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CLINICAL RESEARCH ARTICLE

Assessment of catabolic state in infants with the use of urinary titin N-fragment

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BACKGROUND: Urinary titin N-fragment levels have been used to assess the catabolic state, and we used this biomarker to evaluate the catabolic state of infants.
METHODS: We retrospectively measured urinary titin N-fragment levels of urinary samples. The primary outcome was its changes according to postmenstrual age. The secondary outcomes included differences between gestational age, longitudinal change after birth, influence on growth, and relationship with blood tests.
RESULTS: This study included 219 patients with 414 measurements. Urinary titin N-fragment exponentially declined with postmenstrual age. These values were 12.5 (7.1–19.6), 8.1 (5.1–13.0), 12.8 (6.0–21.3), 26.4 (16.4–52.0), and 81.9 (63.3–106.4) pmol/mg creatinine in full, late, moderate, very, and extremely preterm infants, respectively ($p < 0.01$). After birth, urinary levels of titin N-fragment exponentially declined, and the maximum level within a week was associated with the time to return to birth weight in preterm infants ($\rho = 0.39$, $p < 0.01$). This was correlated with creatine kinase in full-term infants ($\rho = 0.58$, $p < 0.01$) and with blood urea nitrogen in preterm infants ($\rho = 0.50$, $p < 0.01$).
CONCLUSIONS: The catabolic state was increased during the early course of the postmenstrual age and early preterm infants.

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IMPACT:

- Catabolic state in infants, especially in preterm infants, was expected to be increased, but no study has clearly verified this.
- In this retrospective study of 219 patients with 414 urinary titin measurements, the catabolic state was exponentially elevated during the early postmenstrual age.
- The use of the urinary titin N-fragment clarified catabolic state was prominently increased in very and extremely preterm infants.

INTRODUCTION

Global neonatal mortality has declined by 51%; from 5 million in 1990 to 2.5 million in 2017.[1] Advances in perinatal care have also decreased the mortality rates of preterm infants despite its high mortality risk.[2] However, there has not been an equivalent improvement in growth restriction.[3,4] Particularly, restriction of extrauterine growth and delay of the subsequent developmental are common among preterm infants.[5,6] Preterm infants, found in ~10% of births, have been increasing worldwide due to advanced maternal age[7] and multiple gestations assisted by reproductive technology.[8] Hence, the growth restriction of preterm infants is an important issue to be resolved globally.[9]

One of the etiologies of growth restriction is increased protein catabolism during the neonatal period.[10] After the termination of the transplacental nutrient supply, catabolic hormones cause muscle breakdown and gluconeogenesis until sufficient feeding is established.[11] Particularly, very preterm infants (born before 32 weeks gestation) have a slow growth rate during the neonatal period due to various factors including increased catabolism.[12] Nutritional management is essential for the prevention of growth restriction after birth,[13] and early aggressive nutrition has been proposed for the provision of nutrition equivalent to that in the intrauterine environment.[14,15] However, it is difficult to determine the sufficient amount of

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nutrition, because there is no established biomarker to monitor the catabolic state in infants.[16]

Recently, the urinary titin N-fragment has been developed as a biomarker of the catabolic state and the subsequent muscle breakdown.[17] This biomarker is the breakdown product of titin in muscle. Titin, being the largest protein in humans, has 3.0–3.7 MDa encoded in the *TTN* gene on chromosome 2 (2q31.2).[18] This protein interplays the contraction of actin and myosin in the sarcomere to produce elasticity in muscle.[19] The urinary titin N-fragment has recently been reported to reflect the muscle breakdown in patients with muscular dystrophy[20] and those who are critically ill.[21] This biomarker in urine rapidly reflects the muscle breakdown of patients within hours.[22] Furthermore, this biomarker is noninvasive because it is obtained from urine, not blood. Frequent blood tests in infants should be avoided because it often causes iatrogenic anemia.[23] Therefore, urinary titin N-fragment can be a promising biomarker for the evaluation of the catabolic state in infants compared with blood tests such as creatine kinase and blood urea nitrogen.

