Sport-related differences in biomarkers of bone resorption and cartilage degradation in endurance athletes

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Summary

Objective: By measuring urinary cross-linked N-telopeptide (NTx) as a bone resorption marker and urinary C-telopeptide of type II collagen (CTX-II) as a cartilage degradation marker, we asked whether differences in skeletal stresses in college athletes undergoing high-intensity training for diverse types of aerobic sports affect their skeletal metabolism and, if so, differentially or in unison.

Methods: The study was cross-sectional at a Division I college campus with 60 student athletes representing crew, cross-country running and swimming. Controls were 16 non-athlete undergraduates. Urine samples were collected for NTx and CTX-II analysis by enzyme-linked immunosorbent assay, normalizing results to creatinine. Two-way analysis of variance models and pair-wise comparisons were used to test whether biomarker levels differed by sport and the significance when adjusted for body mass index (BMI).

Results: NTx and CTX-II showed significant differences between groups before and after adjusting for BMI. NTx was highest in the rowers, and higher in runners and swimmers than in swimmers or controls. CTX-II was significantly higher in runners than in crew, swimmers or controls, when unadjusted for BMI. After adjusting for BMI, these group differences remained significant except for runners over crew.

Conclusion: Athletes in-training in the three sports show significant differences in these markers of bone resorption and cartilage collagen degradation. The results suggest that crew undergo the highest bone remodeling and runners the highest cartilage degradation. The results also show how these markers can vary physiologically between individuals, at extremes of skeletal exercise.

Key words: Collagen, Cartilage, Cross-links, Biomarkers, Athletes.

Introduction

Rowing, cross-country running, or swimming competitively at the college level requires outstanding aerobic fitness and extensive aerobic training. In all three sports, most of the training is sport-specific. While the cardiovascular challenges for the three sports are similar, the musculoskeletal challenges vary significantly. Runners expose their lower extremity bones and joints to large repetitive axial loads. While crew athletes expose their entire skeleton to both axial and non-axial loads, their lower extremity bones and joints experience lower peak axial loads than those of runners. Swimmers’ bones and joints experience the lowest axial loads. Such extremes of chronic loading might be expected to affect the turnover rate of bone and cartilage in affected parts of the skeleton and be detectable using systemic biomarker assays.

Various molecular markers have been reported as indicators of bone turnover and cartilage metabolism in human subjects and in patients enrolled in clinical studies of bone and joint disorders. The cross-linked N-telopeptide (NTx) of type I collagen in blood or urine has seen extensive use as a marker of systemic bone resorption. The NTx peptide is a neoepitope generated from bone collagen during resorption by osteoclasts, and cathepsin K, a protease abundantly expressed by osteoclasts, has been shown to make the proteolytic cleavage that generates the NTx epitope. NTx levels are elevated in post-menopausal women, in patients with metabolic bone disease and in growing children. Type I collagen in non-mineralized tissues such as skin is degraded by a different proteolytic pathway hence explaining the specificity of urinary NTx as a bone resorption marker. The specificity to bone resorption is seen in the response of NTx to antiresorptive therapies for osteoporosis, for example to the bisphosphonate, alendronate.

In a related approach, a fragment of type II collagen found in urine has been targeted as the basis of a biomarker assay for cartilage collagen breakdown. In this case, the neoepitope was a peptide from the C-telopeptide of type II collagen (CTX-II) of sequence EKGPDP, where the lysine residue (K) is part of a cross-link. Using a monoclonal antibody 2B4 that recognizes the C-terminal sequence (GPDP), the proteolytic epitope (CTX-II) can be detected in synovial fluid, serum, and urine. Various collagen cleavage products, including the pyridinoline cross-links themselves have been pursued as markers of joint pathology in osteoarthritis. Atley et al. found very high levels of the 2B4 epitope in the urine of growing children, presumably from growth plate activity, and levels were higher in patients with rheumatoid arthritis and osteoarthritis than in controls.
In contrast to the many reports on NTx and other collagen degradation markers in metabolic bone disease, there have been few studies on the effects of extremes of normal skeletal stress on NTx, for example in high activity athletes, and none on a cartilage collagen marker. Here, we designed a study to compare whether these two markers were differentially affected in three groups of intercollegiate athletes undertaking training for different aerobic sports and compared with non-athlete controls.

