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N-terminal fragments of titin in urine as a biomarker for eccentric exercise-induced muscle damage

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Abstract

N-terminal fragments of titin in urine have been proposed as a noninvasive marker of muscle damage, but there are no data on its time course after 96 h. The aims of this study were to investigate the time course of urinary titin N-terminal fragment (UTF) following eccentric exercise and the subsequent correlations between UTF and other muscle damage indices. Seventeen healthy young men performed 30 maximal isokinetic eccentric exercises at the elbow flexors. Muscle soreness (SOR), range of motion (ROM), maximal voluntary isometric contraction (MVIC), creatine kinase (CK), and UTF were sampled before, immediately after, and 24–144 h after exercise. The changes and correlations were analyzed with one-way ANOVA and Spearman’s rank correlation analysis. All the measured parameters showed significant differences from their pre-exercise values at 24 h or later after exercise (P < 0.05). UTF showed significant correlation with MVIC (r ≤ −0.485) at 24 h and later, with SOR (r ≥ 0.549) at 48 h and later, with ROM (r ≤ −0.485) at 48 h and later, and with CK (r ≥ 0.647) at all time points. UTF reached peak value at 96 h after the exercise and had not recovered completely at 144 h. Because UTF was highly correlated with the other muscle damage indices, especially CK, throughout the measurement period, it may be as useful an indicator of muscle damage as CK obtained from blood.

Keywords: biomarker, creatine kinase, delayed-onset muscle soreness, eccentric exercise, noninvasive sampling, urine

Introduction

When muscles are exposed to strenuous eccentric contraction, this induces inflammation and damage in the myofibrils, known as exercise-induced muscle damage (EIMD), which results in soreness during movement or compression and reduced muscle strength and range of motion (ROM)1. The muscle soreness (SOR) and restriction of movement can have a negative impact not only on high-intense activity, such as in sports and resistance training, but also on light activity, such as daily work and recreational sports2. Various biochemical markers are used to measure EIMD, especially levels of creatine kinase (CK), myoglobin (Mb), and aldolase (ALD), which have been used in many previous studies3. Recently, Kanda et al.4 identified that it may be possible to measure EIMD using biochemicals released into urine; this has the benefit that the physical and psychological burden for the subject is less than with blood sampling, and any researcher should be able to collect and analyze samples easily. Kanda et al.4 identified N-terminal fragments of titin, one of the proteins that form myofibers, in urine after subjects performed 100 calf-raise exercises that included eccentric contraction. The authors reported that the time course of urinary titin N-terminal fragment (UTF) over 96 hours showed high correlation with other muscle damage indices, including the rate of decline in ROM and maximum isometric contraction. At 96 h after exercise, the UTF level was significantly higher than that at baseline (P < 0.01)4. UTF levels also showed high correlation with CK (r = 0.98), Mb (r = 0.98), ALD (r = 0.98), and SOR (r = 0.91) at 48 h after exercise, and with the rate of decline of ROM (r = −0.76) at 72 h after exercise4. This has been the only study to investigate the time course of UTF levels and the relationship with other muscle damage indices. Thus, the time course of UTF after 96 h and correlations other than those described remain unknown.

Studies of changes in CK levels over time have reported that, although the peak value of CK varied according to the degree of EIMD and the intensity of the exercise used as the fatigue task, the peak generally occurred at 72–120 h after the task5,6. This suggests that it may be necessary to measure UTF levels for 120 h or more to identify the
peak value and the values that correlate highly with CK. Correlations between CK and other muscle damage indices have been reported after eccentric exercise at the elbow flexor, including moderate negative correlations between peak CK and the rate of decline of muscle strength immediately after exercise ($r = -0.473$), and at 24 h ($r = -0.442$), 48 h ($r = -0.533$), 72 h ($r = -0.608$), and 96 h ($r = -0.626$), and weak positive correlations between peak CK and SOR at 48 h ($r = 0.287$), 72 h ($r = 0.334$), and 96 h ($r = 0.320$). Given that UTF has shown strong correlation with CK, it is likely that it would be weakly or moderately correlated with other muscle damage indicators.

The aims of this study were to clarify the timing of the peak level of UTF by tracking it for 144 h after exercise and to characterize UTF by evaluating its correlations with other muscle damage indices. We hypothesized that, like CK, UTF would peak between 72 and 120 h, and that there would be a weak or moderate correlation with other muscle damage indices at times other than 48 and 72 h.

