Virginia J. Vitzthum
Miranda von Dornum
Peter T. Ellison
Irene Sandy

Assessing Salivary Progesterone in Coca-Leaf Chewing Populations

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Virginia J. Vitzthum is an NICHD Post-Doctoral Fellow at the Population Studies Center and an Assistant Research Scientist in the Department of Anthropology, University of Michigan.

Miranca von Dornum is a graduate student in the Department of Anthropology, Harvard University.

Peter T. Ellison is Professor of Anthropology and Director of the Reproductive Ecology Laboratory, Harvard University.

Irene Sandy is a biotechnician with the Instituto Boliviano de Biologia de la Altura, La Paz, Bolivia.

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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 potentially 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. On the basis of this study, an appropriate protocol is developed for the collection of salivary samples in coca-leaf chewing populations. These results highlight the necessity of establishing suitable collection procedures before full field implementation of saliva sampling.

INTRODUCTION

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). 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. The technique allows an assessment of reproductive function that is impossible to obtain through interviews, and the non-invasive, non-disruptive methodology is suitable to typical field conditions. However, coca-leaf chewing—a ubiquitous practice among high altitude Andean populations—is likely to 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 two 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 proposed.

MATERIALS and METHODS

These procedures are based on Ellison (1988) and Lipson and Ellison (1989).

Collection Tubes: Saliva was collected in polystyrene plastic test tubes pretreated with sodium azide (a bacteriocide, ~0.1% concentration) as a preservative.

Stimulation of Saliva Production: Proven promoters—either olfactory (chocolate, gum, or coca leaves are sniffed) or liquid (five drops of citric acid solution placed on the tongue and gently swilled in the mouth)—were used to enhance saliva production.

Storage: After collection, sample tubes were tightly capped and kept at ambient temperature for 4 months until received in the laboratory, then subsequently frozen until assayed.

Laboratory Analyses: 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 for a medium and low pool averaged 12.6% and 16.3% respectively. 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.

Pre-collection 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 was designed to determine the immediate contaminatory effect of coca-leaf chewing on assays of salivary progesterone.

Protocol:

1. Observe pre-collection protocol (see Materials and Methods).
2. Initiate olfactory stimulation of saliva production and collect 10cc saliva in prepared tube.
3. Immediately place coca (~15 leaves; with or without *llipta*) in cheek pouch.
4. Gently smash coca between molars, collecting 10cc saliva in second prepared tube.

Sample: A total of 27 paired trials (15 with *llipta*, 12 without) from five study participants (three female, two male) who have never chewed coca before this experiment.

Results:

Individual data are presented in Table 1 and summarized in Figure 1.

Coca-leaf chewing dramatically elevates the apparent values for salivary progesterone. For 27 trials, the difference in readings before and after chewing coca averages 385 pmol/L, a mean increase of 716%. Variation in readings between individuals or with respect to *llipta* use, sex, or age is not statistically significant.

