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In PCOS, adrenal steroids are regulated differently in the morning vs. in response to nutrient intake

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Abstract

Objective—To investigate adrenal steroid regulation in PCOS

Design—5-hour oral glucose tolerance test (OGTT) and frequently sampled-intravenous GTT

Setting—University research center

Patients—Thirty patients

Intervention—None

Main outcome measures—Anthropometrics, leptin, cortisol, DHEAS, glucose, insulin

Results—Morning cortisol correlated with sensitivity index (SI, r=0.540, p=0.0109), DHEAS correlated inversely with age (r=−0.6359), body mass index (BMI, r=−0.6199), fat mass (r=−0.630) and leptin (r=−0.5676) (p< 0.002 for all). Between the 2nd and 4th hour of OGTT, cortisol changes (Δ) exhibited 3 patterns: I. Responders (n=9, Δ:10.7±1.0μg/dL), II. Non-responders (n=10, Δ:−3.5±0.6μg/dL), III. Intermediates (n=11, Δ:4.3±1.0μg/dL). Compared to non-responders, responders were more obese (BMI: 37.0±1.6 vs. 31.7±1.8kg/m^2, p< 0.05); had higher leptin (28.9±1.7 vs. 24.1±1.1ng/mL, p<0.03) and lower DHEAS (133±12 vs. 236±32ng/mL, p<0.01), higher glucose at 1h of OGTT (195±13 vs. 131±12mg/dL, p< 0.05), higher $AUC_{Glucose}$ (332±20 vs. 265±17mg/dL, p=0.0208), higher $AUC_{Insulin}$ (244±50 vs. 125±30μU/mL, p=0.05) and lower nadir glucose (61±2 vs. 70±2mg/dL, p=0.0002).

Conclusion—Obesity and insulin resistance are associated with lower morning cortisol and DHEAS but increased cortisol and DHEA responses after glucose ingestion. Morning steroid levels may not reflect the day-long exposure.

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Conflict of interest: None.

Keywords

Adrenal steroids; PCOS; obesity; insulin resistance; leptin

In polycystic ovary syndrome (PCOS), the adrenals contribute to the androgen excess by producing excess amounts of dehydroepiandrostenedione (DHEA) and androstenodione (1,2). Although obesity and insulin resistance, that are common in PCOS, influence adrenal function, their role is not straightforward. The entire literature is based on the adrenal steroid measurements obtained in the morning. Our recent research demonstrated that PCOS patients secreted cortisol, DHEA and androstenedione after drinking glucose for oral glucose tolerance testing (OGTT) (3). Those who developed even mild postprandial hypoglycemia had a brisk increase in adrenal steroid secretion. Although hypoglycemia is a known stimulator of adrenal steroids, the standard insulin-induced hypoglycemia test used for this purpose aims to serum glucose below 50 mg/dL. Whereas our PCOS patients secreted the adrenal steroids with serum glucose ≤ 69 mg/dL. Similarly, Spyer et al. (4) had reported increased counter-regulatory hormone secretion with plasma glucose concentrations < 67 mg/dL in well controlled diabetic patients and Solter et al. had demonstrated increased counter regulatory hormones during asymptomatic hypoglycemia in obese subjects (5,6). Thus, it appeared that factors common to PCOS, obesity and type2 diabetes may influence adrenal response. To investigate this possibility, we determined the relationships between the adrenal steroid response and body weight, body composition, insulin secretion and insulin sensitivity in a new group of PCOS patients.

Materials and Methods

Subjects

Thirty patients (23 White, 3 Hispanic, 2 African American and 2 Asian) with PCOS aged 20–45 y and with a body mass index (BMI) of 22–50 kg/m^2 were recruited after signing the informed consents approved by the Institutional Review Board of University of California, Davis. The investigators did not have any conflict of interest. All participants were examined by the principal investigator (SEK-K) who is the director of the PCOS program at the Medical Center of the University of California, Davis. The participants fulfilled the NIH criteria for PCOS (7) by having ovarian dysfunction, as evidenced by amenorrhea (no periods for >6 mo) or oligomenorrhea (<6 periods/y), clinical (hirsutism) or laboratory evidence for hyperandrogenemia (total testosterone >54 ng/dL or free testosterone >9.2 pg/mL), along with the absence of any confounding clinical pathology (i.e. Cushing's disease, 21 hydroxylase deficiency or prolactinoma). Patients were excluded if they used oral contraceptives, antiandrogenic medications, insulin sensitizers, d-chiro inositol, or any other medications or supplements that affect weight or insulin sensitivity during the preceding two months; have impaired glucose tolerance, diabetes mellitus, untreated hypothyroidism, and any other systemic illness such as renal, hepatic, and gastrointestinal disease; smoke; or drink > 2 alcoholic drinks per week.

