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Original Article

Utility of urinary N-titin as a muscle atrophy biomarker in dexamethasone-induced muscle atrophy model mice

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ABSTRACT — Titin is a giant protein that is specifically expressed in striated muscle and essential for the maintenance of sarcomere structure and function. Recently, the N-terminal fragment of the Titin (N-titin) has been reported to show high levels in human urine in patients with muscular diseases and is expected to serve as a diagnostic biomarker for these diseases. In this study, we examined the utility of N-titin as a biomarker to detect muscle atrophy in mice. Male BALB/c mice (6 weeks of age, n=5 per group) were given 10 mg/L dexamethasone (DEX) dissolved in drinking water for 4 weeks. The gastrocnemius muscle (GAS) weight was significantly decreased and mRNA levels of muscle atrophy-related genes (Atrogin-1 and MuRF-1) were increased in the GAS after 4 weeks of DEX treatment. Although there were no degenerative/necrotic changes in the histopathological examination, the muscle fiber cross-sectional area significantly decreased in the GAS. On the other hand, there were no DEX treatment-related changes in the muscle weights and the muscle fiber cross-sectional area in the soleus muscle. These results suggest that 4-week of DEX treatment preferentially caused atrophy of fast-dominant muscle. Under the condition of this study, urinary N-titin/CRN ratio markedly increased from Week 2 of the DEX treatment. From the above results, the urinary N-titin/CRN ratio could be a biomarker for monitoring skeletal muscle atrophy in mice.

Key words: N-titin, Biomarker, Sarcopenia, Muscle atrophy

INTRODUCTION

Sarcopenia, defined by progressive loss of skeletal muscle mass, strength and function which occurs with aging, represents a significant health challenge in elderly populations (Rosenberg, 1989; Doherty, 2003). As the global demographics shifts to an older population, understanding the mechanisms, diagnostic criteria, and potential interventions for sarcopenia has become an important area of research. Identifying reliable and simple biomarkers for sarcopenia is crucial for the diagnosis, prognosis and monitoring treatment efficacy (Cruz-Jentoft *et al*., 2019). Although there are several biomarkers associated with sarcopenia, such as muscle protein turnover biomarkers, inflammation-mediated and redox biomarkers, growth factor biomarkers, neuromuscular junction biomarker and behavior-mediated biomarkers, due to the complexity of sarcopenia's pathophysiology, it is diffi-

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cult to monitor sarcopenia by a single biomarker (Supriya *et al*., 2021). Most standard assessments for muscle mass were considered to be magnetic resonance imaging and computed tomography (Beaudart *et al*., 2016). However, the high cost of the equipment and the lack well defined cut-off values, limits their use in primary care (Beaudart *et al*., 2016). Therefore, development of simple and versatile biomarkers for the diagnosis and prognosis of sarcopenic patients is still required.

Skeletal muscle fibers are mainly classified into two fiber types, slow-twitch oxidative (type 1) and fast-twitch glycolytic (type 2) fibers (Bottinelli and Reggiani, 2000; Schiaffino and Reggiani, 2011) and it is well known that atrophic changes preferentially occur in type 2 fibers in aging and sarcopenia (Lexell *et al*., 1988; Nilwik *et al*., 2013). Type 1 fibers are primarily involved in endurance exercise because of their high oxidative capacity and resistance to fatigue and as they contain an abundance of mitochondria and myoglobin. Conversely, type 2 fibers, especially type 2X fibers which are similar to type 2B fibers in rodents, are primarily dependent on glycolytic metabolism and are appropriate for activities to generate rapid force. Type 2A fibers have characteristics of both type 1 and type 2B fibers (Schiaffino, 2010).

Titin, also known as connectin, is a giant protein that is specifically expressed in striated muscle and is essential for the maintenance of sarcomere structure and function (Labeit and Kolmerer, 1995). Structurally, titin spans the Z-disk to the M-band of the sarcomere and confers elasticity and mechanical stability (Granzier and Labeit, 2006). Functionally, it acts as a molecular spring, contributing to muscle stretching and assembly, while also organizing the signaling complexes involved in muscle growth and adaptation (Granzier and Labeit, 2006; Ibata and Terentjev, 2021). Due to its essential roles in muscle structure and function, titin mutations and dysregulation have been implicated in a wide range of muscular disorders, including muscular dystrophies, cardiomyopathy and neuromuscular disorders (Hackman *et al*., 2002; Herman *et al*., 2012; Savarese *et al*., 2016). Titin is known to be bind with several proteolysis-associated enzymes, such as E3 ubiquitin ligase MuRF, calpain and matrix metalloproteinase (Centner *et al*., 2001; Kontrogianni-Konstantopoulos *et al*., 2009; Ali *et al*., 2010). Furthermore, it is known that these enzymes are partially responsible for the degradation of titin (Müller *et al*., 2021; Beckmann and Spencer, 2008; Ali *et al*., 2010).

Recently, proteomics profiling of urine derived from Duchenne muscular dystrophy patients revealed N-terminal fragments of titin increased in the patients' urine (Rouillon *et al*., 2014). In addition, using a highly sensitive sandwich ELISA system, N-titin has been reported to show high levels in urine from patients with muscular disorders, such as amyotrophic lateral sclerosis and muscular dystrophies, and is expected to serve as a diagnostic biomarker for these diseases (Maruyama *et al*., 2016; Awano *et al*., 2018; Yamada *et al*., 2021). Furthermore, an increase in urinary N-titin levels was noted not only in patients with skeletal muscle diseases, but also healthy human after high-intensity exercise (Maruyama *et al*., 2016; Tanabe *et al*., 2021). Therefore, N-titin has the potential to monitor a wide range of muscle disorders, from relatively mild to severe muscle disorders.