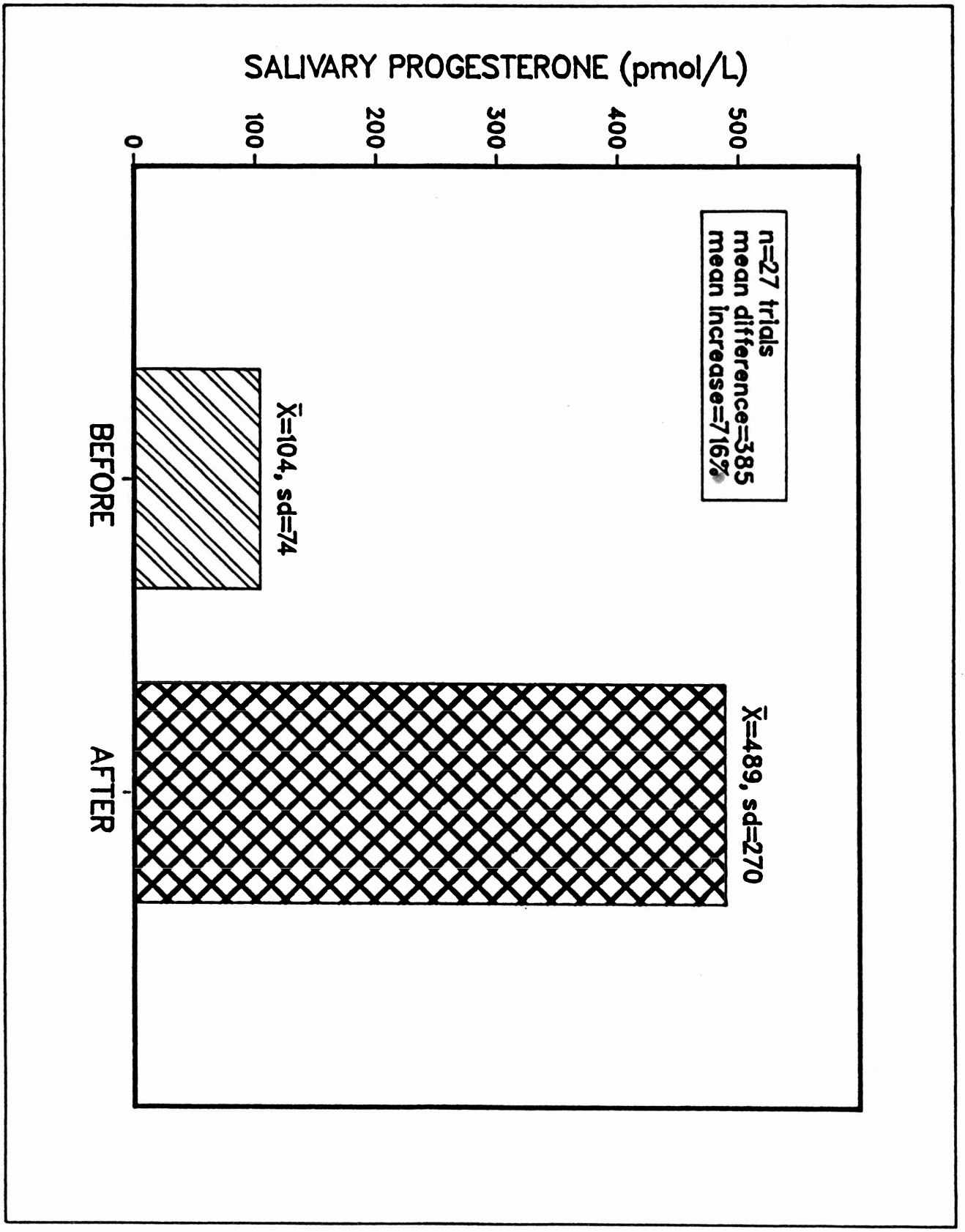
Though the apparent increase in progesterone is substantial, the magnitude of difference due to coca-leaf contamination approximates that separating normal follicular and luteal levels in cycling women. Thus, undetected coca chewing could mistakenly lead to classifying individual samples as luteal and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

Table 1

Salivary Progesterone (pmol/L): Contamination Experiment

Subject	Sex	*Llipta*	Before	After
1	F	N	219	544
1	F	N	248	447
1	F	N	164	943
1	F	N	203	350
1	F	Y	98	327
1	F	N	55	416
1	F	N	71	903
1	F	Y	89	482
1	F	Y	109	775
1	F	N	116	328
2	M	N	175	404
2	M	Y	323	321
2	M	Y	127	598
2	M	Y	82	282
2	M	Y	46	153
2	M	N	29	624
2	M	Y	31	945
2	M	N	39	223
3	F	N	75	587
3	F	N	70	124
4	F	Y	47	473
4	F	Y	40	485
4	F	Y	83	477
4	F	Y	74	1211
4	F	Y	67	136
5	M	Y	25	259
5	M	Y	101	373

FIGURE 1


SALIVARY PROGESTERONE (pmol/L)
0
100
200
300
400
500
n=27 trials
mean difference=385
mean increase=716%
BEFORE
X̄=104, sd=74
AFTER
X̄=489, sd=270

EXPERIMENT 2: TIME SERIES STUDY

This procedure was designed to determine the duration of coca-leaf contamination once chewing has ended.

Protocol:

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

Sample: Twelve women, ranging in age from 23 to 45 years, in three use classes: frequent (chews 15 to 30 times a month; n=3), moderate (chews 1 to 4 times a month; n=3), rare (chews no more than 3 times a year; n=6). Completed series, as outlined in the above protocol, totals 11 tubes for each participant.

