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Salivary progesterone levels and rate of ovulation are significantly lower in poorer than in better-off urban-dwelling Bolivian women

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BACKGROUND: Agriculturalists in less-developed countries (LDC) have lower progesterone levels than urban industrialized populations. However, it is unknown if urban LDC populations are also relatively lower. We tested whether urban Bolivia samples—poorer (Bol-p) and better-off (Bol-b)—have lower progesterone than a Chicago (USA) sample, and whether progesterone and rate of ovulation are lower in Bol-p than in Bol-b. METHODS: Serial salivary samples collected from Bolivians, screened according to strict exclusion criteria during two complete menstrual cycles, were radioimmunoassayed for progesterone; anthropometrics were collected at mid-follicular and mid-luteal phases. RESULTS: Progesterone levels are lower in the Bolivia samples, and higher in the Bol-b than Bol-p; ovulation rate is greater in Bol-b than Bol-p. For only ovulatory cycles, mean-follicular-P (pmol/l), mean-luteal-P (pmol/l), and mean-peak-P (pmol/l) are respectively 65, 142 and 208 in Bol-p; 76, 167 and 232 in Bol-b; and 96, 240 and 330 in Chicago. Principal components representing body-size and progesterone level are positively correlated ($r = 0.404$, $P = 0.005$). CONCLUSIONS: Progesterone levels appear to be influenced by chronic and acute ecological conditions, evidenced by the association with body-size and the probability of ovulation respectively. These findings have implications for understanding cancer aetiology, developing population-appropriate hormonal contraceptives, and modelling the evolution and functioning of the reproductive system.

Key words: anthropometrics/cancer risk/ovulation/reproductive ecology/salivary progesterone

Introduction

Previous work (Seaton and Riad-Fahmy, 1980; Wilson *et al*., 1992; Ellison *et al*., 1993) suggests that levels of salivary progesterone may be naturally and considerably lower in non-industrialized populations compared with samples of women from more developed countries. In a series of studies (Ellison *et al*., 1989; Lipson and Ellison, 1992; Panter-Brick *et al*., 1993; Vitzthum *et al*., 1994, 2000; Jasienska and Ellison, 1998) using comparable field and laboratory protocols, US women in Boston had the highest levels of progesterone (Table I); agriculturalists from Bolivia, Nepal, Poland and the Democratic Republic of Congo (formerly Zaire) were all lower to varying degrees. Age differences in sample composition and the effects of seasonality (likely influencing activity and dietary patterns) contributed to some of the apparent differences (Vitzthum *et al*., 2000). Nonetheless, significant variation is evident even when comparisons are restricted to women observed in comparable seasons who are 25–35 years of age, a period of relative stability in ovarian function (Lipson and Ellison, 1992).

Different models have been proposed to explain this observed interpopulational variation (Ellison, 1990, 1996; Vitzthum, 1990, 1997, 2001; Vitzthum *et al*., 2000). However, it has not been possible to test definitively these hypotheses due, in part, to methodological limitations in past studies. Specifically, sample sizes have been small and especially sparse for women between 23 and 35 years old; some currently menstruating participants may have been recently pregnant or lactating; some participants were known or suspected to have a history and/or current case of sexually transmitted disease

Table I. Previously reported salivary progesterone (P) indices in five populations

	n	Mean-luteal-P (pmol/l)	Mean-mid-luteal-P (pmol/l)			Data source
			Total sample	25–35 years old		
				n		
Bolivia (rural Quechua)	20	179 (18.7)	243 (21.0)			Vitzthum *et al*. (1994, 2000)
Younger (19–21)	4	165 (21.8)	219 (29.8)			
Mid (23–35)	10	200 (13.2)	264 (17.3)	9	251 (17.1)[a]	
Older (39–42)	6	149 (14.4)	203 (21.4)			
Poland						G.Jasienska (personal communication)
Post-harvest	20	226 (9.5)	283 (16.9)	15	299 (19.6)[a]	
Harvest	20	188 (6.9)	224 (12.5)	15	237 (14.7)	
Nepal						Panter-Brick *et al*. (1993)
Winter	21	117 (7.2)[b]	138 (21.0)	5	253 (53.0)[a]	
Monsoon	20		85 (8.0)	5	124 (19.0)	
Zaire	56	132 (11.0)	167 (6.5)		201[c]	P.T.Ellison (personal communication)
Boston	124	232[c]	333[c]	42	373[c]	Lipson and Ellison (1992)

Values are mean (SE).
[a]Comparable seasons.
[b]Based on –1 to –14 days of cycle; original published value was based on –1 to –16 days of cycle.
[c]SE not available.

and possible ovarian dysfunction; some samples have included uncertain numbers of anovulatory cycles; and samples may have been inadvertently biased towards women of lower fecundity because in non-contracepting populations more fecund women can be expected to be pregnant or lactating and thus unavailable for inclusion in a study of currently cycling women (Vitzthum *et al*., 2000).