Study design

Data collection

The OGTT and FS-IVGTT tests were performed at the Clinical and Translational Science Center Clinical Research Center of the University of California, Davis. The subjects were following their habitual diets (1873±104 kcal/d, 34% fat, 50% carbohydrate, 16% protein). They consumed 237±16 g/d carbohydrate prior to testing and they were weight stable.

Anthropometric data—Subjects were seen after an overnight fast. Weight was determined in light clothing using the Tanita BWB800-P Digital Medical Scale. Height without shoes was measured using an Ayrton Model S100 stadiometer. Body composition was determined using bioelectrical impedance (Biostat, British Isles) (8). Because the fluctuations occurring in body-water during menstrual cycles can affect bioelectrical impedance, menstruating women were studied during the first ten days of their cycles.

5-h OGTT—Participants were tested between 0600 and 0900, after an overnight fast. Water intake was permitted. An intravenous catheter was placed into the forearm and kept open with saline. At time point zero, participants drank 75 g of glucose (Glucola ™). Blood samples were obtained at baseline (time point -10 min) and then, after glucose ingestion, at 30-minute intervals for 5 hours. Subjects remained supine throughout the procedure to avoid the confounding effects of physical activity on blood glucose levels. Samples for glucose measurement were collected in Na fluoride containing tubes. Other samples were collected on either serum separation tubes or on tubes containing EDTA or heparin.

3-h FS-IVGTT—Participants were tested between 0600 and 0900, after an overnight fast. An intravenous catheter was placed in their forearm and kept open with normal saline. Heating pads were used in order to maximize blood flow. Three blood samples were obtained at times -15, -10 and -5 minutes. Glucose (0.3u/kg as 25% dextrose) was given intravenously at time 0 min. Intravenous insulin 0.03 u/kg (Humulin Regular: Eli Lilly) was given at time 20 min after the glucose administration. Blood samples were obtained at times 0, 2, 3, 4, 5, 6, 8,10, 12, 14, 16, 19, 22, 23, 24, 25, 27, 30, 40, 50, 60, 70, 90, 100, 120, 140, 160 and 180 min. Blood samples for tests on glucose, insulin, and other parameters were collected as described with the OGTT. Acute insulin response to glucose ($AIR_{Glucose}$), β-cell function, sensitivity index (SI) and disposition index (DI) was calculated using MiniMod Millennium software (Dr. Bergman, Los Angeles, CA).

Clinical symptoms—Our recent study indicated a relationship between adrenal steroid secretion and postprandial hypoglycemia. To determine the symptomatology that relates to adrenal steroid secretion, we used the Hypoglycemia Symptoms Logs (HSL) program developed for hand-held computer in collaboration with William Horn, and Nancy Keim, PhD. The key symptoms related to the autonomic response, neuroglycopenia and malaise (sweating, shaking, hunger, weakness, confusion, drowsiness, behavior, speech difficulty, incoordination, nausea and headache) were recorded hourly on a 0–100 scale. The data were transferred from the hand held computer to an Excel™ spreadsheet for analysis, and were plotted against time as shown in Figure 2.