Results:

Individual raw data are given in Table 2. Standardized data, derived as a percentage of the first sample value, are presented in Table 3. Based on the raw data, Figures 2 and 4 plot the individual values and the sample statistics (mean and standard deviation); interassay variability is responsible for the two very high peaks in Figure 2. Figure 3 omits these two high peaks to reveal the variation among the other ten sample series. Standardized data are plotted in Figures 5 and 6.

The contamination effect of coca-leaf chewing is transitory, as is 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 1046% and 1158% respectively), a finding consistent with the results of Experiment 1.

At 15 minutes after chewing ceases (45 minute sample), salivary progesterone readings approach pre-chewing levels (161% of first sample). By 30 minutes after chewing ceases, values for the remaining sequential samples are nearly identical to the initial reading.

Statistical analyses (ANOVA with repeated measures, Scheffe F-test, Fisher PLSD) confirm that the 15 minute and 30 minute (i.e. *coca-chewing*) samples are not significantly different from each other but both are significantly greater than all other samples, all of which are statistically indistinguishable from each other. Figure 5 reveals that frequent users of coca—compared to

moderate and rare users—have the greatest elevation in progesterone readings upon chewing (2468%, 767%, and 475% of initial value, respectively) though their initial values are no greater. ANOVA confirms that frequent users have significantly greater readings than moderate or rare users for both *coca-chewing* samples; there are no significant differences among the three groups for any other samples. This higher contamination level is likely the result of a larger coca volume and a greater chewing skill among the more experienced users.

Table 2

Salivary Progesterone (pmol/L): Raw Data for Time Series Experiment

Age in Years	SEQUENTIAL SAMPLE #										
	1	2	3	4	5	6	7	8	9	10	11
Rare Coca Users											
25	47	111	149	44	58	32	65	95	65	49	28
25	421	1581	2008	1136	677	457	194	171	178	272	159
28	36	521	375	47	23	35	54	26	0	18	9
35	153	143	752	141	26	156	24	85	115	36	106
37	376	668	987	389	302	261	463	183	310	345	252
41	43	224	157	66	51	0	0	79	47	17	9
Moderate Coca Users											
23	81	378	78	95	36	23	68	51	46	63	44
24	95	423	571	55	36	61	11	37	38	61	47
27	220	3059	2955	407	165	328	254	253	280	396	308
Frequent Coca Users											
32	41	689	835	58	44	13	24	8	16	26	23
43	37	772	1076	91	27	35	0	26	22	28	19
45	27	982	1069	93	31	14	46	39	24	22	32
TOTAL SAMPLE											
mean	131	796*	918*	219	123	118	100	88	95	111	86
s.d.	137	821	838	316	193	151	139	76	105	140	101

* marks coca-chewing samples; these are statistically indistinguishable from each other but significantly greater than all other samples ($p<0.05$).

Table 3

Salivary Progesterone (% of Sample 1): Standardized Data for Time Series Experiment

Age in Years	SEQUENTIAL SAMPLE #										
	1	2	3	4	5	6	7	8	9	10	11
Rare Coca Users											
25	100	236	317	94	123	68	138	202	138	104	60
25	100	376	477	270	161	109	46	41	42	65	38
28	100	1447	1042	131	64	97	150	72	0	50	25
35	100	94	492	92	17	102	16	56	75	24	69
37	100	178	263	104	80	69	123	49	82	92	67
41	100	521	365	154	119	0	0	184	109	40	21
Moderate Coca Users											
23	100	467	96	117	44	28	84	63	57	78	54.3
24	100	445	601	58	38	64	12	39	40	64	50
27	100	1391	1343	185	75	149	116	115	127	180	140
Frequent Coca Users											
32	100	1681	2037	142	107	32	59	20	39	63	56
43	100	2087	2908	246	73	95	0	70	60	76	51
45	100	3637	3959	344	115	52	170	144	89	82	119
TOTAL SAMPLE											
mean	100	1047	1158	161	85	72	76	88	72	76	62
s.d.		1054	1214	85	41	41	62	60	40	39	35

