreased concentrations of myostatin mRNA in skeletal muscles of young chickens [9], but did not influence expression of myostatin mRNA in muscles of humans [10], pigs [11], or rats [12]. However, the expression of myostatin mRNA and protein are both reduced in the first 4 weeks of long-term (22 wk) undernutrition in sheep [13]. These reports do not show a clear relationship between nutrition and the expression of myostatin, but instead suggest that species differences, dissimilar nutritional perturbations, and varying developmental stages result in distinct, but variable responses.

Adding a new layer of complexity to the regulation of myostatin and the development of skeletal muscles is the discovery of MSV [14]. We have shown that MSV protein binds to myostatin and antagonizes the canonical signaling of myostatin to increase myoblast proliferation [14]. Thus, we have proposed that MSV may regulate the bioactivity and/or bioavailability of myostatin to control muscle mass [14].

There is little doubt that IGF1 plays a major role in the development of skeletal muscles at a number of levels. IGF1 stimulates the proliferation and differentiation of myoblasts [15–17]. Concomitantly, IGF1 promotes protein synthesis and suppresses protein degradation, thereby, contributing to the hypertrophy of muscle fibers [18–20]. The regulation of IGF1 by nutrition is well documented (for reviews see: [21,22]). We have previously shown that concentrations of IGF1 in plasma are reduced during fasting of sheep for 24 h to 72 h and that prolonged underfeeding results in a decrease in the concentration of IGF1 mRNA in skeletal muscle [13,23]. At present, it is unclear how MSV, myostatin, and IGF1 respond to and mediate the effect of undernutrition in skeletal muscle.

In light of such contrasting actions for the differentially spliced products of the myostatin gene, the importance of IGF1 on growth and the impact of undernutrition on early growth, we tested the hypothesis that underfeeding of young, growing sheep would decrease the transcription and translation of myostatin, MSV, and IGF1 in skeletal muscles. A limitation of our earlier study [13] was that we observed early changes in our previous study, but collected only 1 sample in the first 4 weeks. Therefore, in the present study, we collected more frequent samples during a 28-d period of undernutrition to better understand the changes in expression of myostatin, MSV, and IGF1.

2. Materials and methods

2.1. Animals

Ninety female New Zealand Romney lambs (*Ovis aries*) were housed indoors and randomly allocated to control and underfed dietary regimes at 8 months of age. The control groups (n = 48) were fed ad libitum a complete pelleted diet (9.75 MJ/kg of dry matter of metabolizable energy containing 15% [w/w] fiber and 17% [w/w] protein; Country Harvest Stockfeed, New Zealand), whereas those in the underfed groups (n = 42) were fed the same pelleted diet to 30% of body maintenance for up to 4 weeks. The 30% of feed was calculated from the metabolizable energy requirements of the control group as follows: the dry matter of feed consumed by the control lambs was recorded daily and used to calculate the average feed consumption on a kilogram per live weight basis. Underfed lambs were offered 30% of the mass of the average feed consumed by the ad libitum fed control lambs each day. At the onset of the study, 6 sheep in the control group were slaughtered. Animals from control (n = 6) and underfed (n = 6) treatment groups were then slaughtered serially at 3, 5, 7, 10, 14, 21, and 28 d of the experiment. The carcasses were weighed, and then, the semitendinosus (ST) muscle was excised and weighed. A sample was systematically collected from a specified midbelly region of each muscle and immediately homogenized to extract the total RNA for quantitative reverse transcription–polymerase chain reaction (qRT-PCR). A second sample from the ST muscle was frozen in liquid nitrogen and stored at −80°C for Western blot analyses. Approval for this study was obtained from the Ruakura Animal Ethics Committee, Hamilton, New Zealand.

2.2. Total RNA isolation and quantitative PCR analyses

One hundred milligrams of ST muscle from each animal were homogenized in 1.0 mL of Trizol Reagent (Life Technologies, CA, USA) for 30 s at 13,500 rpm using an Ultra Turrax homogeniser (Janke & Kunkel, Germany). Debris was removed by centrifugation for 5 min at 11,000*g* at 4°C, and total RNA was isolated according to the manufacturer's protocol (Life Technologies, CA, USA). RNA was resuspended in diethyl pyrocarbonate–treated water, and the concentration was determined by measuring the absorbance at 260 nm (Nanodrop spectrophotometer; Thermo Fisher Scientific, USA). Two micrograms of total RNA isolated from each ST muscle was reverse transcribed into complementary DNA (cDNA) using oligo(dT) primers and the SuperScript III First Strand cDNA Synthesis kit according to the manufacturer's instructions (Life Technologies). All oligonucleotide primers used in this study span across exon–exon boundaries to avoid the amplification of genomic templates. The following primers were used for myostatin (GenBank accession number: DQ530260): 5′-GATCTTGCTGTAACCTTCCC-3′ (forward) and 5′-GTGGAGTGCTCATCACAATC-3′ (reverse); for MSV (GenBank accession number: DL465814): 5′-GCTCAAACAACCTGAATCCAAC-3′ (forward) and 5′-CCATAGGGAGGAGTGTAAAAATG-3′ (reverse); and for IGF1 (GenBank accession number: M31736) 5′-AGGCTCAGAAGGAAGTACATTT-3′ (forward) and 5′-GAGCGGGATAGAGGAACAAG-3′ (reverse). Serial dilutions of pooled RT reactions were used to generate standard curves for each gene of interest. PCR was carried out with the FastStart DNA Master plus SYBR Green I reagent (Roche Diagnostics, Germany) and the following PCR protocol: preamplification denaturation (95°C for 5 min), amplification (95°C for 5 s, 60°C–62°C depending on the gene amplified for 10 s, 72°C for 20 s, with a single fluorescence measurement, 45 cycles), melt curve analysis (60°C–95°C with a heating rate of 0.1°C/s with continuous fluorescence measurement) on a LightCycler 2.0 instrument (Roche Diagnostics). The PCR products were separated in a 1.5% (w/v) agarose gel stained with SYBR Safe (Life Technologies) to confirm their size. Representative PCR products were extracted from the gel and directly sequenced

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