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Brief Communication: Effect of Coca-Leaf Chewing on Salivary Progesterone Assays

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ABSTRACT Although there is evidence for reduced fertility in Andean and Himalayan populations at higher altitudes, factors other than hypoxia may be primarily responsible. A valuable approach in the investigation of these fertility determinants is the use of salivary steroid assays. However, coca-leaf chewing—a ubiquitous practice among high altitude Andean populations—has negative consequences for the accurate measurement of ovarian steroids. This report evaluates the effects of coca-leaf chewing on assays of salivary progesterone. Study participants include naive and habitual users of coca leaf from La Paz and El Alto, Bolivia. Approximately 300 saliva samples were collected immediately before, during, and after coca-leaf chewing. The series includes samples with and without the alkaloid enhancer typically used by coca-leaf chewers. Coca chewing produces false salivary progesterone values that mimic luteal phase values. On the basis of this study, an appropriate protocol is developed for the collection of salivary samples in coca-leaf chewing populations. These results verify the feasibility of salivary assays, even for very difficult field conditions, and highlight the necessity of establishing suitable collection procedures before full field implementation of saliva sampling.

There is evidence for reduced fertility in Andean and Himalayan populations at higher altitudes compared to their counterparts at lower elevations (James, 1966; Baker and Dutt, 1972; Abelson et al., 1974; Hoff and Abelson, 1976; Gupta, 1980), and it appears that conditions at high altitude have a direct and negative bearing on at least some aspects of reproductive function (Clegg and Harrison, 1971; Abelson, 1976; Heath and Williams, 1981). For example, a delay in menarche and a greater incidence of dysmenorrhea and irregular menses are reported among women in these settings (Donayre, 1966; Greksa, 1990). However, factors other than hypoxia may underlie the apparently lowered fecundity. Poor nutrition, inadequate health care, later age at first marriage, and infant feeding practices are all implicated (cf. De Jong, 1970; Weitz et al., 1978; Dutt, 1980; Goldstein et al., 1983, 1984a,b; Abelson, 1984; Hoff, 1984; Kashiwazaki et al., 1988; Vitzthum, 1988, 1989). Because it is likely that several interacting determinants contribute to lowered fertility in high altitude populations, the controversy regarding cause and mechanism continues. Moreover, the extent to which hypoxic conditions affect ovulatory function in humans—particularly those with lifelong residence at high altitude—remains unknown.

The measurement of progesterone in human saliva (Ellison, 1988) can assist in resolving this debate. This technique allows an assessment of reproductive function that

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is impossible to obtain through interviews, and the noninvasive, nondisruptive methodology is suitable to typical field conditions. However, coca-leaf chewing—a ubiquitous practice among high altitude Andean populations—can prevent the accurate measurement of ovarian steroids in saliva if appropriate precautions are not taken during sample collection.

This report evaluates the magnitude and duration of coca-leaf contamination on assays of salivary progesterone by conducting two experiments. The first compares progesterone readings for saliva samples taken immediately before and after chewing; the second collects sequential samples twice while chewing and every 15 minutes for 2 hours after the cessation of chewing. Study participants comprise naive and habitual users of coca leaf from El Alto and La Paz, Bolivia. Samples with and without *llipta,* the alkaloid enhancer typically used by coca-leaf chewers, are included. On the basis of these findings, appropriate protocols for salivary sample collection in populations known to chew coca leaf are developed.

MATERIALS AND METHODS

Salivary samples were collected from volunteers visiting or resident in La Paz and El Alto, Bolivia during August and September 1989 according to procedures derived from Ellison (1988) and Lipson and Ellison (1989). Saliva was collected in polystyrene plastic test tubes pretreated with sodium azide (a bacteriocide, 0.1% concentration) as a preservative. Saliva production was stimulated using proven promoters—either olfactory (chocolate, gum, or coca leaves were sniffed) or liquid (five drops of citric acid solution placed on the tongue and gently swilled in the mouth). After collection, sample tubes were tightly capped and kept at ambient temperature for 4 months until received in the laboratory, then subsequently frozen at −20°C until assayed.

Samples were assayed in the Reproductive Ecology Laboratory at Harvard University under the direction of Peter Ellison by methods previously described (Ellison, 1988). Interassay variability (expressed as the coefficient of variation of replicate pooled samples run in every assay) averaged 12.6% for a luteal pool and 16.3% for a follicular pool. Intraassay variability averaged 13.8%, and the sensitivity limit of the assay was less than 15 pmol/L. All samples from a given individual were run in the same assay to minimize the effects of interassay variability.

