Domestic Animal Endocrinology 57 (2016) 63–70

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

Different dietary energy intake affects skeletal muscle development through an Akt-dependent pathway in Dorper × Small Thin-Tailed crossbred ewe lambs

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ARTICLE INFO

Article history:
Received 6 February 2016
Received in revised form 19 May 2016
Accepted 19 May 2016

Keywords:
Feed restriction
Skeletal muscle
Akt
mTOR
Lamb

ABSTRACT

The objective of this experiment was to investigate the mechanisms through which different levels of dietary energy affect postnatal skeletal muscle development in ewe lambs. Twelve Dorper × Small Thin-Tailed crossbred ewe lambs (100 d of age; 20 ± 0.5 kg BW) were selected randomly and divided into 2 groups in a completely randomized design. Animals were offered identical diets at 100% or 65% of ad libitum intake. Lambs were euthanized when BW in the ad libitum group reached 35 kg and the semitendinosus muscle was sampled. Final BW and skeletal muscle weight were decreased ($P < 0.01$) by feed restriction. Both muscle fiber size distribution and myofibril cross-sectional area were altered by feed restriction. *Insulin-like growth factor 1* (*IGF-1*) messenger RNA (mRNA) content was decreased ($P < 0.05$) when lambs were underfed, whereas no difference for *IGF-2* mRNA expression was observed ($P > 0.05$). Feed restriction altered phosphor-Akt protein abundance ($P < 0.01$). Moreover, the mammalian target of rapamycin (mTOR) pathway was inhibited by feed restriction, which was associated with decreased phosphor-mTOR, phosphorylated eukaryotic initiation factor 4E binding protein 1 (phosphor-4EBP1), and phosphorylated ribosomal protein S6 kinase (phosphor-S6K). Both mRNA expression of *myostatin* and its protein content were elevated in feed-restricted ewe lambs ($P < 0.05$). In addition, mRNA expression of both *muscle RING finger 1* and *muscle atrophy F-box* was increased when ewe lambs were underfed. In summary, feed restriction in young growing ewe lambs attenuates skeletal muscle hypertrophy by inhibiting protein synthesis and increasing protein degradation, which may act through the Akt-dependent pathway.

1. Introduction

Skeletal muscle is a major mass peripheral tissue that accounts for approximately 40% of the total body mass [1]. Meat animals are raised for their skeletal muscle; therefore, understanding mechanisms related to skeletal muscle development and growth is important for the food animal industry. Skeletal myogenesis is a complex process that requires an elaborate interplay of extrinsic and intrinsic regulators and orchestrated signaling pathways [2]. Serine and/or threonine kinase Akt (also known as protein kinase B or PKB) serves as a central node in cell signaling downstream of several growth factors, cytokines, and other cellular stimuli [3]. Akt regulates skeletal muscle development by exerting control at multiple levels via distinct signaling mechanisms. In human and mice, activation of the Akt pathway inhibits protein degradation by phosphorylating and thus repressing the transcription factor of Forkhead box O family (FoxO) and stimulates protein synthesis by phosphorylating mammalian target of rapamycin (mTOR) and glycogen synthase kinase 3 β [4].

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http://dx.doi.org/10.1016/j.domaniend.2016.05.010

Lamb production has substantially increased in several parts of the world in recent years due to the increasing consumption of mutton [5]. The Dorper breed of sheep is a fast-growing meat-producing sheep originating from South Africa and is renowned for its hardiness, lean carcass, and high growth rate [6]. The Small Thin-Tailed sheep is an indigenous sheep breed that demonstrates strong adaptability to local conditions in China [7]. The Dorper sheep has recently been introduced into China to crossbreed with Small Thin-Tailed sheep, and this crossbreed is now dominant for lamb production in Western and Northwestern China. Nevertheless, drought occurs frequently in this region, which results in significant reductions in forage production [8]. Thus, lambs in this area are frequently subjected to a reduced supply of feed. Identification of downstream signaling of undernutrition is important for understanding how skeletal muscle adapts to feed deprivation in lambs. The aim of the present study was to investigate the effects of restricted dietary energy levels on postnatal skeletal muscle mass in semitendinosus (ST) muscle from 1/2 Dorper × 1/2 Small Thin-Tailed ewe lambs and to examine potential signaling pathways within muscle that could mediate these effects.

