ORIGINAL RESEARCH

Trends in serum relaxin concentration among elite collegiate female athletes

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Purpose: This study was designed to investigate the relationship between serum relaxin concentration (SRC) and menstrual history and hormonal contraceptive use among elite collegiate female athletes. Evaluation of SRC in athletes is necessary, because relaxin has been associated with increased knee joint laxity and decreased anterior cruciate ligament (ACL) strength in animal models.

Methods: National Collegiate Athletic Association Division I female athletes participating in sports at high risk for ACL tears – basketball, field hockey, gymnastics, lacrosse, soccer, and volleyball – were invited to participate. All participants completed a questionnaire about their menstrual history and hormonal contraceptive use. Venipuncture was performed to obtain samples of serum progesterone and relaxin. Samples were obtained during the mid-luteal phase from ovulating participants, and between the actual or projected cycle days 21 to 24, from anovulatory participants. Serum concentration of relaxin and progesterone was determined by ELISA and the data were analyzed using SPSS statistical software with significance set at $P = 0.05$.

Results: 169 female athletes participated. The mean SRC among all participants was 3.08 ± 6.66 pg/mL). The mean SRC differed significantly between those participants using hormonal contraceptives (1.41 pg/mL) and those not using hormonal contraceptives (3.08 pg/mL, $P = 0.002$). Mean SRC was lowest among amenorrheic participants (1.02 pg/mL) and highest among oligomenorrheic participants (3.71 pg/mL) and eumenorrheic participants (3.06 pg/mL); these differences were not significant ($P = 0.53$). Mean serum progesterone concentration (SPC) differed significantly between those participants using hormonal contraceptives (2.80 ng/mL), and those not using hormonal contraceptives (6.99 ng/mL, $P < 0.0001$).

Conclusions: There is a positive correlation between serum progesterone and SRC and an attenuation of SRC with hormonal contraceptive use. Our results underscore the significant role that hormonal contraceptives can play in decreasing relaxin levels, if future investigations establish a link between relaxin levels and ligamentous injury among female athletes.

Keywords: hormones, ACL, anterior cruciate ligament, hormonal contraceptives, menstrual cycle

Introduction

Relaxin is a peptide hormone that belongs to the insulin superfamily of hormones.[1,2] Recent research on this hormone has revealed that it has three forms in humans,[3] which have unique sites of production[4–6] and a broad range of physiologic effects throughout the body. The focus of this study is on Relaxin-2, which is produced by the corpus luteum,[7] decidua,[8–10] and placenta[6,10] and is transcriptionally regulated by progesterone and glucocorticoid hormones.[11] Relaxin-2 has historically been studied in pregnant

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women. Because it is also found in the serum of nonpregnant women,[12–14] its effects on nonreproductive tissue merits further investigation.

Research has established that the function of Relaxin-2 is to facilitate the remodeling of collagen through the upregulation of collagenases,[15,16] matrix metalloproteinases,[17] and tissue plasminogen activator (tPA).[15,17] Because of its well-established role as a female reproductive hormone, the collagenolytic effects of Relaxin-2 have been studied primarily with respect to its effect on pregnancy and parturition,[18,19] myometrial pathology,[20] urinary incontinence,[21] and premenstrual dysphoric disorder[22] in middle-aged women. More recently, a number of studies have begun to investigate the role of relaxin in nonreproductive organ and tissue disease processes.[23–26]

Relaxin has been associated with increased knee joint laxity in pregnant women[27] and decreased anterior cruciate ligament (ACL) strength in animal models.[28] The established presence of relaxin receptors on the female ACL[29,30] also underscores that relaxin-mediated remodeling of female ligaments is an important mechanism to investigate. To date, no studies have described the serum relaxin concentration (SRC) among a large sample of young, healthy, nulligravidae elite female athletes. Understanding SRC among this population is important, because relaxin has been shown to increase ligament laxity among women.[27,31,32]

This study was designed to investigate the relationship between SRC and menstrual history and hormonal contraceptive use among elite collegiate female athletes. Our hypothesis is that athletes who are on hormonal contraceptives, or who are amenorrheic, will have significantly lower levels of relaxin than those who are not on hormonal contraceptives or those who are eumenorrheic or oligomenorrheic, because these athletes will be anovulatory and not producing the corpus luteum thought to be necessary to secrete Relaxin-2.

