Absolute Serum Hormone Levels Predict the Magnitude of Change in Anterior Knee Laxity across the Menstrual Cycle

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Shultz, S.J., Gansneder, B.M., Sander, T.C., Kirk, S.E., & Perrin, D.H. (2006). Absolute serum hormone levels predict the magnitude of change in anterior knee laxity across the menstrual cycle. *Journal of Orthopaedic Research*, *24*(2), 124-131. DOI:10.1002/jor.20021.

Abstract:

This study aimed to determine whether absolute sex hormone concentrations predict the magnitude of knee joint laxity changes across the menstrual cycle. Twenty-two females (18–30 years, body mass index ≤30), who reported normal menstrual cycles for the previous 6 months were tested daily across one complete menstrual cycle for serum levels of estradiol (E = pg/mL), progesterone (P = ng/mL), and testosterone (T = ng/dL), and knee joint laxity (K_{Lax} = mm displacement at 134*N*) measured with a standard knee arthrometer. The change in K_{Lax} across the cycle (maximum–minimum), and minimum (early follicular) and peak (postovulatory) hormone concentrations were recorded for each subject. A stepwise linear regression determined if the minimum, peak, or absolute change in hormone concentrations would predict the magnitude of change in K_{Lax} across the cycle. K_{Lax} changed on average 3.2 ± 1.1 mm across the menstrual cycle (range, 1.5–5.3 mm). Minimum levels of E (39.9 ±11.8 pg/mL) and P (0.61 ± 0.27 ng/mL), coupled with peak concentrations of E (199.6 ± 54.9 pg/mL) and T (22.5 ± 10.5 ng/dL) explained 57.6% of the change in K_{Lax} across the cycle. Greater absolutechanges in K_{Lax} were observed in response to peak E and T levels when minimum E concentrations were lower and minimum P concentrations were higher in the early follicular phase. The absolute minimum concentrations of E and P in the early follicular phase appear to be important factors in determining the sensitivity of the knee joint's response to changing hormone levels.

Article:

INTRODUCTION

Anterior knee laxity changes across the menstrual cycle,[1–3] and these changes are largely dependent on changes in sex hormone concentrations.[4] We have found that significant increases in knee laxity occurred between the early follicular phase and early luteal phase, and that estradiol, progesterone, testosterone, and their interactions explained on average ~63% of the change in knee laxity across the cycle when a time delay (~3–4 days) was considered.[3,4] However, the length of the time delay (from when hormone levels change to when knee laxity changes), the relative contribution of each hormone to the variance explained, and the amount of knee laxity change (~1–5 mm) that occurs across the cycle varied widely between subjects. Because these knee laxity changes are fairly transient,[3] this variability makes it difficult to identify a specific day or range of days to represent the same point in the cycle for all females,

and requires almost daily tracking to know when and if a particular female will experience significant knee laxity changes from month to month. This variability may also explain why some studies have failed to show cyclic changes in knee laxity across the menstrual cycle based on a single, representative test day for each phase.[5,6] While this intersubject variability creates challenges both for the clinician and researcher, it is consistent with the variability in hormone profiles between females.[7]

It is well known that sex hormone levels, particularly estrogen and progesterone, fluctuate across the menstrual cycle and vary by phase. Although rarely considered when describing the female menstrual cycle, testosterone concentrations also vary across the cycle, typically peaking during the middle third of the cycle.[8,9] These cyclic variations in estrogen, progesterone, and testosterone can be quite different between individual females in regards to cycle length (both follicular and luteal phases), hormone phasing, day of ovulation, and absolute changes in hormone levels across the cycle.[7,10–12] Hence, it is not surprising that if sex hormones play a primary role in mediating changes in knee laxity, this variability in hormone profiles might explain why some women experience marked changes in knee laxity across the cycle while others do not.

In an effort to better understand the hormonal profile that mediates substantial changes in knee laxity across the menstrual cycle, our purpose was to determine whether the minimum, peak, or the absolute change in hormone levels would explain the intersubject variability in the magnitude of knee laxity changes. We hypothesized that the absolute magnitude of change in hormone levels across the cycle would predict greater changes in knee laxity.

