Serum Relaxin Levels in Young Athletic Men Are Comparable With Those in Women

Jennifer Moriatis Wolf, MD; Kenneth L. Cameron, ATC, PhD; Kari B. Clifton, PhD; Brett D. Owens, MD

Abstract: Relaxin was originally described as a reproductive hormone that mediated joint laxity in pregnant women and has been minimally studied in men. The purpose of this descriptive laboratory and clinical study was to evaluate serum relaxin in a young, primarily male population and compare levels between the sexes. In addition, the authors evaluated the relationship between relaxin and generalized laxity.

Relaxin is a peptide hormone that was first described as a reproductive hormone produced in pregnancy, which acts to loosen pelvic ligaments and soften the cervix in preparation for parturition. Relaxin is also produced in nonpregnant women and has been shown to have multiple physiologic effects in humans, including improvement of cardiac and renal blood flow through vasodilation and prevention of lung fibrosis. In the musculoskeletal system, relaxin’s role in ligamentous laxity has been investigated in the anterior cruciate ligament (ACL) and anterior oblique ligament of the basilar thumb joint. Relaxin secretion has also been demonstrated in men’s reproductive tracts. Relaxin has a key influence on prostate growth and development. Relaxin receptors have also been shown in the ACLs of men, although less consistently than in women. In a study of 9 men and 5 women (mean age, 58 years) with congestive heart failure, serum relaxin levels averaged 20 pg/mL, which was approximately 12-fold lower in age-matched controls. Although normative levels of relaxin have been investigated in pregnant and nonpregnant women, limited data exist on normative serum relaxin levels in men. The majority of reported serum relaxin levels in men are derived from studies of patients with renal or cardiac failure and may be reflective of the effect of chronic disease on this multidimensional hormone. No studies to date have evaluated serum relaxin levels in a cohort of young and physically active men.

The purpose of this study was to evaluate serum relaxin in a large population of young athletic men and to compare serum relaxin levels and the proportion with detectable levels between the sexes. A secondary objective was to evaluate the relationship between relaxin and generalized joint laxity.

Materials and Methods

For this descriptive laboratory and clinical study, the authors obtained serum samples that were derived from blood collected for administrative testing during the admissions process for a military academy. Military cadets are chosen through a rigorous screening process that emphasizes academic excellence, physical fitness and endurance, and the desire to ultimately serve in the military. On entrance, they undergo extensive physical, mental, and academic testing, and the authors obtained serum from blood drawn during this pro-
cess. Blood from 857 men and 135 women was collected during the standard intake physical in the summer of 2006. In addition, a subset of this group was examined during the intake examination using the Beighton-Horan criteria to determine generalized laxity. This study was reviewed and approved by the Keller Army Hospital institutional review board.

Levels of human relaxin-2, the circulating form of relaxin, were determined using enzyme-linked immunosorbent assay (ELISA) sandwich testing (Quantikine Human Relaxin-2 Immunoassay; R&D Systems, Minneapolis, Minnesota). All testing was performed in accordance with the manufacturer’s protocol. Assays were performed in duplicate. Results were determined from the standard curve, extrapolated to 0. The mean minimum detectable dose of this kit is 1 pg/mL (range, 0.26-4.54 pg/mL).

The authors evaluated the primary outcome of interest—serum relaxin levels—similar to the methods used by Dragoo et al. First, 0 values were assigned to all participants who had a serum sample available for analysis but had no detectable serum relaxin values when the sample was analyzed by ELISA. Subsequently, a similar subgroup analysis was performed that included only those participants with detectable serum relaxin levels, defined as a serum relaxin level of 1 pg/mL or more. Means and SDs were calculated for continuous variables, and frequencies and proportions were calculated for categorical variables. Because the relaxin values were not normally distributed, median values and interquartile ranges were also calculated. Pearson’s chi-square test was used to examine whether a greater proportion was found of women with detectable serum relaxin levels compared with men. Finally, between-group differences in serum relaxin values between men and women were also evaluated using the Kruskal-Wallis test. For evaluation of clinical hypermobility testing, Spearman correlations between Beighton scores and serum relaxin levels were performed. All statistical analyses were performed using STATA/SE version 10.1 software (StatCorp, College Station, Texas).