In addition, outside the USA, these studies of salivary progesterone have been restricted to relatively homogeneous rural populations. It is not known if urban dwellers in less-developed countries also have relatively lower progesterone nor is it known if the socioeconomic heterogeneity that typifies urban areas is associated with variation in ovarian function. Worldwide rapid growth of urban areas, often disproportionately among the impoverished, and the accompanying changes in lifestyle that impact health, well being and reproductive patterns, warrant a better-informed understanding of inter- and intrapopulational variation in women's reproductive physiology.

This study tests the hypotheses that progesterone levels are lower in Bolivian than US urban-dwelling women and that, among the former, progesterone and the rate of ovulation are lower in poorer than in better-off women. These predictions derive from empirical, albeit flawed, data suggesting lower progesterone in rural Bolivian Quechua women (Table I) and a theoretical model that posits adaptive responses in reproductive functioning to environmental stressors (Vitzthum, 1990, 1997, 2001). Through strict adherence to exclusion criteria, the study design eliminates or substantially mitigates the methodological limitations noted above.

Materials and methods

Populations and study design

Potential participants were recruited in La Paz, Bolivia, during May and June 1995 through announcements and by word of mouth, from the national medical school (economically better-off sample, Bol-b) and residents of an impoverished periurban neighbourhood (poorer, Bol-p). Informed consent was obtained according to an approved protocol (IRB, University of California, Riverside). Subsequent to a screening interview administered in a woman's native language, samples (Bol-b, $n = 31$; Bol-p, $n = 30$) comprised healthy women (Aymara and/or Spanish ancestry) living at high altitude (>3500 m) since birth or early childhood; 23–35 years old; reporting regular menstrual cycles of 25–35 days duration; free of known previous or current reproductive disorders or sexually transmitted diseases; and (for at least the previous 6 months) not pregnant, lactating, using hormonal contraceptives or prescriptive medications, or experiencing significant body weight changes (± 2 kg).

For two complete consecutive cycles, serial 5 ml saliva samples were collected every other day according to an established protocol (Vitzthum *et al*., 1993) and assayed at Northwestern University for progesterone following published methods (Lu *et al*., 1999). Because salivary progesterone is stable for extended periods, samples collected under field conditions yield accurate values (Lipson and Ellison, 1989). All Bolivian samples had comparable collection, handling, storage, and assay conditions. Coinciding with mid-follicular (days 7–9) and mid-luteal (days 21–23) phases, standard anthropometrics (Lohman *et al*., 1988) were collected by a single observer (Spielvogel). Indices included body mass index (BMI, kg/m^2), total upper arm area, upper arm muscle area, upper arm fat area, and arm fat index (Gurney and Jellife, 1973).

For comparison, a sample of Chicago women attempting to conceive provided daily salivary samples assayed for progesterone in the same laboratory according to identical protocols; recruitment details and sample characteristics have been published (Lu *et al*., 1999).

Data analyses

Progesterone indices

Serial progesterone values were aligned on the first day of the subsequent cycle, and the luteal phase was taken to begin at day –14. Given any span of days from x to y, we defined mean progesterone as ($\int$ of P from x to y)/($y - x$) where P at any time is defined by linear interpolation of the observed progesterone values, the first observation of the subsequent cycle serving as an 'anchor' [a reference

(fiducial) day of 0] for interpolation between the current cycle's final observation and cycle completion. This approach estimates progesterone indices at individual and sample levels, thus affording investigation of the causes of individual hormonal variation.

(i) mean-luteal-P (x = –14.5, y = –0.5): estimates mean progesterone during the final 14 days of the cycle, a span chosen for comparability at the sample level to mean-luteal-P calculated as the grand mean of daily mean progesterone of all women who have contributed a sample on a given day for days –1 to –14 (Ellison, 1988; Ellison *et al*., 1989; Lipson and Ellison, 1992; Panter-Brick *et al*., 1993; Vitzthum *et al*., 1994, 2000; Jasienska and Ellison, 1998).

(ii) mean-peak-P (x = day of peak P – 2.5, y = day of peak P + 2.5): peak P is restricted to the greatest observed progesterone from –2 to –14 days. This index is preferable to those assuming progesterone peaks within a given range of days (e.g. –5 to –9) thereby often underestimating the true magnitude of the luteal rise in progesterone because of the considerable variation among cycles in the timing of the peak.