Biochemical measurements—Plasma and serum samples were obtained after an overnight fast. Glucose was measured using YSI 2300 STAT Plus Glucose & Lactate Analyzer (YSI Life Sciences, Yellow Springs, OH. The coefficient of variation (cv) for glucose was1%. Insulin was measured using RIA kits (Linco Research Inc, St. Charles, MO) with a cv of 8.2%. The homeostatic model assessment (HOMA), a surrogate measure of insulin sensitivity, was calculated using the formula: [fasting insulin (μU/mL) × fasting glucose (mM)]/22.5. Total testosterone, sex hormone binding globulin (SHBG), cortisol and DHEAS were measured by RIA (Diagnostic Systems Laboratories, Webster, TX). The cvs were: 8.3% for testosterone, 4.4% for SHBG, 5.3% for cortisol, 9.6% for DHEAS and 4.9% for DHEA. The reference range for these hormones, measured in a group of lean, healthy women with normal ovarian function (n = 19; age, 40±1 yr; BMI 23.9±1.5 kg/m2) were: testosterone: 0.27±0.029 ng/mL; SHBG, 68.5±6.6 nmol/L, and DHEAS: 116±24 ng/mL.

Statistical Analysis

Statistical analysis was performed using SAS statistical software, version 9.1 (SAS Institute Inc, Cary, NC). Descriptive statistics (mean or adjusted mean, standard error (SE), 95% confidence interval) were calculated for each measurement by the response group (categorized based on change in cortisol) and time point. Pearson's correlation co-efficients and corresponding p values were calculated for the baseline values of the entire group.

Trajectory of 5-hour change in response levels was estimated by a repeated measures analysis of variance. Individual trajectories of glucose, insulin cortisol, DHEA changes over 11 time points, measured every 30 minutes, were estimated from linear random-effects models. Each response level was entered as the dependent variable (Y). The response group, time (min), and response group × time interaction term were entered as independent variables. To account for between subject heterogeneity in the changes of response levels, intercept and time were modeled as random effects.

Analysis of variance (ANOVA) was performed to assess 1) whether each response level was associated with the response group (overall); and 2) there was significant mean difference between responders and non-responders. A two-sided p-value of 0.05 was considered significant.

Results

Definition of the groups based on cortisol response

The adrenal response was defined based on cortisol changes during OGTT. Those subjects who had a minimum increase of 7.2 μg/dl (200 nM) in cortisol were defines as responders--similar to the interpretation of positive cortrysin stimulation test (9). Those who had no change or a decrease in cortisol from the baseline were defined as non-responders. The responders (n= 9) had 10.7±1.0 μg/dL increase in cortisol between the 2^{nd} and 4^{th} hour of OGTT; the non responders (n=10) had 3.5±0.6 μg/dL decrease; the remaining 11 subjects had intermediate responses (Δ = 4.3±1.0 μg/dL). Cortisol responses differed significantly among these 3 groups (p=0.0001).

The changes in DHEA concentrations followed a similar pattern between the 2^{nd} and 4^{th} hour: Δ = 14.4±1.7 ng/mL in the responders, Δ = 0.4±0.9 ng/mL in the non-responders, and Δ = 3.6±1.2 ng/mL in the intermediates. These differences were also significant (p=0.0003).

Baseline characteristics of responders vs. non-responders (Table 1)

Age was did not differ significantly between the responders vs. non-responders (Mean ±SEM: 34±2 vs. 32±2 years).

Anthropometric variables—Responders were more obese (BMI: 37.0±1.6 vs. 31.7±1.8 kg/m^2, $p<0.05$), had larger lean-mass and a borderline increase in fat-mass. Since body compartment measurements by bioelectrical impedance relate to each other (8), an independent indicator of fat mass was sought. It is well established that serum leptin directly correlates with fat-mass (10). Thus, serum leptin was also measured. Responders had higher serum leptin than non-responders (28.9 vs. 24.1 ng/mL, p=0.0270).

Insulin resistance parameters—Fasting glucose, insulin and HOMA did not differ.

Steroid hormones—Morning DHEAS was lower in responders than in the non-responders (133±12 vs. 236±32 ng/mL, p=0.0099). DHEA tended to be lower also in responders (9.9±2.8 vs. 16.3±2.6 ng/mL, p=0.101). Cortisol and testosterone concentrations

were not different. Responders had lower serum SHBG (33.9±3.1 vs. 58.6±6.7 nmol/L, p=0.0220).

Relationships between morning levels of adrenal steroids and anthropometric parameters, insulin resistance, pancreatic β-cell function

Morning cortisol correlated directly with SI (r=0.5405, p=0.002), DI (r=0.3509, p=0.0573), and inversely with β-cell function (r=−0.3969, p=0.0299) indicating that insulin sensitivity was associated with higher serum cortisol in the morning. Cortisol showed a weak inverse correlation with leptin (r=−0.3310, p=0.0740).