FIGURE 2

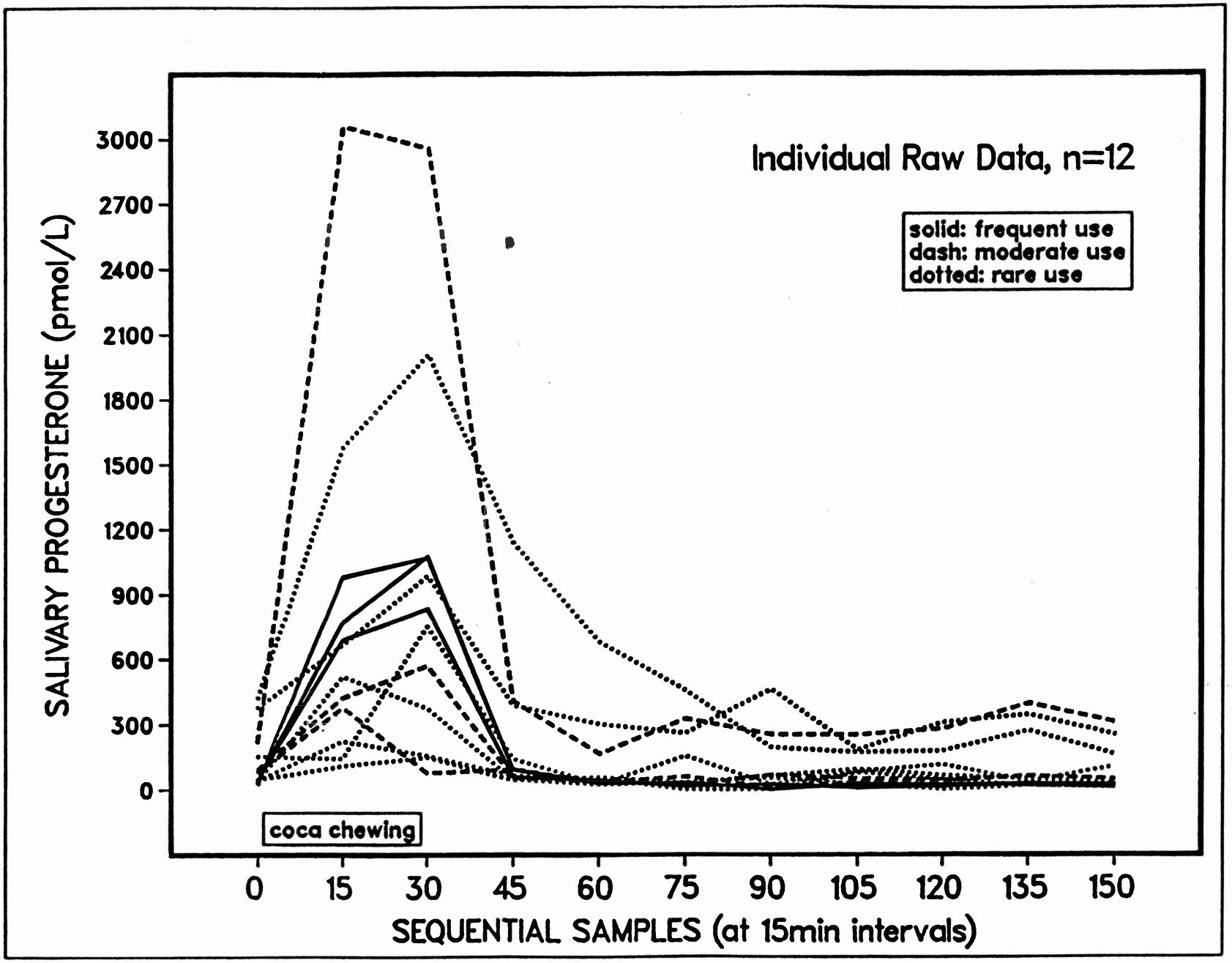

Individual Raw Data, n=12
solid: frequent use
dash: moderate use
dotted: rare use
SALIVARY PROGESTERONE (pmol/L)
3000
2700
2400
2100
1800
1500
1200
900
600
300
0
coca chewing
0
15
30
45
60
75
90
105
120
135
150
SEQUENTIAL SAMPLES (at 15min intervals)

