

## COMMENTS

# Correction of Hyperandrogenemia by Laparoscopic Ovarian Cautery in Women with Polycystic Ovarian Syndrome Is Not Accompanied by Improved Insulin Sensitivity or Lipid-Lipoprotein Levels\*

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### ABSTRACT

Polycystic ovarian syndrome (PCOS) is a common disorder associated with hyperandrogenemia and infertility. Abdominal obesity, insulin resistance, and dyslipoproteinemias are other common metabolic disorders typically found in women with PCOS. The cause-effect relationship between hyperandrogenemia and insulin resistance-dyslipoproteinemia remains unclear. In this study, we have investigated the changes in androgenemia, insulin sensitivity, and plasma lipid-lipoprotein levels after laparoscopic ovarian cautery (LOC) for ovulation induction in eight infertile women with clomiphene citrate-resistant PCOS. After LOC, significant decreases in androstenedione (43%), testosterone (48%), and free testosterone (48%) concentrations were observed ( $P < 0.05$ ). Glucose utilization

during an euglycemic-hyperinsulinemic clamp did not change after LOC. In addition, no significant changes after the surgical procedure were observed for cholesterol, triglycerides, and apolipoprotein concentrations measured in total plasma and in different lipoprotein fractions. In conclusion, within the short duration of observation of this study, our findings demonstrate that insulin resistance and lipoprotein abnormalities associated with PCOS are not secondary to hyperandrogenemia. The clinician, therefore, must be cognizant of the persistence of these metabolic risk factors for cardiovascular disease once successful ovulation and fertility is restored, and institute appropriate monitoring, counseling, and medical intervention as required. (*J Clin Endocrinol Metab* 84: 4278–4282, 1999)

**P**OLYCYSTIC ovarian syndrome (PCOS) is an endocrine abnormality that affects 5–10% of women in the reproductive age group (1, 2). Women with PCOS often demonstrate abdominal obesity (3, 4), insulin resistance (4–6), and dyslipoproteinemias (7) and therefore present an increased risk of developing type 2 diabetes and cardiovascular disease (5, 7–10).

In women with PCOS as well as in those without PCOS, androgen levels have been shown to be correlated with insulin sensitivity and variables of the lipid-lipoprotein profile (5, 11–13). The cause-effect relationship between hyperandrogenemia and insulin resistance-dyslipoproteinemias remains unclear, however, and elucidating this relationship was the major purpose of the present study. Some studies that have induced a decrease in androgen concentrations

with a GnRH analog in women with PCOS have failed to improve insulin sensitivity or the lipid profile (14–16), whereas others have demonstrated improvements in the metabolic profile after normalization of the concentrations of circulating androgens with spironolactone (17) or Lupron (a GnRH agonist) (18). Differences in strategies used to decrease androgen levels may partially explain these conflicting results, and differences observed between studies may reflect an effect of the particular pharmacological agent used.

Studies conducted in women with PCOS in which the insulin-sensitizing agent, troglitazone, was used to enhance insulin action have demonstrated that improved insulin sensitivity is accompanied by significant decreases in circulating androgens without changes in body weight or body fat distribution (19, 20). These studies support the idea that insulin resistance contributes causally to the hyperandrogenemia in women with PCOS.

Some mechanisms have been proposed to explain the effect of insulin on androgen concentrations (for a review, see Ref. 21). Briefly, studies have demonstrated that insulin has a stimulatory effect on the production of androgens by human ovarian stroma and theca. Insulin may act not only through insulin receptors, but also through insulin-like growth factor receptors. It has also been suggested that in some insulin-resistant states such as PCOS, the ovary would

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remain sensitive to the action of insulin, acting through either its own receptor or an insulin-like growth factor receptor, and this could explain how hyperinsulinemia associated with insulin resistance is associated with increased androgen production.

In the present study, laparoscopic ovarian cautery (LOC) was performed to treat ovulatory infertility in women with PCOS who had previously failed clomiphene citrate therapy. LOC is an endoscopic procedure that is associated with a fall in circulating androgen levels and with a restoration of ovulation in 80–85% of women with PCOS (see Ref. 22 for a review) through mechanisms that are not well resolved. Apart from the effects of LOC on androgen levels and ovulation that have already been documented (23), this study was designed to evaluate the impact of LOC on insulin sensitivity (as determined by the euglycemic-hyperinsulinemic clamp) and variables of the plasma lipid-lipoprotein profile. We hypothesized that nonpharmacological reduction of serum androgen levels in patients with PCOS would have no effect on plasma lipids/lipoproteins or insulin sensitivity.

