Enhanced Post-Receptor Insulin Effects in Women Following Dehydroepiandrosterone Infusion

Eldon D. Schriock, Cynthia K. Buffington, James R. Givens, and John E. Buster

OBJECTIVE: *We hypothesized that intravenous dehydroepiandrosterone (DHEA) would decrease insulin resistance in normal and insulin-resistant women.*

METHODS: *Five insulin-resistant women diagnosed as having polycystic ovaries (PCO) with elevated testosterone and normal dehydroepiandrosterone sulfate (DHEAS) with amenorrhea were recruited. Obese controls (OC) with normal menses and normal testosterone and DHEAS were recruited and matched to each PCO woman for age and weight. The PCO women had a mean testosterone of 3.2 ± 0.4 nmol/L, fasting serum insulin level of 330 ± 55 pmol/L, and DHEAS level of 3.4 ± 1.3 μmol/L. An oral glucose tolerance test (OGTT) was performed at 8 AM after an overnight fast. A DHEA infusion (1 mg/hour for 17 hours) was begun at 6 PM and continued until the completion of the second OGTT performed the following morning at 8 AM. T-lymphocytes were drawn at 8 AM each morning.*

RESULTS: *The DHEA infusion had no significant effect on any of the in vivo indices of insulin sensitivity, ie, basal and OGTT insulin, C-peptide, and ratios of insulin/glucose. In vitro, DHEA significantly increased insulin binding to T-lymphocytes of PCO women but caused no significant change in OC women. There was, however, marked enhancement of T-lymphocyte pyruvate dehydrogenase (PDH) activities in both groups of study subjects following DHEA.*

CONCLUSION: *We conclude that a 17-hour infusion of DHEA enhanced T-lymphocyte insulin binding and PDH activity while producing no detectable improvements in in vivo indices of insulin sensitivity.* (J Soc Gynecol Invest 1994;1:74–8)

KEY WORDS: DHEA, insulin, polycystic ovary syndrome.

Although insulin resistance is commonly associated with the hyperandrogenism of polycystic ovary syndrome (PCO), we recently identified a group of obese women with elevated levels of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) who were not insulin resistant despite obesity and elevated circulating testosterone.[1,2] Furthermore, we found that in women in general, indices of insulin sensitivity are positively correlated to circulating DHEA, negatively correlated with testosterone, and more strongly associated with each individual's ratio of DHEA/testosterone than to either of these androgens alone.[1–3] These studies suggest that DHEA may have certain salutary effects on insulin sensitivity.

To test this hypothesis, we examined the effects of DHEA infusion on insulin sensitivity in vivo and in vitro in patients with PCO and insulin resistance and in weight-matched obese controls (OC) with indices of normal insulin effect. The first aim of this study was to assess in vivo the DHEA effect on insulin sensitivity by comparing a 17-hour DHEA infusion against a saline control and by measuring basal insulin with C-peptide and oral glucose-challenged insulin and C-peptide. The second aim was to examine in vitro insulin receptor and post-receptor actions by comparing saline against DHEA infusion and determining phytohemagglutinin (PHA)-activated T-lymphocyte insulin binding and the activation state of pyruvate dehydrogenase (PDH), the regulatory enzyme of overall carbohydrate metabolism.

MATERIALS AND METHODS

The study population consisted of two groups of women representing a wide range of insulin resistance: 1) five women with PCO and insulin resistance and 2) five OC women without clinical hyperandrogenism. Women with PCO were defined by being clinically hyperandrogenic with seborrhea, acne, or hirsutism; with long-

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standing histories of oligomenorrhea or amenorrhea; with serum prolactin of 20 ng/mL or less; and with basal FSH of 10 mIU/mL or less. The PCO and OC women were otherwise matched for age, weight, and race. All women were screened for impaired glucose tolerance based on the criteria of the National Diabetes Data Group.[4] All women were between 18–36 years of age and 130–210% of ideal body weight. Ovulatory function in the OC women was confirmed by luteal phase progesterone concentrations (greater than 10 ng/mL or 31.8 nmol/L). The protocol was approved by the University of Tennessee, Memphis, Institutional Review Board.