However, little is known about the urinary titin N-fragment in infants. To adjust kidney function and various physiological conditions, the urinary titin N-fragment is corrected by urinary creatinine (pmol/mg creatinine [Cr]). Therefore, there was no difference in the standard level of the urinary titin N-fragment in healthy 3-year-old children: 2.2 ± 4.1 pmol/mg Cr[24] and healthy adults: 2.2 ± 1.4 pmol/mg Cr.[17] We considered that this test can be applied to infants, and hypothesized that the urinary titin N-fragment is increased during the early postmenstrual age and in preterm infants. Therefore, we retrospectively measured prospectively collected urine samples in neonatal intensive care units and the follow-up.

METHODS

Study design

We conducted a retrospective analysis of prospectively collected urine samples at Kobe University Hospital and Tokushima University Hospital between August 2018 and December 2020, respectively. This study was approved by the Institutional Review Board of Kobe University (#B200211) and Tokushima University (#1425), and retrospectively registered as a clinical trial (UMIN-Clinical Trials Registry: 000042755). Informed consent for this study was obtained in the form of opt-out on the website at Kobe University and opt-in at Tokushima University. Written informed consent was obtained from all parents for the use of personal medical data for research. This study was reported according to the STROBE statement.

Urine sample

We included prospectively collected residual urine samples from different studies. At Kobe University Hospital, urine samples were originally collected for the assessment of the risk of congenital cytomegalovirus infection[25] and rickets, follow-up of 33 weeks of gestation, and some urine tests for neonates after urethral catheter insertion. At Tokushima University Hospital, the urine samples were collected for research related to urinary angiotensinogen in low-birth-weight infants.[26] These samples were stored at −20 °C until the measurements. We excluded urine samples collected on the same day from the same patients. Urine samples were measured using an ELISA kit (27900 Titin N-Fragment Assay Kit, Immuno-Biological Laboratories Co. Ltd., Japan), which can measure the N-terminal fragment of titin breakdown (25 kDa).[17] In ELISA, we tested all samples in duplicate in a blinded manner, and used the average values for the analysis. Urinary titin N-fragment was corrected by urinary creatinine to adjust for kidney function and various physiological conditions. Urinary creatinine was measured via LabAssay Creatinine (Wako Pure Chemical Industries, Ltd., Osaka, Japan) at Kobe University Hospital and via L-Type Creatinine M (LSI medience Corporation, Tokyo, Japan) at Tokushima University Hospital.

Outcomes

The primary outcome of this study was to describe the time course of the urinary level of titin N-fragment during the postmenstrual age. We included all data in each postmenstrual week. We also analyzed the time course in preterm or full-term infants. The secondary outcome was to investigate the urinary level of titin N-fragment mainly in four parts: differences among gestational ages, longitudinal change after birth, influence on growth, and relationship with blood tests (Supplementary Fig. S1). The secondary analyses included urine samples applicable for these analyses.

Data collection of secondary outcomes

Among different gestational ages, we compared the urinary titin N-fragment level among full-term and preterm birth including late, moderate, very, extremely preterm infants, defined as 34–36, 32–33, 28–31, and <28 weeks of gestation, respectively. We used a urine sample within a week of collection for comparison. Older urine samples were used in multiple measurements from the same infant.

We investigated the longitudinal change of the urinary titin N-fragment to assess the change of the urinary titin N-fragment after birth, wherein the day of birth was defined as day 0. We included data that had at least two consecutive measurements from a single patient.

Concerning the influence on growth, we investigated the relationship between the urinary titin N-fragment and the time to return to birth weight, which is reported to reflect growth velocity.[27] We used urinary samples collected within a week after birth for comparison. In the case of multiple measurements, we used the maximum urinary level of titin N-fragment for the comparison. The analysis was conducted separately in full-term or preterm infants to analyze the growth restriction in preterm infants.