Morning DHEAS correlated inversely with age (r=−0.6359, p=0.0002), weight (r=−0.5663, p=0.0011), BMI (r = −0.6199, p = 0.0003), fat-mass (r=−0.06295, p=0.0002), leptin (r= −0.5676, p=0.0011) and fasting insulin (r=−0.4017, p=0.0278). DHEAS correlated directly with SI (r=0.3664, p=0.0464).

Morning DHEA correlated inversely with obesity indicators such as weight, BMI, fat-mass, leptin, fasting glucose and fasting insulin, similarly to DHEAS. DHEA correlated inversely with β-cell function (r=−0.4128, p=0.0234) and directly with SI (r=0.5283, p=0.0027).

Other noteworthy correlations were observed between testosterone and DHEAS and DHEA; between cortisol and DHEAS and DHEA (Table 2). Serum SHBG correlated inversely with leptin (r=−0.4257, p=0.0190) and weight (r=−0.3593, p=0.0512), but directly with SI (r = 0.3535, p=0.0553) and adiponectin (r=0.3369, p=0.0687). Serum leptin correlated with weight (r=0.6727, p<0.0001), BMI (r=0.7814, p<0.0001) and fat mass (r=0.7784, p<0.0001).

Glucose and insulin changes during OGTT in responders vs. non-responders (Figure 1)

Responders had higher glucose at 1h (194±13 vs. 131±12 mg/dL, p< 0.05), and higher area under the curve ($AUC_{Glucose}$) during the first 2h (332±20 vs. 265±17 mg/dL, p< 0.03). In contrast, responders had lower nadir glucose (61.4±2.2 vs. 70.2±2.3 mg/dL, p=0.0002), 3h-glucose (75.10±8.93 vs. 104.04±8.47 mg/dL, p<0.03) and 4h-glucose (72±4 vs. 87±4 mg/dL, p< 0.02). Responders had also higher $AUC_{Insulin}$ during the first 2h (244±50 vs. 125±30 mU/mL, p=0.05) and higher serum insulin at 2h (159±31 vs. 54±29 mU/mL, p<0.05).

DHEAS and cortisol changes during OGTT in responders vs. non-responders (Figure 1)

Although baseline serum cortisol levels were similar in responders vs. non-responders, they started to diverge during 3.5 h of OGTT. By the 4^{th} hour, cortisol increased from 10.9±2.1 to 16.0±1.7 ng/mL in responders, but had decreased from 10.7±2.0 to 6.2±1.6 ng/mL) in non-responders (p<0.001).

Baseline DHEA concentrations tended to be lower in responders (9.9±2.8 vs. 16.3±2.6 ng/mL, p=0.101). In responders, DHEA increased from 9.9±2.8 to 24.1±3.2 ng/mL, whereas in non-responders, it did not change (from 16.3±2.6 to 16.9±3.1 ng/mL).

Clinical symptoms in responders vs. non-responders (Figure 2)

Starting at the 3^{rd} h, the clinical symptoms diverged: Responders had higher scores in shakiness, sweatiness, weakness and hunger.

Discussion

This study indicated that adrenal steroids are regulated differently in the morning vs. after nutrient intake.