**Materials and Methods**

**Subjects and study design.** Subjects included 17 healthy young men (age, 23.2 ± 1.4 years; height, 172.3 ± 6.1 cm; weight, 69.6 ± 8.5 kg; body fat, 17.1 ± 3.9 %) with no muscle injury of the upper limbs. All provided informed consent to participate in the study. The sample size was calculated on the basis of an α level of 0.05, power (1 – β) of 0.8, and an effect size of 0.6, given that a previous study reported that UTF levels were highly correlated with SOR ($r = 0.91$), the rate of decline of ROM ($r = -0.76$), and CK ($r = 0.98$). The protocol of the present study was conducted in accordance with the Declaration of Helsinki and was approved by Waseda University Ethics Committee (2017-162). We frequently reminded the subjects to abstain from performing any unaccustomed or strenuous physical activities, and to refrain from taking any anti-inflammatory medication during the experiment. We measured body weight and body fat using a multifrequency impedance body composition analyzer (InBody720, Inbody Japan Inc., Tokyo, Japan), and body height using a wall-mounted stadiometer (SECA213, Yamaguchi S, et al.).

**Eccentric exercise procedure.** A previous study did not find any significant difference between the dominant and nondominant arms in the response to muscle damage resulting from eccentric exercise. In the present study, therefore, subjects used only their dominant arm, the one more accustomed to exerting muscle strength, on the first day. The subject sat on the chair of an isokinetic dynamometer (Biodex System 3, Biodex Medical Systems, Shirley, NY, USA), and the shoulder joint angle was set at 45° flexion with 0° abduction. The subject was asked to supinate the forearm and to grasp the attachment connected to the lever arm of the dynamometer. The subject then performed three sets of 10 repetitions of eccentric exercise of the elbow flexors using an isokinetic dynamometer, with a 60-s rest between sets. We forcibly extended the elbow joint from flexion (90°, starting position) to extension (180°, end position) in 3 s. To ensure that the subject performed only eccentric exercise actions, we ensured using the dynamometer that flexion was conducted passively from the end position to the start position over 3 s. To create an environment in which the subject performed maximal exercise, we encouraged him verbally during the exercise, and we calculated the peak torque and work of each contraction using Biode software.

**Maximal voluntary isometric contraction torque.** We measured MVIC torque of the elbow flexors using the same isokinetic dynamometer as for the eccentric exercise, with the subject in the same position. We recorded the positions of the dynamometer and each attachment on the first day of the experiment and measured each subject’s MVIC values at the same position on subsequent days. The subject performed three 5-s MVICs at an elbow joint angle of 110°, with a 60-s rest between contractions. The highest of the three measurements of torque was used in further analysis. In addition, MVIC was measured at the end of all measurement items to occlude the influence of MVIC on the other measurements.

**Range of motion.** To determine elbow joint angle, the center of the acromion, lateral epicondyle, and ulnar styloid, were labelled with a semi-permanent ink marker and the arm was photographed with relaxed and flexed elbow joint angles. Using ImageJ (version 1.39, NIH, USA), we measured the angle between the line connecting the center of the acromion and the lateral epicondyle and the line connecting the lateral epicondylitis and the ulnar styloid. We subtracted the relaxed angle from the flexed angle and used this as the ROM of the elbow joint.

**Muscle soreness.** To evaluate SOR, subjects actively moved their arms and reported the levels of extension and flexion pain using a 100-mm visual analogue scale (VAS) in which 0 indicated no pain and 100 represented extreme pain. We asked each subject to sit on a chair holding his shoulder joint at a flexion angle of 90°, and to mark the level of perceived soreness on the VAS.

**Blood sample analyses.** We took blood samples (approximately 5 ml) from the antecubital vein by a standard venipuncture technique using serum separation tubes. The serum separation tubes were left to clot at room tem-
perature for 30 min and the serum was then centrifuged at 3,000 × g for 10 min. The serum samples were stored at −80 °C for later analyses. We estimated serum CK activity using an automated analyzer (Model 747-400, Hitachi, Tokyo, Japan).