Methods

After institutional review board approval was obtained, National Collegiate Athletic Association Division I female athletes who participate in sports identified as high risk for ACL tears – basketball, field hockey, gymnastics, lacrosse, soccer, and volleyball – were recruited to participate. Athletes with a history of pregnancy were ineligible to participate. Of the approximately 190 eligible athletes, 169 agreed to participate (89% response rate). The desired sample size was at least 90, to reach 80% power to detect a 10% difference in mean SRC between 2 groups.

All participants completed a questionnaire on their demographic information, menstrual history, pregnancy history, and hormonal contraceptive use. This information was verified by review of participant medical records. Urine luteinizing hormone (LH) testing was used to confirm when to obtain blood samples from the participants, because samples had to be obtained during the mid-luteal phase of the menstrual cycle when relaxin is known to peak.[8,13] Participants who reported being eumenorrheic (10–12 menstrual periods per year) or oligomenorrheic (5–9 menstrual periods per year), and who were not on any form of hormonal contraception, underwent self-testing with urine LH detection tests (Ovulation Midstream Tests, Early-pregnancy-tests.com, Bellingham, WA, USA) to determine the date of their LH surge. LH testing was conducted for 5 days on average starting 2 days before the anticipated date of ovulation. If a positive LH test was not obtained during the first round of testing, more tests were given for a longer timeframe during the subsequent menstrual cycle and the testing start date was adjusted, based on the newly determined cycle length. Once participants had reported a positive LH test, they were scheduled for a blood draw 7 to 10 days after the date of the positive LH test, which is the mid-luteal window when the relaxin surge is expected to occur.

Participants who reported being amenorrheic (4 or fewer menstrual cycles per year) did not undergo LH testing since they were assumed to be anovulatory. Instead, amenorrheic participants were scheduled for a blood draw 21 to 24 days after they were projected to have had their last menstrual period (LMP). The projected day zero of their LMP was based on a forward calculation of assuming regular cycles from their last confirmed LMP. Participants who reported being on a form of hormonal contraception to suppress ovulation, regardless of their menstrual history, were scheduled to have their blood taken 21 to 24 days after the start of their LMP. Urine pregnancy tests (hCG Midstream Tests, Early-pregnancy-tests.com, Bellingham, WA, USA) were given when blood was taken, to verify that participants were not pregnant.

Venipuncture was performed using a BD Vacutainer Blood Collection System and approximately 3 to 4 mL of blood was collected. The plasma sample was left standing undisturbed at room temperature for 30 minutes to allow for adequate clotting. The sample was then placed in a centrifuge (Quest Diagnostics VanGuard V6500, Madison, NJ, USA) and spun for 15 minutes at 3000 rpm to separate the serum and blood cell layers. After centrifugation, the serum was extracted by pipette and aliquotted into two 1-mL

samples in Nalgene vials. All serum samples were coded with unique identifiers to ensure that the lab technician was blinded to their content. All samples were stored at 80°C until analysis.

A Quantikine Human Relaxin-2 Immunoassay (DRL200, R&D Systems Inc., Minneapolis, MN, USA) was used according to the manufacturer's protocol to determine the SRC of the samples. The intra-assay and inter-assay coefficients of variation for mean SRC (34.4 pg/mL and 40.8 pg/mL) were 4.7% and 10.2%, respectively. The mean minimum detectable dose was 1 pg/mL. All samples were run in triplicate and serum relaxin concentration values were calculated based on the standard curve, including the zero dose standard. The assay utilized the standard quantitative sandwich enzyme immunoassay technique.

A Progesterone ELISA (BQ 129S, BioQuant, San Diego, CA, USA) was used to determine the progesterone concentrations in the samples in order to verify that those who were on hormonal contraceptives did not have ovulatory cycles. The intra-assay and inter-assay coefficients of variation for mean progesterone concentration (1.72 ng/mL and 2.16 ng/mL) were 5.36% and 9.68%, respectively. All samples were run in duplicate according to the manufacturer's protocol. The threshold for determining was a progesterone concentration of 3.3 ng/mL, while 4.4 ng/mL was deemed the cut-off for determining mid-luteal phase.

