Biochemical and Biophysical Research Communications 388 (2009) 112–116

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Upregulation of myostatin gene expression in streptozotocin-induced type 1 diabetes mice is attenuated by insulin

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ARTICLE INFO

Article history:
Received 9 July 2009
Available online 30 July 2009

Keywords:
Myostatin
Insulin
Type 1 diabetes
Muscle atrophy
Atrogin-1

ABSTRACT

Myostatin is a strong inhibitor of muscle growth, and its expression is increased in several types of muscle atrophy. However, whether or not myostatin expression is altered in muscle atrophy associated with type 1 diabetes (T1D) remains uncertain. In this study, we provided experimental evidence to show that myostatin mRNA increased in the early stage of T1D but came back to control levels later on. This expression pattern was closely correlated with the loss of body weight and atrogin-1 expression. Furthermore, induction of myostatin expression could be attenuated by insulin in T1D mice. Taken together, our findings indicate that the upregulation of myostatin expression most likely contributes to the muscle atrophy process during insulin deficiency.

Introduction

Myostatin belongs to the transforming growth factor-β superfamily and plays an essential role in the regulation of skeletal muscle mass. As its name indicates, the major role of myostatin is to inhibit skeletal muscle growth. This is supported by evidence that inhibition of myostatin dramatically increases muscle mass in various animal species [1–4] and humans [5]. Conversely, administration of myostatin can induce profound muscle atrophy and a cachectic state as observed in transgenic mice overexpressing myostatin [6] or in mice transplanted with Chinese hamster ovary cells stably expressing myostatin [7]. Moreover, increased myostatin expression was observed in several types of muscle atrophy such as muscle inactivity [8–10], age [11], denervation [11–13], glucocorticoid treatment [14] and cancer cachexia [15]. However, whether myostatin expression is altered in muscle atrophy resulting from insulin deficiency remains uncertain.

In this report, the potential role of myostatin in the pathological progression of STZ-induced T1D in mice was investigated by examining its expression. We found that the level of myostatin mRNA increased in STZ-induced T1D mice and that this increased expression could be attenuated by insulin. Most interestingly, the expression pattern of myostatin was closely correlated with the severity of muscle wasting in T1D.

Materials and methods

Animals. Seven-week-old C57BL/6J male mice were purchased from Beijing Vitalriver Laboratory Animal Inc. (Beijing, China). Animals were housed in a special pathogen-free environment with constant temperature at 22 °C, 50% humidity and a 12:12 h light-dark cycle in the Animal Facility of the Peking Union Medical College. All animals were fed a standard commercial chow diet and had free access to water *ad libitum*. All protocols of the animal experimental study were approved by the Animal Care Committee of Peking Union Medical College.

Multiple low dose of STZ (mld-STZ)-induced T1D. After one week of accommodation, mice were rendered diabetic by administration of an intraperitoneal injection of 45 mg/kg body weight STZ (Sigma, USA) for 5 days. STZ was dissolved in 0.1 M chilled sodium citrate buffer (pH 4.5) just before injection. In the control group, mice were intraperitoneally injected with an equal volume of sodium citrate buffer. Body weight and fasting plasma glucose were monitored after STZ injection. Fasting plasma glucose was measured by OneTouch Ultra Glucometer (LifeScan, USA). After onset of diabetes, mice were sacrificed by cervical dislocation. Gastrocnemius muscles from each mouse were removed and immediately frozen by liquid nitrogen. For examining myostatin expression patterns in the pathological progression of T1D, mice from both STZ and control groups were sacrificed at 1, 2, 3, 4 and 8 weeks after STZ injection. Body weight and fasting plasma glucose were measured at each time point. For the insulin therapy experiment, diabetic mice received a subcutaneous slow acting insulin (4 IU, Novolin N, Denmark) injection each day for 3 consecutive days. Five

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doi:10.1016/j.bbrc.2009.07.129

Table 1
Primer sequences for PCR.

Gene name	Forward primer	Reverse primer
For semi-quantitative RT-PCR		
Myostatin	5′-TGTTGCAAAATTGGCTCAAA-3′	5′-GCACAAGATGAGTATGCGGA-3′
GAPDH	5′-GTCTTCACCACCATGGAGAAG GC-3′	5′-ATTCATTGTCATACCAGGAAA-3′
For real time PCR		
Myostatin	5′-AACCTTCCCAGGACCAGGAG-3′	5′-CGCAGTCAAGCCCAAAGTCT-3′
Atrogin-1	5′-AAGCTTGTGCGATGTTACCCA-3′	5′-CATGGATGGTCAGTGCCCTT-3′
GAPDH	5′-TGGAGAAACCTGCCAAGTATGA-3′	5′-CTGTTGAAGTCGCAGGAGA CA-3′

animals were randomly allocated in each group in all the experiments as indicated.

High dose STZ (HD-STZ)-induced T1D. To generate an acute T1D model, mice were intraperitoneally injected with a single high dose of STZ (180 mg/kg body weight). In the control group, mice received an equal volume of citrate buffer. Four days after STZ injection, body weight and fasting plasma glucose were determined before the mice were sacrificed. Gastrocnemius muscles were collected according to the protocol mentioned above.