After 3 days on a high carbohydrate diet, all subjects were admitted to the University of Tennessee, Memphis, Clinical Research Center. An intravenous catheter was inserted at 6 PM on day 1 (Figure 1). Both PCO and OC women were randomly infused with either normal saline (50 mL/hour) or DHEA at a rate of 1 mg/hour as outlined in Figure 1. At 8 AM on the morning of day 2, and while the DHEA or saline infusion continued, an oral glucose tolerance test (OGTT) was performed with 75 g of glucose. Blood samples for the determination of insulin, C-peptide, glucose, DHEA, DHEAS, androstenedione (Δ^4A), and testosterone were collected at −15, 0, 30, 60, 90, 120, and 180 minutes after the beginning of the test; levels were measured with commercial kits or in-house radioimmunoassay as described by us previously.[1,2] Following the test, the patients were given a mixed meal containing at least 200 g carbohydrate and underwent an overnight fast. The infusion of either saline or DHEA was then restarted at 6 PM on day 2, and the OGTT was repeated on admission day 3 at 8 AM in the previous manner.

Blood samples for T-lymphocyte insulin binding and PDH were drawn at 8 AM on days 2 and 3. T-lymphocytes were separated from whole blood using sheep-rosetting as previously described.[2,4] Cells were then cultured for 72 hours with 1 μg/mL PHA at 37°C under 95% O_2/5% CO_2. The cells were then harvested and specific insulin binding was determined according to our methods published previously.[2,5,6] T-lymphocyte PDH actual (a) and total (t) activities were measured in the extracts of frozen cellular pellets using 1 mmol/L pyruvate, according to our procedures.[2,5,6] Total PDH was assessed under conditions that led to maximal activation of the enzyme, ie, inhibition of PDH kinase and stimulation of PDH phosphatase.[2,4] Values were expressed as nmol $^{14}CO_2$ produced/minute/number of cells, and the activation state of the enzyme was calculated from the ratio of PDHa/PDHt.

Statistical comparisons were done by analysis of variance or multiple *t* tests with Bonferroni correction for repeated measures. Statistical correlations were tested using multiple linear regressions.

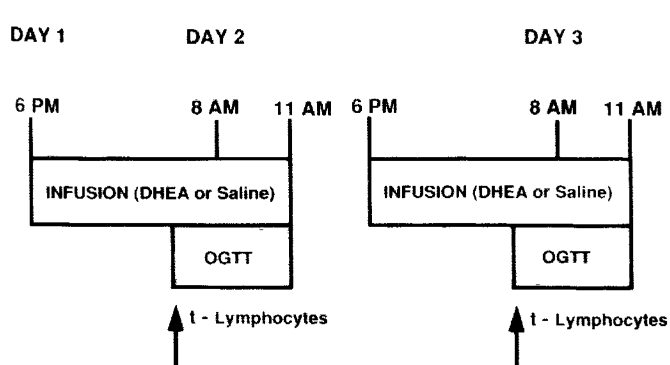

Figure 1. Study protocol: dehydroepiandrosterone (DHEA) or saline was given in random sequence. OGTT = oral glucose tolerance test.

RESULTS

Table 1 lists the basal steroid measurements and clinical characteristics of the PCO patients and the weight-matched OC women. The groups were similar except for basal testosterone and Δ^4A levels in the PCO group, which were 2.0–2.5-fold above those in the OC women ($P < .05$). Figure 2 summarizes the basal and oral glucose-challenged insulin levels, insulin/glucose ratios, and PHA-activated T-lymphocyte insulin binding. The PCO subjects were significantly above controls ($P < .05$) with respect to basal insulin, oral glucose-challenged insulin area under the curve (AUC), and insulin/glucose, and significantly ($P < .05$) below controls for PHA-activated T-lymphocyte insulin binding. These data are consistent with greater impairment of insulin sensitivity in the PCO women.