ANOVA analysis was used to determine the difference between mean SRC and mean progesterone, based on menstrual history (eumenorrheic, oligomenorrheic, and amenorrheic) and t tests were used to determine differences between groups based on hormonal contraceptive use and ovulatory status. Pearson correlations were done to assess the relationship between relaxin and progesterone, as well as between body mass index (BMI) and relaxin. Significance was set at $P = 0.05$.

Results

All 169 participants (23 from basketball, 24 from field hockey, 39 from gymnastics, 38 from lacrosse, 24 from soccer, and 21 from volleyball) provided adequate blood samples for analysis. The mean age of participants was 19.49 ± 1.34 years. The mean BMI was 22.42 ± 1.83. There was no significant correlation between BMI and SRC ($r = 0.02$, $P = 0.81$) and no significant correlation between BMI and progesterone levels ($r = 0.03$, $P = 0.67$). Overall, the majority (123, 72.8%) of participants were eumenorrheic, 36 (21.3%) were oligomenorrheic, and 10 (5.9%) were amenorrheic. The majority (99, 58.6%) of participants

were not using a hormonal form of contraceptive compared with the 70 (41.4%) who were using a form of hormonal contraceptive. Of the women on hormonal contraceptives, the vast majority used combination oral contraceptive (COC) pills, 2 were using the NuvaRing, and 1 was on a progesterone-only contraceptive pill (Nora-BE). Of participants on COCs, 66% were on either Yasmin, Orthotricyclen Lo, Loestrin, or Trinessa, and the remainder were on either Zovia, Alesse, Desogen, Enpresse, Kavina, Diane-35, Ovcon, Fecon, Microgestin, OrthoEvra, Mycrogynon, TriSpintec, or Junel. Within the hormonal contraceptive-using group, 4 (6%) were amenorrheic, 19 (29%) were oligomenorrheic, and 43 (65%) were eumenorrheic.

The mean SRC among all participants was 3.08 ± 6.66 pg/mL (range, 0–50.38 pg/mL). Three participants had SRCs greater than 1 standard deviation (SD) above the mean (>9.11 pg/mL), 3 had SRCs greater than 2 SDs above the mean (>15.77 pg/mL), and 2 had SRCs greater than 3 SDs above the mean (>22.43). As outlined in Table 1, there was a significant difference between the mean SRC among participants using hormonal contraceptives (1.41 ± 3.50 pg/mL) compared with those not using hormonal contraceptives (4.25 ± 8.02 pg/mL, $P = 0.002$). ANOVA analysis revealed no statistically significant difference in mean SRC between the participants based on menstrual history ($P = 0.53$). However, the mean SRC was lowest among amenorrheic participants (1.02 ± 2.47 pg/mL) and highest among oligomenorrheic participants (3.71 ± 8.86 pg/mL) and eumenorrheic participants (3.06 ± 6.14 pg/mL). Among the women not using hormonal contraceptives, there was a significant difference in SRC ($P = 0.003$) and in serum progesterone concentration (SPC) ($P < 0.0001$) when analyzed by ovulatory status.

The mean serum progesterone concentration (SPC) among all participants was 5.25 ± 4.58 ng/mL (range, 1.09–18.84 ng/mL). There was a significant difference between mean SPC among participants using hormonal contraceptives (2.80 ± 1.76 ng/mL) versus participants not using hormonal contraceptives (6.99 ± 5.15 ng/mL, $P < 0.0001$). ANOVA analysis revealed no significant difference in mean SPC between participants based on menstrual history alone ($P = 0.45$). The mean SPC was lowest among amenorrheic participants (3.53 ± 2.20 ng/mL), second lowest among oligomenorrheic participants (5.13 ± 4.93 ng/mL), and highest among eumenorrheic participants (5.43 ± 4.62 ng/mL). In the hormonal contraceptive group, 21 participants (30%) had SPCs above the 3.3 ng/mL threshold for determining ovulation. Of the participants not using hormonal contraception,

Table 1 Mean serum relaxin concentration, serum progesterone concentration, body mass index (BMI), and participant age