## Subjects and Methods

### Subjects

Seventeen women with PCOS who had failed to ovulate or failed to conceive with at least six cycles of clomiphene citrate, up to a dose of 200 mg/day for 5 consecutive days (with or without hCG), were offered LOC. In addition, five of these women had already attempted and failed to conceive after gonadotropin therapy, three had failed to conceive after complications of gonadotropin therapy, and two had previously failed *in vitro* fertilization. Aside from PCOS, all women were otherwise healthy and taking no medications. Diabetes was excluded in all patients by performing a 3-h oral glucose tolerance test. Baseline measurements of serum androgen concentrations, insulin sensitivity, and plasma lipid-lipoprotein profile were performed. This study was approved by The Toronto Hospital committee for research on human subjects, and all subjects gave their written informed consent before their participation in the study.

### Laparoscopic ovarian cautery

Laparoscopic ovarian cautery was performed under general anesthetic, on an out-patient basis, using a standard three-puncture technique and video guidance as previously described (23). All patients were discharged the same day, and there were no intraoperative complications. All patients maintained menstrual cycle calendars and basal body temperature charts immediately after LOC. Serum progesterone concentrations were used to confirm ovulation.

### Follow-up

Three of the 17 women who underwent LOC failed to ovulate for at least 3 cycles postoperatively. Fourteen of the 17 women (82%) initiated regular ovulatory cycles (25–35 days) after LOC. Six of them conceived within 3 ovulatory cycles and therefore could not undergo postoperative metabolic evaluation. Eight women who ovulated for at least 3 cycles postoperatively, but who had not yet conceived, were available for postoperative assessment. All postoperative metabolic evaluations occurred between days 2 and 7 of their fourth menstrual cycle, on the same day as the euglycemic hyperinsulinemic clamp study (preoperatively the women were not cycling). Measurements performed at baseline and at follow-up are described in the following sections.

### Euglycemic-hyperinsulinemic clamp

The euglycemic-hyperinsulinemic clamp was performed after a 12-h overnight fast. An antecubital arm vein was cannulated with a catheter for infusion of insulin and 20% dextrose. A hand vein from the con-

tralateral arm was cannulated, the cannula was attached to a three-way stopcock, and the hand was placed in a thermoregulated Plexiglas box maintained at 55 C to permit sampling of arterialized blood for determination of plasma insulin and glucose levels. A primed continuous infusion of insulin (Humulin, 40 mU/m<sup>2</sup>·min) was begun and continued for 2 h (24). Blood was withdrawn every 5 min for measurements of plasma glucose, and measurement of insulin levels was performed every 15 min. The dextrose infusion rate was adjusted to maintain euglycemia. The glucose disposal rate was calculated from the mean glucose infusion rate measured during the last 30 min of the test. The validity of these measurements assumes that the endogenous glucose production has been completely suppressed during the insulin infusion. We assume that endogenous glucose production was more than 90% suppressed, as complete suppression using a similar insulin infusion rate, and [<sup>3</sup>-<sup>3</sup>H]glucose turnover methodology has been reported by others in obese subjects. (25).

Plasma glucose concentrations were measured at the bedside using an automated glucose analyzer (Glucose Analyser II, Beckman Coulter, Inc., Fullerton, CA), whereas plasma insulin levels were determined by RIA, using a double antibody separation method (Pharmacia Diagnostics, Uppsala, Sweden) (26).

### Plasma lipid-lipoprotein profile

Fasting blood samples were drawn into Na<sub>2</sub> ethylenediamine tetraacetate (1 mg/mL) and chilled to 4 C. The plasma was adjusted at a density of 1.019 g/mL and spun at 39,000 rpm in a 70.1 Ti rotor (Beckman Coulter, Inc., Palo Alto, CA) for 18 h at 4 C to obtain the S<sub>f</sub> >12 fraction [intermediate density lipoprotein (IDL) and very low density lipoprotein (VLDL) fractions]. The S<sub>f</sub> >12 was overlaid with a solution of density 1.006 g/mL and spun at 29,000 rpm in an SW-40 bucket rotor (Beckman Coulter, Inc.) for 2 h at 15 C to obtain the VLDL (top) and the IDL (bottom) fractions. The high density lipoprotein (HDL) fraction was separated after manganese/heparin precipitation of plasma apolipoprotein B (Apo B)-containing lipoproteins (27).