FIGURE 3

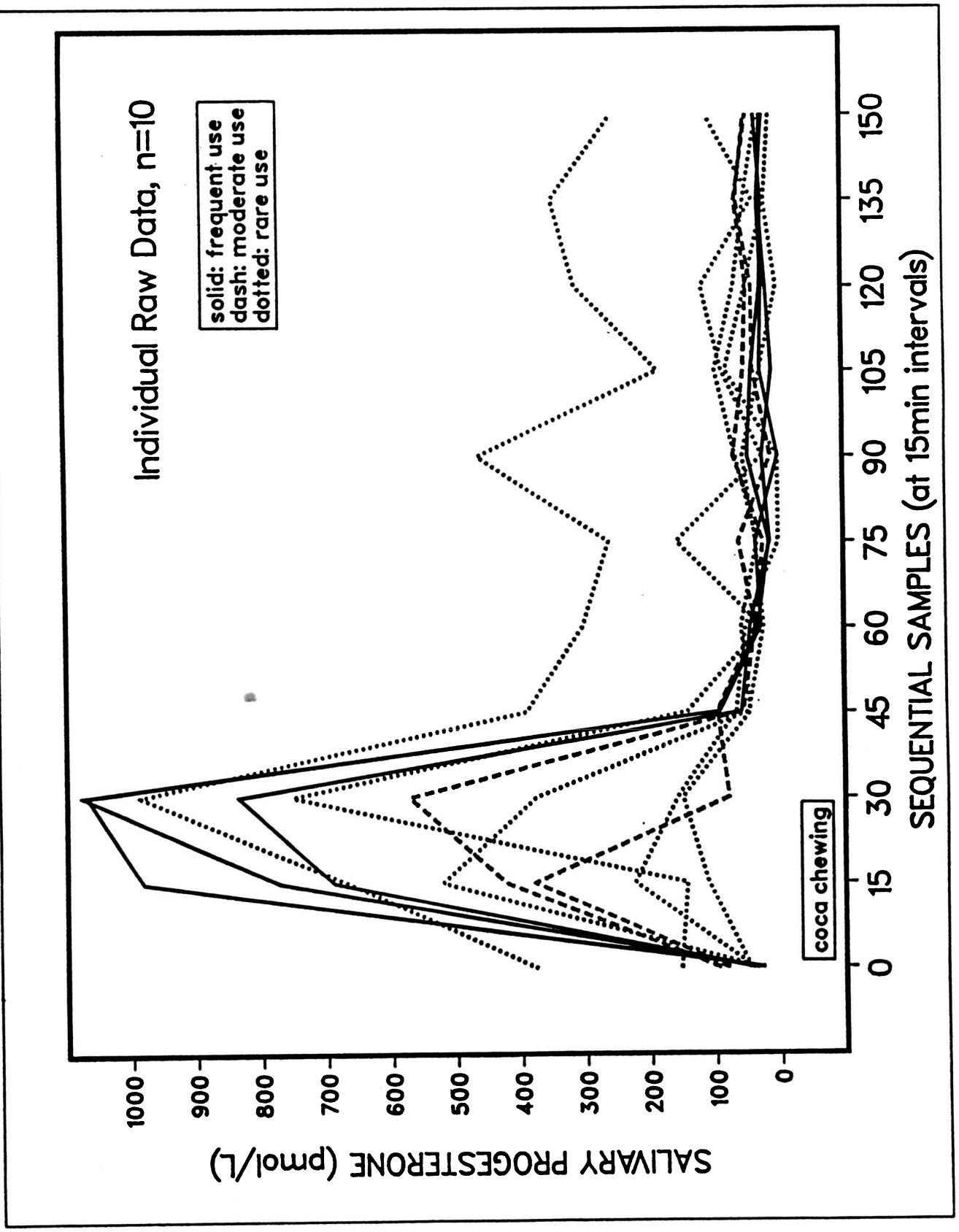