**Methods**

**SUBJECTS**

The study was approved by the Human Subjects Review Board at the University of Washington with participant informed consent. Sixty collegiate athletes (NCAA Division I) representing crew, cross-country runners, and swimmers were recruited at their routine fall physical to participate in the study. Three groups of athletes each consisting of 10 males and 10 females were enrolled. All athletes had been actively training for their sports for several weeks before specimen collection. In addition, one swimmer, one cross-country runner, and six rowers participated in some cross training. Individual heights and weights were measured to determine body mass index (BMI). In the control group were 16 University of Washington college students, 8 males and 8 females, recruited as volunteers and randomly selected from the university student population. None of the controls participated in collegiate athletics, and they participated in sports-related activity ≤ 3 times per week.

**PROCEDURES**

Spot urine samples were collected in the afternoon and stored at −20°C before assay as a batch. NTx was measured in urine using a commercial enzyme-linked immuno-sorbent assay (ELISA) kit (Osteomark®, Ostex International) and the values were normalized to creatinine measured by a commercial assay based on the Jaffe reaction (Sigma Diagnostics). Intra- and inter-assay coefficients of variation for the NTx/Cr assay are <5% and <8%, respectively.

The CTx-II assay is based on a monoclonal antibody, 2B4, which recognizes a peptide sequence, EKGPDP, derived from the C-telopeptide domain of type II collagen and in which the lysine residue (K) is part of a pyridinoline cross-link. The epitope is a proteolytic neoepitope, in that the C-terminal sequence PDP is required for antibody binding. Various matrix metalloproteinases are able to make this cleavage in vitro from intact type II collagen but the tissue source(s) and pathway of origin of the collagen type II peptides measured in urine are not yet clearly defined. The assay is a competition ELISA and the results are expressed as ng of peptide equivalents per mg of creatinine in urine. Intra- and inter-assay coefficients of variation for the CTx-II/Cr assay were <6% and <13%, respectively.

**STATISTICAL ANALYSIS**

The study was cross-sectional in design. Spearman rank correlation coefficients were used to determine any association between the athlete characteristics (height, weight, BMI, and age) and biomarker measures. Using the Kolmogorov–Smirnov test, both NTx/Cr and CTx-II/Cr data sets passed the test of normality. Two-way analysis of variance (ANOVA) models were used to test whether biomarker levels differed by sport. When significant differences were found, further pair-wise comparisons were made. Differences in athlete characteristics by sport were assessed by the Kruskal–Wallis test. ANOVA was used to assess the significance of group biomarker values adjusted by BMI.

**Results**

Table I shows the mean (±SD) height, weight, BMI and age profiles of the athlete and control groups. There are no significant differences in age among the male or female groups. There are small differences in height between the groups and larger differences in weight and subsequently BMI.

The biomarker data were examined for confounders that included known injuries at the time of sample collection, concurrent illness, recent surgeries, history of amenorrhea, use of medications including oral contraceptive pills, and history of recent fractures. None of these potential confounders appeared to have any influence on the data.

Mean NTx/creatinine values were significantly different between sports groups for both male and female athletes [Fig. 1, Table II(a)]. The male rowers had a higher NTx excretion than all other groups (cross-country: P < 0.01). NTx for the swim group remained significantly lower than all other groups (cross-country: P < 0.01, control: P < 0.01). After adjustment for BMI, NTx excretion of the male rowers remained significantly higher than the male swimmers (P < 0.01), and male controls (P < 0.01) but were not significantly different from the cross-country group. For female rowers, NTx was higher than for the swimmers (P < 0.01) and the controls (P < 0.01). NTx for the swim group was significantly lower

<table>
<thead>
<tr>
<th>Table I</th>
<th>Mean (±SD) height, weight, BMI and age profiles of the athlete and control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cross-country</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>No. of participants</td>
<td>10</td>
</tr>
<tr>
<td>Height</td>
<td>71.5 (2.5)</td>
</tr>
<tr>
<td>Weight</td>
<td>153.8 (14.1)</td>
</tr>
<tr>
<td>BMI</td>
<td>21.2 (1.2)</td>
</tr>
<tr>
<td>Age</td>
<td>18.7 (0.9)</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>No. of participants</td>
<td>10</td>
</tr>
<tr>
<td>Height</td>
<td>66.4 (2.4)</td>
</tr>
<tr>
<td>Weight</td>
<td>121.2 (11.2)</td>
</tr>
<tr>
<td>BMI</td>
<td>19.2 (1.2)</td>
</tr>
<tr>
<td>Age</td>
<td>19.8 (1.4)</td>
</tr>
</tbody>
</table>

Kruskal–Wallis non-parametric test used to test for group differences. *Significance of variability across all groups.
than for the cross-country runners ($P = 0.2$). Pair-wise analysis showed that the differences remained statistically significant after adjusting for BMI ($P < 0.01$). When combined male and female NTx results were adjusted for BMI, the cross-country group was significantly higher than the swimmer and control groups and rowers were higher than swimmers and controls [Table II(b)].