Cholesterol (Chol) and triglycerides (TG) were measured enzymatically in the plasma and in the VLDL, IDL, and HDL fractions (Roche Molecular Biochemicals, Laval, Canada). LDL-cholesterol and LDL-TG concentrations were obtained by difference. Apo B was quantified in whole plasma and in VLDL and IDL fractions by an electroimmunoassay procedure (28). The LDL-Apo B was then calculated by subtraction. Apo A1 was determined in whole plasma by a rocket electroimmunoassay using the Sebia Hydrigel Apo A1B kit (Gelman Sciences, Toronto, Canada; catalog no. 4050) according to the method of Laurell (29).

Nondenaturing 2–16% polyacrylamide gel (purchased from David Rainwater, Southwest Foundation for Biomedical Research, San Antonio, TX) electrophoresis was performed on whole plasma to determine LDL particle size, according to the procedure of Krauss *et al.* (30). A volume of 5  $\mu$ L plasma was applied to the gel, and electrophoresis was performed at 125 V for 16 h. A mixture of standard molecular weight proteins of known diameter (HMW Calibration Kit, Pharmacia Biotech, Piscataway, NJ) consisting of thyroglobulin, ferritin, catalase, lactate dehydrogenase, and albumin was applied to a separate lane in each gel along with Lp(a) and LDL standards for which diameters were determined by electron microscopy. Gels were stained for proteins with Coomassie brilliant blue G-250 (Eastman Kodak Co., Rochester, NY) in perchloric acid (0.1% stain and 5% perchloric acid) for 24 h and destained for 2 days in 7% acetic acid. The diameters of the particles were assessed with a densitometer (ImageMaster DTS, Pharmacia Biotech, Uppsala, Sweden) and with Image Master computer software (Pharmacia Biotech, Uppsala, Sweden) based on the distance of migration relative to the standards.

### Steroid profile

Testosterone, free testosterone, and 17 $\alpha$ -hydroxyprogesterone were measured by RIA using kits supplied by Diagnostic Products (Los Angeles, CA). The androstenedione RIA kit was supplied by Diagnostics Systems Laboratories, Inc. (Webster, TX). Dehydroepiandrosterone sulfate (DHEAS) was measured by RIA using the Coat-A-Count DHEAS commercial kit (Diagnostic Products).

### Statistical analyses

The significance of changes in variables measured before and after LOC were tested with the paired Student's *t* test. Wilcoxon signed rank tests were performed when values were not normally distributed. For insulin concentrations measured during the euglycemic-hyperinsulinemic clamp, ANOVA on repeated measurements was performed to determine whether insulin levels remained in steady state during the euglycemic-hyperinsulinemic clamp. Statistical analyses were performed with SigmaStat software (SigmaStat for Windows, version 1.0, Jandel Corp., San Rafael, CA). Data are presented throughout the manuscript as the mean  $\pm$  SD.  $P < 0.05$  was assumed to be significant.

### Results

Women who completed the two phases of the study had a mean age of  $31 \pm 2.7$  ( $\pm$ SD) yr. They were generally obese, with a mean body mass index of  $31.7 \pm 4.4$  kg/m<sup>2</sup> (ranging from 23.1–39.1 kg/m<sup>2</sup>), and no change in body mass index was found after LOC. The baseline demographic and biochemical data from the nine subjects who were screened but who did not complete both phases of the study were analyzed, and there were no significant differences compared to the eight subjects reported.

Figure 1 shows that significant decreases in serum concentrations of androstenedione (44%), testosterone (48%), free testosterone (48%), and 17 $\alpha$ -hydroxyprogesterone (53%) were observed after LOC ( $P < 0.05$ ), whereas no significant change in DHEAS (2% decrease) was seen.