METHODS

Subjects

Data for this study were from a larger project on gender, hormones, and knee laxity,[3,4] and consisted of 22 females [23.0 ± 3.5 years; 163.4 ± 5.6 cm; 65.0 ± 11.6 kg; 24.3 ± 3.5 body mass index (BMI); 2.7 ± 2.1 h of physical activity per week), who reported normal menstrual cycles (28–32 days) over the previous 6 months. A sample size of 20 subjects was determined a priori through pilot analysis, and inclusion criteria were no history of pregnancy, no use of oral contraceptives or other hormone-stimulating medications for 6 months, nonsmoking behavior, two healthy knees with no prior history of joint injury or surgery, no medical conditions affecting the connective tissue (e.g., Marfan's syndrome, Ehlers–Danlos disease, rheumatoid arthritis, etc.), and physical activity limited to 7 h or less per week to reduce the likelihood of irregular or anovulatory menstrual cycles that can occur with high volume or high intensity training.[13,14] Participants were excluded if they experienced an anovulatory cycle (ovulation kit did not test positive or progesterone levels rose <3 ng/mL[15,16]) or if they missed 3 or more consecutive days of testing during the cycle. Because of the daily data collection needs of this project, a total of 25 subjects were originally recruited to insure 20 subjects completed the study. Two subjects were lost due to exclusionary criteria (one experienced an anovulatory cycle and one lost more than 3 days of data collection due to an equipment failure), and one subject voluntarily withdrew prior to completing the study. All subjects were informed of the study and associated risks, and signed an informed consent approved by the University Health System's Human Investigation Committee and General Clinical Research Center's Research Advisory Committee.

Procedure

Participants were instructed to begin using a commercially available ovulation kit {CVS One Step Ovulation Predictor [sensitivity, 20 mIU/mL luteinizing hormone (LH); accuracy 99%]; CVS Corporation, Woonsocket, RI} on day 8 of their menstrual cycle, and were asked to report to the research study coordinator the day the test became positive. Day of ovulation was confirmed to (1) insure an ovulatory menstrual cycle had occurred; (2) provide a common reference point by which to counterbalance participants and to mark the beginning and ending of data collection; and (3) provide indirect confirmation that female subjects were not pregnant.

Hormone assays and knee joint testing were performed in the University's General Clinical Research Center. Participants were tested daily across one complete menstrual cycle, undergoing the same data collection procedures on each day of testing. Testing was performed in the morning (8:00 A.M.–12:00 P.M.) to obtain the most stable concentrations[17] and to control for diurnal fluctuations in hormone levels.[18] Within this window, every attempt was made to bring subjects in at the same time each day. However, some flexibility was needed to accommodate participant's class and work schedules given the daily data collection requirements. Participants were counterbalanced to begin and end data collection either at ovulation (ovulation kit detecting the leutinizing hormone surge), or the onset of menses (self-report of the first day of menstrual bleeding). The test examiner was blinded to the participant's time in the cycle.

On each day of testing, 5–7 cc of venous blood were drawn to assay serum levels of estradiol (pg/mL), progesterone (ng/mL), and testosterone (ng/dL). Estradiol was analyzed using a double-antibody RIA Assay (DSL-4400; Diagnostic Systems Laboratories, Webster, TX). Progesterone and testosterone levels were analyzed using chemiluminescence assays (Coat-A-count; Diagnostic Products Corporation, Los Angeles, CA). Mean percentage coefficient of variations (% CV) ranged from 3.9%–14.1% (intra-assay) and 2.8%–16.3% (interassay) for estradiol, 3.4%–10.0% (intra-assay) and 3.8%–12.0% (interassay) for progesterone, and 4.5%–11.3% (intra-assay) and 5.2%–13.8% (interassay) for testosterone. Assay sensitivities for estradiol, progesterone, and testosterone were 1.5 pg/mL, 0.1 ng/mL, and 10 ng/dL, respectively.