Glucocorticoids are known to promote protein catabolism and stimulate production of free amino acids in the skeletal muscle. Muscle atrophy due to excessive activation of glucocorticoid signals is known as steroid myopathy, which is characterized by muscle weakness and atrophic changes in type 2B muscle fibers (Pereira and Freire de Carvalho, 2011). Glucocorticoids are considered to lead to muscle atrophy by inhibiting protein synthesis and inducing proteolysis and the ubiquitin-proteasome system is considered to play a key role in muscle degradation (Schakman *et al*., 2013). Based on the pathological similarity, dexamethasone (DEX)-induced muscle atrophy models have been used as animal models for sarcopenia (Xie *et al*., 2021; Wang *et al*., 2022).

In this study, we demonstrated the utility of N-titin as a biomarker to detect muscle atrophy using DEX-induced muscle atrophy mice.

MATERIALS AND METHODS

Animal Experiments

Animals

All animal experiments were conducted in accordance with the guidelines of the Tokyo University of Agriculture and approved by the Institutional Animal Care and Use Committee of the university. Ten male BALB/c mice at 6 weeks of age were obtained from Japan SLC Inc (Shizuoka, Japan). The animals were housed in an environment-controlled room with a temperature of 23 ± 3°C, a relative humidity of 55 ± 15%, and a 12-hr light/dark cycle and were allowed free access to a commercial diet (CE-2, CLEA Japan Inc., Tokyo, Japan). The animals were quarantined and acclimated for 1 week and allocated to 2 groups (five animals per group), based on the body weights measured on the day of allocation so that the initial mean body weights of each group were equivalent.

Animal experiment procedures

10 mg of Dexamethasone (DEX., FUJIFILM Wako

Pure Chemical Corporation, Osaka, Japan), completely dissolved in 400 μL of dimethyl sulfoxide (DMSO., Nacalai Tesque, Inc., Kyoto, Japan), was mixed with Milli-Q water and brought to 1 L. The 10 mg/L DEX water was provided *ad libitum* to a DEX group for 4 weeks. 0.04% DMSO containing Milli-Q water was given to the control group in the same manner. 10 mg/L of DEX was selected because marked decreases in body weights were noted in mice treated with 30 mg/L of DEX for 4 weeks (data not shown).

During the dosing period, body weights, food consumption and water intake were measured. On the day after the last dosing (Day 29), animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia.

Urine and tissue sampling

Urine was collected for approximately 6 hr in a metabolism cage for each animal before the initiation of dosing with DEX and on Days 7, 14, 21, and 28 during the dosing period. Since a sufficient volume of urine could not be collected from some animals on Day 28, urine in the urinary bladder was collected at the time of euthanasia on Day 29 and combined with the urine from Day 28.

On the day of necropsy (Day 29), the gastrocnemius muscle (GAS) and soleus muscle (SOL) were collected as samples of fast muscle and slow muscle, respectively, from both hindlimbs and the bilateral muscles were weighed together. The relative weight of each muscle to the body weight on day of necropsy was calculated. One side of the soleus muscle was fixed in 10% neutral buffered formalin solution, and another side was soaked in a RNAlater™ Stabilization solution (Thermo Fisher Scientific Inc., MA, USA). One side gastrocnemius muscles were fixed in 10% neutral buffered formalin, and the other side was frozen with liquid nitrogen.

Determination of mRNA levels of the genes associated to muscle atrophy

mRNA levels of MuRF-1 and Atrogin-1 were determined by quantitative RT-PCR (qRT-PCR). Total RNA was isolated from the gastrocnemius and soleus muscles by Sepasol®-RNA I Super G (Nacalai Tesque, Inc.) and chloroform in accordance with the manufacturer's instructions. cDNA was synthesized by ReverTra Ace® qPCR RT Master Mix (Toyobo Co., Ltd., Osaka, Japan) according to the manufacturer's instruction. qRT-PCR was conducted by Thermal Cycler Dice Real Time System Multiplate RQ (Takara Bio Inc., Shiga, Japan) using THUNDERBIRD Next SYBR qPCR Mix (Toyobo Co., Ltd.). Comparative quantification was performed by the delta-delta CT method using Cyclophilin as a normalizer. Primers were custom synthesized by Eurofins Genomics K.K. (Tokyo, Japan) and used for the qRT-PCR (Table 1).

Measurements of Urinary N-titin and Creatinine Concentrations

Urinary N-titin concentrations were measured using a Mouse Titin N-Fragment (Urine) ELISA Kit (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) according to the manufacturer's instructions. For the measurements and analysis, the Infinite NANO (Tecan Group Ltd., Zürich, Switzerland) and Magellan Pro (Tecan Group Ltd.) were used, respectively. Urinary Creatinine Concentrations were measured by an enzymatic method using Determiner L CRE (Minaris Medical Co., LTD., Tokyo, Japan) with a TBA-120FR automated analyzer (TOSHIBA CORPORATION, Tokyo, Japan). Urinary N-titin concentrations were corrected by the urinary creatinine concentration.

Pathological examination

Fixed muscles (GAS and SOL) were prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin (HE), and were examined microscopically. Histopathological sections of the muscles were prepared in cross-section. In addition, the muscle fiber cross-sectional area (FCSA) was measured for both GAS and SOL using HE stained sections. Mean FCSA of 200 fibers/animal and 100 fibers/animal were calculated for GAS and SOL, respectively, using the virtual slide scanner NanoZoomer S360 and image viewer NDP.view2 (Hamamatsu Photonics K.K., Shizuoka, Japan) under a magnification of × 400.

Table 1. Sequences of primers used for qRT-PCR.

Gene	Forward primer	Reverse primer
MuRF-1	5'-GAGTGAGACACGCTCTGGAC-3'	5'-GGAGCCCTATGCTAGTCCCT-3'
Atrogin-1	5'-GCCCTCCACACTAGTTGACC-3'	5'-GACGGATTGACAGCCAGGAA-3'
Cyclophilin	5'-TGGCTCACAGTTCTTCATAACCA-3'	5'-ATGACATCCTTCAGTGGCTTGTC-3'

Statistical analysis

The mean values and standard deviations (S.D.) in each group were calculated for each parameter. Student's t-test was performed using Microsoft Excel (Microsoft Corporation, Washington, USA). The levels of significance were set at 5% and 1% (two-tailed). Log values were used for the evaluation of the S.D. and statistical analysis for comparative quantification of mRNA levels.