Table III(a) and Fig. 2 show the differences in collagen type II peptide excretion (CTx-II) between sport activities.

For males, the cross-country group had higher levels than both the swimmers ($P < 0.01$) and controls ($P = 0.02$). After adjusting the CTx-II of males for BMI, however, there were no statistically significant sport group differences. But for females, there were significant group differences in CTx-II, even after adjusting for BMI, and in the same pattern as the trend for males. From pair-wise analysis, the female cross-country group had significantly higher CTx-II values than all three other groups (swimmers $P < 0.01$, crew $P = 0.02$, and control $P < 0.01$) in unadjusted analyses. After adjusting for BMI, the cross-country CTx-II was still significantly higher than for swimmers ($P < 0.01$) and controls ($P < 0.01$), but not for crew. The combined male and female results [Table III(b)] showed significant group differences in CTx-II both before and after adjusting for BMI. The cross-country runners had significantly higher CTx-II than each of the three other groups (swimmers $P < 0.01$, crew $P = 0.01$, control $P < 0.01$) in unadjusted analyses. These pair-wise differences remained statistically significant (at $P = 0.02$) when adjusted for BMI, except for the cross-country to crew comparison. The combined male and female analyses presumably reflect the larger sample size and hence increased statistical power. The sport-related effects on both biomarker levels also remained highly significant when the data were log transformed or a non-parametric test was applied (not shown).

**Discussion**

Both biomarkers, for bone and cartilage degradation, showed significant differences among the three sports. The high NTx values in crew athletes are probably a result of the high axial and non-axial forces applied to the entire skeleton. Similarly, the high NTx values in runners compared with controls, swimmers and the published normal range ($39 \pm 3^{13}$, probably result from the high amplitude loading of the lower skeleton. Swimming clearly stresses the entire body, but apparently not the skeleton in a way that stimulates bone remodeling. Although we have

![Figure 1](https://example.com/figure1.png)  
**Fig. 1.** Differences between sport groups in the bone collagen resorption marker, urinary NTx. Results are plotted by sex for each sport and control group as medians (bar), second and third quartiles (extent of box), bottom and top quartiles (whiskers) and outlier points.

![Table II](https://example.com/table2.png)  
**Table II**  
(Urinary NTx/creatinine levels for sport activity)  
(a) Mean (s.e.m.) for each sport activity (nmol/mmol creatinine)

<table>
<thead>
<tr>
<th></th>
<th>Cross-country</th>
<th>Swimming</th>
<th>Crew</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Unadjusted</td>
<td>61.7 (10.1)</td>
<td>45.9 (10.1)</td>
<td>92.1 (10.1)</td>
<td>46.9 (11.3)</td>
</tr>
<tr>
<td>Male Adjusted for BMI</td>
<td>66.9 (13.4)</td>
<td>44.9 (10.3)</td>
<td>89.8 (10.8)</td>
<td>44.4 (12.1)</td>
</tr>
<tr>
<td>Female Unadjusted</td>
<td>62.5 (10.3)</td>
<td>31.7 (8.5)</td>
<td>79.6 (11.0)</td>
<td>37.0 (7.8)</td>
</tr>
<tr>
<td>Female Adjusted for BMI</td>
<td>61.1 (10.5)</td>
<td>32.1 (8.8)</td>
<td>80.8 (10.1)</td>
<td>36.8 (9.7)</td>
</tr>
<tr>
<td>Combined Unadjusted</td>
<td>62.1 (6.5)</td>
<td>38.8 (6.5)</td>
<td>85.8 (6.5)</td>
<td>41.9 (7.3)</td>
</tr>
<tr>
<td>Combined Adjusted for BMI</td>
<td>65.1 (7.9)</td>
<td>38.1 (6.6)</td>
<td>83.8 (7.1)</td>
<td>41.4 (7.4)</td>
</tr>
</tbody>
</table>

(b) Statistical significance of pair-wise comparison for different sports and controls (combined males and females)

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted for BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>XC, Swimming</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>XC, Crew</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>XC, Controls</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Swimming, Controls</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Swimming, Crew</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Crew, Controls</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

NS = Not statistically significant.