We found that the morning concentrations of the adrenal steroids correlated inversely with age, obesity and insulin resistance. Serum DHEAS and DHEA concentrations related to anthropometric variables much more strongly than serum cortisol. These results are consistent with the observations of Moran at al. who noted that younger and thinner PCOS patients had higher DHEAS levels (11). Age correlated inversely with DHEAS levels in both White and African American women (2). This inverse relationship between DHEAS and obesity and insulin resistance is somewhat unexpected for several reasons. First, it has been hypothesized that a single pathology, namely an overactive serine kinase, may cause both insulin resistance and increased androgens because serine phosphorylation of insulin receptorβ inhibits insulin signaling while serine phosphorylation of P450c17, 17,20-lyase, stimulates DHEA production (12). In this case, serum DHEAS should have correlated with insulin resistance parameters.; our findings showed the opposite. Second, insulin sensitizers lower androgen levels (13–16). However, a closer inspection of the data indicates that this has not been a consistent finding: In one report, metformin treatment did not decrease, but in fact tended to increase morning cortisol levels (p=0.07) and did not affect DHEAS (17). In another, DHEAS and cortisol levels were not reported (16). In yet in another, only serum testosterone decreased, DHEAS did not change, and cortisol levels were not included (13). In addition, these studies showed that the effects of metformin may differ from those of the thiozalidinediones (TZD) (18). In general, TZD caused larger reductions in androgens (13,15–17,19,20). An intriguing question is whether the differential effects of insulin sensitizers relate to their effects on weight; whether TZD lower androgens more than metformin because TZD promote weight gain whereas metformin promotes weight loss (21,22). Unfortunately, not all studies using insulin sensitizers reported changes in weight.

Recent evidence indicates that the effect of obesity on steroid synthesis may be mediated by leptin. Leptin is an adipose tissue protein. Serum leptin levels correlate directly with fat-mass (23,24). In humans, serum leptin correlated inversely with cortisol (25). In leptin deficient mice, leptin administration reduced corticosterone levels (26). In our study, serum leptin correlated directly with weight, BMI and fat mass, and inversely with DHEAS and DHEA. There was also a weak inverse correlation between leptin and cortisol ($p = 0.0740$). It appears that leptin inhibits steroid synthesis by down regulating the rate limiting step–the cholesterol side chain cleavage enzyme (P450scc) (27,28). It is conceivable that changes in leptin may account for the differential effects of insulin sensitizers on androgens: TZD treatment, which suppresses androgen levels, increases leptin, whereas metformin, which does not affect androgen levels, has no effect on leptin (29,30).

We investigated dynamic responses of adrenal steroids by measuring cortisol and DHEA during OGTT. We did not measure DHEAS because it has a long half life and does not change promptly during testing. We did not measure androstenodione either because we had demonstrated previously that androstenodione exhibits smaller increases than DHEA during OGTT (3). Similarly, Farah-Eways et al. reported that after ACTH stimulation serum DHEA increased by 222% while androstenodione increased by 31% in PCOS patients, and DHEA increased by 266% while androstenodione increased by 68% in healthy control women (31).

We observed that those individuals with increased cortisol and DHEA responses were more obese and hyperinsulinemic than non-responders. The responders had higher glucose and insulin levels during the first two hours of OGTT, but lower glucose levels afterwards. These findings are consistent with the report of Gambineri et al. (32) that showed that PCOS patients with hyperglycemia and hyperinsulinemia during OGTT exhibited exaggerated cortisol and androstenedione responses to ACTH stimulation. It appears that early hyperinsulinemia led to postprandial hypoglycemia and triggered the hypothalamic-pituitary-adrenal axis. It is possible that obesity augments the adrenal response through two different but complementary mechanisms: First obesity causes hyperinsulinemia and

postprandial hypoglycemia, and thus triggers the hypothalamic-pituitary component of the axis. In addition, obesity increases steroid synthesis in the adrenals directly. Several studies that bypassed the hypothalamus and the pituitary and tested only the adrenals by injecting ACTH found increased steroid response in obesity (33–37). Although the underlying mechanism is not yet clear, it is known that rapid steroid response to provocative stimuli is mediated by the steroidogenic acute regulatory protein (StAR) which facilitates the movement of cholesterol from the outer to the inner mitochondrial membrane (38). Recent research indicates that adipose tissue produces signaling molecules that induce the transcription of the StAR promoter and this may account for the effects of obesity (39).

Finally, we wanted to relate the clinical symptomatology to adrenal steroid secretion. It has been recognized that a significant number of PCOS patients complain of symptoms suggestive of hypoglycemia after consuming sweets and simple sugars (3,40). Our findings indicate that symptoms like shakiness, sweating, weakness and excessive hunger are associated with increased adrenal steroid response, and emphasize the importance of questioning patients specifically for these symptoms.