	Serum relaxin concentration (pg/mL)	Serum progesterone concentration (ng/mL)	BMI	Participant age (years)
All participants (N = 169)	**3.08 ± 6.66**	**5.25 ± 4.58**	**22.42 ± 1.83**	**19.49 ± 1.34**
No hormonal contraceptive (N = 99)	**4.25 ± 8.02***	**6.99 ± 5.15****	**22.35 ± 1.80**	**19.11 ± 1.20**
Ovulatory (71)	5.30 ± 9.08[a]	8.79 ± 5.04[b]	22.28 ± 1.78	19.17 ± 1.27
Nonovulatory (28)	1.61 ± 3.08[a]	2.42 ± 0.49[b]	22.27 ± 1.86	18.96 ± 1.04
Hormonal contraceptive (N = 70)	**1.41 ± 3.50***	**2.80 ± 1.76****	**22.53 ± 1.90**	**20.01 ± 1.35**
Ovulatory (21)	2.12 ± 5.39	4.88 ± 1.85[c]	21.99 ± 1.96	19.62 ± 1.28
Nonovulatory (49)	1.11 ± 2.18	1.91 ± 0.54[c]	22.77 ± 1.84	20.18 ± 1.35
Amenorrheic (N = 10)	**1.02 ± 2.47**	**3.53 ± 2.20**	**22.15 ± 2.22**	**19.10 ± 1.66**
Ovulatory (4)	0.0 ± 0.0	5.47 ± 2.40	22.15 ± 2.52	19.0 ± 2.0
Nonovulatory (6)	1.70 ± 3.09	2.23 ± 0.46	22.51 ± 2.24	19.17 ± 1.60
Oligomenorrheic (N = 36)	**3.71 ± 8.86**	**5.13 ± 4.93**	**21.98 ± 1.61**	**19.42 ± 1.25**
Ovulatory (17)	6.67 ± 12.13	8.48 ± 5.51[d]	21.86 ± 1.49	19.53 ± 1.28
Nonovulatory (19)	1.05 ± 2.45	2.14 ± 0.55[d]	22.09 ± 1.75	19.32 ± 1.25
Eumenorrheic (N = 123)	**3.06 ± 6.14**	**5.43 ± 4.62**	**22.58 ± 1.85**	**19.54 ± 1.34**
Ovulatory (71)	4.32 ± 7.56[e]	7.89 ± 4.72[f]	22.40 ± 1.86	19.23 ± 1.24
Nonovulatory (52)	1.33 ± 2.54[e]	2.06 ± 0.60[f]	22.82 ± 1.83	19.96 ± 1.36

Notes: *$P = 0.002$, difference in mean serum relaxin concentration (SRC); **$P < 0.0001$, difference in mean serum progesterone concentration (SPC); [a]$P = 0.003$, difference in mean SRC; [b]$P < 0.0001$, difference in mean SPC; [c]$P < 0.0001$, difference in mean SPC; [d]$P < 0.0001$, difference in mean SPC; [e]$P = 0.003$, difference in mean SRC; [f]$P < 0.0001$, difference in mean SPC.

28% had SPCs below the 3.3 ng/mL cutoff for ovulatory cycles; 2 amenorrheic, 14 oligomenorrheic, and 17 eumenorrheic participants had SPCs below the 4.4 ng/ML threshold for determining mid-luteal phase. Lastly, as depicted in Table 2, there was a significant correlation overall, and among noncontracepting, ovluatory, oligomenorrheic and eumennorheic participants, between serum progesterone and relaxin concentrations.

Discussion

This is the largest study to assess SRC among young, healthy, nulligravidae, elite collegiate female athletes. It is

Table 2 Pearson correlation between serum relaxin concentration and serum progesterone concentration

	Correlation coefficient (R)	P-value
Overall	0.49	<0.01
Amenorrheic	−0.13	0.71
Oligomenorrheic	0.63	<0.01
Eumenorrheic	0.44	<0.01
Hormonal contraceptive	0.01	0.93
No hormonal contraceptive	0.50	<0.01
Ovulatory	0.45	<0.01
Nonovulatory	0.21	0.07

important to understand the SRC in this population because of the possible effect of relaxin on ligament stability among athletes. Since relaxin is involved in collagen remodeling, and collagen is an important structural element of ligaments, it is plausible that women with increased relaxin levels may have an increased risk of ligament injury, which could then make them more susceptible to injuries such as ACL rupture. Before investigating any correlation between these types of injuries and relaxin levels, however, further investigation of relaxin concentrations in this population is necessary.

