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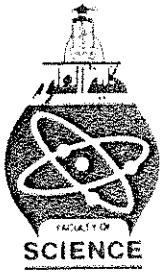

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نفيدكم بقبول البحث المقدم من قبلكم للنشر بمجلة جامعة الملك عبد العزيز « العلوم » ،
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وسنوافيكم بالمسودة لتصحيحها واعادتها الينا بأسرع ما يمكن .

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Dehydroepiandrosterone and menstrual cycle hormones in obese and non-obese Saudi women

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Running title: Dehydroepiandrosterone and menstrual cycle hormones

Abstract:

The purpose of this study was to determine the relationship of plasma dehydroepiandrosterone (DHEA) and its metabolite (DHEA-S) to the menstrual cycle hormones in obese and non-obese Saudi women. Sixty-five healthy volunteers between 19–39 years of age with regular menstrual cycle and proven fertility were recruited from King Abdul-Aziz University, (Jeddah, KSA). The women were grouped into obese (n=26) and non-obese (n=39) groups according to their BMI. Blood samples were collected after an overnight fast during days 3, 10, 17, and 24 of the menstrual cycle from all volunteers. Anthropometrics measurements were recorded and DHEA, DHEA-S, progesterone, estradiol, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) levels were measured.

No significant differences were found on comparing the levels of DHEA between the obese and non-obese women. The analysis also revealed no significant correlation between the level of DHEA and BMI in both groups. In obese and non-obese groups, DHEA levels were highly significantly correlated to progesterone at day 10 ($r=0.945$, $p<0.005$; $r=0.494$, $p<0.0001$, respectively), to FSH at day 24 ($r=0.929$, $p<0.01$; $r=0.353$, $p<0.05$, respectively), and to LH at day 24 ($r=0.707$, $p<0.05$, $r=0.483$, $p<0.001$, respectively). DHEA-S levels failed to show any correlation with sex hormones. The results suggest a close functional relationship

between plasma DHEA and serum progesterone, FSH, and LH which may indicate a regulatory role of DHEA towards menstrual cycle hormones.

Introduction:

Dehydroepiandrosterone (DHEA) was isolated from urine in 1934, and its sulfate form was identified 10 years later (1, 2). It took another decade to identify DHEA and DHEA-S in peripheral blood (3). Since then, thousands of studies on the metabolic effects of DHEA have been published. DHEA is the most abundant circulating seriod hormone in humans (4). It is secreted primarily by the adrenal glands and to a lesser extent by the brain, skin, testes, and ovaries (5-10). After being secreted by the adrenal glands, it circulates in the bloodstream as DHEA-sulfate (DHEA-S); which is generally metabolically inactive. DHEA and DHEA-S are inter-convertible in peripheral and adrenal tissues (11). As cells take DHEA-S from the blood, they reconvert it into DHEA, and possibly to other metabolites. About 64%-74% of the DHEA-S produced each day is converted to DHEA; but only a small amount (13%) of the DHEA gets converted by sulfatransferase in the liver and kidney to DHEA-S creating equilibrium between the two forms of the steroid (12).

DHEA has been referred to as the "mother steroid", because it is a precursor for many other steroid hormones (13). It is converted peripherally to androgens; testosterone, dihyrotestosterone, androstendione and also to estrogens (14). It is estimated that more than 90% of total estrogen in postmenopausal women is derived from peripheral conversion of DHEA/DHEA-S (15). Although sex hormones serve

well-known functions, the exact mechanism of action and clinical role of DHEA itself and its sulfate is not so well understood.

Many researches have reported low levels of DHEA in degenerative diseases, suggesting the higher levels of DHEA and DHEA-S may protect against the development of diabetes, high cholesterol, and obesity (16, 17). These observations have lead to the hypothesis that DHEA may play a regulatory role in obesity (18). Yen and his group demonstrated that, the administration of DHEA significantly inhibited obese mice to gain weight. In 1986, Cleary and Zisk treated lean and obese female rats with DHEA and showed that adipose tissue weights were lowered in DHEA treated obese and lean rats compared with untreated control group rats (19). These mechanisms could explain DHEA anti-obesity effects.