An important consideration is long-term consequences of repeated stimulation of the hypothalamic-pituitary-adrenal axis by nutrients. It is conceivable that ingestion of simple sugars can cause postprandial hypoglycemia and stimulate secretion of adrenal steroids, which in turn causes further weight gain and insulin resistance, thus creating a vicious cycle. Consistent with this concept we recently demonstrated that PCOS patients who consumed a simple-sugar supplement lost less weight than those who consumed a protein supplement (41). In addition, an earlier study demonstrated that PCOS patients have lower day-long glucose levels as compared to control women (42). Since hypoglycemia stimulates growth hormone secretion, this study monitored growth hormone and found that PCOS patients had higher growth hormone levels throughout the day as well. Recently, Mai et al. reported that infusion of lipid and heparin increased serum levels of free fatty acids and androgens in healthy control women (43). Taken altogether, these studies support that nutrients influence adrenal function. Hormone levels obtained in the morning may not reflect the day-long exposure. Clinical significance of the nutrient-regulated hormone secretion needs to be further investigated.

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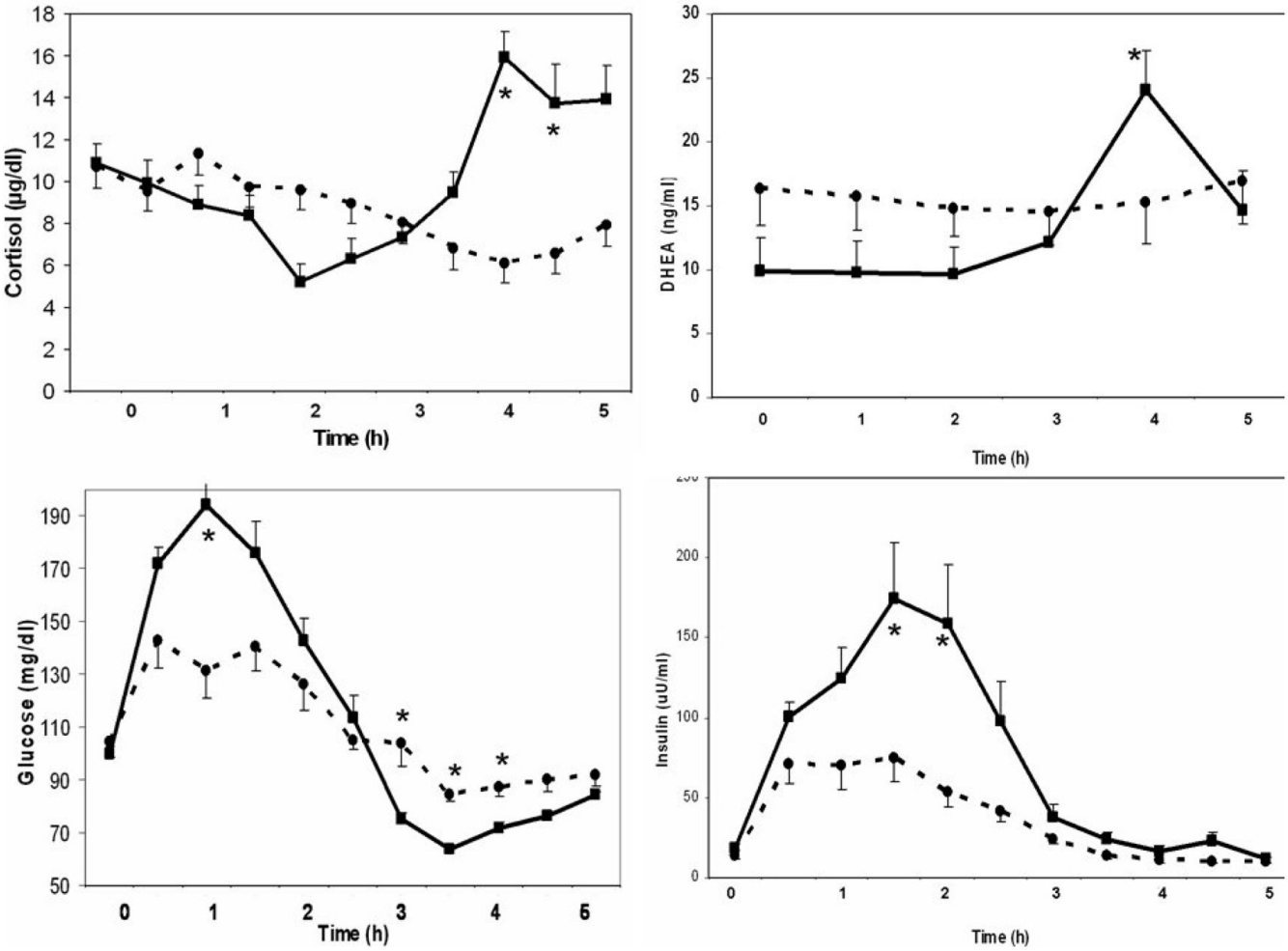