We investigated the relationship between the urinary titin N-fragment and blood tests. This analysis was conducted only in patients who had urinary and blood tests on the same day. All applicable tests at different timings and from the same patients were included in the analysis due to the limited sample size. Included blood tests were creatine kinase, blood urea nitrogen, lactate dehydrogenase, creatinine, aspartate aminotransferase, glucose, ionized calcium, and lactate. These analyses were conducted in the overall population, and full or preterm infants.

Statistical analysis

Continuous data are presented as mean ± standard deviation or median (interquartile range), as appropriate, whereas categorical data are presented as number (%). Variables were compared using *t*-test or the Mann–Whitney *U*-test for two-group comparisons, and one-way analysis of variance or the Kruskal–Wallis test for multiple comparisons. Post-hoc correction for multiple comparisons was performed with Tukey's or Steel–Dwass test. An exponential curve was assessed to evaluate longitudinal changes over time. The Spearman correlation coefficient was used to investigate relationships between the urinary titin N-fragment and time to birth weight or blood tests. Missing data were not complemented. The sample size was not determined a priori due to the exploratory nature of this research. Data analyses were conducted using JMP version 13.1.0 (SAS Institute Inc., Cary, NC). All statistical tests were two-tailed, and the chosen type 1 error rate was a p-value of <0.05.

RESULTS

This study included 219 patients with 414 urinary titin N-fragment measurements. In all measurements, the median gestational age was 31.6 (29.0–36.6) weeks, and we conducted measurements at 37.0 (33.6–40.0) weeks of postmenstrual age and 27 (4–60) days after birth. The median urinary titin N-fragment was 10.8 (6.4–20.3) pmol/mg Cr (Supplementary Table S1). Among 219 patients, males were 120 (55%), and caesarian sections were performed in 163 (74%) cases (Supplementary Table S2).

All data until 60 week's postmenstrual age are shown (Fig. 1). There was an exponential decline in the urinary titin N-fragment with postmenstrual age (urinary titin N-fragment [pmol/mg Cr] = 8126.9 × exp [−0.174 × week], $r^2 = 0.36$). The median urinary titin N-fragment was 64.0 (34.4–94.5), 11.8 (7.6–19.8), 8.4 (5.0–15.1), 5.5 (4.0–7.4), and 4.0 (2.9–7.5) pmol/mg Cr during 22–29, 30–39, 40–49, 50–59, and ≥60 weeks, respectively ($n = 26$, 281, 58, 20, and 29). The exponential decline was more prominent in preterm infants compared with that in full-term infants ($r^2 = 0.48$ vs. 0.06; Supplementary Figs. S2 and S3). The decline was observed from extremely to late preterm infants (Supplementary Figs. S4–S7).

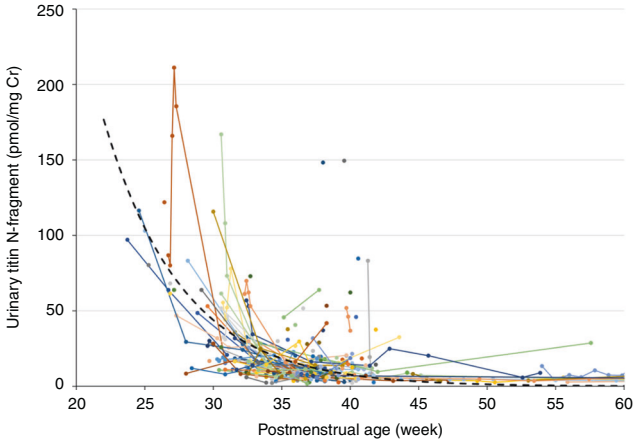

Fig. 1 Urinary levels of titin N-fragment during postmenstrual age. All data are shown until 60 weeks postmenstrual age, these include 219 patients with 414 measurements. There was an exponential decline of urinary titin N-fragment with postmenstrual age (urinary titin N-fragment [pmol/mg Cr] = 8126.9 × exp [−0.174 × week], $r^2 = 0.36$). Different colored lines represent individual patients.