(iii) mean-follicular-P (x = first day of cycle + 2, y = –15.5). The chosen span of days avoids inclusion of (a) the relatively high progesterone levels at cycle commencement that properly represent the falling hormone levels of the previous luteal phase and (b) the rising progesterone associated with the subsequent luteal phase.

Ascribing ovulation

Although a luteal rise in progesterone is characteristic of normal ovulation, there is no definitive threshold of salivary progesterone below which ovulation can be said not to have occurred (Ellison, 1988). One algorithm (Ellison, 1988; Panter-Brick *et al*., 1993) infers ovulation when at least one observed luteal progesterone value is >2 SD above the mean-follicular-P levels (i.e. there is <5% probability that a given luteal progesterone value falls within the distribution of follicular progesterone). However, for a given cycle, the chance probability of observing one luteal value above this threshold rises with the number of luteal observations. Even without a systematic rise in progesterone due to ovulation, this chance probability is 0.51 ($= 1 - 0.95^{14}$) in daily sampling (luteal observations = 14) and 0.30 ($= 1 - 0.95^{7}$) in every-other-day sampling (the typical protocol in samples from non-industrialized populations). Hence, the comparison of ovulatory rates in samples having different sampling regimes is biased by chance alone, and the comparison of those having identical sampling regimes entails a large probability that anovulatory cycles will be misclassified as ovulatory.

Because each progesterone index has a continuous distribution, any threshold used to distinguish ovulatory cycles is somewhat arbitrary. With this caveat, we have ascribed ovulation to any cycle having a mean-peak-P >110 pmol/l. To determine this cut-off, each cycle's profile (without regard to the sample from which it was drawn) was visually inspected for an apparent luteal rise in progesterone relative to the previous follicular phase, and if this rise was absent the cycle was designated anovulatory. The distribution of mean-peak-P was then examined to ascertain that cut-off value between visually designated ovulatory and anovulatory cycles that reflected a reasonable compromise between the probabilities of Type I and Type II errors (i.e. false positives versus false negatives). At mean-peak-P = 110 pmol/l, all visually designated anovulatory cycles were still classified as anovulatory, and only three visually designated ovulatory cycles were reclassified as anovulatory. This procedure has the benefit of being replicable as well as internally consistent for the samples compared in this study. In addition, the magnitude of difference in ovulatory rates between Bol-p and Bol-b (see Results) is so large as to suggest that whatever misclassification may have occurred, its effect is not substantial in this study.

Table II. Bolivian sample characteristics [mean (SD) or proportion]

	Poorer (n = 29)	Better-off (n = 26)
Age (years)	28.9 (3.7)	28.1 (4.1)
Age at menarche	13.2 (1.5)	13.1 (1.8)
Previous pregnancies (%)**		
None	7	65
>0	93	35
Age at first birth	20.6 (2.6)	22.0 (4.7)
Current male partner (%)*		
Yes	72	35
No	28	65
Current occupation (%)**		
Professional/ university student	0	100
Household	24	0
Manual labour	55	0
Educational assistant	21	0

χ^2: *$P < 0.03$; **$P < 0.001$.

Principal components analyses

No single anthropometric variable can adequately convey the extent of variation in body shape and fat deposition patterns, nor can one progesterone index adequately express the totality of progesterone levels throughout the cycle. Principal components analysis (PCA) is used here to extract those main components of body-size and composition reflected in the anthropometrics, and those features of the progesterone profile represented by the progesterone indices. These principal components (factors) are included in correlation analyses evaluating the relationships between progesterone levels and body-size and composition.

Analytical samples

Consistent with the exclusion criteria, observed cycles of <25 days or >35 days were omitted from analyses, yielding 29 Bol-p, 26 Bol-b and 22 Chicago women having at least one observed cycle length of 25–35 days, inclusive. The mode number of cycles/woman was 2 in the Bolivian samples and 1 in Chicago (with a range of 1–4). To construct a sample of independent data points, each woman contributed a single 'representative cycle' (i.e. a woman's average index for her cycles meeting inclusion criteria). Samples comprised (i) cycles without regard to ascribed ovulation status; and (ii) only ovulatory cycles. One woman from Bol-b was excluded from all analyses because her progesterone profiles strongly suggested a conception, followed by fetal loss and a non-ovulatory segment before the resumption of normal menses.

