Stress Fracture Influences Bone Resorption marker (u-NTX) in Female Long Distance Runners

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ABSTRACT
In this study, we aim to clarify the influence based on bone resorption markers at onset of stress fracture. Also, we will clarify the state of the bone resorption markers of female long distance runners who have a history of stress fracture and also ones who routinely practices running long distances. Participants comprised 19 female long distance athletes. The survey period was 2011–2014, and we measured u-NTX as a bone resorption marker at least twice a year, taking the mean ± SD of the periodic measured values without stress fracture as the mean value. Measurements were collected sample when stress fractures developed. 132 u-NTX measurements were taken from 19 participants. As a result, the average was 41.03 ± 12.31 nmolBCE/mmolCRE (Q1: 33.15, Q2: 40.55, Q3: 47.95). In six of the 19 participants, u-NTX could be measured following a stress fracture. The mean value of u-NTX for those participants was 40.16 ± 9.10 nmolBCE/mmolCRE, increasing to 64.08 ± 16.07 nmolBCE/mmol CRE with the stress fracture (p < 0.01). The findings showed that, in adult female long distance runners, u-NTX values when there was no stress fracture were within the standard value for mean premenopausal women, but increased when the athletes suffered from a stress fracture.

Introduction
A stress fracture is a break in bone tissue caused by repeated minor external mechanical stress caused by activities such as running that can occasionally lead to a complete fracture. A stress fracture is a serious injury because it takes a long time to completely heal [2, 3] and prevents athletes from training. Many female long-distance runners compete while suffering from menstrual disorders; the incidence of stress fractures among such women is much higher than for athletes of other sports [4, 12, 16]. To achieve good results through continuous training, it is important to find an indicator for the prevention and early detection of stress fractures in female athletes.

Bone strength is explained by bone density and bone quality (bone metabolism and collagen cross-linking) [21]. It has been reported that low bone density increases the risk of a stress fracture [5, 10, 25]. However, as results based on bone density reflect nutritional condition and mechanical stress over several previous months, they are not suitable for the early detection of stress fractures. In contrast, bone metabolism—bone quality—reflects the condition of bone in a timely manner, and bone metabolism has an
effect on subsequent bone density. If the balance of bone resorption and bone formation is maintained (coupling), bone mass is maintained. However, when uncoupling occurs and bone resorption becomes more dominant, bone density decreases. Bone metabolism can be evaluated using bone metabolism markers measured in serum and urine.

The mechanism underlying stress fractures is that repeated mechanical stresses on the bone repeatedly cause microdamage, and as bone repair cannot keep up, bone mass decreases locally [24]. Bone resorption is believed to be accelerated before and after the occurrence of a stress fracture. However, there is insufficient study on bone metabolism during stress fractures. In addition, it was shown that bone resorption is enhanced by continuous running for long periods, such as during a marathon [7, 15]. Thus, long-distance runners who repeatedly run may already be suffering from enhanced bone resorption. In addition, bone resorption marker is high in athletes with a history of stress fracture compared to athletes who do not [27]. From these facts, there is a possibility that the bone resorption marker is elevated when a stress fracture develops. However, there is a consideration that bone resorption markers may be elevated in long-distance runners practicing on a daily basis and athletes with a history of stress fracture may have an elevated marker even when there is no stress fracture.

In this study, we aim to clarify the influence based on bone resorption markers at onset of stress fracture. Also, we will clarify the state of the bone resorption markers of female long-distance runners who have a history of stress fracture as well as ones who routinely practices running long distances.

**Methods**

**Participants**

Participants consisted of 25 female long-distance runners, ages 19 to 34 years old (avg 23.99 ± 4.11). This study was approved by the ethical committee of Juntendo University (21–11). Participants and their team instructors were given explanations of the experiment orally and in writing before written consent was obtained. This study was conducted according to the ethical standards of International Journal of Sports Medicine [13].

**Measurement item**

Bone metabolism was evaluated noninvasively by measuring type 1 collagen crosslinked N-telopeptide in urine (u-NTX). Participants answered the preliminary questionnaire. The contents of the questionnaire were physical characteristics, experience of irregular menstrual or amenorrhea in the past, or whether they have a past history of stress fracture diagnosed by a doctor. In addition, the same questionnaire was answered each time measurements were taken. We investigated the total distance run per month and injury situation.