RESULTS

Effects on the skeletal muscle weights and body weights

After 4 weeks of DEX treatment in mice, skeletal muscle weights (sum of the right and left muscles) were weighed for both GAS and SOL to investigate the effects on the skeletal muscles. In the DEX group, GAS weights relative to the body weights on Day 29 were decreased (Fig. 1A), and absolute GAS weights were also decreased in the DEX group (Fig. 1C). However, there were no changes in the relative SOL weights (Fig. 1B). Although the absolute SOL weights were decreased in DEX group (Fig. 1D), they might be secondary changes due to the continuous decrease in body weights noted from Day 6 onward (Fig. 2). These results indicates that the effects of DEX on the muscle weights were more prominent in GAS than in SOL under the conditions of this study.

mRNA levels of the muscle atrophy related genes

mRNA levels of MuRF-1 and Atrogin-1, which are known as key regulators of ubiquitin mediated protein degradation in the skeletal muscles, were measured as an indicator of proteolysis in the skeletal muscles. mRNA levels of MuRF-1 increased in DEX groups in both GAS and SOL when compared with the control group. (Fig. 3A). Although there was no statistical significance ($p = 0.052$), mRNA levels of Atrogin-1 tended to be

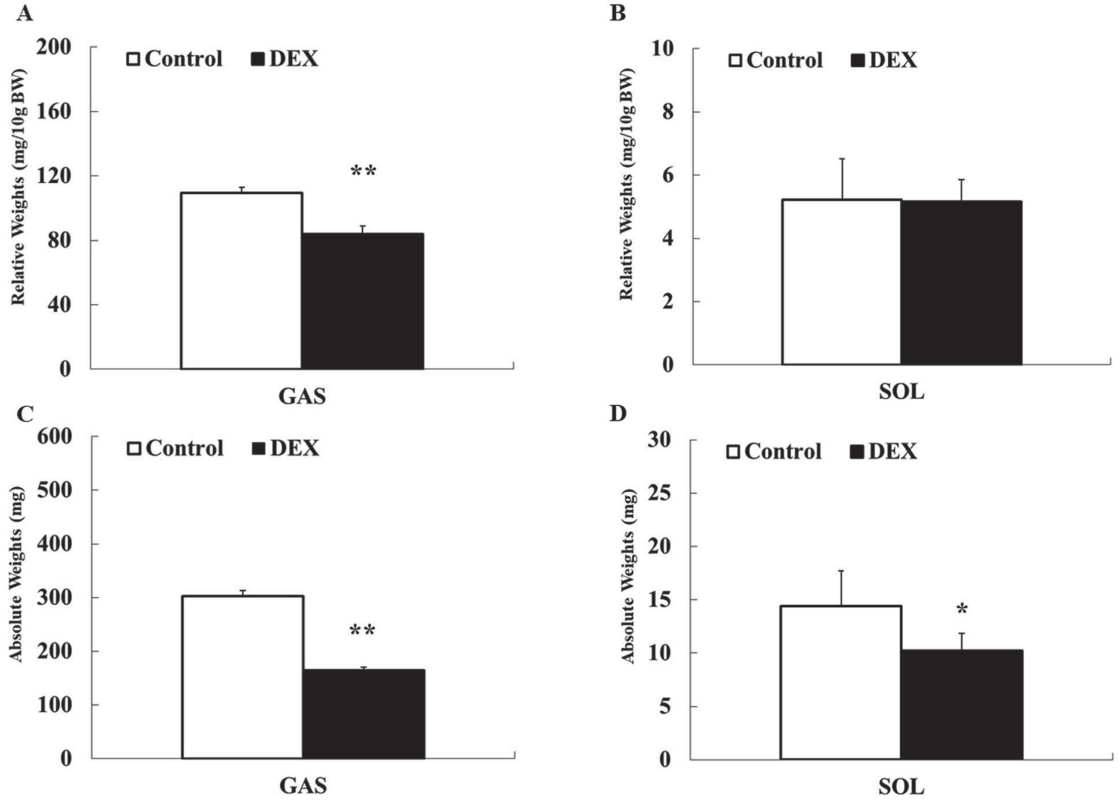

Fig. 1. Absolute and relative skeletal muscle weights after 4 weeks of DEX treatment. (A) GAS weights relative to the body weights on Day 29 (B) SOL weights relative to the body weights on Day 29 (C) Absolute GAS weights (D) Absolute SOL weights Data are shown as mean ± S.D. *: $p<0.05$, **: $p<0.01$; compared with the control group (t-test).

increased in the DEX groups (Fig. 3B). However, there were no changes in the Atrogin-1 mRNA levels in the SOL. The magnitude of the changes in the mRNA levels of MuRF-1 and Atrogin-1 in DEX groups were both greater in GAS than in SOL.

Effects of DEX treatment on the urinary N-titin levels

In the DEX groups, urinary N-titin/CRN levels were markedly increased from Day 14 when compared with the control group (Fig. 4A). The values in the control group remained between 4.8 to 41.9 pmol/mg CRN during the treatment period, while those in the DEX groups

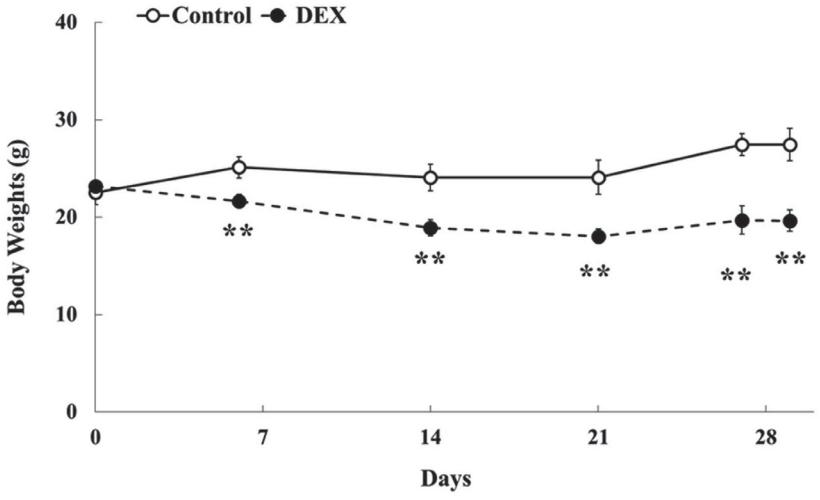

Fig. 2. Body weights during the treatment period. Data are shown as mean ± S.D. **: $p<0.01$; compared with the control group (t-test).