Figure 1.

Changes in cortisol, DHEA, glucose and insulin in responders (n = 9, solid line) and non-responders (n = 10, broken line) during oral glucose tolerance test (Mean±SEM, *; $p < 0.05$ when responders and non-responders are compared to each other).

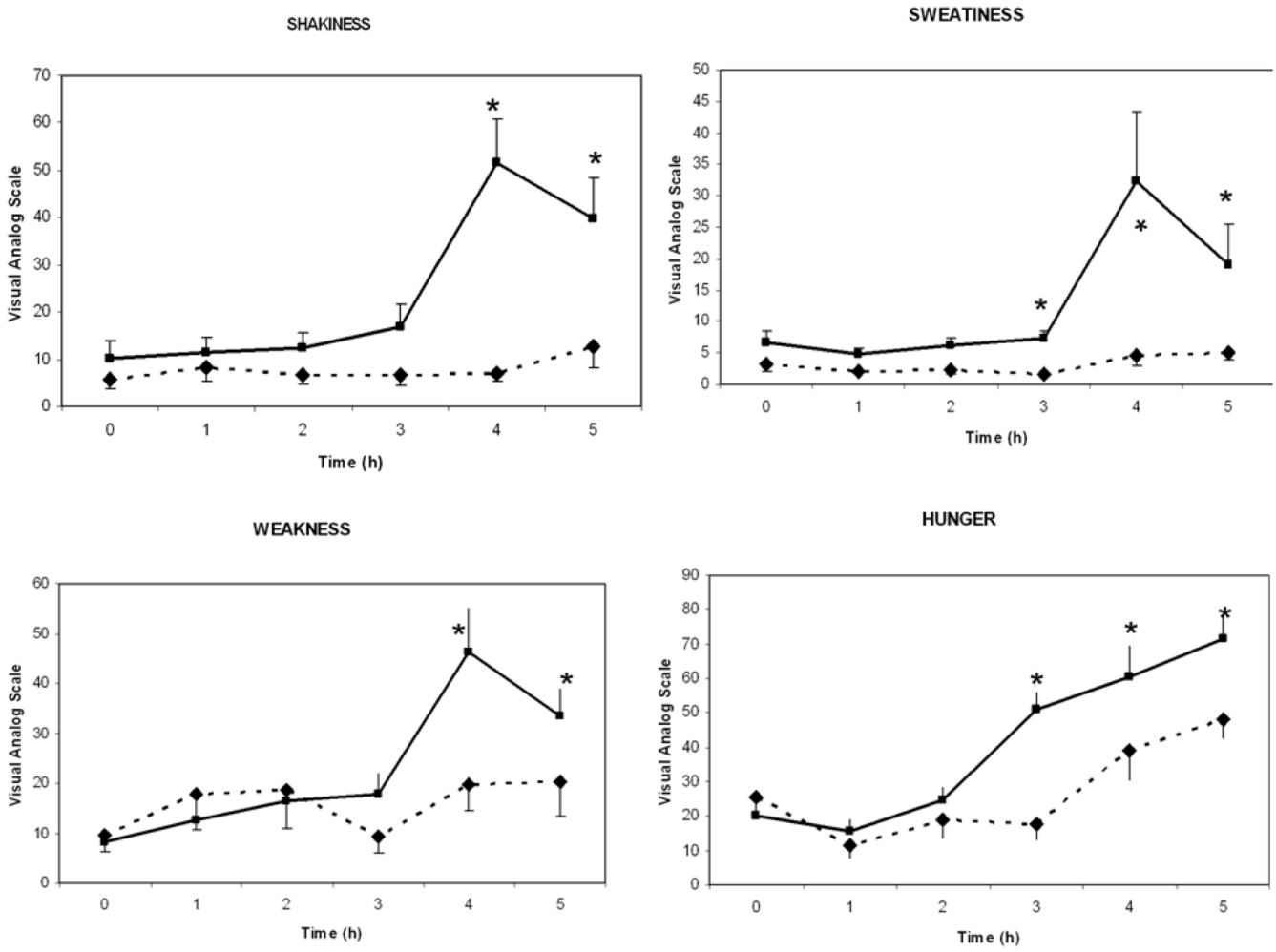

Figure 2.

Clinical symptoms in responders (n = 9, solid line) and non-responders (n = 10, broken line) during oral glucose tolerance test (Mean±SEM, *; $p < 0.05$ when responders and non-responders are compared to each other).

Table 1

Baseline variables of the PCOS patient who demonstrated increased cortisol response during oral glucose tolerance test (responders) and those who did not have an increase (non-responders).

	Responder (n = 9)	Non-responder (n = 10)	p
Anthropometric			
Weight (kg)	105.5 ± 4.9	87.5 ± 5.4	**0.0245**
BMI (kg/m^2)	37.0 ± 1.6	31.7 ± 1.8	**0.0458**
Fat-mass (kg)	49.1 ± 3.1	37.2 ± 4.9	0.0616
Lean-mass (kg)	57.0 ± 2.9	49.8 ± 1.3	**0.0307**
Leptin (ng/mL)	28.9 ± 1.7	24.1±1.1	**0.0270**
Insulin resistance			
Fasting			
Glucose (mg/dL)	99.7 ± 5.7	104.4 ± 5.4	0.5689
Insulin (μU/mL)	18.4 ± 3.0	14.0 ± 2.9	0.2662
HOMA	4.01 ± 1.1	3.22 ± 1.2	0.3241
OGTT			
$AUC_{Glucose}$	332 ± 20	265 ± 17	**0.0208**