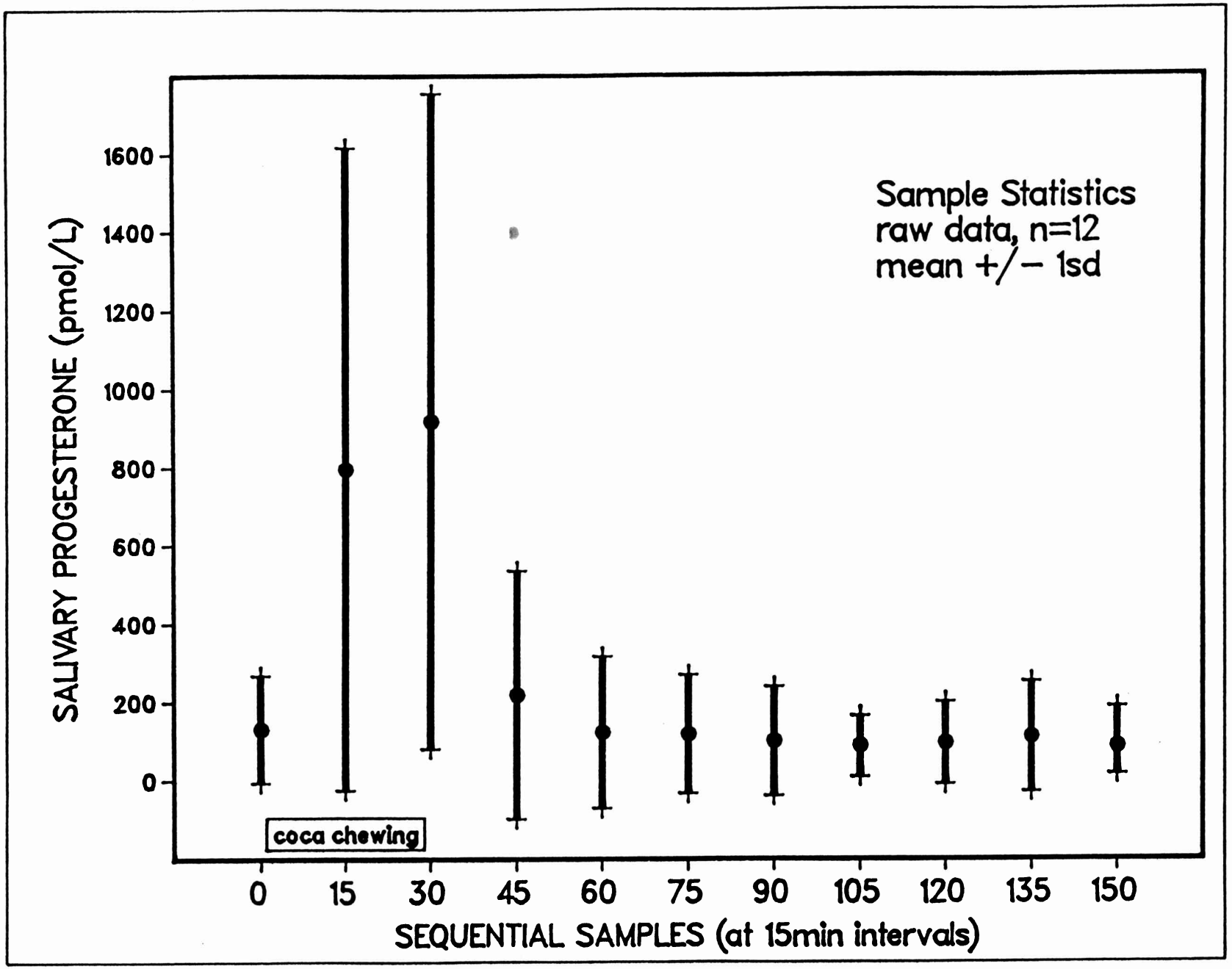