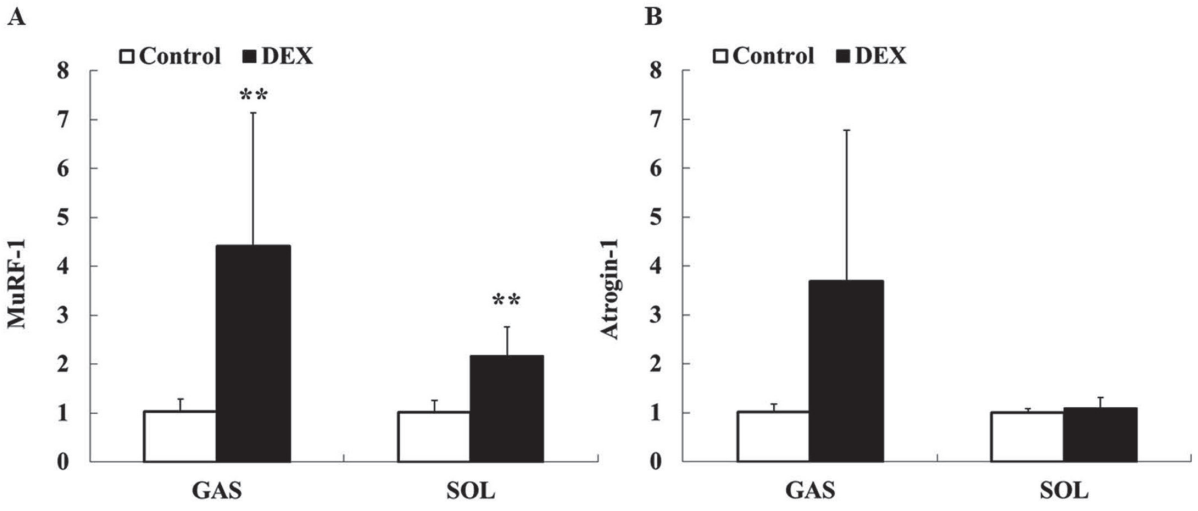

Fig. 3. mRNA levels of the muscle atrophy related genes after 4 weeks of DEX treatment. (A) MuRF-1 mRNA levels in the GAS and SOL (B) Atrogin-1 mRNA levels in the GAS and SOL. Data are shown as mean ± S.D.**: $p<0.01$; compared with the control group (t-test using log values).

reached 280.7 to 712.1 pmol/mg CRN after Day 14. The change was most prominent on Days 14 to Day 21 and was 115 to 125-fold higher than that of the control group and tended to decrease by Day 28/29. Although the blood/urine CRN levels generally reflect total skeletal muscle mass and are known to decrease with muscle atrophy (Baxmann *et al*., 2008), there were no changes in the urinary CRN concentrations in the DEX groups throughout the treatment period (Fig. 4B). Therefore, the increase in urinary N-titin/CRN levels was not due to decreased urinary CRN concentrations associated with decreases in total skeletal muscle mass.

Histopathological examination of the skeletal muscles

Histopathological examination was performed using the HE stained specimens of the skeletal muscles collected after 4 weeks of DEX treatment. In the histopathological examination, no degenerative/necrotic changes were observed in the GAS or SOL (Fig. 5A-D). On the other hands, the mean FCSA of the GAS in DEX group decreased by 42% compared with that in the control group (Fig. 6A). In contrast, there were no DEX-treatment related changes in the mean FCSA of the SOL (Fig. 6B).