Knee laxity was measured as the amount of anterior tibial displacement at 133*N* with a KT 2000™ knee arthrometer (MEDmetric® Corp; San Diego, CA). Using manufacturer's guidelines, subjects were positioned in supine with (1) a thigh support placed just proximal to the popliteal fossa to support the knees in 25° of flexion, (2) the ankles placed in the manufacturer-provided foot cradle, and (3) a Velcro strap placed around the thighs to control rotation of the lower extremity. With the KT-2000™ properly positioned on the anterior tibia of the lower extremity (side counterbalanced between participants), the participants were instructed to relax the thigh muscles, and an anterior-to-posterior directed force was applied to the anterior tibia to identify a stable neutral point, followed by an anterior directed force just over 133*N*. A bubble level was affixed to the device to insure a direct, anterior line of pull. Five trials were collected on each test day, and the average of the middle three trials was recorded as the participant's knee laxity measure. While a single investigator performed the majority of knee laxity measures throughout the study, a second investigator was trained and utilized to perform knee laxity measures when the primary tester was unavailable. Excellent intertester and intratester reliability for these investigators was established and previously reported.[4]

Data Analysis

Hormone concentrations (estradiol, progesterone, testosterone) and anterior knee laxity at 133*N* were recorded for each day of the menstrual cycle. The range in knee laxity values for each female was determined by subtracting the minimum from the maximum values obtained across their cycle. Minimum and maximum values for estradiol, progesterone, and testosterone were also obtained during the early follicular (first 7 days of the cycle when sex hormones are generally lowest) and the luteal phase (postovulation), respectively. A stepwise multilinear regression was used to determine whether the minimum, maximum, or the absolute change in hormone levels from follicular to luteal phases would predict the magnitude of change in knee laxity across the cycle. With this procedure, the hormone variable with the highest correlation with knee laxity change was entered first, followed by the next hormone variable with the highest significant partial correlation with knee laxity change once the effects of the previous variable was accounted for. We continued this process until the addition of further variables did not significantly improve the prediction equation. This process maximized the prediction accuracy with the smallest number of predictors, and insured that all variables entered in the model were significant contributors to the model.

RESULTS

Table 1 provides the descriptive statistics for knee laxity change and minimum and maximum serum estradiol, progesterone, and testosterone levels. All minimum and peak hormone concentrations were within normal ranges.[19] Tables 2 and and33 list the Pearson (bivariate, zero-order) correlations and the regression model summary results, respectively, from the stepwise regression analysis. Minimum levels of estradiol (Est_{min}) and progesterone ($Prog_{min}$) during the follicular phase and peak levels of estradiol (Est_{peak}) and testosterone ($Test_{peak}$) each contributed significantly to the regression model and collectively accounted for 57.6% of the variance in knee laxity change across the menstrual cycle (Table 3). In the stepwise regression, the $Prog_{min}$ entered the model first with the highest zero-order correlation with knee laxity change (0.491), explaining 20% of the variance in the knee laxity change across the cycle. Once $Prog_{min}$ was accounted for, Est_{peak} had the highest partial correlation ($r_{partial} = 0.535$) with knee laxity change and entered the model next, followed by Est_{min} ($r_{partial} = -0.422$), which together explained an additional 27.7% of the variance. $Test_{peak}$ ($r_{partial} = 0.479$) was the last to enter, and increased the variance explained by another 9.5% (Table 3). $Test_{min}$ and $Prog_{peak}$ did not contribute significantly to the regression model and were therefore excluded. The regression equation for the four predictor model is as follows:

Table 1: Means ± Standard Deviation and Range for Minimum and Peak Estradiol, Progesterone, and Testosterone Levels, and Knee Laxity Change across the Menstrual Cycle (*N* = 22)

	Means ± SD	Range
Est_{Min} (pg/mL)	39.91 ± 11.82	23.30–57.50
$Prog_{Min}$ (ng/mL)	0.61 ± 0.27	0.30–1.10
$Test_{Min}$ (ng/dL)	22.50 ± 10.49	10.00–48.00
Est_{Peak} (pg/mL)	203.02 ± 53.60	85.57–295.00
$Prog_{Peak}$ (ng/mL)	12.32 ± 4.30	4.70–20.70
$Test_{Peak}$ (ng/dL)	66.00 ± 6.56	37.00–115.0
Knee Laxity Change (mm)	3.19 ± 1.09	1.51–5.31

Min, minimum concentration; Peak, peak concentration; Est, estradiol; Prog, progesterone; Test, testosterone.