*Significance of variability across all groups.
measured only NTx, a specific marker of bone resorption, not a formation marker, the two processes tend to be tightly coupled in the long term whether the net result is a gain (adolescence) or loss (menopause) in bone mass. Therefore, NTx can be interpreted as a systemic index of bone remodeling activity. Many studies have reported a positive association between load-bearing exercise and bone mineral density, implying that high NTx values in this young adult population likely reflect net increases in bone mass.

The collagen CTx-II results suggest that swimming, compared with rowing and running, minimally stresses joints, similar to the conclusion from the NTx results for bone. This is consistent with other studies suggesting that sports involving high bone and joint stresses such as distance running and rowing cause cartilage remodeling or degradation beyond that seen in swimming and control subjects. The CTx-II marker is less well characterized than NTx in terms of tissue and processing origins, but elevations in clinical studies are consistent with higher cartilage degradation, for example from active growth plates in children or joints in adults with rheumatoid or osteoarthritis. In the present study, the higher CTx-II levels are presumably from accelerated remodeling in stressed joints. Whether from collagen type II forming the framework of the articular cartilage bearing surfaces, or primarily from the deep calcified interface with bone as we suspect, is unknown. It has been shown that in patients with osteoarthritis, those with higher CTx-II measured by a different antibody have more rapidly progressive joint destruction.

Remodeling of joint tissues probably occurs in response to the effects of normal mechanical loading and injuries from high loads, the latter potentially leading to subsequent degenerative changes. In interpreting the CTx-II results, however, it is important to recognize that joint cartilages account for only a small fraction of systemic type II collagen. Articular cartilage was 8% of the total cartilage in young dogs. The respiratory tract, discs, ribs and other tissues are also major sources. Although respiratory tract cartilages conceivably may be stimulated by high aerobic activity, any such effect might be expected to be similar in all three sports.

Studies quantifying bone-loading patterns during rowing and running are lacking but qualitative differences between the sports are clear. Runners load one leg at a time resulting in axial stresses with high amplitude and frequency. Rowers push off both legs simultaneously so the stress amplitude to each leg is lower than in running. Stride cadence is generally higher than stroke rate so the frequency of loading is higher in running. Non-axial skeletal loads, particularly above the pelvis, are higher in rowing. These biomechanical differences may explain biomarker findings suggesting that rowing is more osteo-stimulatory, but less damaging to joints. Studies in an avian ulna model in vivo...
suggest that lower frequency loading may be more osteogenic than higher frequency loading\textsuperscript{23}. Clearly, this animal experiment is not directly comparable to rowing, but the findings call into question the traditional prescription of high frequency, high amplitude loading as the best method to build bone. If this is true, it has implications for exercise counseling in elderly individuals interested in gaining bone density but wanting to minimize progression of osteoarthritis of weight-bearing joints. A longitudinal study has shown that walking exercise in post-menopausal women decreased NTx levels at 3 months and increased spine BMD\textsuperscript{50}. In a previous report on urinary NTx in college-age male and female track athletes monitored over 12 months, no differences in NTx were seen between those who developed stress fractures and those who did not\textsuperscript{25}. In another study, bone markers and bone mineral density scans by DEXA were compared between groups of female college athletes entered in high-impact (basketball and volleyball), medium-impact (soccer and track), and non-impact (swimming) sports vs sedentary controls\textsuperscript{14}. Higher BMD levels were found in the high-impact group but no differences in NTx. The low subject numbers (7–14 in each group) and other differences in study design prevent meaningful comparison with the present study.

One limitation of the present study is that the molecular and cellular origins of CTx-II in urine, though specific to type II collagen, are less well understood than for NTx as a bone resorption marker. Also, it is known that urinary NTx can show a significant circadian variation with higher levels at night and early morning and lower levels in the afternoon\textsuperscript{30}. The markers might also be influenced by hormonal status, dietary intake and genetic effects\textsuperscript{25}. Short-term effects on the markers from the last period of exercise and time before sample collection are also possible. However, in a study of the acute effects on bone biomarkers of moderate exercise in untrained young men (22 ± 1 year), serum NTx levels fell in the hours after exercise indicating an acute decrease in bone resorption activity, but 24-h urine NTx values did not differ from pre-exercise or control values\textsuperscript{27}. In conclusion, we interpret the observed differences between groups as being primarily due to the effect on skeletal metabolism of chronic training in the individual sports.

Acknowledgements

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References