$AUC_{Insulin}$	244 ± 50	125 ± 30	**0.0500**
$Glucose_{Nadir}$ (mg/dL)	61.4 ± 2.2	70.2 ±2.3	**0.0002**
FS-IVGTT			
$AIR_{Glucose}$	843 ± 270	504 ± 175	0.2972
β-cell function	287.4 ± 33.0	207.6 ± 32.8	0.1057
SI	2.72 ± 0.62	4.91 ± 1.38	0.1827
DI	1927 ± 510	1584 ± 272	0.5486
Steroid hormones			
Cortisol (μg/dL)	10.9 ± 2.1	10.7± 2.0	0.7384
DHEA (ng/mL)	9.9 ± 2.8	16.3 ± 2.6	0.101
DHEAS (ng/mL)	133 ± 12	236 ±32	**0.0099**
Testosterone (ng/mL)	0.75±0.05	0.80±0.08	0.7529
SHBG (nmol/L)	33.9±3.1	58.6±6.7	**0.0220**

Table 2

Correlations between morning cortisol, DHEAS, DHEA concentrations and anthropometric and insulin resistance variables (n = 30).

	Cortisol	$p_{Cortisol}$	DHEAS	p_{DHEAS}	DHEA	P_{DHEA}
Age			−0.6359	0.0002		
Anthropometric						
Weight			−0.5663	0.0011	−0.4579	0.0109
BMI			−0.6199	0.0003	−0.3525	0.0560
Fat-mass					−0.3851	0.0356
Leptin	−0.3310	0.0740	−0.5676	0.0011	−0.5297	0.0026
Insulin resistance						
Fasting						
Glucose					−0.3442	0.0625
Insulin			−0.4017	0.0278	−0.3581	0.0520
FS-IVGTT						
β-cell funct.	−0.3969	0.0299			−0.4128	0.0234
SI	0.5405	0.0020	0.3664	0.0465	0.5283	0.0027
DI	0.3509	0.0573				
Other						
Cortisol			0.4523	0.0121	0.6101	0.0003
DHEAS					0.7008	<0.0001
Testosterone			0.4762	0.0078	0.523	0.0030