Table 2: Pearson Correlations for Variables Entered in the Stepwise Regression Model

	Knee Laxity$_{Change}$	Est$_{Min}$	Prog$_{Min}$	Test$_{Min}$	Est$_{Peak}$	Prog$_{Peak}$	Test$_{Peak}$
Knee Laxity$_{Change}$	1.000	−0.428*	0.491*	0.004	−0.005	−0.078	0.326
Est$_{Min}$		1.000	−0.068	0.345	−0.093	0.190	0.282
Prog$_{Min}$			1.000	0.092	−0.693*	−0.043	0.011
Test$_{Min}$				1.000	0.155	0.114	0.293
Est$_{Peak}$					1.000	0.030	0.223
Prog$_{Peak}$						1.000	0.108
Test$_{Peak}$							1.000

Min, minimum concentration; Peak, peak concentration; Est, estradiol; Prog, progesterone; Test, testosterone. *$P \leq 0.05$.

Table 3: Stepwise Regression Model Summary

					Change Statistics				
Model	***R***	***R*^{2}**	**Adjusted *R*^{2}**	**Standard Error of the Estimate**	***R*^{2} Change**	***F* Change**	**df$_1$**	**df$_2$**	**Significant F Change**
1	0.491	0.242	0.204	0.969	0.242	6.369	1	20	0.020
2	0.677	0.459	0.402	0.840	0.217	7.618	1	19	0.012
3	0.745	0.555	0.481	0.782	0.096	3.896	1	18	0.064
4	0.811	0.657	0.576	0.707	0.102	5.054	1	17	0.038

Min, minimum concentration; Peak, peak concentration; Est, estradiol; Prog, progesterone; Test, testosterone.
[11]Predictors: (Constant), Prog$_{Min}$.
[22]Predictors: (Constant), Prog$_{Min}$, Est$_{Peak}$.
[33]Predictors: (Constant), Prog$_{Min}$, Est$_{Peak}$, Est$_{Min}$.
[44]Predictors: (Constant), Prog$_{Min}$, Est$_{Peak}$, Est$_{Min}$, Test$_{Peak}$.

$$\text{Laxity Change} = 0.03572 + 2.82900\,(\text{Prog}_{\text{min}}) + 0.00738\,(\text{Est}_{\text{peak}}) - 0.04115(\text{Est}_{\text{min}}) + 0.02381(\text{Test}_{\text{peak}})$$

Examination of the regression coefficients revealed that when minimum progesterone concentrations were higher and minimum estradiol concentrations were lower during the early follicular phase, females experienced greater increases in knee laxity across the menstrual cycle with attainment of peak estradiol and testosterone levels postovulation. While the zero-order correlation showed no relationship between peak estradiol concentrations and knee laxity change values (Table 2; r = −0.005), their partial correlation increased considerably ($r_{partial}$ = 0.535), once minimum progesterone concentrations were accounted for.

DISCUSSION

Our earlier report indicated that sex hormones explain, on average, 63% of the change in knee laxity across the menstrual cycle within individual females.[4] However, we could not determine from these data why some females experienced marked changes in knee laxity while others did not. Because females varied considerably in their nadir and peak hormone concentrations, we sought to determine in the current analysis the extent to which these concentrations may predict the magnitude of knee laxity change each female experienced. Our primary findings revealed that when progesterone levels were higher and estrogen levels were lower during the early follicular phase (i.e., a more androgenic environment), females experienced a greater increase in knee laxity once estrogen and testosterone levels peaked. Although simple bivariate correlations revealed no relationship between peak estradiol levels and knee laxity change, the relationship between these variables was much stronger once minimum progesterone levels were accounted

for. These findings suggest that the absolute minimum concentrations of estradiol and progesterone in the early follicular phase play an important role in determining the threshold of the knee joint's sensitivity to rising estrogen and testosterone levels. Because both absolute minimum and maximum values were found to play a significant role in predicting knee laxity changes, we believe our hypothesis was in large part supported.