Individual Raw Data, n=10
solid: frequent use
dash: moderate use
dotted: rare use
coca chewing
SALIVARY PROGESTERONE (pmol/L)
SEQUENTIAL SAMPLES (at 15min intervals)
0
100
200
300
400
500
600
700
800
900
1000
0
15
30
45
60
75
90
105
120
135
150

FIGURE 4


SALIVARY PROGESTERONE (pmol/L)
1600
1400
1200
1000
800
600
400
200
0
Sample Statistics
raw data, n=12
mean +/- 1sd
coca chewing
0
15
30
45
60
75
90
105
120
135
150
SEQUENTIAL SAMPLES (at 15min intervals)

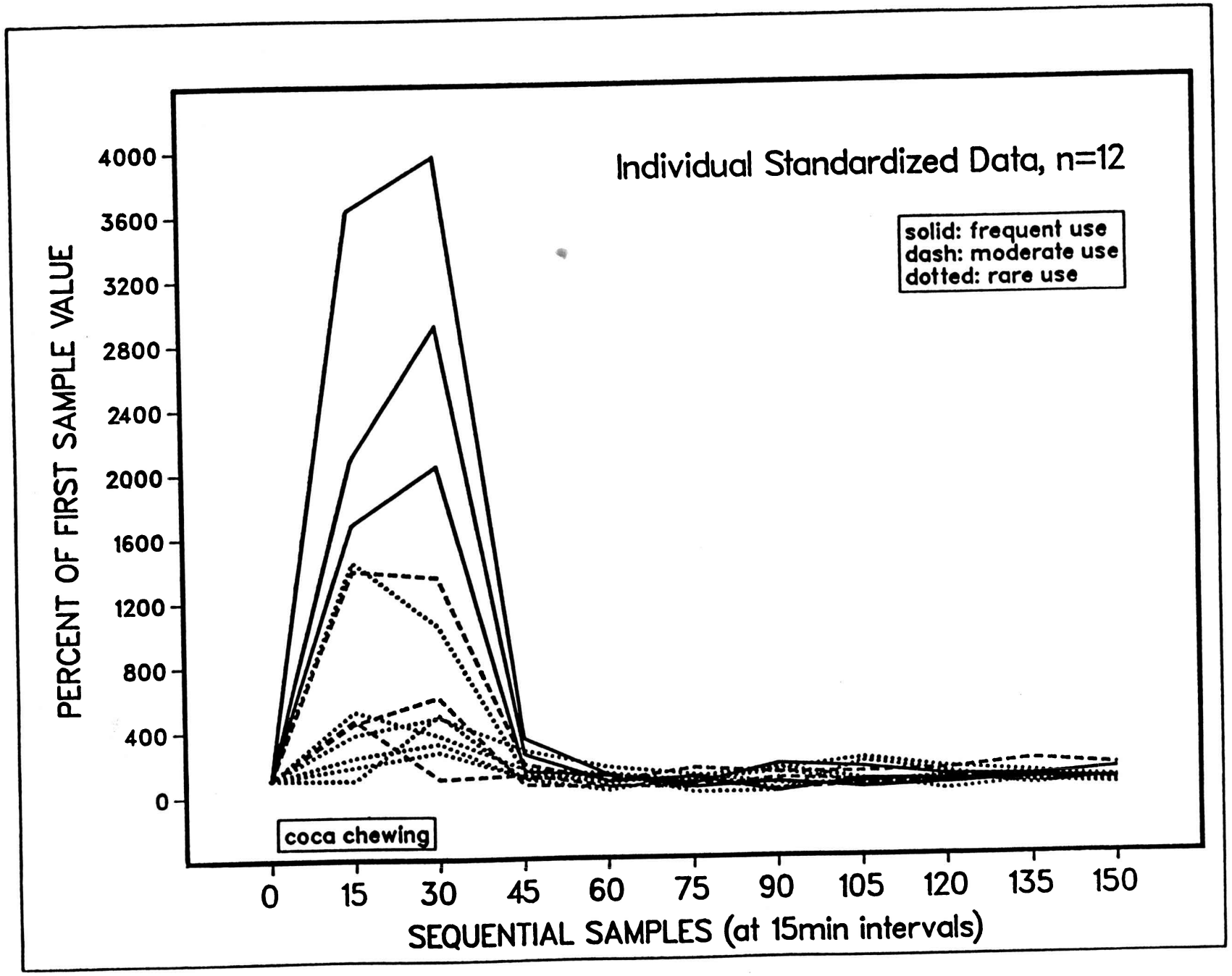

Individual Standardized Data, n=12
solid: frequent use
dash: moderate use
dotted: rare use
PERCENT OF FIRST SAMPLE VALUE
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
coca chewing
0
15
30
45
60
75
90
105
120
135
150
SEQUENTIAL SAMPLES (at 15min intervals)