**Measurement methods**

Generally, when measuring bone metabolism markers, both bone resorption and formation are taken. But because the participants were professional athletes, they were uncooperative with blood sample collection. Therefore, to avoid diurnal and daily variations, the second urine of the morning was sampled for the u-NTX measurement. This was analyzed using the ELISA method (Osteomark; Alere Medical Co. Chiba, Japan). To eliminate any effects of the kidney, the creatinine conversion factor was used for the analysis. Results were expressed in nmol bone collagen equivalents (BCE)/mmol creatinine (CRE). All measurements were outsourced to Hoken Kagaku Kenkyujo laboratory.

**Measurement period**

To measure the normal condition, which is the condition without stress fracture and able to participate in full practices, we measured each athlete’s u-NTX 11 times, including three times in 2011, twice in 2012, twice in 2013, and four times in 2014. The measurements were taken at the following months and practice periods:

In April and July 2011, measurements were taken in the regular practice period. The measurement in April 2012 was taken during a regular practice period, and in October during a performance-enhancement practice period. A performance-enhancement practice period is when athletes attend training camp. Measurements in February and October, 2013 were taken during performance-enhancement practice periods. Measurements in May and June, 2014 were taken during regular practices; August was performance-enhancement practice period. The u-NTX was taken and assessed by the amount of practice on weekly running distances. As measured values of u-NTX can show considerable variation in an individual, we used the mean value of the measurements obtained during the period without any stress fracture as the normal value. If a stress fracture occurred during the survey period or before, measurements were obtained during the examination, at which time it was determined that a stress fracture had occurred. Stress fractures were diagnosed using radiographic inspection (i.e., X ray) by orthopedic surgeons. Also, bone metabolism marker was taken at the diagnosis. The onset date of the stress fracture was defined as when the participants felt pain at the injured site. The date of onset and the date of measurement of bone metabolism markers are shown in Table 1.

**Exclusion criteria and grouping**

Out of 25 participants, 6 participants with u-NTX measurements less than 3 times were excluded from this study; therefore, 19 participants were included in this study (▶ Fig. 1). Among them, 6 participants with measurement data of u-NTX when stress fracture occurred were selected as the SF group, and other participants were selected as the Control group. In the SF group, the values of measurement when stress fracture occurred were compared with the values of measurement without stress fracture. Based on the preliminary questionnaire, participants were grouped into two groups with or without the history of stress fracture, and a comparison was made between the two groups.

**Data analysis methods**

The measured values were presented as mean ± standard deviation (SD) or median (interquartile rage). To decide a normal value for individual participants, a mean value and SD of measurements without stress fracture of each participant were calculated and used as a “normal value” for each participant.
Table 1 Details of stress fracture, menstruation condition and u-NTX data in SF group.

<table>
<thead>
<tr>
<th>Location of an injury</th>
<th>Date of onset</th>
<th>Urine sample</th>
<th>Normal value</th>
<th>Stress fracture</th>
<th>Urine sample from date of onset</th>
<th>Menstruation situation</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Nov. 9, 2002</td>
<td>Dec. 16, 2002</td>
<td>36.8 ± 14.2</td>
<td>67.9</td>
<td>37 days later</td>
<td>Normal</td>
<td>30</td>
</tr>
<tr>
<td>B</td>
<td>May 14, 2010</td>
<td>June 6, 2010</td>
<td>35.8 ± 8.6</td>
<td>54.6</td>
<td>19 days later</td>
<td>Irregular menstruation</td>
<td>20</td>
</tr>
<tr>
<td>C</td>
<td>Sept. 22, 2012</td>
<td>Oct. 30, 2012</td>
<td>40.0 ± 5.2</td>
<td>41.5</td>
<td>6 weeks later</td>
<td>Normal</td>
<td>24</td>
</tr>
<tr>
<td>D</td>
<td>Nov. 15, 2011</td>
<td>Dec. 9, 2009</td>
<td>40.3 ± 7.7</td>
<td>70.2</td>
<td>25 days later</td>
<td>Amenorrhea</td>
<td>29</td>
</tr>
<tr>
<td>E</td>
<td>Mar. 20, 2010</td>
<td>Apr. 20, 2010</td>
<td>30.7 ± 9.9</td>
<td>61.1</td>
<td>3–5 weeks later</td>
<td>Primary amenorrhea</td>
<td>22</td>
</tr>
<tr>
<td>F</td>
<td>Late Mar. 2010</td>
<td>Apr. 21, 2010</td>
<td>57.3 ± 10.3</td>
<td>89.2</td>
<td>3–4 weeks later</td>
<td>Irregular menstruation</td>
<td>21</td>
</tr>
</tbody>
</table>