Pulsatile secretion is characteristic of many hormones, and is critical to the triggering of hormone-dependent responses to target cells.[19,20] For example, a fall in plasma estradiol is necessary to trigger normal menstrual cycle events, specifically the rise in follicle stimulating hormone (FSH) that initiates follicular recruitment at the beginning of the menstrual cycle.[10,19] In the case of growth hormone, continuous versus intermittent presence of growth hormone dictates the responsiveness of tissues and, ultimately, their sex-differentiated gene expression and growth characteristics.[20] The effects of parathyroid hormone are also dependent on dose and duration of exposure. Continuous treatment with parathyroid hormone increases mRNA for differentiation of osteoclasts that increases bone absorption, while intermittent treatment with parathyroid hormone increases the production of osteoblasts, leading to bone accumulation.[21]

A tissue-dependent response to hormone pulsatility has also been demonstrated in human anterior cruciate ligament fibroblasts in vitro. Yu and colleagues[22] noted that type I procollagen synthesis decreased in a dose-dependent manner with increasing estradiol concentrations. However, this response was most pronounced in early periods of estradiol exposure (days 1 and 3), then began to attenuate within 7 days of exposure. This attenuation was thought to either be due to a saturation of the estrogen receptors, or perhaps a downregulation of the receptor response with prolonged exposure.[22] While the literature supports the presence of estrogen and progesterone receptors in the human anterior cruciate ligament (ACL)[23] and that sex hormones may have a direct influence on collagen tissue,[24–28] the results by Yu and colleagues suggest the degree of pulsatility of these hormones can modify the response by the receptors and influence collagen structure and metabolism.

In the present study, higher estrogen and lower progesterone levels during menses were related to a greater stability in knee laxity values across the menstrual cycle. Based on the aforementioned examples of hormone pulsatility effects, nadir estrogen levels may stay sufficiently high during the early follicular phase in some females so as to "dampen" the collagen receptors response to rising estradiol and testosterone levels, resulting in no substantial change in ligament behavior. Conversely, those females who experienced a more androgenic profile (lower estradiol, higher progesterone) experienced a marked increase in joint laxity following peak ovulatory levels. While it is difficult to compare these results with other studies, it is interesting to note that in two studies that did not identify changes in knee laxity across select days of the menstrual cycle,[5,6] average estradiol levels were near the upper limits of normal ranges at menses (56 and 73 pg/mL) and considerably below the normal ranges postovulation (137 and 120 pg/mL) using similar hormone assays. Hence, their results would actually be consistent with our current findings of little or no change in knee laxity in subjects who maintain higher nadir and lower peak estradiol levels. However, because the values reported were only sampled on a single representative day for each phase, one cannot be sure whether they truly represent peak and nadir values, or whether other factors explain the lack of cyclic knee laxity changes observed.[5]

In contrast to our earlier findings that did not identify a clear relationship between changes in progesterone concentrations and knee laxity changes,[4] the current analyses appear to indicate that nadir progesterone concentrations may be a primary mediator of changes in knee laxity. The bivariate correlations between progesterone and knee laxity change was moderately high (r = 0.491), and was the first variable to enter the regression model, explaining 20% of the variance in knee laxity alone. Once minimum progesterone levels were accounted for, peak and minimum estradiol levels explained an additional 28% of the variance in knee laxity. It is important to note, however, that exploratory regression analyses revealed that peak estradiol concentrations would not have entered the model without first accounting for minimum progesterone levels, but minimum estradiol levels would have still entered and independently explained 14% of the variance. These findings suggest that the sensitivity of the knee joint to greater absolute changes in estradiol levels from the early follicular to early luteal phase is largely dependent on the nadir levels of estradiol and progesterone. This would appear to be consistent with basic science studies that have demonstrated an interactive effect between estradiol and progesterone in modifying collagen structure.[24,27] Romani and colleagues[29] also found stronger correlations between ACL stiffness and estradiol concentrations once the effects of other hormones were accounted for.