Correlations between basal DHEA/testosterone ratios of all subjects ($n = 10$) and indices of insulin sensitivity were consistent with our previous findings[2]: DHEA/testosterone ratios correlated inversely to basal insulin ($P < .0021$) and C-peptide ($P < .05$), insulin/glucose indices ($P < .001$), insulin ($P < .008$), and C-peptide AUC ($P < .005$), and correlated positively to PHA-activated T-lymphocyte insulin binding. Infusions of DHEA led to a 4.5- and 2.3-fold increase in levels of DHEA for the OC and

Table 1. Basal Measurements and Clinical Characteristics of Study Subjects

Index	OC subjects (n = 5)	PCO subjects (n = 5)
Age (y)	33.4 ± 2.2	31.4 ± 2.1
% ideal body weight	161.1 ± 9.3	173.6 ± 12.5
Glucose (nmol/L)	5.3 ± 0.2	5.2 ± 0.1
DHEA (nmol/L)	7.9 ± 1.2	10.3 ± 4.4
DHEAS (μmol/L)	3.3 ± 0.4	3.4 ± 1.3
Testosterone (nmol/L)	1.3 ± 0.2	3.2 ± 0.4*
Androstenedione (nmol/L)	4.8 ± 0.5	8.5 ± 1.1*
Cortisol (nmol/L)	22 ± 4	30 ± 8

OC = obese controls; PCO = polycystic ovary syndrome; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate.
Data are presented as mean ± SEM of five PCO women and five age-, weight-, and race-matched OC subjects. There were no significant differences between the groups except as noted.
* $P < .05$.

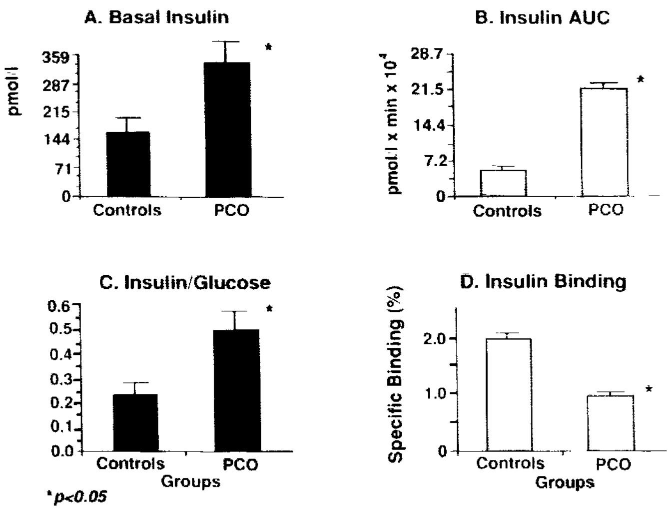

Figure 2. Baseline indices of insulin sensitivity. Basal insulin levels, oral glucose-challenged insulin areas under the curve (AUC), basal insulin/glucose ratios, and PHA-activated T-lymphocyte insulin binding are illustrated by panels A, B, C, and D, respectively. Values represent mean ± SEM of five women with polycystic ovaries (PCO) and five weight-matched OC women.

PCO subjects, respectively (Figure 3A). Levels of DHEAS, testosterone, and Δ^4A did not change significantly with DHEA infusion in the PCO patients (Figure 3, B–D). However, in the OC subjects, DHEA infusion resulted in significant increases in testosterone and Δ^4A (Figure 3, C–D).

Table 2 lists the in vivo indices of serum insulin in PCO and OC women. Although DHEA infusion increased DHEA levels and DHEA/testosterone ratios of all study subjects, there was no significant effect on basal or glucose-challenged insulin, C-peptide, or insulin/glucose indices. In vitro, DHEA did not significantly alter T-lymphocyte insulin binding in OC subjects but did produce a 41% increase in binding activities in the cells of PCO subjects ($P < .05$). In addition, DHEA enhanced the activation state of PDH in lymphocytes from both groups of subjects, ie, 58 and 94% for OC and PCO cells, respectively (Figure 4) ($P < .05$).