We found that our sample of collegiate female athletes had a relatively low range of SRCs (0–50.83 pg/mL) compared with the SRCs reported by Arnold et al among a similar population of athletes (291–616 pg/mL).[33] The SRCs among our athletes were also lower than those reported by Stewart[13] among women with nonconceptive cycles (30–150 pg/mL). Additionally, these SRCs were lower than those reported among older parous women by Binder et al (260–620 pg/mL, Santa Cruz Biotechnology Inc. assay),[23] by Pehrrson et al (144–4500 pg/mL, Immunodiagnostix assay),[22] and by Wreje et al (20–255 ng/L, Genentech reagents).[34] Just as all these studies reported 1 to several participants with unexpectedly high SRCs, our study also revealed outliers. Four athletes had values greater than 1 SD

above the mean (>9.38 pg/mL) and 3 had values greater than 2 SDs above the mean (>16.22 pg/mL). However, all samples had an SRC <150 pg/mL, which is consistent with the SRCs previously reported for nonconceptive menstrual cycles.[13] It is possible that the variation between our SRCs and those previously reported could be explained by a difference in the specificity of the various assays that have been used in these different studies. The relatively new R&D Systems assay (launched in August 2007) used in this study may be more specific for Relaxin-2, which would explain why our levels were lower than those previously reported. The R&D assay reports no significant cross-reactivity or interference with human insulinlike growth factor (IGF)-1, IGF-2, Insulin (aa 25–110), Insulin (pro) K (aa 25–110) N His, IGF-3, Relaxin-1, Relaxin-3. However, the majority of previously used assays do not report their level of specificity or cross-reactivity profiles. Only a couple have reported having no cross reactivity with insulin,[22,23] or IGF-1.[23] Lastly, this difference could also reflect a sustained increase in SRC among primi- or multiparous women, which makes their SRCs significantly higher than those of nulligravidae women.

Also as expected, there was a significant difference between mean SRC and SPC among participants who used hormonal contraceptives and those who did not. Hormonal contraceptives suppress ovulation and therefore prevent the formation of a corpus luteum and subsequently prevent the production of relaxin and progesterone. Consequently, it is logical that women on hormonal contraceptives would have lower levels of relaxin and progesterone.

The primary challenge of this study was the timing of the venipuncture to ensure that the serum hormone concentration results could be compared across groups. We attempted to standardize this process by having a set window (days 21–24) for venipuncture among the hormonal contraceptive group and days 7 to 10 after a positive LH test in those not on hormonal contraceptives. However, given that approximately 28% of the participants in the group not on hormonal contraceptives had SPCs below the 3.3 ng/mL threshold for determining ovulation, it is likely that they were either having anovulatory cycles or that the samples were drawn in the follicular phase. Conversely, it was also interesting to find that approximately 30% of participants on hormonal contraceptives had ovulatory cycles as indicated by SPC >3.3 ng/mL. This could represent a failure rate in the use of the hormonal contraceptives which, however, would be twice as high as typical use failure rates and is therefore unlikely to be explained completely by COC misuse. It is more likely that some of the higher SPCs in this group were from adrenally produced progesterone.

Conclusions

This study has reinforced the strong correlation between progesterone and relaxin levels and corpus luteum production. The finding that hormonal contraceptive use attenuates serum progesterone and relaxin levels is also significant. Should future investigations establish a link between relaxin levels and ligamentous injury among women, our results underscore the significant role that hormonal contraceptives can play in decreasing relaxin levels. Future studies should continue to characterize the levels and behavior of relaxin among young nulligravidae women in addition to the variation in cross-reactivity and sensitivity among relaxin assays to improve the uniformity of SRC quantitation.

Acknowledgments

Disclosure

The authors declare no conflicts of interest.

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