Motivated by this background, this study was undertaken to assess the relationship of DHEA and it's metabolite to the menstrual cycle hormones in obese and non-obese Saudi women.

Material and methods

Subjects: Sixty-five healthy Saudi female volunteers ranged in age from 19-39 years of age were included in this study. All women were non-pregnant with normal regular menstrual cycle between 27 and 30 days. Their ovulatory cycles were assessed according to the information recorded about their last 8 menstrual cycles. They were divided into two groups according to their BMI. Obese women of BMI ≥ 27 kg/m^2, (n=26), ranged between 27-39 kg/m^2. Non-obese women of BMI < 27 kg/m^2, (n=39), ranged between 20-26 kg/m^2. All subjects were of good health according to their medical history and the performed routine laboratory tests. None of the women were taking any medication. All the women in the study provided informed consent for all procedures.

Assay: Blood samples were collected after an overnight fast, on day 3, 10, 17, and 24 from the beginning of menstruation. All blood samples were withdrawn between 08:00 and 10:00 a.m. after an overnight fast. Information was recorded for all subjects, including weight and height. Serum cholesterol, high-density lipoproteins (HDL), low-density lipoprotein (LDL), and triglyceride were determined by enzymatic methods (Boehringer Mannheim GmbH, Mannheim, Germany) on a Hitachi 917 blood chemistry analyzer (Tokyo, Japan). Plasma DHEA and DHEA-S were determined using a commercial direct ELISA kits (DRG International, Germany). Serum progesterone, estradiol, FSH, and LH were measured by the

electrochemiluminescence ECLIA methods (Roche Diagnostics GmbH, Mannheim, Germany) on the Roche Elecsys 2010 Immunoassay Analyzer (Tokyo, Japan). All measurements from each individual were run in duplicate in a single assay. As DHEA and DHEA-S levels do not vary with menstrual cycle (20), the serum levels were measured using the samples collected on day 3 only.

Statistical analysis: The analysis was performed using the student t-test of the statistical package for Social Sciences 10 for Windows. The results were expressed as the means ± standard deviation. Correlations were studied using Pearson's method. Statistical significance was defined at $p < 0.05$ or higher.

Results:

Serum DHEA does not show any significant differences between the two groups. The mean DHEA and DHEA-S levels in obese women ranged between 10.74-18.15ng/ml and 1285.20-2047.34ng/ml respectively. In the non-obese group, the mean DHEA and DHEA-S levels ranged between 15.24-24.42ng/ml and 1846.11-2514.57ng/ml, respectively (Table 1). The levels of DHEA-S exceeded DHEA levels in both groups and the ratio of DHEA to DHEA-S in both groups was found to be of approximately 1:100. The lipid profile, presented in table 1, revealed that there are significant differences between the obese and non-obese groups in the levels of triglyceride and HDL. The obese group had significantly higher levels of triglyceride (1.03±0.60 vs. 0.71±0.40 nmol/L, $p<0.05$), and lower HDL (1.23±0.32 vs. 1.50±0.23mmol/L, $p<0.01$).Table 2 shows the serum levels of menstrual cycle hormones at days 3, 10, 17, and 24 of the cycle. Normal pattern of progesterone, FSH, LH, and estradiol was noted. The level of progesterone (day 3), estradiol (day 3), and LH (day 10) demonstrated a significant differences between the obese and non-obese women.

To assess associations between the variables, the Pearson r.test was used. Table 3 shows the correlations between DHEA and menstrual cycle hormones for the obese and non-obese women. The analysis revealed a highly significant correlation between

the levels of DHEA in the obese and non-obese groups with progesterone at day 10 ($r=0.945$, $p<0.005$; $r=0.494$, $p<0.001$, respectively). At day 24, DHEA level in obese and non-obese subjects was significantly and positively related to FSH and LH ($r=0.929$, $p<0.01$; $r=0.353$, $p<0.05$) ($r=0.707$, $p<0.05$; $r=0.483$, $p<0.001$) respectively. Progesterone, FSH, LH, and estradiol were failed to show any significant correlation with DHEA-S. The analysis showed that DHEA-S levels were related negatively with BMI in obese group only ($p<0.05$; $r=-0.25$). DHEA levels in obese and non-obese failed to show any correlation with BMI. Moreover, neither DHEA nor DHEA-S showed any correlations with lipid profile.