**Determination of biomarkers in urine.** Approximately 3-ml samples of urine were collected and the level of UTF was measured by an enzyme-linked immunosorbent assay (ELISA) system using a kit (Titin N-terminal Fragment Assay Kit; Immuno-Biological Laboratories Co. Ltd., Fujioka, Japan), as described in previous studies\(^\text{13,14}\). Samples were stored at −20 °C for later analyses. We diluted thawed urine samples with dilution solution to obtain concentrations of 1:5 to 1:350, so that the diluted samples were in the linear detection range. Diluted samples and standard solutions were added to antibody-coated wells on microplates, which were incubated at 37 °C for 60 min. After washing the microplates four times with wash buffer, labeled antibodies were added to the wells and the microplates were incubated at 37 °C for 30 min. After washing five times, we incubated the sample with tetramethylbenzidine solution at room temperature for 30 min. For the final ELISA procedure, we added stop solution to each well to stop the reaction and measured the absorbance at a main wavelength of 450 nm and a sub-wavelength of 650 nm with a microplate reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA). We calculated UTF levels from a linear regression model, and estimated the urinary creatinine using an automated analyzer (Bio Majesty JCA-BM8060, JEOL, Tokyo, Japan). We normalized UTF values relative to urine creatinine\(^\text{26}\).

**Statistical analysis.** Values are presented as means ± SE. Before correlation analysis, we used the Shapiro–Wilk test to check that the data were normally distributed. Spearman’s rank correlation analysis was applied to evaluate correlations between UTF and other muscle damage indices at each time point. In addition, we also evaluated correlations between the peak value of UTF for all the time periods and the other muscle damage indices; this avoided potential problems such as with the divergence of the time course. Because the Shapiro–Wilk test indicated the UTF and CK data were not normally distributed, we applied a logarithmic transformation (log\(_10\)) before ANOVA\(^\text{15}\). One-way ANOVA was used to evaluate the changes in SOR, MVIC, ROM, UTF, and CK after eccentric exercise, as well as any change in the work volume between sets during eccentric exercise. Post hoc tests using Bonferroni’s method were used to identify significant differences between times and sets. The statistical significance level was set at \(P < 0.05\). Statistical analyses were performed using Predictive Analytics Software (PASW) version 25.0 for Windows (SPSS Japan Inc., Tokyo, Japan).

**Results**

**Work volume during eccentric exercise.** Work volume during the first, second, and third sets of eccentric exercise was 532.8 ± 95.6 J, 429.5 ± 89.5 J, and 359.0 ± 97.7 J, respectively. Compared to the first set, the work volumes of the second and third sets were significantly lower (both \(P < 0.001\)), and the work volume of the third set was significantly lower than that of the second (\(P < 0.001\)).

**Changes in each measure after eccentric exercise.** Table 1 and Fig. 1 summarize the changes in each measure immediately after eccentric exercise (0 h) and at 24, 48, 72, 96, and 120 h later. MVIC decreased significantly at 0 h and gradually increased to a non-significant level by 96 h. SOR increased significantly at 0 h, was at its peak at 48 h, and then decreased to a non-significant level at 144 h. ROM decreased significantly at 0 h and gradually returned to a non-significant level by 120 h. CK increased significantly at 24 h, reaching a peak at 96 h and then declining, though it remained significantly high at 144 h. UTF followed a similar pattern, with a significant increase at 24 h, a peak at 96 h, and a subsequent decrease up to 144 h, at which time it was still significantly high.

**Correlations between UTF and other muscle damage indices.** Table 2 presents the correlation coefficients between UTF and the other markers at each time point after eccentric exercise. There were strong correlations between UTF and CK values throughout the whole experimental period, with very strong correlations \((r = 0.868–0.973)\) from 24 h to 144 h. UTF and MVIC showed a moderate negative correlation at 24 h and a strong negative correlation \((r = -0.603 to -0.681)\) from 48 h to 144 h. UTF and SOR showed moderate to strong correlations \((r = 0.509–0.724)\) from 48 h to 144 h. UTF and ROM showed strong negative correlations \((r = -0.607 to -0.781)\) from 48 h to 144 h.