Semi-quantitative RT-PCR and real time PCR for gene expression assays. Total RNA extraction from gastrocnemius was performed using TRIzol (Invitrogen, USA) according to the manufacturer's instructions. The integrity of RNA was checked on 2% agarose gels, and total RNA concentration was determined by a spectrophotometer (Eppendorf, Germany). Reverse transcription was carried out with 2 μg of total RNA using M-MLV reverse transcriptase (Promega, USA). Semi-quantitative RT-PCR was performed as described previously [16]. Briefly, 100 ng of cDNA templates were used in

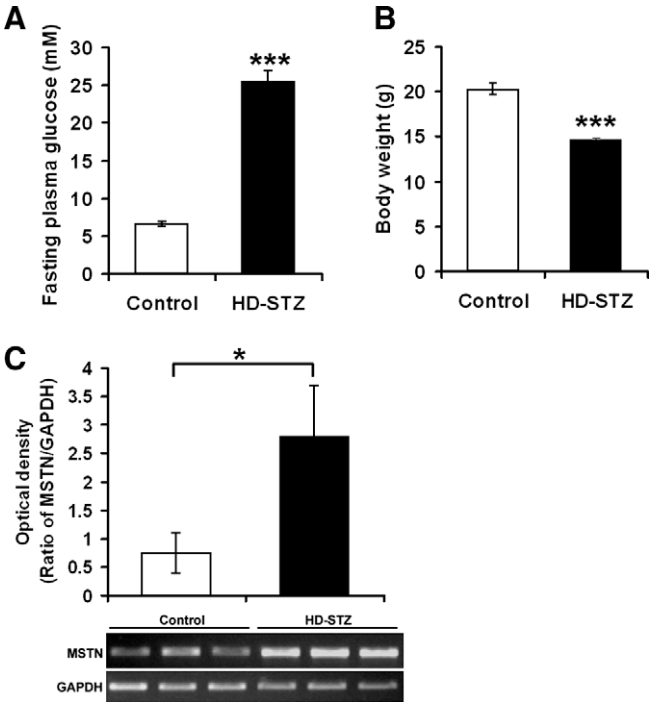

Fig. 2. Myostatin expression in skeletal muscle of the HD-STZ-induced T1D model. (A and B) Fasting plasma glucose and body weight were measured at 4 days after STZ injection, respectively. (C) Four days after STZ injection, total RNA from gastrocnemius muscles of each mouse was extracted, and myostatin expression was determined by semi-quantitative RT-PCR and normalized to GAPDH expression. Quantitative results were calculated by optical density. Values are means ± SEM ($n = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group).

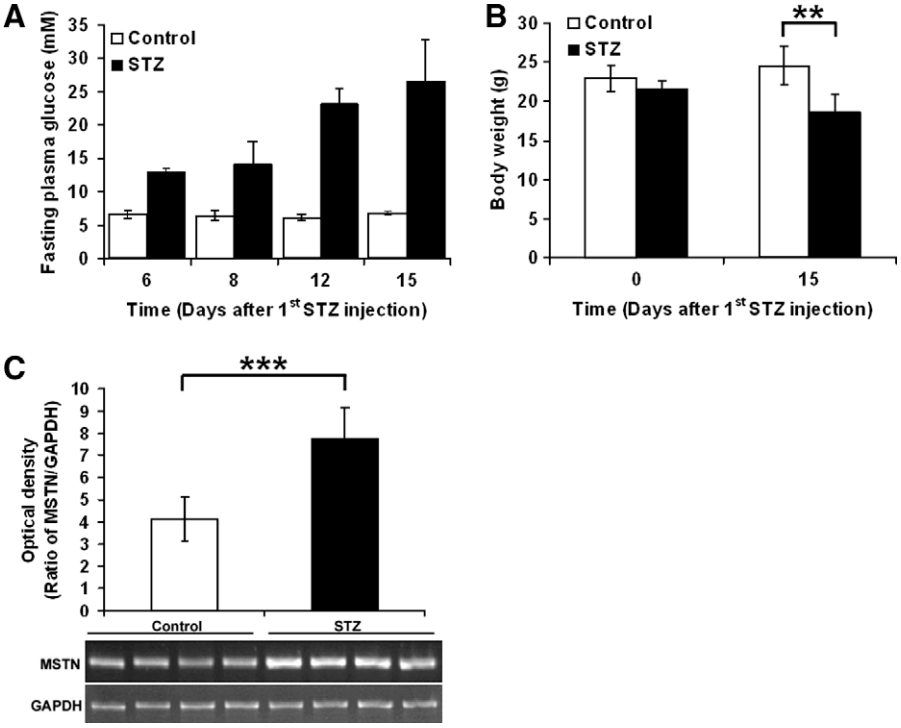

Fig. 1. Expression pattern of myostatin (MSTN) in skeletal muscle of the mld-STZ-induced T1D model. (A) Fasting plasma glucose was measured on the indicated days after first STZ injection. (B) Body weight was measured before and 15 days after STZ injection. (C) Total RNA from gastrocnemius muscles at day 15 after STZ injection was extracted, and myostatin mRNA level was determined by semi-quantitative RT-PCR and normalized to GAPDH expression. Quantitative results were calculated by optical density. Values are means ± SEM ($n = 5$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with control group).