Discussion

The purpose of this work, in which Saudi women were considered, was to measure serum levels of DHEA and assesses their relationship to the levels of menstrual cycle hormones. To our knowledge this study is the first to examine the relationship of DHEA with levels of menstrual cycle hormones in Saudi women.

Previously conducted studies tested the effect of DHEA on the levels of sex hormones in women through treatment with exogenous DHEA. It was shown that the percutaneous administration of DHEA in postmenopausal women caused an increase level of estradiol sulfate and estrone sulfate (21). Another study showed increased in serum estradiol and estrone levels in postmenopausal women after a larger dose of DHEA (22). Stomati *et al.*, (2000), treated 18 postmenopausal women with oral DHEA and showed that, estradiol and esterone levels were elevated, while progesterone levels remained constant (23). In contrast to the previous studies, Morales *et al.*, (1994), reported that estrogens were not significantly altered after treatment of the women with 50 mg of DHEA (24). The positive correlation between DHEA and estradiol, which was found by these authors, may be explained by considering that all these studies administrated DHEA to postmenopausal women, where the ovary has ceased; and the only source for estradiol formed in the peripheral tissues is the serum DHEA.

In this study, the correlation between DHEA and estradiol failed to show any relationship. This result can be explained by the fact that serum estradiol reflects ovarian steroid secretion and the important contribution of the adrenal DHEA is not accurately reflected in circulation levels of estradiol. The adrenal DHEA is converted in tissue into the active estrogens and is metabolized locally into inactive glucuronidated and sulfated metabolites, which in turn could be measured in the circulation (21).

Concerning the relationship between DHEA, progesterone, FSH, and LH, limited information is available. Our data showed a correlation to progesterone at day 10 and at day 24 with FSH and LH. Progesterone and DHEA both are derived from cholesterol and at day 10 of the menstrual cycle, progesterone begins to elevate. Day 24, late luteal phase, is also characterized by increasing FSH and LH. The positive correlation between DHEA with progesterone, FSH, and LH may possibly be explained by the fact that DHEA may regulate progesterone, FSH, and LH production.

Regarding the relationship between DHEA and BMI in women, little is known with conflicting data. In 1991, De Pergola *et al.*, reported a significant inverse correlation between DHEA serum levels and BMI (25). Another investigator showed that DHEA levels were weakly and positively associated with BMI (26). The reason for the discrepancy is not clear, but could refer to differences in subject selections. Our study failed to show any correlation between DHEA and BMI. This result can be

explained by the fact that there were differences in the ranges of BMI between all the studies. Also in the present study, all of the volunteers were with BMI less than 40kg/m^2. So we can not rule out the possibility that the range of BMI could be the reason. This is obviously a matter that needs to be taken into consideration in interpreting this type of data.

Considering the relationship between DHEA and lipid profile, limited information is available. Our data showed that DHEA and DHEA-S levels did not correlate with lipid profiles. Counteracting this result, Casson *et al.*, (1998) showed that DHEA administration decreased high-density lipoprotein, but did not change serum LDL and Triglyceride in postmenopausal women (27). Other investigators found that, DHEA-S levels are negatively associated with total cholesterol and triglyceride in obese women (28). Further study showed that, HDL levels declined after treatment of the premenopausal women with 50mg of DHEA (29).

In conclusion, the results suggest a close functional relationship between plasma DHEA and serum progesterone, FSH, and LH which may indicate a regulatory role of DHEA towards menstrual cycle hormones.

References:

1. Butenandt A, Tscherning K. Uber Androsteron, Krystallisiertes mannliches sexual hormone. ***Physiol Chem*** 1934; 229: 167-184.

2. Munson PL, Gallagher TF, Koch FC. Isolation of dehydroepiandrosterone sulfate from normal urine. ***J Biol Chem*** 1944; 152: 67-77.