Results

Participant characteristics

Although the Bolivian samples did not differ significantly in current age or reported age at menarche (Table II), their reproductive histories and lifestyles were substantially different. The better-off women were all professionals and/or university students. Only about a third currently had a male partner, and only about a third had ever been pregnant, the average age at first birth among parous women being 22 years. In contrast, almost three-quarters of Bol-p had a current male partner, and all but two had previously been pregnant. Among parous women, age at first birth (20.6 years) was non-significantly younger than Bol-b. In neither sample were ages at menarche or first birth correlated with anthropometric or progesterone variables. The majority of the Bol-p supported

Table III. Anthropometrics [mean (SD)]

	Poorer (n = 27)		Better-off (n = 26)
Height (cm)	149.1 (4.9)	***	154.2 (5.3)
Weight (kg)	57.1 (6.9)	*	53.4 (6.2)
Body mass index (kg/m^2)	25.6 (2.7)	***	22.4 (2.3)
Skinfolds			
Biceps (mm)	10.8 (2.9)		10.9 (3.5)
Triceps (mm)	18.8 (5.2)		18.0 (3.2)
Subscapular (mm)	19.1 (5.6)		16.7 (5.1)
Suprailiac (mm)	22.2 (5.8)	*	18.3 (5.3)
Circumferences			
Mid-arm (cm)	26.5 (1.7)	**	25.1 (1.8)
Calf (cm)	33.9 (2.8)		33.2 (1.9)
Composition indices			
Total upper-arm area (cm^2)	55.8 (7.2)	**	50.5 (7.0)
Arm muscle area (cm^2)	33.7 (4.1)	**	30.3 (3.9)
Arm fat area (cm^2)	22.1 (6.5)		20.2 (4.4)
Arm fat index (%)	39.1 (8.1)		39.7 (4.8)
Haemoglobin (g/l)	154 (12)	*	161 (11)

t-Test (two-sided): *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$.

their households through manual labour including clearing large stones or laying cobbles for road building, hauling crops and other products for market, hand laundry and house-cleaning. Those without any current means of monetary income (working in the household only) typically also engaged in manual labour including hauling water and fuel, and occasionally traded manual labour for food.

Paired t-tests revealed no significant differences in anthropometrics during the follicular and luteal phases, hence subsequent analyses used the mean of an individual's measurements (Table III). Poorer women were on average >5 cm shorter than better-off women, a difference that probably reflected chronic undernutrition throughout childhood (Bogin, 1999). This shorter stature coupled with the greater weight among Bol-p women yielded a mean BMI of 25.6 kg/m^2 versus 22.4 kg/m^2 in the Bol-b (two-sided t-test, $P < 0.001$). These relative BMI values lend themselves to the misinterpretation that the poorer women were at a nutritional advantage compared with Bol-b. However, except for the suprailiac skinfold, mean skinfolds and limb circumferences did not differ significantly between the samples. Body composition indices also indicated that fat levels did not differ significantly, but that mean arm muscle area (reflective of total body muscle mass) was significantly greater among Bol-p. In other words, the greater manual labour engaged in by poorer women was reflected in a relatively greater muscle mass that tended to inflate BMI. In addition, BMI is known to be affected by body proportions, being artificially exaggerated in shorter individuals and in those with relatively short limbs (Norgan, 1994). Both as a whole, and especially for the poorer women, the BMI gives a false impression of an elevated nutritional status in Andean women (Aguayo and Vitzthum, 1997).

Anovulatory rates

Comparing anovulatory rates among these samples is not straightforward because of differences in numbers of cycles per woman and participant inclusion criteria. In addition, the Chicago women were seeking to conceive, a criterion that may have resulted in non-participation of women who opt-out (self-select) because of a known or suspected pattern of anovulatory cycles.

With these caveats in mind, for samples comprising only each woman's first observed cycle, anovulatory rate is defined as the proportion of cycles having mean-peak-P <110 pmol/l. Anovulatory rates did not differ ($\chi^2 = 0.104$, two-sided P = not significant) between Chicago (2/22 = 9%) and Bol-b (3/25 = 12%) but were significantly higher ($\chi^2 = 10.97$, $P = 0.001$) in Bol-p (16/29 = 55%) than Bol-b.

Progesterone levels

Table IV (top panel) compares progesterone indices for the samples comprising all representative cycles. Peak-day did not differ significantly between the samples. Mean-luteal-P and mean-peak-P each varied significantly, being greatest for Chicago (227 and 311 pmol/l respectively) and lowest in the Bol-p (92 and 132 pmol/l respectively), Bol-b falling midway (162 and 223 pmol/l respectively). The same pattern was observed for mean-follicular-P, although Chicago and Bol-b were not significantly different.