**Correlations between peak UTF and other muscle damage indices.** Peak UTF showed a moderate negative correlation with peak MVIC \((r = -0.596, P < 0.05)\), a strong negative correlation with peak ROM \((r = -0.738, P < 0.01)\), a strong positive correlation with peak SOR \((r = 0.626, P < 0.01)\), and a very strong positive correlation with peak CK \((r = 0.966, P < 0.01)\) (Fig. 2).

**Discussion**

It has recently been proposed that levels of N-terminal fragments of titin in urine could be used as a noninvasive biochemical marker for EIMD\(^\text{4}\). In the present study, we investigated the time course of UTF at 96–144 h after eccentric exercise, a later time period than that studied previously by Kanda et al.\(^\text{20}\). We also established the time at which UTF reached its peak value, and we evaluated cor-
relations between UTF and other muscle damage indices throughout the 144 h period. The findings of this study showed that the peak value of UTF occurred at 96 h after eccentric exercise and that UTF had not returned to its pre-exercise value even after 144 h. We also demonstrated moderate to strong correlations between UTF and MVIC, SOR, and ROM from 24 or 48 h after the exercise, and very strong correlations with levels of CK throughout the entire experimental period. Table 3 shows the actual UTF values in each subject in the present study.

The work volume of eccentric exercise performed in this study decreased significantly from the first set to the third set. These results were similar to those of a previous study in which eccentric exercise was performed by subjects who did not exercise daily\(^9,16\). The significant changes in MVIC, SOR, and ROM after eccentric exercise were also consistent with those reported previously\(^9,17,18\). In one of the previous studies\(^9\), MVIC, ROM, and SOR

### Table 1. Changes in muscle damage markers for 144 h following eccentric exercise

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
<th>144 h</th>
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</thead>
<tbody>
<tr>
<td>MVIC (Nm)</td>
<td>56.1 ± 1.8</td>
<td>38.7 ± 1.9</td>
<td>39.0 ± 2.5</td>
<td>41.8 ± 2.8</td>
<td>44.3 ± 2.8</td>
<td>48.2 ± 3.1</td>
<td>50.0 ± 3.0</td>
<td>51.0 ± 2.9</td>
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<tr>
<td>SOR (mm)</td>
<td>0.0 ± 0.0</td>
<td>13.7 ± 3.7</td>
<td>44.5 ± 3.7</td>
<td>50.5 ± 4.5</td>
<td>44.3 ± 5.5</td>
<td>31.9 ± 5.2</td>
<td>17.5 ± 4.1</td>
<td>9.9 ± 3.6</td>
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<td>ROM (°)</td>
<td>120.4 ± 1.9</td>
<td>105.3 ± 1.9</td>
<td>109.4 ± 1.5</td>
<td>107.0 ± 2.4</td>
<td>107.1 ± 3.2</td>
<td>110.7 ± 2.2</td>
<td>113.8 ± 1.7</td>
<td>115.2 ± 2.0</td>
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<tr>
<td>CK (IU/L)</td>
<td>144.2 ± 16.8</td>
<td>152.8 ± 17.1</td>
<td>260.3 ± 33.8</td>
<td>636.1 ± 161.8</td>
<td>2164.3 ± 736.1</td>
<td>3382.5 ± 1138.2</td>
<td>2776.9 ± 986.0</td>
<td>1746.5 ± 548.3</td>
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</tr>
<tr>
<td>UTF (pmol/mg/dl)</td>
<td>2.3 ± 0.4</td>
<td>2.6 ± 0.6</td>
<td>4.9 ± 0.8</td>
<td>15.2 ± 4.5</td>
<td>54.3 ± 17.4</td>
<td>69.0 ± 25.7</td>
<td>59.5 ± 17.7</td>
<td>40.0 ± 11.3</td>
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</table>

Data are presented as mean ± SE. Pre, before exercise; Post, immediately after exercise; MVIC, maximum isometric voluntary contraction; SOR, muscle soreness; ROM, range of motion; CK, creatine kinase; UTF, urinary titin N-terminal fragment. * \(P < 0.05\), ** \(P < 0.01\) relative to the pre-exercise value.