3. Migeon CJ, Plager JE. Identification and isolation of dehydroepiandrosterone from peripheral human plasma. ***J Biol Chem*** 1954; 209: 767-772.

4. Ebeling P, Koivisto VA. Physiological importance of dehydroepiandrosterone. ***Lancet***. 343, 1479-81.

5. Kishimoto Y, Hoshi M. (1972). Dehydroepiandrosterone sulphate in rat brain: incorporation from blood and metabolism in vivo. ***J Neuro chem*** 1994; 19: 2207-2215.

6. Vermeulen A. Adrenal androgens and aging. Raven Publisher. New York. 1980; PP. 207-217.

7. Majewska MD, Demirgoren S, Spivak CE. The neurosteroid dehydroepiandrosterone sulfate is an allosteric antagonist of the GABA A receptor. ***Brain Res*** 1990; 526: 143-146.

8. Baulieu EE. Neurosteroids: of the nervous system, by the nervous system. ***Recent Prog Horm Res*** 1997; 52: 1-32.

9. Kroboth PD, Salek FS, Pittenger AL, Fabian TJ, Frye RF. DHEA and DHEA-S: Review. *J Clin Pharmacology* 1999; 39: 327-348.

10. Wolf OT, Kirshbaum C. Action of dehydroepiandrosterone and its sulfate in the central nervous system: effect on congnition and emotion in animals and humans. *Brain Res review* 1999; 30: 264-288.

11. Longcope C. Dehydroepiandrosterone metabolism. *J Endocrinol* 1996; 150:S125-S127.

12. Sharp S, Barker EV, Coughtrie MW, Lowenstein PR, Hume R. Immunochemical characterization of dehydroepiandrosterone sulfotransferase in rats and humans. *Eur J Biochem* 1993; 211: 539-548.

13. Regelson W, Loria R, Kalimi M. Dehydroepiandrosterone (DHEA)-the "mother steroid": Immunologica action. *Ann N Y Acad Sci* 1994; 719: 553-563.

14. Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgens metabolites during aging. *J Clin Endocrinol Metab*1997; 82: 2396-2402

15. Baulieu EE. Dehydroepiandrosterone (DHEA): fountain of youth? *J Clin Endocrinol Metab* 1996; 81: 3147-3151.

16. Young J, Couzinet B, Nahoul K, Brailly S, Chanson P, Baulieu EE, Schaison G. Panhypopituitarism as a model to study the metabolism of Dehydroepiandrosterone (DHEA) in humans. ***J Clin Endocrinol Metab*** 1997; 82: 2578-2585.

17. Williams JR. The effects of dehydroepiandrosterone on carcinogenesis, obesity, the immune system, and aging. ***Lipids*** 2000; 35: 325-331.

18. Yen TT, Allan JA, Person DV, Acton JM, Greenberg MM. Prevention of obesity in *Avyla* mice by dehydroepiandrosterone. ***Lipids***. 1977; 12:409-413.

19. Cleary MP, Zisk JF. Anti-obesity effect of two different level of dehydroepianderosterone in lean and obese middle-aged female *Zucker* rat . ***Int J Obes.*** 1986; 10:139-204.

20. Abraham GE. Ovarian and adrenal contribution to peripheral androgens during the menstrual cycle. ***J Clin Endocrinol Metab*** 1974; 39:240-246.

21. Labrie F, Belanger A, Cusan L, Gomez JL, Candas B. Physiological changes in Dehydroepiandrosterone are not reflected by serum levels of active androgens but of their metabolites: intracrinology. ***J Clin Endocrinol Metab*** 1997; 82: 2403-2409.

22. Mortola JF, Yen SS. The effects of oral dehydroepiandrosterone on endocrine-metabolic parameters in postmenopausal women. *J Clin Endocrinol Metab* 1990; 71: 696-704.

23. Stomati M, Monteleone P, Casarosa E, Quirici E, Puccetti S, Baernardi F, Genazzani AD, Rovati L, Luisi M, Genazzani AD. Six-month oral dehydroepiandrosterone supplementation in early and late postmenopause. *Gynecol Endocrinol* 2000; 14: 342-363.