In samples comprising only ovulatory cycles (Table IV, bottom panel), Chicago and Bol-b indices changed little as both samples had a low anovulation rate. In stark contrast, progesterone indices for the Bol-p were raised substantially by the exclusion of anovulatory cycles. Mean-follicular-P increased by 23% (53 to 65 pmol/l), mean-luteal-P by 54% (92 to 142 pmol/l), and mean-peak-P by 58% (132 to 208 pmol/l). ANOVA for mean-peak-P and mean-luteal-P revealed that both Bol-p and Bol-b remained significantly lower than Chicago, but the two Bolivia samples were not significantly different (but see results from PCA, below). For mean-follicular-P, only Chicago versus Bol-p was significantly different.

Figure 1 summarizes these findings. The Bol-p profile for all cycles was exceedingly low with a peak progesterone that barely rose above the anovulatory cut-off of 110 pmol/l. Removing the anovulatory cycles, represented by the virtually flat profile, revealed a profile for Bol-p ovulatory cycles much closer to that of Bol-b. Both Bolivia samples of ovulatory cycles were substantially lower than ovulatory cycles in Chicago women during the follicular as well as luteal phase of the cycle.

The final column of Table IV presents sample averages for mean-luteal-P as calculated in previous studies of salivary progesterone (Ellison *et al*., 1989; Lipson and Ellison, 1992; Panter-Brick *et al*., 1993; Vitzthum *et al*., 1994, 2000; Jasienska and Ellison, 1998). The values were quite similar to the index as estimated by our procedure, verifying the appropriateness of comparing the sample-level index across different studies.

Correlations of progesterone level in ovulatory cycles with body-size and composition

The analytical sample for PCA (Table V) comprises representative ovulatory cycles from all the Bolivian women (n = 40). For the anthropometrics, two factors having eigenvalues >1.0 were extracted. The first, accounting for 60% of the total

Table IV. Progesterone indices (pmol/l)

	n	Mean-follicular-P	Peak-day	Mean-peak-P	Mean-luteal-P	Mean-luteal-P
Bolivia, poorer (Bol-p)	29	53[a] (7.3)	–7.8 (0.47)	132[b] (14.9)	92[c] (11.1)	95
Bolivia, better-off (Bol-b)	26	78[a] (5.4)	–7.3 (0.39)	223[b] (14.0)	162[c] (8.5)	164
Chicago	22	91[a] (10.3)	–7.9 (0.49)	311[b] (28.2)	227[c] (21.2)	233
Ovulatory cycles only						
Bolivia, poorer (Bol-p)	16	65[d] (12.4)	–6.7 (0.59)	208[e] (16.9)	142[f] (12.1)	149
Bolivia, better-off (Bol-b)	24	76[d] (3.6)	–7.2 (0.41)	232[e] (14.0)	167[f] (8.3)	170
Chicago	21	96[d] (9.5)	–7.9 (0.51)	330[e] (28.3)	240[f] (21.9)	244

Values are mean (SE). Signifiance levels for pairwise comparisons are Bonferroni-adjusted.
[a]ANOVA, $F = 6.49$, $P = 0.003$; $P \leqslant 0.05$ for all pairwise comparisons except Bol-b versus Chicago.
[b]ANOVA, $F = 22.17$, $P \leqslant 0.0001$; $P \leqslant 0.01$ for all pairwise comparisons.
[c]ANOVA, $F = 23.95$, $P \leqslant 0.000$; $P \leqslant 0.01$ for all pairwise comparisons.
[d]ANOVA, $F = 3.49$, $P = 0.037$; Bol-p versus Chicago $P = 0.040$, all other pairwise comparisons not significant.
[e]ANOVA, $F = 9.26$, $P < 0.0001$; $P \leqslant 0.01$ for all pairwise comparisons except Bol-p versus Bol-b.
[f]ANOVA, $F = 10.58$, $P < 0.0001$; $P \leqslant 0.01$ for all pairwise comparisons except Bol-p versus Bol-b.

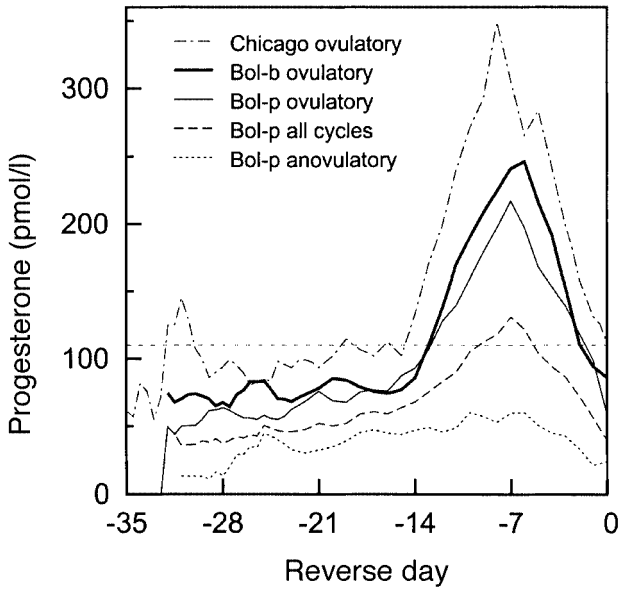

Figure 1. Progesterone profiles in Chicago, better-off Bolivian (Bol-b) and poorer Bolivian (Bol-p) samples. Cycles are aligned on the first day of the subsequent cycle, and days are numbered backwards from that point (Reverse Day).