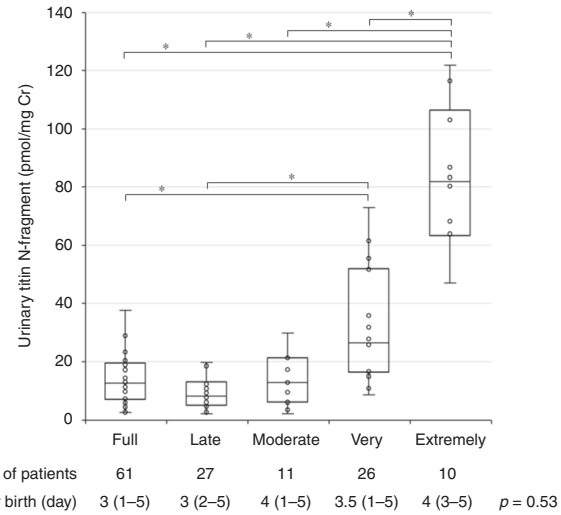

Fig. 2 Urinary levels of titin N-fragment in full-term and preterm infants. The data includes 135 patients with 135 measurements. There was no significant difference regarding different days of measurement ($p = 0.53$), but there was a difference in the urinary levels of titin N-fragment among the different gestational ages ($p < 0.01$). *Significance set at $p < 0.01$ based on post-hoc Steel–Dwass test.

Analyses of different gestational ages included 135 patients with 135 measurements (Fig. 2). Full, late, moderately, very, and extremely preterm were 61, 27, 11, 26, and 10 patients, wherein the measurement days after birth were not different at 3 (1–5), 3 (2–5), 4 (1–5), 3.5 (1–5), and 4 (3–5) days ($p = 0.53$). Urinary levels of titin N-fragment levels were 12.5 (7.1–19.6), 8.1 (5.1–13.0), 12.8 (6.0–21.3), 26.4 (16.4–52.0), and 81.9 (63.3–106.4) pmol/mg Cr in full, late, moderate, very, and extremely preterm infants, respectively. There were statistically significant differences among the groups (Kruskal–Wallis test, $p < 0.01$). Post-hoc analysis showed that very and extremely preterm infants had a significant difference to full and late preterm infants (Steel–Dwass test, $p < 0.01$). Among preterm births, the difference in body weight at birth was 2832 (2415–3099), 2180 (1952–2278), 1690 (1622–1868), 1396 (1188–1675), and 688 (644–936) g (Kruskal–Wallis test and Steel–Dwass test, $p < 0.01$). In this population, the urinary level of titin N-fragment did not vary between caesarian section and vaginal delivery (13.2 [7.8–27.8] vs. 12.7 [7.0–41.8] pmol/mg Cr in 98 caesarian sections and 37 vaginal deliveries, $p = 0.93$).

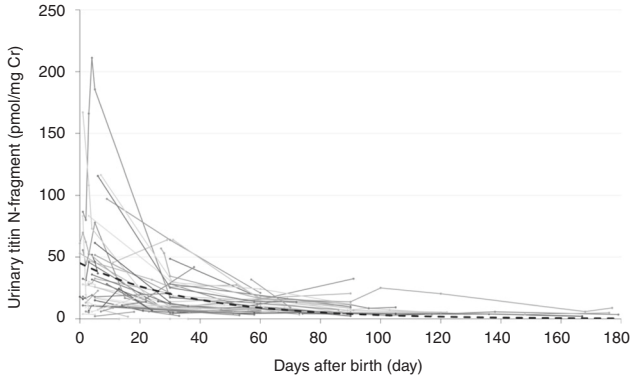

Fig. 3 Longitudinal change in urinary levels of titin N-fragment after birth. All consecutive data are shown until 180 days after birth. Data includes 74 patients with 269 measurements. Urinary titin N-fragment levels exponentially declined during the postmenstrual age (urinary titin N-fragment [pmol/mg Cr] = 45.2 × exp [−0.027 × day], $r^2 = 0.22$).