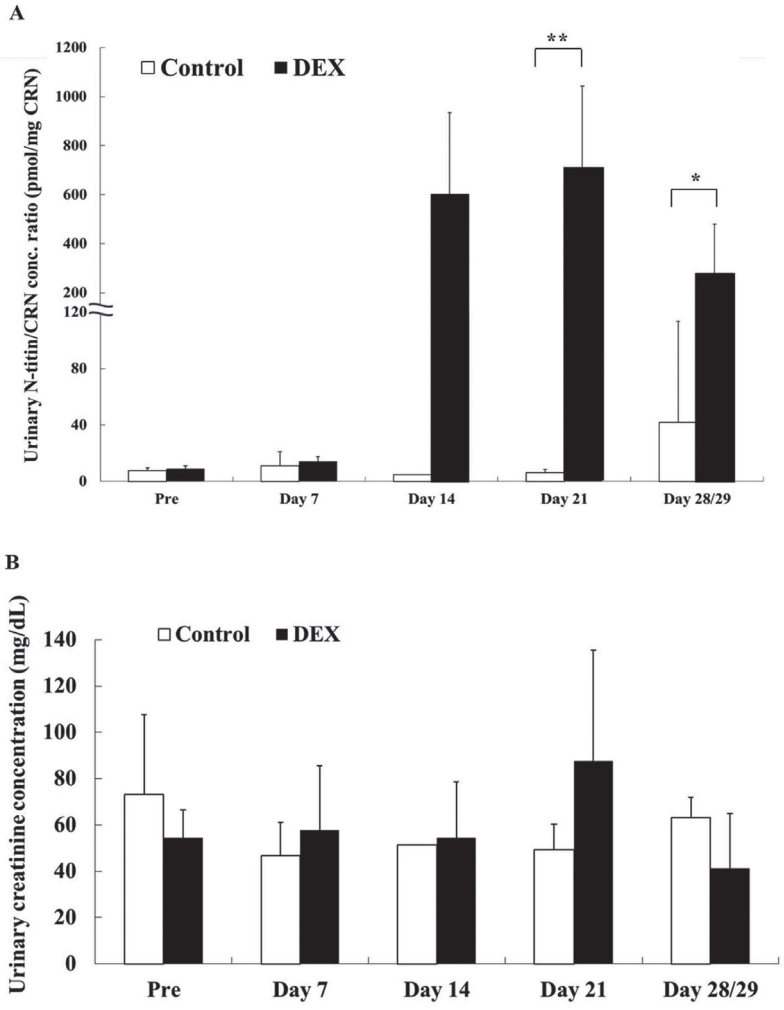

Fig. 4. Urinary N-titin excretion and creatinine concentrations. (A) Urinary N-titin concentration corrected by urinary creatinine concentration (B) Urinary creatinine concentration, As only 2 urine samples were collected from the control group on Day 14, S.D. value could not be calculated and statistical analysis was not performed on Day 14. Data are shown as mean ± S.D. *: $p<0.05$, **: $p<0.01$; compared with the control group (t-test).

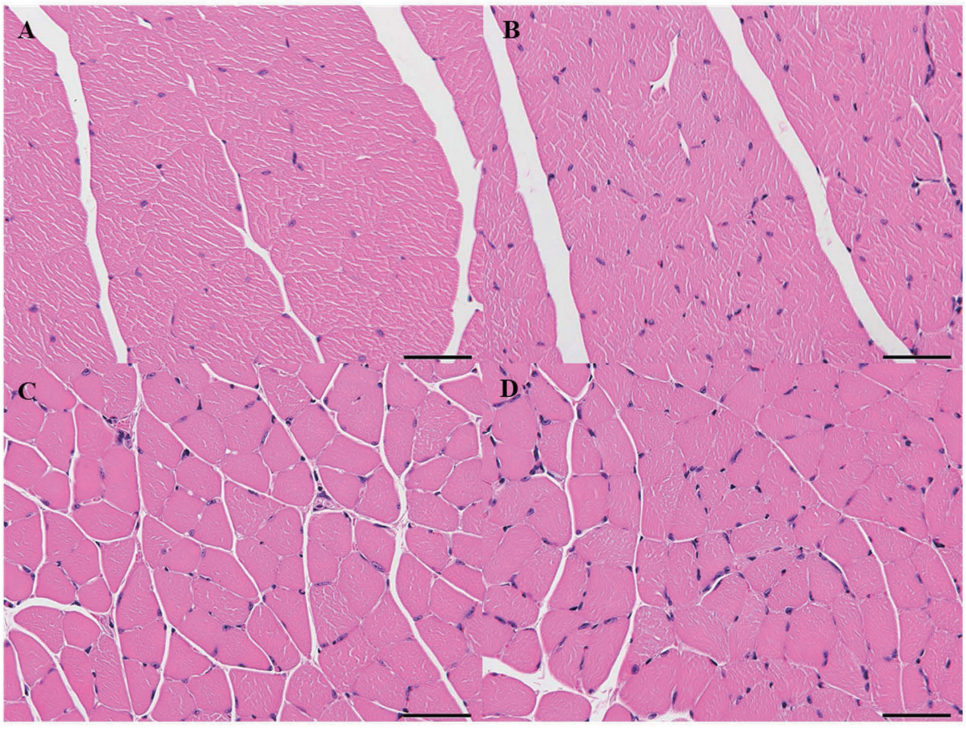

Fig. 5. Histology of the HE-stained skeletal muscles (Cross-section). (A) GAS in the control group (B) GAS in the DEX group (C) SOL in the control group (D) SOL in the DEX group Bar = 50 μm.

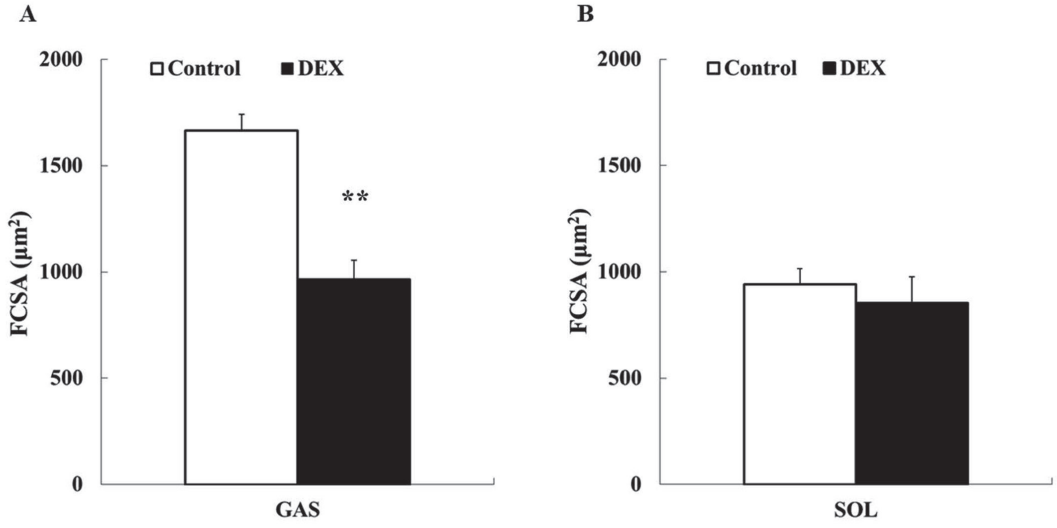

Fig. 6. Morphometric analysis of FCSA in the GAS and SOL. (A) Mean FCSA of the GAS (B) Mean FCSA of the SOL. Data are shown as mean ± S.D. **: $p<0.01$; compared with the control group (t-test).