variance, loaded heavily on all skinfolds as well as weight and thus represented variation in overall body-fatness (names of factors are hyphenated throughout). The second factor, accounting for 16% of the total variance, loaded principally on height and represented variation in overall body-size. For the progesterone indices, representing hormone levels at different parts of the ovarian cycle, it is as expected, that PCA extracted a single factor that represented variation in overall progesterone level (subsequently called 'P-level') and accounted for 73% of the total variance. It is noteworthy that the factor loaded strongly on mean-follicular-P albeit to a lesser extent than on the luteal indices.

All progesterone indices were significantly positively correlated with one another (a complete Pearson correlation matrix may be obtained from the authors). All skinfolds were significantly positively correlated with one another as well as with weight and BMI, but not with height. This pattern of correlations among the anthropometrics was consistent with the designation of the first anthropometric factor as body-fatness, and with the second factor as body-size.

No single progesterone index was significantly correlated with any single anthropometric variable, nor with BMI, except for a positive correlation between mean-luteal-P and height ($r = 0.35$, $P = 0.015$). In contrast, 'P-level' and body-size were significantly positively correlated ($r = 0.40$, $P = 0.005$). Body-size was also significantly positively correlated with mean-luteal-P ($r = 0.45$, $P = 0.002$) and mean-peak-P ($r = 0.35$, $P = 0.015$). Other than the correlation with body-size, there were no significant correlations between 'P-level' and any single anthropometric variable nor with BMI ($r = 0.02$, $P = 0.46$) or body-fatness ($r = 0.063$, $P = 0.35$).

Figure 2 plots 'P-level' against body-size, distinguishing Bol-p from Bol-b. It is evident that the observed correlation was not a spurious result of the shorter Bol-p women clustering apart from the taller Bol-b women. Correlation analyses for each of the samples revealed the same relationship, albeit with less statistical significance because of the smaller sample sizes.

Bol-p and Bol-b significantly differed in 'P-level' (Kolmogorov–Smirnov test, $Z = 1.420$, two-sided $P = 0.035$) and body-size ($Z = 1.536$, two-sided $P = 0.018$), but not in body-fatness ($Z = 0.893$, $P = 0.402$). This pattern of difference and similarity in the anthropometric factors was paralleled by the differences and similarities previously described for the anthropometrics (Table III).

Discussion

Ovulatory rates were comparable in the Chicago and Bol-b samples but far lower in the Bol-p sample. Nonetheless, relative to Chicago, progesterone (as represented by the principal component, 'P-level') in ovulatory cycles was significantly lower in Bol-b and lower still in Bol-p. In ovulatory cycles of Bolivian women, there was a significant correlation between P-level and body-size, but not with body-fatness, BMI, nor any single anthropometric variable.

Table V. Principal components analyses for anthropometrics and progesterone in ovulatory cycles (n = 40 women)

Anthropometrics			Progesterone indices	
Component matrix	Component		Component matrix	Component 1: 'P-level'
	1: body fatness	2: body size		
Height	–0.030	0.957	Mean-peak-P	0.888
Weight	0.917	0.215	Mean-luteal-P	0.965
Arm circumference	0.876	0.008	Mean-follicular-P	0.686
Calf circumference	0.809	0.242		
Subscapular skinfold	0.805	–0.297		
Suprailiac skinfold	0.763	–0.407		
Triceps skinfold	0.771	0.147		
Biceps skinfold	0.850	0.076		
Initial eigenvalues	4.811	1.301		2.189
% of variance	60.14	16.26		72.98
Cumulative %	76.40			72.98