The longitudinal change after birth included 74 patients with 269 measurements. Data until 180 days after birth are shown in Fig. 3. There was an exponential decline of the urinary titin N-fragment during the postmenstrual age (urinary titin N-fragment [pmol/mg Cr] = 45.2 × exp [−0.027 × day], $r^2 = 0.22$). The median urinary titin N-fragment was 26.8 (11.0–52.2), 11.9 (7.6–20.9), 8.2 (5.2–11.3), 9.7 (7.0–16.3), and 6.4 (3.9–9.7) pmol/mg Cr for 0–19, 20–39, 40–59, 60–79, and ≥80 days, respectively ($n = 86$, 63, 16, 34, and 70).

Influence on growth included 87 patients with 87 measurements (Fig. 4). The maximum urinary titin N-fragment was associated with the time to return to birth weight in preterm infants ($\rho = 0.38$, $p < 0.01$, $n = 60$), but not in full-term infants ($\rho = -0.07$, $p = 0.73$, $n = 27$).

We investigated the correlation between the urinary titin N-fragment and blood tests in 120 patients with 204 measurements (Table 1). Creatine kinase was correlated with the urinary titin N-fragment in full-term infants ($\rho = 0.58$, $p < 0.01$), but not in preterm infants ($\rho = -0.12$, $p = 0.31$), whereas blood urea nitrogen was correlated in preterm infants ($\rho = 0.50$, $p < 0.01$), but not in full-term infants ($\rho = -0.04$, $p = 0.79$). Lactate dehydrogenase and serum creatinine were correlated with the urinary titin N-fragment in overall, full-term, and preterm infants. The details of correlation are shown in the supplemental file (Supplementary Figs. S8–S15).

DISCUSSION

In our study using the urinary titin N-fragment, we found that the catabolic state of infants was increased at the early postmenstrual age, and exponentially declined after birth. Very and extremely preterm infant, defined as <32 gestational weeks, had a prominently increased catabolic state, and the maximum urinary level of titin N-fragment within a week was associated with growth restriction during the early phase after birth. The role of the urinary titin N-fragment may be different between full and preterm infants due to different relationships with creatine kinase and blood urea nitrogen.

During the early phase of the postmenstrual age, the urinary level of titin N-fragment was 64.0 (34.4–94.5) pmol/mg Cr during

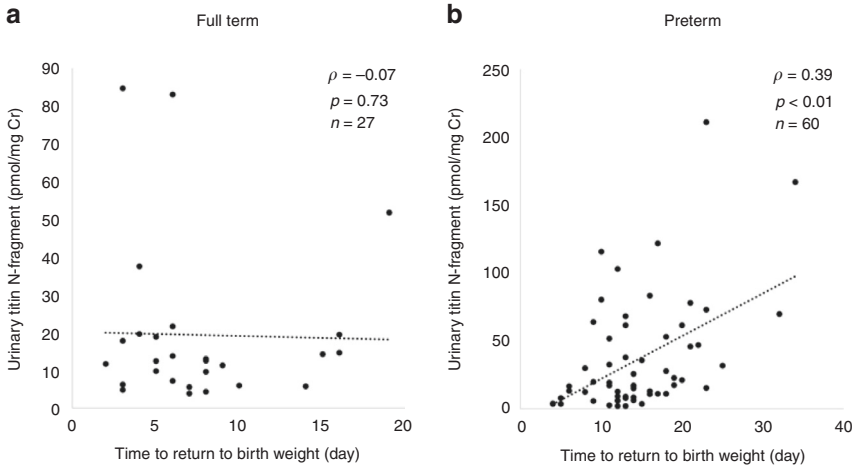

Fig. 4 Relationship between urinary titin N-fragment and the time to return to birth weight. a Full-term infants. **b** Preterm infants. The maximum urinary titin N-fragment within a week was associated with the time to return to birth weight in preterm infants ($\rho = 0.39$, $p < 0.01$, $n = 60$), but not in full-term infants ($\rho = -0.07$, $p = 0.73$, $n = 27$).

Table 1. Correlations between urinary titin N-fragment and blood tests.