The additional contribution of testosterone to the equation is less clear. Although concentrations of testosterone are substantially lower in females (<100 ng/dL) than males (300–1000 ng/dL), the ovaries secrete testosterone during the course of the menstrual cycle,[19] and these concentrations can increase as much as two- to threefold around the LH surge midcycle.[8,9,19] We chose to include testosterone in the current analysis because these values also vary considerably between females as well as within females across their cycle (see Table 1), and our earlier work indicated a positive relationship between testosterone and changes in knee laxity that were independent of the effects of estrogen and progesterone.[4] This positive relationship was upheld in the current analysis, with peak testosterone concentrations accounting for an additional 10% of the variance in knee laxity change. However, minimum testosterone levels did not contribute to knee laxity changes, suggesting there is no specific minimum threshold on which the effects of testosterone are dependent. Of interest, these relationships remained unchanged when estrogen and progesterone were excluded from the model. The physiological importance of the positive relationship between peak testosterone concentrations and knee laxity change is currently unknown, and is difficult to speculate based on previous findings. While Hamlet and colleagues[30] identified androgen receptors on the human ACL in young adult males, they did not observe the presence of these receptors in females. Further, the effects of testosterone on collagen tissue have consistently been found to be anabolic in nature, increasing collagen content and fiber diameter[24,27] and decreasing elastin content.[31] This would suggest testosterone would have more of a stiffening effect on the capsuloligamentous structures, rather than contributing to increased knee laxity as we observed. Further research is needed to understand the physiological importance of rising testosterone levels in mediating changes in knee laxity.

Clinical Relevance

Mounting evidence suggests that sex differences in knee laxity are largely dependent on sex hormone concentrations and are greatest when females are in the early luteal phase of their menstrual cycle.[1–4] In some females, these changes in knee laxity exceed 3 mm, an amount that

is considered to be diagnostic of ACL injury,[32] while other females experience relatively little variations in knee laxity across the cycle. This variable knee laxity response among females may be an important risk factor consideration, as recent epidemiology suggests that increased knee laxity is a relevant factor in predicting ACL injury risk in females,[37] and has the potential to alter the passive restraints, articular surface congruity and contact, and active muscle forces that contribute to knee stability.[33–36] Therefore, understanding those factors that can modify knee laxity, and being able to identify which females may experience significant changes in knee laxity as a result of these factors would have clinical value.

The current study identified that minimum concentrations of estrogen and progesterone during days of menses are critical factors in predicting the magnitude of knee laxity increase with rising estrogen and testosterone levels. These findings further clarify the complex, variable relationships between sex hormones and changes in knee laxity that we previously reported among individual females.[4] Confirmation of the current findings would be clinically beneficial in that it would greatly simplify methods for identifying females as "hormone responders" (experiencing transient changes in knee laxity across the cycle) or "hormone non-responders" (knee laxity remaining fairly stable across the cycle), should transient changes in knee laxity represent a variable injury risk factor among women. Obtaining measures during the menstrual phase and for 2–3 days following ovulation (when estrogen is known to peak) may be sufficient to identify clinically important changes in knee laxity, without having to track females across their entire cycle. These findings may also be valuable to researchers and clinicians when examining knee laxity over time (e.g., monitoring joint laxity following ACL reconstruction), depending on the time in the cycle when these measures are taken. Work is ongoing to further characterize the hormone profile that produces increased knee laxity in females, and determine the extent to which both absolute and transient increases in knee laxity impact weight bearing knee joint neuromechanics and injury risk.

Limitations

While our study represents an initial step in describing hormone profile(s) that dictate knee laxity changes in women, our findings are limited to a relatively small sample size and measurements obtained over a single cycle. In our regression model, we used six predictors with a sample size of 22, which has the potential to inflate the correlations. Generally, 10 subjects per predictor (in this case, 60 subjects) are recommended. However, had we examined minimum versus maximum hormone concentrations separately, we would not have identified the interdependency between the two. To address this concern and provide a more accurate reflection of the strength of the regression, we reported the more conservative adjusted R^2 value to account for the percentage of variance that may be due to chance.[38] Even with this conservative approach, we believe it is important to confirm these findings in a larger sample in future studies. It is also not known from these data whether females will experience consistent changes in knee laxity from month to month. Females do vary somewhat in their cycle from month to month (although substantially less than the variability between females),[39] so this variability should also be explored in future work. While the ideal study may involve direct control and manipulation of the subject's sex steroids by a GnRH antagonist, the risk–benefit ratio with the currently available agents is not favorable.

ACKNOWLEDGMENTS

Support was provided by NIAMS/NIH RO3 AR 47178, and in part from the University of Virginia General Clinical Research Center [NIH MO1 RR00847], and a cooperative agreement [NICHD/NIH U54 HD28934] as part of the Specialized Cooperative Centers Program in Reproductive Research. The authors thank Christopher R. Carcia, PhD, for his assistance with data collection, and Laurie Wideman, PhD, for her suggestions on the manuscript. The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Navy or the Department of Defense.

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