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**Fig. 1** Time course of each measurement items for 144 hours after eccentric exercise at elbow flexor. Maximum isometric voluntary contraction (MVIC), range of motion (ROM), muscle soreness (SOR), creatine kinase (CK), urinary titin N-terminal fragment (UTF), before (b) and after (a).
Table 2. Spearman’s correlation coefficients between UTF and other muscle damage markers at each time point after eccentric exercise

<table>
<thead>
<tr>
<th></th>
<th>Pre</th>
<th>Post</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
<th>120 h</th>
<th>144 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>UTF (pmol/mg/dl)</td>
<td></td>
<td></td>
<td>0.056</td>
<td>-0.485 *</td>
<td>-0.610 **</td>
<td>-0.603 **</td>
<td>-0.679 **</td>
<td>-0.681 **</td>
</tr>
<tr>
<td>MVIC (%)</td>
<td>-</td>
<td></td>
<td>0.112</td>
<td>-0.047</td>
<td>0.549 *</td>
<td>0.633 **</td>
<td>0.704 **</td>
<td>0.630 **</td>
</tr>
<tr>
<td>SOR (mm)</td>
<td>-</td>
<td></td>
<td>0.221</td>
<td>-0.336</td>
<td>-0.696 **</td>
<td>-0.747 **</td>
<td>-0.772 **</td>
<td>-0.781 **</td>
</tr>
<tr>
<td>ROM (%)</td>
<td>0.675 **</td>
<td>0.647 **</td>
<td>0.917 **</td>
<td>0.868 **</td>
<td>0.973 **</td>
<td>0.966 **</td>
<td>0.939 **</td>
<td>0.961 **</td>
</tr>
<tr>
<td>CK (IU/L)</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

UTF, urinary titin N-terminal fragment; Pre, before exercise; Post, immediately after exercise; MVIC, maximum isometric voluntary contraction; SOR, muscle soreness; ROM, range of motion; CK, creatine kinase. * P < 0.05, ** P < 0.01.

Fig. 2  Correlation between peak value of UTF and other muscle indices. The relationship between peak urinary titin N-terminal fragment (UTF) and peak maximum isometric voluntary contraction (MVIC, a), peak range of motion (ROM, b), peak muscle soreness (SOR, c) and peak creatine kinase (CK, d). We showed significant correlation following; * P < 0.05, ** P < 0.01.
were tracked until 120 h after 30 eccentric contractions at the elbow flexor using a dumbbell. It was reported that ROM was reduced by about 20% immediately after exercise, and then recovered to about 95% of the pre-exercise value; MVIC decreased to about 65% of the pre-exercise value immediately after exercise and recovered to only about 80% of the pre-value even at 120 h; and SOR was scored as 40 on the VAS at 48 h, and remained at a score of about 20 even after 120 h\(^9\). In the present study, the ROM decreased to about 13% of the pre-exercise value immediately after exercise, and recovered to about 95% of the pre-exercise value at 120 h. MVIC decreased to about 68% of the pre-exercise value immediately after exercise and did not recover completely, even at 120 h. SOR was scored as about 50 on the VAS at 48 h, with some pain remaining at 120 h. These results were similar to those of the previous studies, indicating that the exercise task in the present study resulted in EIMD similar to that of the previous studies.

In the present study, UTF was significantly higher than its pre-exercise value at 24 h after exercise. This was earlier than in the study by Kanda et al.\(^4\), in which UTF reached a significantly higher value than the pre-exercise level at 96 h after exercise. The difference in results may have been because elbow flexors were used in the present study, whereas plantar flexors were used in the previous study. A different study\(^9\) that compared the optimal angle, ROM, MVIC, SOR, muscle circumference, plasma CK and myoglobin levels, and ultrasound echo intensity between elbow flexors and knee extensors after an extension contraction exercise reported that the knee extensors showed significantly lower values for all the measured items compared to the flexor muscles. Chen et al.\(^20\), who compared sensitivity to EIMD among nine muscles (elbow flexors, elbow extensors, pectoralis, knee extensors, knee flexors, plantar flexors, latissimus, abdomenis, and erector spinae), reported that the elbow flexor was the muscle most susceptible to pain, whereas the plantar flexor muscle came sixth in the list. Another previous study\(^21\) reported that the degree of EIMD was influenced by the frequency of exposure to eccentric contraction on a daily basis; thus, the EIMD of lower limb muscles, which are used on a daily basis for eccentric loads such as running, jumping, and walking down stairs, show significantly less EIMD compared to the upper limbs. Kanda et al.’s study\(^4\) used the plantar flexor muscles, which are less likely to develop EIMD, so it may have taken time for the UTF to reach a significantly increased level.