Figure 2 shows that no changes in fasting plasma glucose were observed after LOC (from  $5.3 \pm 0.6$  to  $5.2 \pm 0.6$  mmol/L). Although the group mean of fasting plasma insulin levels after LOC ( $112.3 \pm 76.2$  pmol/L) was lower than that before ( $132.1 \pm 156.2$  pmol/L), there was a high degree of variability between individuals, and by paired analysis, there was no significant change. The glucose disposal rate after LOC was unaltered (from  $3.2 \pm 0.8$  to  $3.3 \pm 1.1$  mg glucose/kg·min). In Fig. 3, it can be seen that insulin concentrations (before and after LOC) reached a steady state after 30 min and remained constant for the following 90 min. In addition, steady state insulin concentrations during the clamp before LOC were well matched with values measured during the postoperative clamp.

Table 1 presents the plasma lipid-lipoprotein levels, before and after LOC. Cholesterol concentrations measured in total plasma or in VLDL, LDL, or HDL subfractions and the ratio of total chol/HDL-chol did not change significantly after LOC. Similarly, no change in the plasma TG concentration was observed. Apo A1 and Apo B levels as well as LDL particle size did not change significantly in response to LOC. Finally, the content of chol, TG, and Apo B of the IDL fraction was not significantly altered after LOC (results not shown).

### Discussion

In the present study, LOC was performed in infertile women with clomiphene citrate-resistant PCOS. The LOC procedure resulted in successful restoration of the ovulatory cycles and in a marked reduction of serum androgen levels, but failed to correct insulin sensitivity or improve the plasma lipid-lipoprotein profile, therefore suggesting that androgens are not related in a causal fashion to insulin sensitivity and plasma lipid-lipoprotein levels. Our study differs from

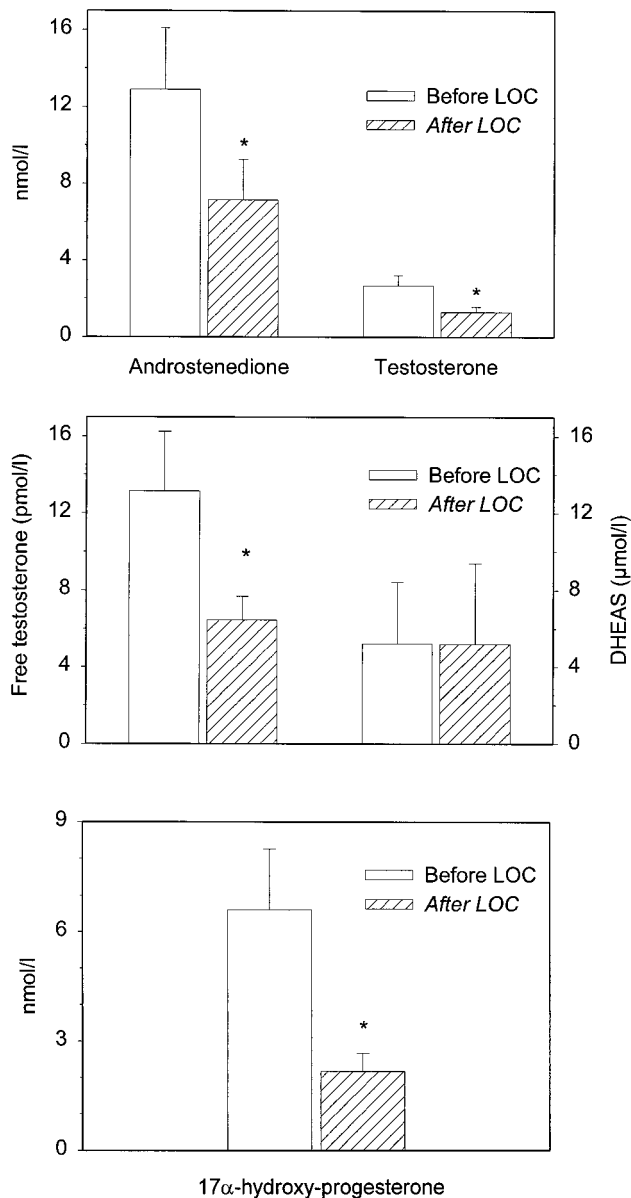


FIG. 1. Androstenedione, testosterone, free testosterone, DHEAS, and 17 $\alpha$ -hydroxyprogesterone levels before and after LOC. \*, Significantly different from the value measured before LOC,  $P < 0.05$ .

most others that have examined the relationship between serum androgen levels and insulin resistance, as we used a nonpharmacological method to reduce androgen levels in women with PCOS. To our knowledge, there is only one other study that has examined the effect of LOC on fasting insulin concentrations (31). In accordance with our results, the researchers reported that fasting insulin levels were not changed 1 month after LOC in a group of 10 women with PCOS.