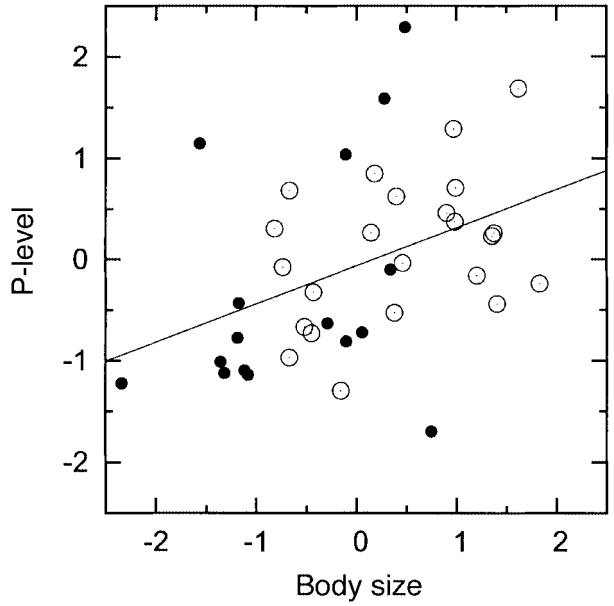

Figure 2. Correlation (r = 0.40, P = 0.005) of progesterone ('P-level') factor score and body-size factor score in Bolivian ovulatory cycles; solid = poorer Bolivian (Bol-p); open = better-off Bolivian (Bol-b); n = 40 women.

The Bolivian participants selected for this study were carefully screened and, among Bol-b, progesterone indices and anthropometrics did not differ between nulliparous and parous women (i.e. those of 'unproven fertility' did not have progesterone levels lower than those of 'proven' fertility). Nearly all the Bol-p women are of 'proven fertility' despite the lower progesterone levels characterizing their ovulatory cycles. Hence, the observed progesterone levels are very unlikely to be the result of a sampling bias towards women of lower fecundity.

The progesterone indices and method of ascribing ovulation proposed here avoid some of the methodological limitations of previous approaches. The factors derived from PCA better represent variation in progesterone levels and body-size and composition than any one index or single anthropometric variable. For example, in ovulatory cycles, individual progesterone indices are not significantly different between the Bolivian samples, but 'P-level' (the factor capturing the totality of the progesterone profile) is significantly different.

Although tempting, comparisons of progesterone levels in these Bolivia samples with those observed in previous studies (Table I) are spurious because those participants had not met comparable exclusion criteria and the samples contained unknown numbers of ovulatory cycles, making it impossible to ascertain the significance of differences and similarities in progesterone levels.

The results of this study are consistent with hypotheses that current variation in progesterone, and fecundity in general, reflects both current (acute) and longstanding (chronic) variations in energy intake and expenditure as well as other potential determinants of reproductive success (Ellison, 1990, 1996; Vitzthum, 1990, 1997, 2001; Vitzthum *et al*., 2000). It is a fundamental ecological principle that in adults of reproductive age, energy must be allocated to maintenance/repair or reproduction. Lifetime reproductive success in a given set of conditions may be increased if energy is efficiently allocated such that waste is minimized. Thus it may prove advantageous, over a lifetime, to temporarily suspend investment in reproduction if current conditions are such that a conception has a relatively poorer probability of resulting in a live birth and the survival of the child.

The Bol-p women had a higher parity than Bol-b women, although reported ages at menarche and first birth were comparable. Given this indisputable evidence of normal ovarian function over the long-term in the poorer Bolivian women, the observed high rate of anovulation is reasonably attributable principally to stressors acting at the time of the study. Specifically, these data were collected during the Bolivian winter months, a period when some of the very poorest urban dwellers can experience a temporary loss of food supplies from relatives on farms. Rather than pathological, the reduction in fecundity evidenced by the high rate of anovulation is appropriately viewed as an adaptive response to these short-term (acute) relatively limited resources.

Adaptation to long-term (chronic) conditions, on the other hand, may explain the lower progesterone levels, compared with Chicago, observed in ovulatory cycles of both Bolivian samples, and the relatively lower 'P-level' of Bol-p compared with Bol-b. While not definitive, it may be assumed that the majority of these women were raised in conditions roughly similar to those of their adult economic status. Ample evidence (Bogin, 1999) argues that chronic nutritional deprivation during childhood and/or adolescence influences immune responsiveness and reproductive functioning, and results in relatively shorter adult stature. Significant anthropometric differences between Bol-b and Bol-p in height, body-size, and arm musculature all lend support to the presumption that Bol-p experienced relatively greater nutritional deprivation and perhaps greater physical labour during childhood. Given that variations in body-size seen both within and between the samples of Bolivian women are probably the result of differences in ecological conditions during childhood, the significantly positive correlation found between body-size and 'P-level' suggests that variation in 'P-level' in ovulatory cycles of adult women may also reflect such differences in developmental conditions.