FIGURE 5

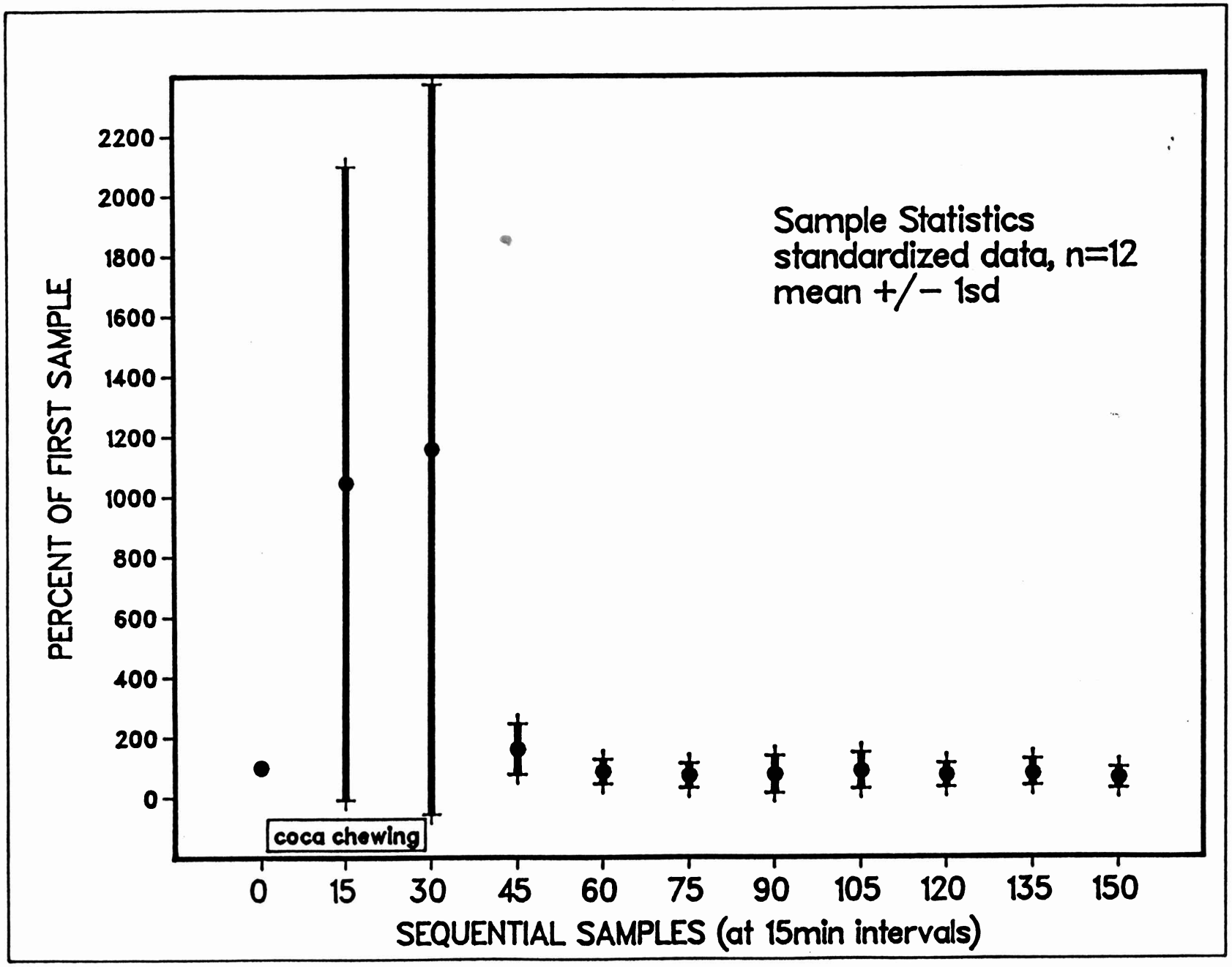

PERCENT OF FIRST SAMPLE
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Sample Statistics
standardized data, n=12
mean +/- 1sd
coca chewing
0
15
30
45
60
75
90
105
120
135
150
SEQUENTIAL SAMPLES (at 15min intervals)

FIGURE 6

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, pers. comm.).

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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