At baseline, our sample of hyperandrogenic women was representative of many women with PCOS, as they were generally obese and showed insulin resistance. The glucose disposal rate, a measure of whole body insulin sensitivity, was about half the value previously reported in control women of reproductive age (5, 25). Although the women in

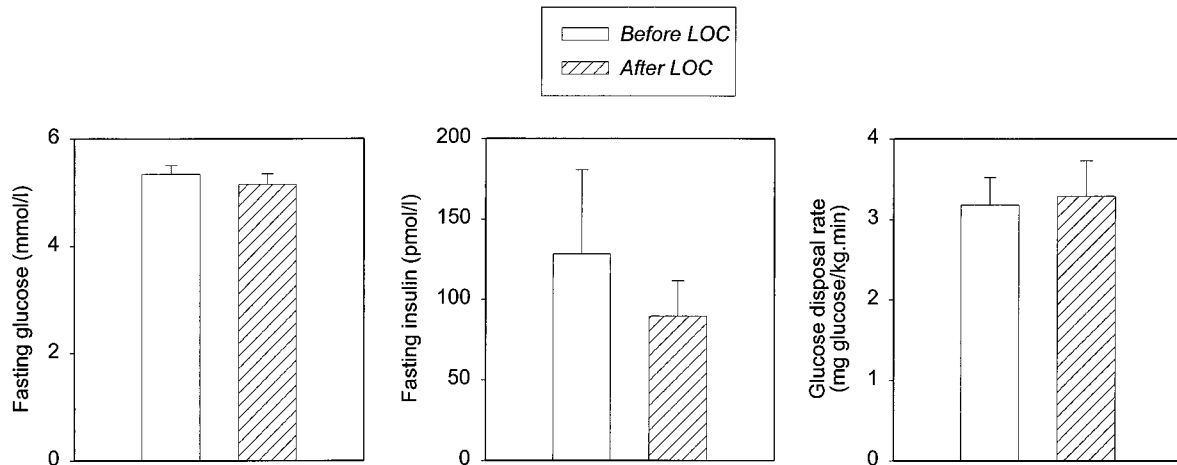


FIG. 2. Concentrations of fasting plasma glucose and insulin and glucose utilization during an euglycemic-hyperinsulinemic clamp before and after LOC. Glucose utilization was measured during the last 30 min of the euglycemic-hyperinsulinemic clamp. Values of fasting insulin levels were not normally distributed and were therefore compared using Wilcoxon's signed rank test. Differences were not statistically significant.

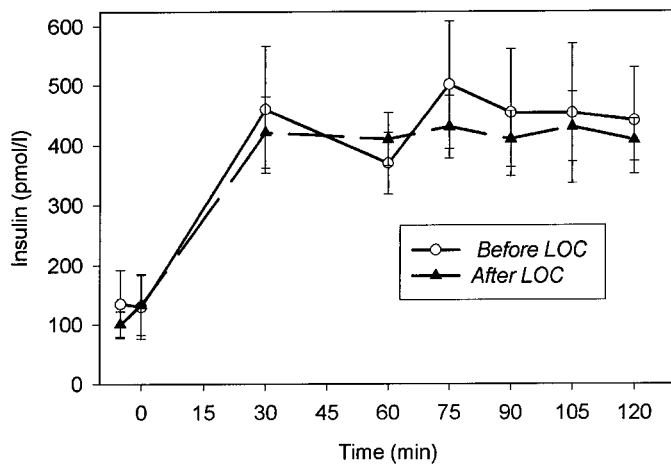


FIG. 3. Plasma insulin concentrations measured during the euglycemic-hyperinsulinemic clamp before and after LOC.