2. Materials and methods

2.1. Care and use of animals

All animal procedures were approved by the Shanxi Agricultural University Animal Care and Ethics Committee. A total of twelve 1/2 Dorper × 1/2 Small Thin-Tailed ewe lambs (100 d of age; 20 ± 0.5 kg of BW) were selected randomly and housed in individual stalls (3.0 m × 0.8 m) equipped with feeders and a water source. Ivermectin was used to treat for internal parasites before the experiment at a dosage of 0.2 mg/kg BW. Before the experiment, the ewe lambs were fed an adaptation diet for 10 d ad libitum. Animals were fed individually twice daily with either a diet (Table 1) containing 100% National Research Council (NRC, 2007) recommendation for energy (ad libitum) or 65% of NRC's recommendation at 8:00 AM and 6:00 PM and had ad libitum access to water. Diets were expected to yield BW gains of approximately 300 g/d and 150 g/d, respectively. Feed supplied to the ad libitum group was adjusted daily in the morning according to dry matter intake (DMI) of the previous day to make sure 10% of the feed remained, and feed offered to restricted groups was adjusted based on DMI of the ad libitum group from the previous day. When the BW in the ad libitum group reached 35 kg (60 d after initiation of treatment), all lambs were anesthetized by inhaled CO_2. Total skeletal muscle and the ST muscle from the left side were dissected and weighed. One piece of ST muscle was sampled at the anatomic center and snap-frozen in liquid nitrogen. A second piece was fixed in 4% paraformaldehyde (PFA) for paraffin embedding.

Table 1
Ingredient and chemical composition of the mixed diet.

Category	%
Dietary ingredient	
Corn stalk	34.0
Sunflower seed null	11.0
Corn grain	27.3
Soybean meal	9.0
Rapeseed dregs	5.2
Distiller's dried grain with soluble	6.1
Wheat bran	5.0
Salt	0.6
Mineral/vitamin premix	1.8
Total	100.0
Chemical composition (determined)	
DM, % as fed	91.9
GE (MJ/kg of DM)	17.3
CP, % of DM	7.8
NDF, % of DM	41.3
Ash, % DM	5.8
Ca, % of DM	0.5
P, % of DM	0.4

Abbreviations: CP, crude proteins; DM, dry matter; GE, gross energy; NDF, neutral detergent fiber.

2.2. Hematoxylin and eosin (H&E) staining and muscle fiber size analysis

Unless stated otherwise, all chemicals were of analytical grade. Briefly, ST muscle from all animals fixed by 4% PFA (pH 7.4) was serially dehydrated in ethanol and xylene and embedded in paraffin. Each block was cut with a microtome using 7-μm sections (Leica, German). Sections were dewaxed and rehydrated serially by incubation with xylene and different concentrations of ethanol followed by H&E staining. Myofibril cross-sectional area analysis was performed as previously described [9]. Briefly, microscopic images were taken at a × 100 magnification using a Nikon ECLIPSE Ti microscope (Nikon, Japan) and digitally analyzed for muscle fiber cross-sectional area using Image J software (NIH, Bethesda, MD, USA). Approximately, 200 fibers were traced per animal in a blinded fashion. All fibers in the cross-sectional images were quantified, unless the sarcolemma was not intact.

2.3. Real-time quantitative PCR (RT-PCR)

Total RNA in ST muscle sample was extracted using Trizol reagent (Sigma, Saint Louis, MO, USA), and the integrity of RNA samples was determined by electrophoresis with 2% agarose gel. A cDNA was synthesized using a reverse transcription kit (TaKaRa Co, Ltd Dalian, China), with RT-PCR performed using the CFX RT-PCR detection system (Bio-Rad, Hercules, CA, USA) and a SYBR Green RT-PCR kit (TaKaRa Co, Ltd). Primers used are listed in Table 2. The PCR cycle parameters were as follows: 36 3-step cycles of 95°C, 20 s; 55°C, 20 s; and 72°C, 20 s. After amplification, a melting curve (0.01°C/s) was carried out for confirmation of products purity, and agarose gel electrophoresis was used to confirm the targeted size. Relative messenger RNA (mRNA) content was normalized to the *RPL13* content [10].

2.4. Antibodies

Antibodies against Akt (no. 9272), phosphor-Akt (no. 4056), AMPK (no. 2532), phosphor-AMPK (no. 2535), mTOR