- Mean: 40.2 ± 9.3, SD: 9.9 ± 3.0, 64.1

Amenorrhea was defined as a state without menstruation for more than 3 months, and irregular menstruation was defined as not occurring within the regular menstrual cycle (28–38 days).

Fig. 1 Exclusion criteria and grouping.

The Wilcoxon signed-rank test was used to compare the difference between the value at the time of stress fracture and the normal value. Unpaired t-test was used to compare the difference between the groups with and without the history of stress fracture. Statistical analysis was done using nonparametric Kruskal-Wallis test comparing the difference among the average weekly running distance measuring u-NTX.

Furthermore, changes of u-NTX at the time of stress fracture were investigated using the normal values and SD. "Rate of over" was calculated for SF and NSF group using the normal value ± SD of each participant, and the extent of changes of u-NTX values when stress fracture occurred was analyzed. "Rate of over" in the SF group was defined as the rate of participants whose u-NTX values at the time of stress fracture were over 1 SD, 1.5 SD or 2 SD of the normal value. "Rate of over" in the NSF group was defined as the rate of participants whose highest u-NTX values were over 1 SD, 1.5 SD or 2 SD of the normal value. Fisher’s exact test was used to compare the difference in the “rate of over” of the two groups.

The effect size (ES) and power in post hoc tests were calculated using Gpower software (Version 3.1) [11]. The ES between the 2 groups (with and without the history of stress fracture) and 2 conditions (values at stress fracture and normal value) were calculated using ES (d). The evaluations of the ES strength are: small (d < 0.40), moderate (0.40 < d < 0.80), large (d > 0.80). The ES among the average weekly running distances measuring u-NTX were calculated using ES (f). The evaluations of the ES strength are: small (f < 0.25), moderate (0.25 ≤ f < 0.40), large (f ≥ 0.40). The ES between 2 groups (SF group and NSF group) considered as “rate of over" was calculated using ES (w). The evaluations of the ES strength are: small (w < 0.10), moderate (0.10 ≤ w < 0.30), large (w ≥ 0.30). α error was set to p < 0.05, and β error was set to (1-β) > 0.80.

Results

Participants’ average physical and other characteristics were as follows: height 159.91 ± 6.36 cm, weight 46.13 ± 3.93 kg, body mass index (BMI) 18.02 ± 1.05 kg/m², weekly running distance 121.7 ± 49.4 km, and time for 5000-m run 15:45.9 ± 23.9. In this study, a total of 132 u-NTX measurements were taken from 19 participants. As a result, the average was 41.03 ± 12.31 nmol BCE/mmol CRE (Q1: 33.15, Q2: 40.55, Q3: 47.95).

The weekly running distance when u-NTX was measured is shown in Table 2. There was no significant difference in the weekly running distance among measurements (p = 0.36, ES (f) = 0.29, 1-β = 0.91).

Comparison of u-NTX values between with and without history of stress fracture

Out of the 19 participants, nine had a history of stress fracture (height 159.67 ± 7.55 cm, weight 44.89 ± 4.78 kg, BMI 17.55 ± 0.66) and 10 did not (height 160.14 ± 5.48 cm, weight 47.25 ± 2.78 kg, BMI 18.45 ± 1.19). Although u-NTX values were 36.51 ± 9.86 nmol BCE/mmol CRE for the group with a history of stress fracture and 44.01 ± 8.06 nmol BCE/mmol CRE for the group without, this difference was not statistically significant (p = 0.08, ES (d) = 0.834, 1-β = 0.508).
Table 2 The mean distance per month indicated by weekly unit when measuring u-NTX.