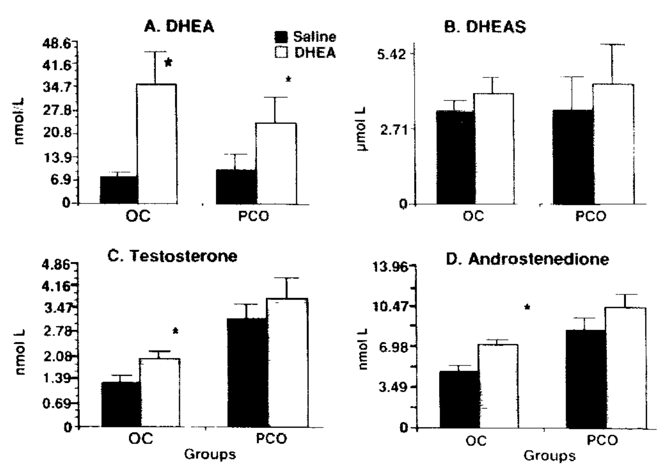

Figure 3. Infusion of dehydroepiandrosterone (DHEA) and the androgen response it produces. Panels A, B, C, and D illustrate DHEA, DHEAS, testosterone, and Δ^4A levels, respectively, with 17-hour saline and DHEA infusions. Values represent mean ± SEM of five obese controls (OC) and five subjects with polycystic ovaries (PCO).

DISCUSSION

We have reported previously that patients with elevated DHEAS and DHEA/testosterone ratios have basal and oral glucose-challenged insulin levels significantly below weight-matched control values.[1,2] Similarly, hypoglycemic responses to intravenous insulin as well as erythrocyte and T-lymphocyte insulin binding and PDH activities were described as significantly different from weight-matched control values.[1,2] These studies led us to conclude that DHEA may have salutary actions on insulin sensitivity and glucose metabolism. Subsequent reports, however, have been conflicting. Nestler et al[7,8] found no changes in glucose disposal or insulin sensitivity in either lean or obese males treated with high doses of DHEA for 1 month. Welle et al[9] found no changes in metabolic rates and total energy expenditure in healthy males administered DHEA for a comparable time period. Furthermore, Mortola and Yen,[10] studying postmenopausal women, noted a deterioration of insulin sensitivity following 28 days of DHEA in high doses. Thus, we were prompted to conduct the above study to determine the effects of shorter-term and more physiologic DHEA on insulin sensitivity in premenopausal women using both in vivo and in vitro indices of insulin effect.

Consistent with our previous studies, we found that the DHEA/testosterone ratios were inversely correlated to basal insulin and C-peptide, insulin/glucose indices, insulin, and C-peptide, and positively correlated to PHA-activated T-lymphocyte insulin binding.[2] However, DHEA infusion, which increased plasma DHEA levels 2.5-fold, did not significantly alter basal or glucose-challenged insulin and C-peptide or insulin/glucose indices. We conclude therefore that infusion of physiologic DHEA over the short term of this study design has no detectable effect on insulin sensitivity in vivo.

In vitro, the DHEA infusion did enhance insulin action. We chose activated T-lymphocytes for our in vitro assessment because they are a readily available tissue that binds and degrades insulin[5,6,11,12] and are responsive to insulin with regard to glucose uptake, amino acid uptake,[11,13] glucose oxidation,[11,13] and PDH activity.[4,14,15] In addition, metabolic defects in PDH found in skeletal muscle, adipose tissue, and kidney are also recognized in T-lymphocytes.[16] Pyruvate dehydrogenase in T-lymphocytes reflects the degree of glucose intolerance of hyperandrogenic women, and in diabetic patients, PDH sensitivity is impaired.[14,17] Finally, T-lymphocytes reflect both acute and chronic changes in in vivo insulin

Table 2. In Vivo Indices of Insulin Sensitivity During Infusion

Index	OC subjects		PCO subjects	
	Saline	DHEA	Saline	DHEA
Basal glucose (nmol/L)	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.3
Glucose AUC (nmol/min/L)	211.5 ± 60	183 ± 81.9	385 ± 36.5	487.0 ± 44.7
Basal insulin (pmol/L)	157 ± 40	171 ± 34	330 ± 55	293 ± 40
Insulin AUC (nmol/min/L)	52.3 ± 7.1	73.1 ± 18.2	215.5 ± 59.6	258.2 ± 67.6
Basal C-peptide (pmol/L)	311 ± 83	274 ± 23	446 ± 90	390 ± 55
C-peptide AUC (nmol/min/L)	67.9 ± 3.1	73.8 ± 11.8	110.0 ± 12.3	105.0 ± 23.8
Insulin/glucose	0.23 ± 0.05	0.26 ± 0.05	0.50 ± 0.08	0.45 ± 0.09
Insulin/glucose AUC	3.15 ± 1.13	3.68 ± 1.01	4.53 ± 1.24	4.06 ± 0.91