DISCUSSION

Sarcopenia, characterized by age-related progressive loss of skeletal muscle mass, strength and function, represents a significant health challenge in elderly populations (Doherty, 2003). With global demographics shifting towards an older population, understanding the mechanisms, diagnostic criteria, and potential interventions for sarcopenia has become a critical area of research. There are several biomarkers associated with sarcopenia, such as muscle protein turnover biomarkers, inflammation-mediated and redox biomarkers, growth factors biomarkers, neuromuscular junction biomarker and behavior-mediated biomarkers (Supriya *et al*., 2021). However, due to the complex pathophysiology of sarcopenia, it is difficult to diagnose and monitor sarcopenia using a single biomarker (Cruz-Jentoft *et al*., 2019). Recently, the N-terminal fragment of the Titin (N-titin) has been reported to show high levels in human urine in patients with muscular disorders such as amyotrophic lateral sclerosis and muscular dystrophies (Awano *et al*., 2018; Yamada *et al*., 2021). In addition, urinary N-titin has been reported to increase in patients with GI tract and hepatobiliary pancreatic malignancies and with sarcopenia (Miyoshi *et al*., 2020). Therefore, N-titin is expected to serve as a diagnostic biomarker for these diseases. In this study, we demonstrated the usefulness of N-titin as a biomarker to detect muscle disorders not only in humans but also in animal models using DEX-induced muscle atrophy model mice.

After 4 weeks of DEX treatment to mice, changes indicating muscle atrophy were observed in the GAS. On histopathological examination, no obvious abnormalities were observed in either GAS or SOL in DEX group. However, GAS weights and mean FCSA of the GAS significantly decreased after DEX treatment. Generally, sarcopenia is characterized as the loss of muscle mass, often caused by decreases in the number and size of the muscle fibers, and their function, however, as the disease progresses, degenerative changes are also seen in the skeletal muscles (Chen *et al*., 2022). Since those changes were not observed under the conditions of this study, it was considered that weak, or an early pathological stage of, skeletal muscle atrophy was seen in this study.

In this study, we found that the urinary N-titin/CRN concentration ratio significantly increased in the DEX-induced muscle atrophy mice. The N-titin/CRN concentration ratio increased after 2 weeks of DEX treatment onward. It is known that blood or urine CRN levels are a reflection of total skeletal muscle mass and decrease with muscle atrophy (Baxmann *et al*., 2008). However, there were no changes in the urinary CRN concentrations in DEX group in this study. Therefore, the increases in urinary N-titin/CRN concentration ratio were considered to reflect increased urinary N-titin excretion.

DEX-treatment also induced the muscle atrophy-related genes (MuRF-1 and Atrogin-1) in the GAS. Among several known pathways of proteolysis in muscle, the ubiquitin-proteasome system is known as the representative pathway that promotes the induction of muscle atrophy (Pang *et al*., 2023; Schakman *et al*., 2013). MuRF-1 and Atrogin-1 are known as E3 ubiquitin ligases expressed in the skeletal muscles. It has been reported that the expression levels of MuRF-1 and Atorgin-1 are markedly increased by muscle atrophy and plays an important role in muscle protein degradation (Clavel *et al*., 2006; Gumucio and Mendias, 2013). As for titin, it is known to bind with several proteolysis-associated enzymes, such as matrix metalloproteinase or calpain (Ali *et al*., 2010; Kontrogianni-Konstantopoulos *et al*., 2009). In particular, MuRF-1 is also known to bind to titin (Centner *et al*., 2001). The mechanism for the generation of N-titin as a degradation product of titin is not fully understood, but several mechanisms are involved, including the ubiquitin-proteasome system and MuRF-1 and Atrogin-1 could play a role in N-titin production.

In the DEX groups, the magnitude of the changes in the muscle weights, mean FCSA and mRNA levels of muscle atrophy related genes was greater in GAS than in SOL. Skeletal muscle fibers are classified into fiber types, mainly slow-twitch (type 1) and fast-twitch (type 2) fibers and it is known that certain muscle diseases predominantly affect particular muscle fibers (Talbot and Maves, 2016). GAS is known as fast muscle and is predominantly composed by type 2 (type 2B) fibers, while SOL is known as slow muscle composed by both type 1 and type 2 (type 2A) fibers in mice (Augusto *et al*., 2004). As a pathological feature of sarcopenia, it is known that atrophic changes preferentially occur in type 2 fibers (Lexell *et al*., 1988; Nilwik *et al*., 2013). On the other hand, atrophic changes are predominantly observed in type 1 fibers in disuse muscle atrophy (Ciciliot *et al*., 2013; Gallagher *et al*., 2005). In addition, sensitivity to chemically-induced muscle atrophy often varies depending on the type and proportion of muscle fibers constituting the muscle. Generally, glucocorticoids predominantly cause atrophic changes to the fast muscles, especially type 2B fibers (Pereira and Freire de Carvalho, 2011). In this study, fast muscles of GAS showed more severe atrophic changes than the slow muscle of SOL with DEX treatment, which is consistent with previous reports. In addition, these results were also consistent to the path-

ological feature of sarcopenia. Although the reason for these differences is not fully understood, some reports suggest that they are due to differences in the expression of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) between fast and slow muscles (Ciciliot *et al*., 2013). PGC-1α, known to prevent FoxO3 to promote MuRF-1 and Atrogin-1 expression, is more abundant in type 1 fibers than in type 2 fibers and prevents type 1 muscle in certain diseases becoming atrophy (Sandri *et al*., 2006). Other reports also suggest that the differences in mitochondrial pathways may be partly responsible for the muscle type-specific selectivity to muscle atrophy (Calvani *et al*., 2013).