In the present study, UTF reached its peak value at 96 h and its time course was very similar to that of CK. There are two mechanisms underlying the onset of EIMD: mechanical stress due to the muscle being elongated forcibly by eccentric contraction, and metabolic stress due to protease produced after eccentric contraction\(^1\). High e-

<table>
<thead>
<tr>
<th>Subject</th>
<th>Baseline UTF (pmol/mg/dl)</th>
<th>Peak UTF (pmol/mg/dl)</th>
<th>Baseline CK (IU/L)</th>
<th>Peak CK (IU/L)</th>
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</thead>
<tbody>
<tr>
<td>A</td>
<td>1.1</td>
<td>449.7</td>
<td>43</td>
<td>16459</td>
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<td>B</td>
<td>1.3</td>
<td>221.3</td>
<td>106</td>
<td>13243</td>
</tr>
<tr>
<td>C</td>
<td>1.7</td>
<td>151.4</td>
<td>117</td>
<td>5778</td>
</tr>
<tr>
<td>D</td>
<td>7.6</td>
<td>138.0</td>
<td>183</td>
<td>3909</td>
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<tr>
<td>E</td>
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<td>85.7</td>
<td>170</td>
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</tr>
<tr>
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<td>119</td>
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<td>G</td>
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<td>161</td>
<td>2359</td>
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<tr>
<td>H</td>
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<td>47.8</td>
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<tr>
<td>I</td>
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<td>99</td>
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<tr>
<td>J</td>
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<td>14.5</td>
<td>97</td>
<td>378</td>
</tr>
<tr>
<td>K</td>
<td>4.9</td>
<td>12.7</td>
<td>248</td>
<td>394</td>
</tr>
<tr>
<td>L</td>
<td>1.7</td>
<td>10.6</td>
<td>105</td>
<td>369</td>
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<tr>
<td>M</td>
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<td>Q</td>
<td>1.3</td>
<td>4.0</td>
<td>105</td>
<td>168</td>
</tr>
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</table>

Mean ± SD  2.3 ± 1.6  77.4 ± 111.4  137.9 ± 53.9  3414.1 ± 4692.6

UTF, urinary titin N-terminal fragment; CK, creatine kinase. Data are presented as mean ± SD.
centric tension is a burden on each muscle fiber, resulting in the destruction of muscle cell membranes and myofibrils\(^22\). This destruction has been confirmed immediately after exercise, but proteases subsequently cause further degradation of the myofibrils\(^23\). The protease calpain 3 is mainly expressed in skeletal muscle and exists in a connecting state with titin\(^24\). It has been reported that calpain 3 specifically cuts or degrades titin, and this phenomenon has been confirmed to induce muscle damage after eccentric contraction\(^25\). UTF is thought that titin was degraded by the action of this calpain that then leaked into the urine. However, UTF is not the only marker to peak after 96 h. Kanda et al.\(^26\) reported that CK, lactate dehydrogenase, alanine aminotransferase, aspartate transferase, and aldolase also showed peaks 96 h after EIMD. Even though the functions, production, and degradation processes differ between the various enzymes and proteins regarded as biomarkers for evaluating EIMD, the mechanisms relating to their release from muscle cells may be similar. However, as yet there is no direct evidence to support this.

Although Kanda et al.’s study showed a strong positive correlation between UTF and SOR (48 h after exercise), they found no correlation with ROM\(^4\). Conversely, they found no correlation between UTF and SOR at 72 h, whereas there was a strong negative correlation with ROM\(^4\). The results of the present study were consistent with some of the results of Kanda et al.’s study, but there were also some differences, such as the time points at which correlations were found and differences in the strengths of the correlations. This may have been due to differences in sample size and statistical analysis methods. In the present study, we calculated the required sample size before the experiment and confirmed that a sample of 17 subjects was appropriate. Kanda et al.’s study included only nine subjects, which may not have been sufficient for investigating EIMD correlations, increasing the likelihood of type II errors (i.e., failing to detect relationships because of the small sample size). Also, Pearson’s correlation coefficients were used in the previous study; however, biomarkers are not suitable for parametric testing because their values are often not normally distributed\(^11,26\). Kim et al. reported that Spearman’s rank correlation analysis produced higher correlation coefficients than Pearson’s correlation analysis for relationships between CK and muscle strength and muscle pain. Thus, the differences in the results of the previous and present studies may be due to the sample size and the selected statistical methods. The results of the present study, based on a larger sample, may be more reliable.