	Difference at gestational age								
	Overall			Full-term			Preterm		
Variables	***n***	***ρ***	***p*-value**	***n***	***ρ***	***p*-value**	***n***	***ρ***	***p*-value**
Creatine kinase	120	0.06	0.50	46	0.58	<0.01	74	−0.12	0.31
Blood urea nitrogen	162	0.39	<0.01	47	−0.04	0.79	115	0.50	<0.01
Lactate dehydrogenase	151	0.48	<0.01	49	0.65	<0.01	102	0.49	<0.01
Creatinine	160	0.70	<0.01	47	0.76	<0.01	113	0.71	<0.01
Aspartate aminotransferase	164	−0.01	0.85	50	0.11	0.46	114	0.01	0.91
Glucose	141	0.04	0.61	33	−0.09	0.61	108	0.06	0.55
Ionized calcium	141	0.02	0.82	33	−0.20	0.26	108	0.10	0.30
Lactate	141	−0.13	0.12	33	−0.10	0.60	108	−0.11	0.26

We evaluated the urine and blood tests conducted on the same day, and 120 patients with 204 measurements were included in this analysis. The Spearman correlation coefficient was used to investigate the relationship between urinary titin N-fragment and blood tests.

22–29 weeks, and the level exponentially declined. These levels were higher than the average urinary level of titin N-fragment of healthy 3-year-old children: 2.2 ± 4.1 pmol/mg Cr.[24] Surprisingly, the increased level after birth was almost equivalent to that of the median level in patients with Becker muscular dystrophy: 43.4 pmol/mg Cr [20] or the peak of critically ill adults: 67.9 (35.7–116.2 pmol/mg Cr).[21] Given that the urinary titin N-fragment reflects muscle breakdown,[21] these results indicate that infants during the early postmenstrual age are exposed to an increased catabolism and subsequent muscle breakdown. This is theoretically reasonable for two reasons. First, newborns are under excessive stress during the transition from the intrauterine to the extrauterine environment, wherein they must adjust without placental circulation.[28] Newborns gradually adjust to circulation, respiration, and nutritional intake after birth. Indeed, the urinary titin N-fragment gradually decreased after birth. Second, delivery stress may accelerate the catabolism reaction. Delivery stress, especially in vaginal delivery, is associated with an increased level of cortisol even at eight weeks after birth.[29] The increased cortisol concentration may contribute to the prominent catabolism during the early phase after birth. However, vaginal delivery was less common in only 26% of the cases in our study population. Therefore, the increased urinary titin N-fragment may be due to the adjustment to the new environment of newborns.

Among preterm births, very and extremely preterm infants had prominently high urinary levels of titin N-fragment. In contrast, there was no statistically significant difference between full term, late, or moderate preterm infants, possibly because measurements were taken slightly earlier in full-term infants (3 [1–5] days in full-term vs. 3 [2–5] or 4 [1–5] days in late or moderate preterm infants). These results indicate that very and extremely preterm infants are exposed to a prominently increased catabolic state, which is consistent with the previous findings that very preterm infants, those born before 32 weeks' of gestation, have a slow growth rate during the neonatal period.[12,30] Interestingly, the maximum urinary titin N-fragment within a week was associated with growth restriction in preterm infants. This biomarker may be useful to evaluate growth restriction in preterm infants, particularly in very and extremely preterm infants because urinary levels of titin N-fragment levels were exponentially elevated during earlier postmenstrual age in preterm infants compared with that in full-term infants ($r^2 =$ 0.48 vs. 0.06; Supplementary Figs. S2 and S3).

There is no established biomarker to assess catabolism in infants.[16] Creatine kinase may be used to assess muscle breakdown.[31] In the current study, creatine kinase correlated with the urinary titin N-fragment in full-term infants, but not in preterm infants. This difference may be derived from the difference in