The urinary N-titin levels reached a peak in Weeks 2 and 3 of DEX treatment and tended to decrease to Week 4. The cause of this is unclear. Since N-titin is a degraded product of titin, urinary excretion levels of N-titin were considered to be more prominent during the progression of muscle atrophy than during the slow progression of the pathological condition. Under the conditions of this study, the progression of muscle atrophy due to DEX may have slowed in Week 4.

In conclusion, we demonstrated that the urinary excretion of N-titin is potentially a novel non-invasive biomarker which can detect skeletal muscle atrophy early in the DEX-induced muscle atrophy model mice. These results indicates that urinary N-titin could serve as an indicator of disease progression and effectiveness in research on sarcopenia. In addition, urinary N-titin has shown to be elevated in patients with other muscle diseases such as muscle dystrophies, therefore, N-titin may become a useful biomarker in animal models of other muscle diseases as well.

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Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Ali, M.A., Cho, W.J., Hudson, B., Kassiri, Z., Granzier, H. and Schulz, R. (2010): Titin is a target of matrix metalloproteinase-2: implications in myocardial ischemia/reperfusion injury. Circulation, **122**, 2039-2047.

Augusto, V., Padovani, C.R. and Campos, G.E. (2004): Skeletal muscle fiber types in C57BL6J mice. J. morphol. Sci., **21**, 89-94.

Awano, H., Matsumoto, M., Nagai, M., Shirakawa, T., Maruyama, N., Iijima, K., Nabeshima, Y.I. and Matsuo, M. (2018): Diagnostic and clinical significance of the titin fragment in urine of Duchenne muscular dystrophy patients. Clin. Chim. Acta, **476**, 111-116.

Baxmann, A.C., Ahmed, M.S., Marques, N.C., Menon, V.B., Pereira, A.B., Kirsztajn, G.M. and Heilberg, I.P. (2008): Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. Clin. J. Am. Soc. Nephrol., **3**, 348-354.

Beaudart, C., McCloskey, E., Bruyère, O., Cesari, M., Rolland, Y., Rizzoli, R., Araujo de Carvalho, I., Amuthavalli Thiyagarajan, J., Bautmans, I., Bertière, M.C., Brandi, M.L., Al-Daghri, N.M., Burlet, N., Cavalier, E., Cerreta, F., Cherubini, A., Fielding, R., Gielen, E., Landi, F., Petermans, J., Reginster, J.Y., Visser, M., Kanis, J. and Cooper, C. (2016): Sarcopenia in daily practice: assessment and management. BMC Geriatr., **16**, 170.

Beckmann, J.S. and Spencer, M. (2008): Calpain 3, the "gatekeeper" of proper sarcomere assembly, turnover and maintenance. Neuromuscul. Disord., **18**, 913-921.

Bottinelli, R. and Reggiani, C. (2000): Human skeletal muscle fibres: molecular and functional diversity. Prog. Biophys. Mol. Biol., **73**, 195-262.

Calvani, R., Joseph, A.M., Adhihetty, P.J., Miccheli, A., Bossola, M., Leeuwenburgh, C., Bernabei, R. and Marzetti, E. (2013): Mitochondrial pathways in sarcopenia of aging and disuse muscle atrophy. Biol. Chem., **394**, 393-414.

Centner, T., Yano, J., Kimura, E., McElhinny, A.S., Pelin, K., Witt, C.C., Bang, M.L., Trombitas, K., Granzier, H., Gregorio, C.C., Sorimachi, H. and Labeit, S. (2001): Identification of muscle specific ring finger proteins as potential regulators of the titin kinase domain. J. Mol. Biol., **306**, 717-726.

Chen, W., You, W., Valencak, T.G. and Shan, T. (2022): Bidirectional roles of skeletal muscle fibro-adipogenic progenitors in homeostasis and disease. Ageing Res. Rev., **80**, 101682.

Ciciliot, S., Rossi, A.C., Dyar, K.A., Blaauw, B. and Schiaffino, S. (2013): Muscle type and fiber type specificity in muscle wasting. Int. J. Biochem. Cell Biol., **45**, 2191-2199.

Clavel, S., Coldefy, A.S., Kurkdjian, E., Salles, J., Margaritis, I. and Derijard, B. (2006): Atrophy-related ubiquitin ligases, atrogin-1 and MuRF1 are up-regulated in aged rat Tibialis Anterior muscle. Mech. Ageing Dev., **127**, 794-801.

Cruz-Jentoft, A.J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A.A., Schneider, S.M., Sieber, C.C., Topinkova, E., Vandewoude, M., Visser, M. and Zamboni, M.; Writing Group for the European Working Group on Sarcopenia in Older People 2 (EWGSOP2), and the Extended Group for EWGSOP2. (2019): Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing, **48**, 16-31.

Doherty, TJ. (2003): Invited review: Aging and sarcopenia. J. Appl. Physiol. (1985). **95**, 1717-1727.

Gallagher, P., Trappe, S., Harber, M., Creer, A., Mazzetti, S., Trappe, T., Alkner, B. and Tesch, P. (2005): Effects of 84-days of bedrest and resistance training on single muscle fibre myosin heavy chain distribution in human vastus lateralis and soleus muscles. Acta Physiol. Scand., **185**, 61-69.

Granzier, H.L. and Labeit, S. (2006): The giant muscle protein titin is an adjustable molecular spring. Exerc. Sport Sci. Rev., **34**, 50-53.

Gumucio, J.P. and Mendias, C.L. (2013): Atrogin-1, MuRF-1, and sarcopenia. Endocrine, **43**, 12-21.

Hackman, P., Vihola, A., Haravuori, H., Marchand, S., Sarparanta, J., De Seze, J., Labeit, S., Witt, C., Peltonen, L., Richard, I. and Udd, B. (2002): Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. Am. J. Hum. Genet., **71**, 492-500.