RESULTS

The authors evaluated serum relaxin in 857 men and 135 women, for a total of 992 samples tested. This study population had an average age of 18.26±0.99 years, average height of 176.09±8.70 cm, and average weight of 73.24±12.96 kg. Descriptive values by sex for age, height, weight, and race are presented in Table 1.

Mean average serum relaxin level was 0.50 pg/mL (range, 0-11.54) in men and 0.75 pg/mL (range, 0-14.06) in women (Table 2). These calculations included 0 values in participants in whom circulating relaxin was not measurable. Relaxin was detectable by ELISA in 16% (141/857) of men and 21% (28/135) of women (χ²=1.52; df=1; P=.218). In this subgroup, mean relaxin value was 3.01 pg/mL in men and 3.60 pg/mL in women (Table 2). The proportion of men and women who had detectable serum relaxin levels was similar. Median values were compared by sex for detectable serum relaxin concentrations, which were not significantly different between the groups (χ²=0.001; df=1; P=.978). The Figure provides a box plot comparing serum relaxin levels between men and women who have detectable concentrations.

Clinical joint hypermobility testing was performed in 681 participants, including 598 men and 83 women. Mean Beighton score in this subset was 0.35 (range, 0-7), with a mean score of 0.26 in men and 0.98 in women (P<.001). The Beighton score did not correlate with serum relaxin levels among those with detectable levels only (R=-0.02; P=.82) or for all tested subjects with nondetectable levels set as 0 (R=0.04; P=.28). Similar results were observed when the association between Beighton score and serum relaxin levels was examined for men and women independently.

DISCUSSION

Relaxin was originally described as a reproductive hormone regulating laxity in pregnancy but has been shown to play a wider role in physiological function outside pregnancy. Based on a study in mice where the relaxin gene was absent, relaxin is believed to regulate collagen turnover and have a protective effect against fibrosis in multiple tissues. Previous studies have identified serum relaxin values as a prospective risk factor for subsequent ACL injury in female collegiate athletes. The goals of the current study were to investigate whether circulating relaxin was measurable in young men and to compare relaxin levels between the sexes.

### Table 1

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Mean age, y</td>
<td>18.30±1.02</td>
<td>18.08±0.78</td>
</tr>
<tr>
<td>Mean height, cm</td>
<td>177.97±7.41</td>
<td>164.16±6.58</td>
</tr>
<tr>
<td>Mean weight, kg</td>
<td>75.1±12.56</td>
<td>61.39±8.46</td>
</tr>
<tr>
<td>Race, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>667 (78.19)</td>
<td>99 (73.33)</td>
</tr>
<tr>
<td>Black</td>
<td>33 (3.87)</td>
<td>10 (7.41)</td>
</tr>
<tr>
<td>Asian</td>
<td>38 (4.45)</td>
<td>4 (2.96)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>62 (7.27)</td>
<td>14 (10.37)</td>
</tr>
<tr>
<td>Other</td>
<td>53 (6.21)</td>
<td>8 (5.93)</td>
</tr>
</tbody>
</table>

*Race information missing for 4 men.*
The authors examined serum relaxin concentrations in a young, athletic group of men and women who were entering military college. Relaxin was detectable in this population of young athletic men, with a mean value of detectable serum relaxin of 3.01 pg/mL. To the authors’ knowledge, this is the first study to report reference values for serum relaxin levels in a large group of young, athletic men. Multiple studies have examined relaxin values in women, but the majority of these have focused on pregnancy or are related to reproduction.