skeletal muscle mass between full-term and preterm infants.[32] Creatine kinase was associated with the urinary titin N-fragment in muscular dystrophy ($r^2 = 0.497$, $p < 0.0001$),[20] exercise-induced muscle damage ($r \geq 0.647$),[33] and critically ill adults.[34] Thus, the immature muscle mass in preterm infants may have resulted in the dissociation of the urinary titin N-fragment and creatine kinase. In general, blood urea nitrogen reflects the catabolic state,[35] and blood urea nitrogen correlated with the urinary titin N-fragment in preterm infants. However, it did not correlate in full-term infants possibly due to the nutritional influence. Blood urea nitrogen is affected by nutritional amino acid intake.[36] Serum blood urea nitrogen level is not affected by parenteral,[37] but is affected by enteral nutrition.[38] Due to intestinal tolerance, full-term infants generally take more enteral nutrition compared with preterm infants from the early phase. Thus, the amino acid from enteral nutrition may increase the serum blood urea nitrogen level in full-term infants, leading to the dissociation from the muscle-derived urinary titin N-fragment. Given that lactate dehydrogenase generally exists in cells,[39] it is likely correlated with urinary levels of titin N-fragment as a result of muscle cell breakdown. In contrast, serum creatinine is likely correlated with urinary levels of titin N-fragment because urinary creatinine was used to correct the urinary levels of titin N-fragment (pmol/mg Cr).[40] However, because aspartate aminotransferase, glucose, calcium, and lactate all exist in various other tissues, these were not correlated with urinary levels of titin N-fragment levels.

Given that post-natal growth restriction influences future disease risk, it is important to manage increased catabolism during the neonatal period.[41,42] Nutritional management suppresses the increased catabolic state and the subsequent muscle breakdown.[43,44] At present, it is unclear how much energy and protein are required in infants, especially in preterm infants.[45,46] The assessment of sufficient nutrition is difficult to determine.[47] Infants may require protein levels of 1.0–2.0 g/kg/day, or 3.0–4.0 g/kg/day as early aggressive nutrition.[14,48] At present, the current study cannot provide the necessary nutritional amount in infants, but at least suggests how the catabolic state is increased in infants. Further research is warranted to find interventional studies that can manage the increased catabolic state and muscle breakdown in infants.

LIMITATIONS

First, it is unclear whether the urinary titin N-fragment crosses the placental barrier. The labor stress of mothers may affect the urinary level of titin N-fragment of newborns. However, at least, the delivery process did not affect the urinary titin N-fragment level because of no difference between caesarian section and vaginal delivery. Second, this is a retrospective study, and we used urine samples collected from healthy and sick infants at various times for different purposes. The patients' background at the time of collection may affect the results. Thus, further investigation is needed before generalizations can be made. Third, we included the same infant multiple times due to the limited sample size (Supplementary Table S3), another limitation that requires further studies. Fourth, body weight was not routinely measured in infants. Thus, body weight change is affected by the timing to measure body weight. Fifth, it was difficult to assess the influence of nutritional intake on the urinary titin N-fragment because we retrospectively measured urine samples from different days.

CONCLUSIONS

In this retrospective study, we investigated the catabolic state of infants using the urinary titin N-fragment. This novel biomarker clarified the catabolic state in infants. The urinary level of titin N-fragment was increased during the early course of the post-menstrual age, and then declined exponentially after birth. Furthermore, the urinary level of titin N-fragment was prominently increased in very and extremely preterm infants.

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AUTHOR CONTRIBUTIONS

S.F. and N.N. equally contributed to conception and design, acquisition of data, analysis, interpretation of data, and drafting of the manuscript as first authors. K.F. and K.S. equally contributed to all aspects of this study as second authors. T.S., K.O., K. H., and R.T. performed laboratory testing and analysis of data. M.U., R.N., and H.A. were involved in the acquisition of the data and drafting of the manuscript. J.O., H.S., and K.I. supervised all aspects of this study. M.M. supervised and contributed equally to all aspects of this study including drafting the article and revising it critically for important intellectual content as a corresponding author. All authors read and approved the final version to be published.

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COMPETING INTERESTS

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CONSENT STATEMENT

Ethics approval was obtained from the Institutional Review Board of Kobe University (#B200211) and Tokushima University (#1425). Opt-out and opt-in informed consent at the Kobe University and Tokushima University, respectively, was obtained for this study. Written informed consent was obtained from all parents for the use of their personal medical data in research.

ADDITIONAL INFORMATION

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