Precollection protocol

1. Prior to initial collection, refrain from coca-leaf chewing for at least 2 hours; from food, drink (except water), tooth brushing, and exertion for at least 30 minutes.
2. Rinse mouth clean of debris.
3. Five minutes before the initial collection, rinse mouth with cold water and deposit in a clean cup; test for blood (a contaminant) using Hemastix.
4. If negative, continue. If positive, repeat step 3; if still positive, stop.

EXPERIMENT 1: CONTAMINATION STUDY

This procedure determined the immediate contaminatory effect of coca-leaf chewing on assays of salivary progesterone. The sample comprised 27 paired trials (15 with *llipta,* 12 without) from 5 study participants (3 females, 2 males) who had never chewed coca before this experiment.

Protocol

1. Observe precollection protocol.
2. Initiate olfactory stimulation of saliva production and collect 10 cc saliva in prepared tube.
3. Immediately place coca (15 leaves; with or without *llipta*) in cheek pouch.
4. Gently smash coca between molars for approximately 3–6 minutes, immediately collecting 10 cc saliva in second prepared tube.

Results

Individual data are summarized in Figure 1 (original data are available upon request from VJV). Coca-leaf chewing dramatically elevates the apparent values for salivary progesterone; the difference in readings before and after chewing coca averages 385 pmol/L, a mean increase of 716% (paired

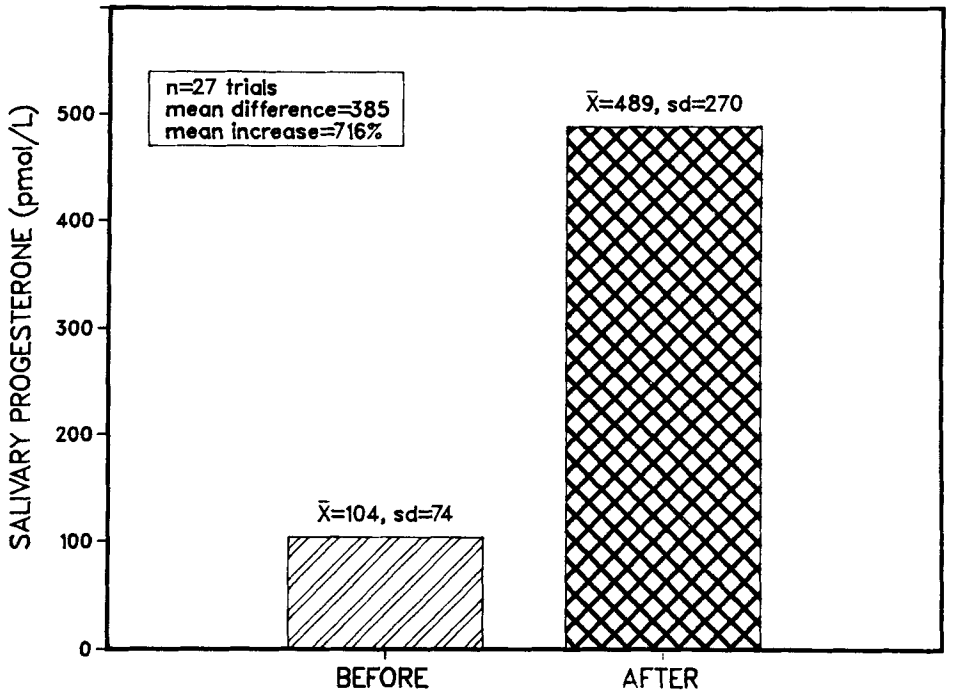

Fig. 1. Experiment 1: Salivary progesterone readings before and after chewing coca leaf. Coca-leaf chewing falsely elevates salivary progesterone reading; a cycle could mistakingly be considered ovulatory.

t-test: $t = -7.44$, $P < 0.00001$). ANOVA confirms that variations in progesterone readings (before, after, and difference) among individuals or with respect to *llipta* use, sex, or age are not statistically significant (in all ANOVAs, $P > 0.26$).

Though the apparent increase in progesterone is substantial, the magnitude of difference due to coca-leaf contamination approximates that separating normal follicular and luteal levels in cycling women. Thus, undetected coca chewing could mistakenly lead to classifying individual samples as luteal and cycles as ovulatory.

EXPERIMENT 2: TIME SERIES STUDY

This procedure determined the duration of coca-leaf contamination once chewing had ended. The sample comprised 12 women, ranging in age from 23 to 45 years, in three use classes: frequent (chews 15–30 times a month; n = 3), moderate (chews 1–4 times a month; n = 3), and rare (chews no more than 3 times a year; n = 6). The completed series, as outlined in the following protocol, totals 11 tubes for each participant.