<table>
<thead>
<tr>
<th>Date</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>May, 2011</td>
<td>125.8 ± 55.3</td>
</tr>
<tr>
<td>Jul., 2011</td>
<td>100.6 ± 59.0</td>
</tr>
<tr>
<td>Apr., 2012</td>
<td>94.8 ± 45.3</td>
</tr>
<tr>
<td>Oct., 2012</td>
<td>126.1 ± 71.0</td>
</tr>
<tr>
<td>Feb., 2013</td>
<td>112.5 ± 70.3</td>
</tr>
<tr>
<td>Oct., 2013</td>
<td>147.9 ± 50.2</td>
</tr>
<tr>
<td>May, 2014</td>
<td>127.4 ± 29.1</td>
</tr>
<tr>
<td>Jun., 2014</td>
<td>119.2 ± 31.8</td>
</tr>
<tr>
<td>Aug., 2014</td>
<td>120.1 ± 43.6</td>
</tr>
<tr>
<td>Oct., 2014</td>
<td>137.8 ± 32.8</td>
</tr>
</tbody>
</table>

Comparison of u-NTX value in SF group between measurement with stress fracture and normal value

Data from the time of a stress fracture were available for six participants (Table 1). The mean value for u-NTX after a stress fracture was 64.08 ± 16.07 nmol BCE/mmol CRE compared with the normal value of 40.16 ± 9.10 nmol BCE/mmol CRE; this difference was statistically significant (p < 0.01, ES (d) = 1.989, 1-β = 0.969). In addition, in four of these six participants, menstrual condition when stress fracture occurred was irregular or with no menstruation.

Changes in u-NTX values at stress fracture

Changes in u-NTX values that were +2 SD or more were observed in five out of six (rate of over: 82%) in the SF group and three out of 13 (rate of over: 23.1%) in the NSF group, which represents a significant difference. Changes of +1 SD or more were more common in the SF group (p < 0.05, ES (w) = 1.597, 1-β = 0.616, odds ratio = 16.6). Five out of six (rate of over: 82%) of the SF group showed a change of +2 SD, a significantly greater proportion than in the NSF group (1/13, rate of over: 7.7%; p < 0.01, ES (w) = 2.023, 1-β = 0.786, odds ratio = 60.0).

Discussion

In this study, we regularly measured u-NTX in 19 female long-distance runners. For six of these participants, measurements were obtained when a stress fracture occurred.

It was found that u-NTX at the time of stress fracture showed a higher value than when there was no stress fracture, indicating enhanced bone resorption.

The underlying mechanism for stress fractures involves repeated mechanical stresses on bones causing repeated microdamage with which bone repair cannot keep up, leading to a localized reduction in bone mass [24]. In animal experiments, when microdamage accumulates, bone remodeling is locally enhanced to repair the damage, and the remodeling space on the bone resorption surface increases [8]. In the present study, although there was a problem that the amount of training was not constant, the mean u-NTX value in multiple measurements obtained during the time without stress fractures was within the standard value for normal premenopausal women of 9.3–54.3 nmol BCE/mmol CRE [17]. In this study, even a history of stress fracture did not lead to increased u-NTX values. The previous study investigated u-NTX values from different sports. The age and u-NTX values of athletes performing high-impact sports (basketball and volleyball), medium-impact sports (soccer and track) and non-impact sports (swimming) were 19.9 ± 0.3 years old; 72.9 ± 11.4 nmolBCE/mmolCRE, 20.6 ± 3.3 years old; 62.5 ± 7.6 nmolBCE/mmolCRE and 19.4 ± 0.3 years old; 80.0 ± 9.2 nmolBCE/mmolCRE, respectively [9]. The value of u-NTX for female cross-country athletes with an average age of 19.8 years similar to the sports category of this study was 62.5 ± 10.3 nmol BCE/mmol CRE [18]. In contrast, the average u-NTX was 41.03 ± 12.31 nmolBCE/mmolCRE in the present study. In the previous studies, the average age was 20 years or younger, whereas the participants of this study were 23 years old or older. It is known that bone metabolism is more active in younger population [19, 26]. In addition, measurements of u-NTX obtained the day after moderate exercise was reported to be no different from measurements obtained before exercise [28]. We therefore assume that u-NTX would show normal values regardless of the amount of exercise when there is no stress fracture, but with a stress fracture it would show a high value because of the accumulation of excessive microdamage in adult female long-distance runners.

Table 3 Changes in u-NTX during stress fracture.