No significant difference was observed in serum relaxin levels between men and women when all samples were analyzed or in the subset of participants who had detectable levels of serum relaxin. In addition, the proportion of men and women with detectable serum relaxin levels was similar. However, the majority of relaxin values from ELISA analysis were undetectable in both groups. This may indicate that physiologic relaxin values are far lower than is detectable by current technology or that relaxin is episodically secreted from the reproductive system.

Although previous studies reported relaxin levels as high as 474 pg/mL in nonpregnant women using radioimmunoassay techniques, more recent ELISA techniques have shown lower levels in the current study and others. Other potential effects on serum relaxin in men and women include chronic disease, which is expected to be minimal to none in a military cadet population, or other medications. The authors were unable to demonstrate a relationship between serum relaxin and generalized joint laxity. Other studies have similarly been unable to draw a direct correlation between relaxin level and measures of joint mobility. This may indicate that relaxin indirectly affects the ligaments. Whether serum relaxin levels are a risk factor for ACL and other musculoskeletal injuries in men has yet to be evaluated. Faryniarz et al. noted that relaxin receptors were present in 4 of 5 female ACL samples compared with 1 of 5 male ACL samples using

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of Participants</th>
<th>Mean ± SD Relaxin Value (Range), pg/mL</th>
<th>Percentiles</th>
<th>IQR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10%</td>
<td>25%</td>
</tr>
<tr>
<td>Men</td>
<td>Relaxin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>857</td>
<td>0.50±1.34 (0.00-11.54)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Detectable relaxin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>141</td>
<td>3.01±1.82 (1.03-11.54)</td>
<td>1.25</td>
</tr>
<tr>
<td>Women</td>
<td>Relaxin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>135</td>
<td>0.75±2.06 (0.00-14.06)</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>Detectable relaxin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>28</td>
<td>3.60±3.21 (1.03-14.06)</td>
<td>1.03</td>
</tr>
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</table>

Abbreviation: IQR, interquartile range.

<sup>a</sup>Analysis of serum relaxin measurements in the entire population, including 0 values.

<sup>b</sup>Subgroup analysis for only participants with detectable serum relaxin levels.

The authors were unable to demonstrate a relationship between serum relaxin and generalized joint laxity. Other studies have similarly been unable to draw a direct correlation between relaxin level and joint mobility. This may indicate that relaxin indirectly affects the ligaments. Whether serum relaxin levels are a risk factor for ACL and other musculoskeletal injuries in men has yet to be evaluated. Faryniarz et al. noted that relaxin receptors were present in 4 of 5 female ACL samples compared with 1 of 5 male ACL samples using
radioimmunoassay analysis, and other investigators found no relaxin receptors in the male ACL tissue.\textsuperscript{21}

The results of the current study suggest that no differences exist between men and women in either the proportion with detectable serum relaxin levels or in the median serum relaxin concentrations. As a result, it is possible that young, athletic men with detectable serum relaxin concentrations may also be at increased risk for ACL injury, as has been shown in women,\textsuperscript{6} as well as other ligamentous injuries to the joints; however, further prospective study is needed to evaluate this hypothesis. Serum relaxin levels have also been shown to correlate with decreased patellar tendon stiffness in young women, but this has not been studied in men.

A limitation of this study was the small proportion of participants with detectable relaxin; these numbers may not have sufficient power to determine a difference between sexes. Also, the timing of blood sampling, which was not linked to the menstrual cycle in women, may affect the ability to detect relaxin levels in women. An additional limitation is that the authors did not have the ability to link serum samples to participants’ medical histories or medications, which may have affected relaxin levels.

**Conclusion**

The authors noted detectable serum relaxin levels in a subset of participants drawn from a young, athletic military cadet cohort including men and women, with nonsignificant sex differences. In a subset of two-thirds of this group, the authors found no correlation between relaxin level and generalized joint laxity. These findings provide support to a broader role for relaxin as a hormonal regulator of collagen homeostasis rather than simply a reproductive hormone.\textsuperscript{8}

**References**