TABLE 1. Plasma lipid-lipoprotein before and after LOC (n = 8)

Variable	Pre-LOC	Post-LOC	P value
Cholesterol (mmol/L)	3.91 ± 0.64	4.07 ± 0.53	0.41
LDL-chol (mmol/L)	2.32 ± 0.58	2.48 ± 0.59	0.39
HDL-chol (mmol/L)	1.08 ± 0.34	1.07 ± 0.17	0.85
VLDL-chol (mmol/L)	0.10 ± 0.06	0.11 ± 0.05	0.61
Chol/HDL-chol	3.87 ± 1.06	3.88 ± 0.71	0.94
Triglyceride (mmol/L)	1.32 ± 0.60	1.31 ± 0.47	0.95
Apo B (mg/dL)	88.6 ± 8.5	76.3 ± 20.0	0.11
LDL-apoB (mg/dL)	65.5 ± 14.4	59.9 ± 16.3	0.41
Apo A1 (mg/dL)	108.9 ± 18.4	107.3 ± 9.7	0.68
LDL size (nm) <sup>a</sup>	24.2 ± 0.7	24.4 ± 0.4	0.46

Values are the mean ± SD.

<sup>a</sup> Wilcoxon signed rank test was used for LDL size.

the present study were not frankly dyslipidemic, they had TG levels close to the 90th percentile, HDL-chol concentrations between the 10th and 25th percentile, Apo B concentrations in the 75th percentile, and Apo A1 levels between the 5th and the 10th percentile according to the distribution of these variables reported in similar age groups (32–34). Our group of women also had small dense LDL, as their mean

LDL diameter was below the cut-off point (25.5 nm) proposed for the identification of the small, dense LDL phenotype (35). Plasma total and LDL-chol concentrations were normal in these subjects, as is typically the case in insulin-resistant syndromes (36). Our group of women with PCOS, therefore, presented with dyslipoproteinemia typical of individuals with the insulin resistance syndrome (37). Similar findings have been described in weight-matched women with and without PCOS, demonstrating that the dyslipidemia that accompanies the hyperandrogenemia in women with PCOS is not explained by obesity alone (38).

Fourteen of the 17 women who underwent the LOC procedure initiated regular ovulatory cycles. As expected and as previously reported (23), restoration of ovulation was accompanied by a fall in androgen levels toward normal values, except for DHEAS, which did not change after LOC. This latter finding was not surprising, as DHEAS is mainly secreted by the adrenals rather than by the ovary. The mechanisms of action by which LOC alters the androgen profile and restores ovulation in women with PCOS remain uncertain, but is likely to involve destruction of theca and stroma, the main ovarian compartment contributing to androgen concentrations (39). It has been shown in most studies that after LOC a consistent decrease in peripheral androgen levels was the earliest and most reproducible hormonal change, with a more delayed decline in LH (39–42). This is true regardless of whether ovarian cauterization is performed unilaterally or bilaterally (43). Unilateral ovarian cauterization induces comparable decreases in LH as well as bilateral ovulation, as does bilateral ovarian cauterization. This suggests that the decrease in ovarian androgen production, secondary to destruction of stromal tissue, in turn leads to correction of disturbed ovarian-pituitary feedback that is most clearly manifested as hypersecretion of LH (43). This theory is supported by the work of Sumioki *et al.*, who demonstrated a marked reduction of LH pulse amplitude (but not frequency), mean LH levels, and pituitary LH responsiveness to GnRH stimulation concomitant with the fall in circulating androgen levels after LOC (40).

In conclusion, our results clearly demonstrated that within

the short duration of observation of this study, the lowering of androgen levels and restoration of ovulatory cycles in women with PCOS after LOC is not accompanied by improvements in insulin sensitivity or in the plasma lipid-lipoprotein profile. Infertility is usually a major concern for women with PCOS who are seeking medical help. Although LOC restores ovulation in women with PCOS, it does not seem to improve cardiovascular risk factors and type 2 diabetes risk. Therefore, in addition to medical therapy or surgery aimed at reestablishing ovulation, women with PCOS should be offered additional therapeutic strategies to improve insulin sensitivity and lipid-lipoprotein levels to reduce their risk of type 2 diabetes and cardiovascular disease. Finally, although our results have shown that a normalization of circulating androgens is not associated with improvements in features of the insulin resistance syndrome in women with PCOS over short term observation, further studies will be needed to delineate the potential long term effects and the possible contribution of altered androgen levels to insulin resistance in other populations.

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