Kim et al.\(^7\) investigated the correlations between peak CK and MVIC and SOR after 50 eccentric contractions and found a strong negative correlation between CK and MVIC at 96 h, and a weak positive correlation between CK and SOR at 72 h. The correlation coefficients in the present study were higher for almost all the measurement items than those of Kim et al. The blood level of CK is thought to increase following the onset of EIMD due to damage to muscle cell membranes or deviation into the blood because of cell permeability\(^3\). Thus, CK, which is used to evaluate muscle damage, may actually be an evaluation of muscle cell membrane damage; and it may not be possible to use it to evaluate myofibrillar damage related to muscle contraction\(^3\). UTF could be a more sensitive biomarker of EIMD than CK because it can directly measure the degree of damage to titin filaments related to muscle contraction\(^27\). This may explain why the correlation coefficients of the present study, based on UTF levels, were higher than those of the previous study based on CK. However, another study that investigated the correlations between CK and other muscle damage indices after 12 or 24 h eccentric contractions at maximum effort, reported a weak correlation between CK and muscle strength, and no correlation between CK and SOR or ROM\(^8\). Thus, there is not yet consistency regarding the presence or absence of associations between biomarkers and other muscle damage indicators. This may be because there is a large variation in the degree of EIMD due to characteristics of the subjects. For example, the degree of EIMD is influenced by gender\(^29\), age\(^30\), and daily exercise habits\(^31\). In particular, exercise experience has been reported to promote adaptation to EIMD (known as the repeated bout effect [RBE]), which can result in large differences in the degree of EIMD experienced\(^31\). These observations suggest it is necessary in future studies to investigate the correlations between UTF and other muscle damage indices for women, elderly people, and subjects with different exercise experiences.

A previous study evaluated EIMD using magnetic resonance imaging\(^22\), ultrasound diagnostic imaging\(^33\), and optimal angle\(^34\). Previous studies reported high correlations between T2 values and CK\(^35,36\); it is conceivable that UTF may also correlate with T2 values. It is understandable why UTF levels would correlate with muscle strength and ROM, which are directly affected by the titin filament, but it is less clear why there was a correlation with SOR. Mizumura et al.\(^37\) reported that there was no direct relationship between SOR and damage to muscle fibers, so further studies are needed to investigate the reason for the strong correlation between SOR and UTF shown in the present study. Also, as shown in Table 3, a large difference in baseline and peak UTF levels of each subject was confirmed in the present study. Regarding the baseline, Maruyama et al. (2016)\(^14\) reported that the daily level of UTF in healthy subjects was 1.47 to 7.14 pmol/mg/dl; thus, the baseline of the present study (0.9 to 7.6 pmol/mg/dl) is considered to be at the daily level. However, this wouldn’t apply to the case in view of the peak values after eccentric exercise. Previous studies have reported that gender, age, genetic factors, ethnicity, contraction duration, effort, intensity, number of contractions, muscle length, selected muscle groups, frequency of daily
exposure to eccentric contractions, and training frequency can cause differences in EIMD symptoms among individuals.\(^ {2,1,3} \) In the present study, because all factors except for genetics and frequency of exposure to eccentric contractions on a daily basis are the same, the cause of the difference in UTF level may be this one factor or both. The subjects in this study had limited resistance training for six months, but had no control on the frequency of exposure to eccentric contractions on a daily basis. There are also reports that genetic differences among individuals affect the degree of EIMD\(^ {39} \). Therefore, in the future, it will be possible to find out the factors that affect UTF by examining the frequency of eccentric exercise exposed daily and the genetics of the individual subjects.

In conclusion, this study investigated UTF concentration before and after eccentric exercise and evaluated correlations between UTF and other muscle damage indices. This study showed that UTF is highly correlated with muscle damage indices used in many previous studies, UTF reached a peak 96 h after eccentric exercise, and had not returned to its pre-exercise value even at 144 h after the exercise.

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Conflict of Interests

The authors declare that there is no conflict of interests. The authors declare that the results of this study are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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