<table>
<thead>
<tr>
<th>Rate of over (%)</th>
<th>SF group (n = 6)</th>
<th>NSF group (n = 13)</th>
<th>Fisher’s exact test</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal value + 1 SD</td>
<td>83.3</td>
<td>84.6</td>
<td>NS</td>
<td>0.9</td>
</tr>
<tr>
<td>Normal value + 1.5 SD</td>
<td>83.3</td>
<td>23.1</td>
<td>p &lt; 0.05</td>
<td>16.6</td>
</tr>
<tr>
<td>Normal value + 2 SD</td>
<td>83.3</td>
<td>7.7</td>
<td>p &lt; 0.01</td>
<td>60.0</td>
</tr>
</tbody>
</table>

SF: stress fracture, NSF: not stress fracture, NS: non-significance “Rate of over” of the SF group: The percentage of participants whose u-NTX value when stress fracture developed was over the normal value + 1 SD, 1.5 SD or 2 SD. “Rate of over” of the NSF group: The percentage of participants whose highest value in normal value measurement was over normal value + 1 SD, 1.5 SD or 2 SD.
We also observed that, when a stress fracture occurs, u-NTX values reach +1.5 SD or more above the normal value. Because u-NTX is tested in urine samples, it is a noninvasive bone metabolism marker that does not put too much stress on the athletes. In addition, u-NTX is a superior marker for monitoring [1]. Thus, after three measurements of u-NTX, if the value reaches +1.5 SD or more above the normal value, a stress fracture should be suspected. A detailed early examination could help the early detection of stress fractures.

In recent years, tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) has been used as a bone resorption marker for measurements in many studies because it reacts sensitively. In a study that targeted lacrosse players, TRACP-5b measured in athletes with a history of stress fracture was higher than in athletes without stress fracture [27]. TRACP-5b also reflects the impact of exercise in particular, reacting sensitively to temporary changes after exercise [20, 23]. Because it is a more sensitive marker, it is believed to be able to reflect the effects of exercise performed on the day before or immediately before measurement. In contrast, measurements of u-NTX obtained the day after moderate exercise were reported to be no different from measurements obtained before exercise [28]. Although it was reported that bone metabolism markers are not suitable prediction markers for stress fracture [29], the bone metabolism marker used in the previous study was a serum marker (TRACP-5b, CTX), and u-NTX was not measured. Based on previous studies, TRACP-5b increases when there is a history of stress fracture, but it may not be reliable on the onset of stress fracture [27], whereas in our study u-NTX became higher when stress fracture develops, indicating that there are certain characteristic bone resorption markers. To clarify the characteristics of bone resorption markers, further investigation is necessary in the future.

A limitation of this study was that u-NTX was high when stress fractures occurred, but it is unknown whether u-NTX increased prior to the occurrence of stress fracture and how long the u-NTX remains high following stress fracture. A previous study reported high u-NTX values prior to stress fractures [22]. Therefore, by periodically measuring the bone resorption marker to check if the value is abnormally high, which might indicate a stress fracture, these tests may be helpful in detecting a stress fracture in the immature stages. However, because the number of cases was small and the measurement of u-NTX was more frequent than in the present study, a prospective cohort study is needed to examine whether u-NTX values increase before a stress fracture occurs. Another weakness of the current study is that the participants were professional athletes, and we were unable to perform the adequate measurements such as collecting blood samples. Therefore, we were unable to examine bone formation. For bone metabolism, the balance between bone formation and bone resorption (coupling) is important, and bone formation markers should therefore be measured and coupling examined. Also, although intake of calcium and vitamin D is also related to bone density and bone metabolism markers [6, 14], the nutritional condition of our participants is unknown because we did not survey diet in this study. However, all of the athletes were living together in dorms, and breakfast and dinners were provided. Therefore, it is unlikely that there was a significant difference in nutritional status between the athletes, and nutrition probably had little effect on the bone metabolism marker.

The findings of this study showed that, in adult female long-distance runners, u-NTX values without stress fracture were within the standard value for normal premenopausal women but increased when the athletes suffered from a stress fracture. Furthermore, our result showed the possibility that a stress fracture has developed if u-NTX shows a value higher than 1.5 SD from the normal value. These facts suggest that regular measurement of u-NTX and monitoring fluctuations could be a convenient and noninvasive indicator of the development of a stress fracture.

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References