Herman, D.S., Lam, L., Taylor, M.R., Wang, L., Teekakirikul, P., Christodoulou, D., Conner, L., DePalma, S.R., McDonough, B., Sparks, E., Teodorescu, D.L., Cirino, A.L., Banner, N.R., Pennell, D.J., Graw, S., Merlo, M., Di Lenarda, A., Sinagra, G., Bos, J.M., Ackerman, M.J., Mitchell, R.N., Murry, C.E., Lakdawala, N.K., Ho, C.Y., Barton, P.J., Cook, S.A., Mestroni, L., Seidman, J.G. and Seidman, C.E. (2012): Truncations of titin causing dilated cardiomyopathy. N. Engl. J. Med., **366**, 619-628.

Ibata, N. and Terentjev, E.M. (2021): Why exercise builds muscles: titin mechanosensing controls skeletal muscle growth under load. Biophys. J., **120**, 3649-3663.

Kontrogianni-Konstantopoulos, A., Ackermann, M.A., Bowman, A.L., Yap, S.V. and Bloch, R.J. (2009): Muscle giants: molecular scaffolds in sarcomerogenesis. Physiol. Rev., **89**, 1217-1267.

Labeit, S. and Kolmerer, B. (1995): Titins: giant proteins in charge of muscle ultrastructure and elasticity. Science, **270**, 293-296.

Lexell, J., Taylor, C.C. and Sjöström, M. (1988): What is the cause of the ageing atrophy? Total number, size and proportion of different fiber types studied in whole vastus lateralis muscle from 15- to 83-year-old men. J. Neurol. Sci., **84**, 275-294.

Maruyama, N., Asai, T., Abe, C., Inada, A., Kawauchi, T., Miyashita, K., Maeda, M., Matsuo, M. and Nabeshima, Y.I. (2016): Establishment of a highly sensitive sandwich ELISA for the N-terminal fragment of titin in urine. Sci. Rep., **6**, 39375.

Miyoshi, K., Shimoda, M., Udo, R., Oshiro, Y. and Suzuki, S. (2020): Urinary titin N-terminal fragment concentration is an indicator of preoperative sarcopenia and nutritional status in patients with gastrointestinal tract and hepatobiliary pancreatic malignancies. Nutrition, **79-80**, 110957.

Müller, E., Salcan, S., Bongardt, S., Barbosa, D.M., Krüger, M. and Kötter, S. (2021): E3-ligase knock down revealed differential titin degradation by autopagy and the ubiquitin proteasome system. Sci. Rep., **11**, 21134.

Nilwik, R., Snijders, T., Leenders, M., Groen, B.B., van Kranenburg, J., Verdijk, L.B. and van Loon, L.J. (2013): The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size. Exp. Gerontol., **48**, 492-498.

Pang, X., Zhang, P., Chen, X. and Liu, W. (2023): Ubiquitin-proteasome pathway in skeletal muscle atrophy. Front. Physiol., **14**, 1289537.

Pereira, R.M. and Freire de Carvalho, J. (2011): Glucocorticoid-induced myopathy. Joint Bone Spine, **78**, 41-44.

Rosenberg, I.H. (1989): Summary comments. Am. J. Clin. Nutr., **50**, 1231-1233.

Rouillon, J., Zocevic, A., Leger, T., Garcia, C., Camadro, J.M., Udd, B., Wong, B., Servais, L., Voit, T. and Svinartchouk, F. (2014): Proteomics profiling of urine reveals specific titin fragments as biomarkers of Duchenne muscular dystrophy. Neuromuscul. Disord., **24**, 563-573.

Sandri, M., Lin, J., Handschin, C., Yang, W., Arany, Z.P., Lecker, S.H., Goldberg, A.L. and Spiegelman, B.M. (2006): PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc. Natl. Acad. Sci. USA, **103**, 16260-16265.

Savarese, M., Sarparanta, J., Vihola, A., Udd, B. and Hackman, P. (2016): Increasing Role of Titin Mutations in Neuromuscular Disorders. J. Neuromuscul. Dis., **3**, 293-308.

Schakman, O., Kalista, S., Barbé, C., Loumaye, A. and Thissen, J.P. (2013): Glucocorticoid-induced skeletal muscle atrophy. Int. J. Biochem. Cell Biol., **45**, 2163-2172.

Schiaffino, S. (2010): Fibre types in skeletal muscle: a personal account. Acta Physiol. (Oxf.), **199**, 451-463.

Schiaffino, S. and Reggiani, C. (2011): Fiber types in mammalian skeletal muscles. Physiol. Rev., **91**, 1447-1531.

Supriya, R., Singh, K.P., Gao, Y., Li, F., Dutheil, F. and Baker, J.S. (2021): A Multifactorial Approach for Sarcopenia Assessment: A Literature Review. Biology (Basel), **10**, 1354.

Talbot, J. and Maves, L. (2016): Skeletal muscle fiber type: using insights from muscle developmental biology to dissect targets for susceptibility and resistance to muscle disease. Wiley Interdiscip. Rev. Dev. Biol., **5**, 518-534.

Tanabe, Y., Shimizu, K., Sagayama, H., Fujii, N. and Takahashi, H. (2021): Urinary N-terminal fragment of titin: A surrogate marker of serum creatine kinase activity after exercise-induced severe muscle damage. J. Sports Sci., **39**, 1437-1444.

Wang, B.Y., Hsiao, A.W., Wong, N., Chen, Y.F., Lee, C.W. and Lee, W.Y. (2022): Is dexamethasone-induced muscle atrophy an alternative model for naturally aged sarcopenia model? J. Orthop. Translat., **39**, 12-20.

Xie, W.Q., He, M., Yu, D.J., Wu, Y.X., Wang, X.H., Lv, S., Xiao, W.F. and Li, Y.S. (2021): Mouse models of sarcopenia: classification and evaluation. J. Cachexia Sarcopenia Muscle, **12**, 538-554.

Yamada, S., Hashizume, A., Hijikata, Y., Ito, D., Kishimoto, Y., Iida, M., Koike, H., Hirakawa, A. and Katsuno, M. (2021): Ratio of urinary N-terminal titin fragment to urinary creatinine is a novel biomarker for amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry, **92**, 1072-1079.