Protocol

1. Observe precollection protocol.
2. Initiate citric stimulation of saliva production and collect 10 cc saliva in a prepared tube.
3. Immediately place coca and *llipta* in cheek pouch, chewing as is customary.
4. At 15 minutes, collect 10 cc saliva in a second prepared tube; again, at 30 minutes in the third tube.
5. Empty mouth of coca; collect 10 cc saliva in individual tubes at 15-minute intervals for an additional 2 hours.

Results

Standardized data, derived as a percentage of an individual's first sample value, are plotted for each woman in Figure 2 and for the sample in Figure 3. The contamination effect of coca-leaf chewing is transitory, as is

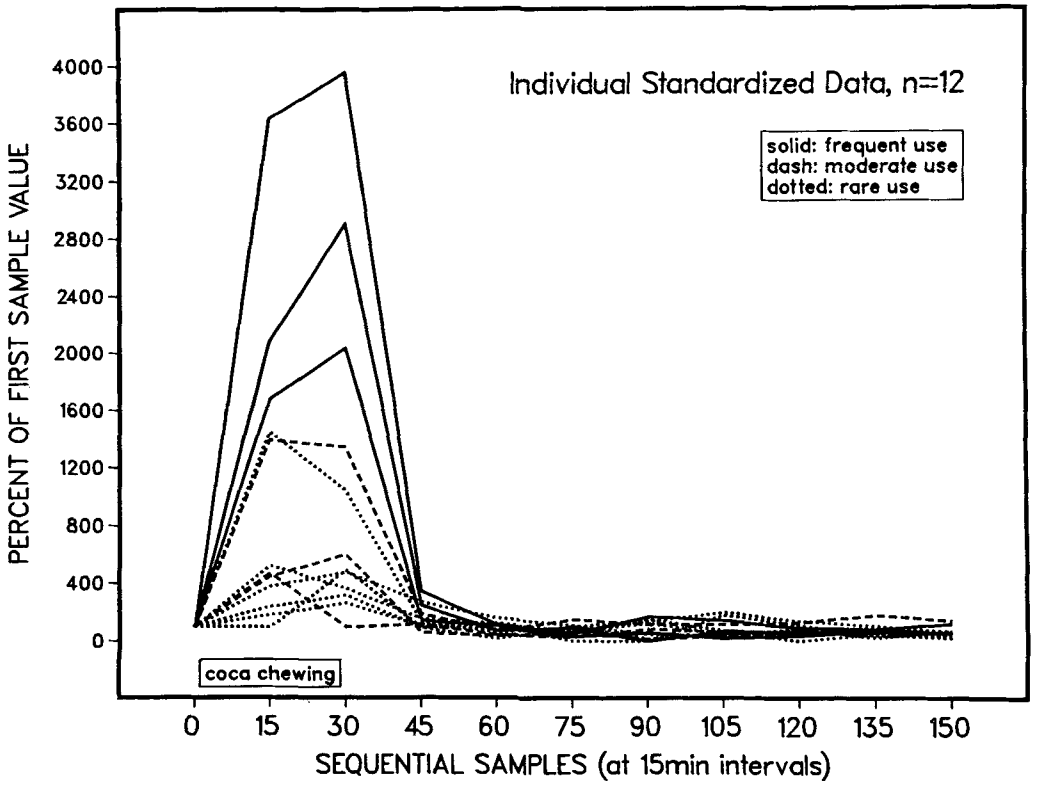

Fig. 2. Experiment 2: Individual standardized data; in all cases the effect of coca-leaf chewing is transitory.

readily seen in the figures. Individually and as an aggregate, salivary samples taken while chewing coca (at 15 and 30 minutes) have markedly elevated progesterone readings (averaging 796 and 918 pmol/L, i.e., rising 1,046% and 1,158% respectively), a finding consistent with the results of Experiment 1.

At 15 minutes after chewing ceases (45-minute sample), salivary progesterone readings approach prechewing levels (161% of first sample). By 30 minutes after chewing ceases, values for the remaining sequential samples are nearly identical to the initial reading. ANOVA with repeated measures tested differences between sequential samples. The 15-minute and 30-minute (i.e., coca-chewing) samples are not significantly different from each other ($F = 0.036$, $P > 0.05$) but both are significantly greater than all other samples (15-minute vs. all but 30-minute sample: $F = 2.2–2.8$, $P \leq 0.05$ for all comparisons; 30-minute vs. all but 15-minute sample: $F = 2.8–3.4$, $P \leq 0.05$ for all comparisons), all of which are statistically indistinguishable from each other ($F = 0.00002–0.028$, $P > 0.05$ for all comparisons).