OC = obese controls; PCO = polycystic ovary syndrome; DHEA = dehydroepiandrosterone; AUC = area under the curve.
Data are presented as mean ± SEM of five PCO women and five OC subjects. There were no significant differences between the groups at $P < .05$.

levels.[14,18] In this system, DHEA stimulated a significant increase in insulin binding to PHA-activated T-lymphocytes of PCO patients; this could be related to increased binding affinity, increased binding sites, or both. Finally, DHEA enhanced PDH activities in both OC and PCO subjects. These findings are in agreement with our previous studies demonstrating a stimulation of pyruvate flux and insulin binding in T-lymphocytes exposed to DHEA for short periods of time, ie, 6–24 hours.[2,19]

Whereas a 17-hour infusion of DHEA was adequate to enhance insulin action in T-lymphocytes in vitro, this may not have been sufficient time to initiate measurable improvements in insulin sensitivity in vivo. It is also possible that our failure to find a significant improvement in insulin sensitivity in vivo resulted from treatment randomization and a carryover action of DHEA into the saline period in some study subjects. The metabolic fate of DHEA when infused for 17 hours may offer still another explanation for our failure to find DHEA-mediated improvements in in vivo indices of insulin sensitivity. In experimental animals, estrogens are known to enhance insulin sensitivity and androgens (testosterone, dihydrotestosterone) to exacerbate insulin resistance.[20–23] Dehydroepiandrosterone is converted to both estrogenic and androgenic metabolites, and it is the estrogenic precursors, ie, DHEAS, and metabolites, ie, etiocholanolones, that are believed to account for the antidiabetic actions of DHEA in animals.[22,23] In the postmenopausal women of Mortola and Yen,[10] the deterioration of insulin sensitivity with DHEA was accompanied by considerable increases in the potent androgens testosterone and dihydrotestosterone. The PCO patients in our study already had high levels of testosterone and Δ^4A, and DHEA infusion resulted in only minor (less than 20%) and nonsignificant increases in these androgens. In contrast, DHEA infusion in the OC patients caused a 60% increase in testosterone and a 50% increase in Δ^4A concentrations. In a recent study, we found enhanced insulin sensitivity and glucose disposal in a hyperandrogenic woman treated orally with DHEA, under conditions in which the treatment increased plasma DHEA and DHEAS and produced little change in testosterone.[24] These observations suggest that the dosing, timing, and metabolic fate of DHEA are important in determining its action.

We conclude that DHEA infusion stimulates in vitro both insulin binding and PDH in T-lymphocytes of PCO women, and PDH only in the cells of weight-matched OC controls. In vivo, exposure to DHEA for time periods greater than 17 hours or under conditions that prevent conversion to more potent androgens may be necessary to induce noticeable improvements in traditional in vivo indices of insulin resistance.

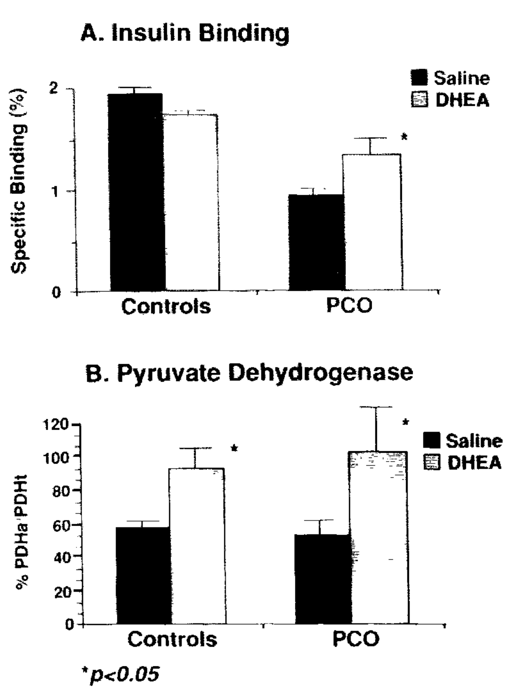

Figure 4. Effects of dehydroepiandrosterone (DHEA) infusion on in vitro indices of T-lymphocyte receptor binding and post-receptor PDH activity. PCO = subjects with polycystic ovaries.

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