The apparent paradox that lower progesterone is not necessarily associated, over the long term, with a reduction of reproductive output is resolved if evaluated from an evolutionary perspective. Based on known attributes of mammalian physiology and functioning and principles of life history theory, the Flexible Response Model (FRM) (Vitzthum, 1990, 1997, 2001; Vitzthum *et al*., 2000) posits that selection favours a flexible reproductive system that reflects developmental conditions and responds to current ecological circumstances. Rather than viewing elevated progesterone in Chicago women as a 'normal' standard below which reproductive functioning can be inferred to be impaired, the FRM hypothesizes that 'normal' progesterone levels are established during pre-adult development as a result of the specific ecological conditions at the time and setting. From the perspective of the organism, the best conditions experienced during development are 'optimal', even if less than ideal, so long as they are minimally adequate to support basic functioning. Therefore, the reproductive system functions 'normally' under those conditions. Individuals unable to function in this way would have relatively low reproductive output and 'lose' in the evolutionary process of natural selection. Hence, the significant difference in ovulatory cycle 'P-level' among the samples is consistent with the hypothesis of the FRM that developmental conditions contribute to variation in adult reproductive functioning.

These findings are also relevant to understanding the aetiology of certain cancers. Changes in reproductive patterns (e.g. earlier menarche, fewer births, and reduced breastfeeding) that increase menstrual cycle frequency have been linked to increased risks of breast, endometrial and ovarian cancer (Eaton *et al*., 1994). US women also have relatively higher levels of reproductive steroids that may exacerbate cancer risks. Explanations for these levels include dietary and activity patterns, but definitive tests of these hypotheses remain elusive. This study suggests that conditions experienced during childhood may be an important aetiological factor underlying elevated reproductive steroids.

For example, the secular trend of increasing height in the US population over the last century is well documented and principally attributed to improved diet and reductions in infectious disease (Bogin, 1999). These Bolivian data predict that an increase in height (and overall body-size) will be associated with an increase in adult reproductive steroids. This relationship can explain, in part, the observation that second generation migrants to the US from populations characterized by relatively low reproductive steroids and shorter stature are found to have both relatively increased stature and elevated reproductive steroids. Also consistent with our findings, epidemiological studies have observed a strong positive association between body-size, particularly height, and risk of breast cancer (Ziegler *et al*., 1996; Friedenreich, 2001), and women in the high Andes have among the lowest age-adjusted risk of breast cancer worldwide (Pan American Health Organization, 1999).

Our findings of naturally lower endogenous progesterone levels in these Bolivian women are also relevant to expressed concerns about appropriate hormonal contraceptive dosages for women in developing countries (Fotherby *et al*., 1980; Bassol *et al*., 1984; Bentley, 1996; Vitzthum and Ringheim, 2000). Hormonal contraceptives that have been designed for women in industrialized countries may be excessively high for those in non-industrialized populations, resulting in an intolerable level of side-effects that leads to discontinuation and, potentially, unplanned pregnancy. While it remains to be determined if lower progesterone results from lower ovarian production or more rapid clearance by the body, during a decade of fieldwork the first author has often heard Bolivian women and health workers express concern about negative experiences with hormonal contraceptives. Contrary to arguments that non-compliance is more a matter of education than biology, the findings of this study suggest that the reports of these women regarding negative sequelae of hormonal contraceptives may well have a physiological basis.

These data contribute to an emerging reconceptualization of the complexity and subtlety of ovarian function. The significant difference in mean-follicular-P in ovulatory cycles between Bol-p and Chicago, and significant loading of 'P-level' on mean-follicular-P in the PCA, challenge the assumption that follicular progesterone levels are uniformly constant and low. It is also noteworthy that all of the women in this study with anovulatory cycles were regularly menstruating. In exercising North Americans, menstrual irregularity or amenorrhoea typically accompanies anovulation (Bullen *et al*., 1985; Chen and Brzyski, 1999), but this is best interpreted as the extreme in a continuum of ovarian response (Prior, 1985; Vitzthum and Smith, 1989; Ellison 1990; Vitzthum, 1990). For example, moderate recreational exercise was found to be associated with suppressed progesterone profiles but not amenorrhea (Ellison and Lager, 1986). Finally, the range of progesterone accompanying normal ovulation in Bolivian women is lower than in Chicago women. Standard clinical practice considers progesterone levels $<40\%$ of 'normal' to be anovulatory (Wilson *et al*., 1998). In the Chicago sample, 40% of mean-peak-P is 132

pmol/l, 20% higher than the threshold of 110 pmol/l mean-peak-P determined in this study. Overall, normal variation in ovarian function is much greater than previously recognized.

A final note of caution is warranted when inferring support from these data for various models regarding cancer aetiology or the evolution of the human reproductive system. The sample sizes are not large, little is known of the developmental circumstances of the participants, nothing is known of the previous trajectory of progesterone levels, and the relative lifetime reproductive output of both poor and better-off samples remains to be realized. The findings, however, do indicate intriguing directions of research worthy of pursuit.

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