Figure 2 reveals that frequent users of coca—compared to moderate and rare users—have the greatest elevation in progesterone readings upon chewing (2,468%, 767%, and 475% of initial value, respectively) though their initial values are no greater. ANOVA confirms that frequent users have significantly greater apparent progesterone readings than moderate or rare users for both samples taken while chewing coca (15-minute sample: $F = 9.3$, $P \leq 0.0063$; 30-minute sample: $F = 19.5$, $P \leq 0.0005$); there are no significant differences among the three groups for any other sam-

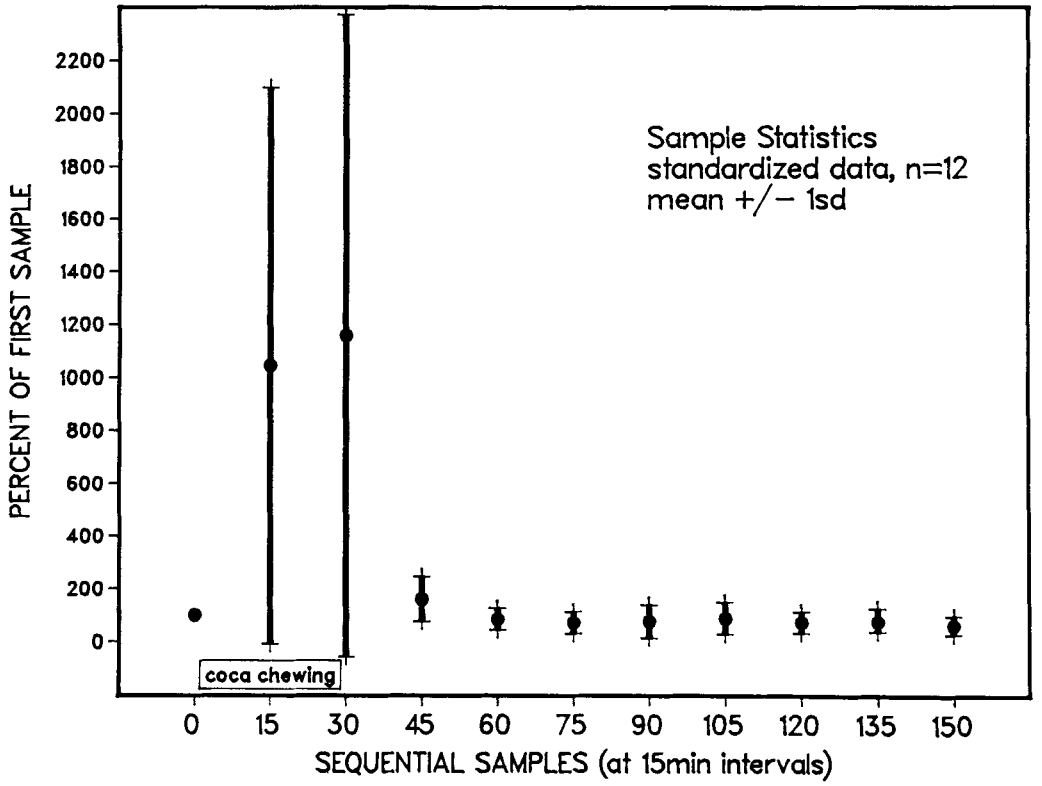

Fig. 3. Experiment 2: Sample standardized data; prechew salivary progesterone levels are reached within 30 minutes after chewing ceases.

ples ($P > 0.05$ in all cases). This higher contamination level is likely the result of a larger coca volume and a greater chewing skill among the more experienced users.

DISCUSSION

Coca-leaf chewing dramatically increases the apparent salivary progesterone levels. Presumably, the contamination is not actually progesterone but rather a substance in the chewing compound that reacts sufficiently with the assay antibody to simulate progesterone. Most importantly, the rise due to coca-leaf chewing is of a magnitude that could be mistaken for normal luteal levels if one were not aware of this practice. These results sharply underscore the essentialness of proper pilot work and protocol testing before using salivary assays in field research. The general necessity of such preliminary work is highlighted by a similar finding that betel nut chewing in Nepal also can contaminate samples if precautions are not taken (Ellison, personal communication).

Fortunately, the effects of coca-leaf chewing are very transitory. Salivary samples can be collected after 30 minutes from the last chew, preferably after the subject has rinsed the mouth with clean water. Having established the feasibility of using this technique in the Andes, and the appropriate protocol for doing so, salivary assays can provide critical data to address several controversies regarding fertility determinants in high altitude populations.

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