24. Morales AJ, Nolan JJ, Nelson JC, Yen SS. Effects of replacement dose of dehydroepiandrosterone in men and women of advancing age. *J Clin Endocrinol Metab* 1994; 78: 1360-1367.

25. De Pergola G, Giagulli VA, Garruti G, Cospite MR, Giorgino F, Cignarelli M, Giorgino R. Low dehydroepiandrosterone circulating levels in premenopausal obese women with very high body mass index. *Metabolism* 1991; 40: 187-190.

26. Barrett-Connor E, Ferrara A. Dehydroepiandrosterone, dehydroepiandrosterone sulfate, obesity, waist-hip ratio, and noninsulin-dependent diabetes in postmenopausal women: the Rancho Bernardo Study. *J Clin Endocrinol Metab* 1996; 81: 59-64.

27. Casson PR, Santoro N, Elkind-Hirsch K, Carson SA, Hornsby PJ, Abraham G, Buster JE. Postmenopausal dehydroepiandrosterone administration increase free

insulin-like growth factor-I and decrease high-density lipoprotein: a six-month trial. ***J Am Soc Rep Med*** 1998; 70: 107-110.

28. Maccario M, Mazza E, Ramunni J, Oleandri SE, Savio P, Grottoli S, Rossetto R, Procopio M, Gauna C, Ghigo E. Relationships between dehudroepiandrosterone-sulphate and anthropometric, metabolic and hormonal variables in a large cohort of obese women. ***Clin Endocrinol*** 1999; 50: 595-600

29. Barnhart KT, Freeman E, Grisso JA, Rader DJ, Sammel M, Kapoor S, Nestler JE. The effect of dehydroepiandrosterone supplementation to symptomatic perimenopausal women on serum endocrine profiles, lipid parameters, and health-related quality of life. ***J Clin Endocrinol Metab*** 1999; 84: 3896-3902.

Table 1: Comparison of physical and metabolic characteristics of obese and non-obese women, (mean ±S.D).

Variables	Obese n=26	Non-obese n=39
BMI (kg/m^2)	31.68±3.43**	21.67± 2.65
DHEA (ng/ml)	14.45± 9.17	19.83± 14.15
DHEA-S (ng/ml)	1666.27± 943.44*	2180.30± 1016.90
LDL (mmol/L)	1.65± 0.82	1.29± 0.76
HDL (mmol/L)	1.23± 0.32**	1.50± 0.23
Cholesterol (mmol/L)	4.43± 0.66	4.38± 0.75
Triglycerides (mmol/L)	1.03± 0.60	0.71± 0.40

DHEA = Dehydroepiandrosterone

DHEA-S = Dehydroepiandrosterone-sulfate.

LDL = Low-density lipoprotein.

HDL = High-density lipoprotein.

*$p < 0.05$, **$p < 0.01$ comparing with non-obese

Table 2: Serum levels of Progesterone, Estradiol, FSH, and LH at days 3, 10, 17, and 24 of the menstrual cycle for obese and non-obese women, (mean ±S.D).

Days of menstrual cycle	Group	Progesterone (nmol/L)	Estradiol (pmol/L)	FSH (mIU/ml)	LH (mIU/ml)
Day 3	Obese	5.92±9.94*	255.98±185.42**	6.59±7.05	4.52 ±2.50
	Non-obese	2.42±1.10	128.86±62.63	7.06± 2.58	5.65± 2.74
Day 10	Obese	1.53±0.89	352.81±172.97	4.96±0.86	6.67±2.28*
	Non-obese	2.07±1.39	493.91±392.87	5.72± 3.13	10.47±11.02
Day 17	Obese	2.84± 4.13	881.76±522.30	5.08±2.42	14.61±9.18
	Non-obese	6.23±8.47	829.83±610.50	4.58±2.07	15.85±15.45
Day 24	Obese	9.51±6.44	744.05±280.90	3.88±2.71	9.31±8.10
	Non-obese	8.91 ±7.87	636.30±405.12	3.69±2.23	9.02±8.83

LH = Leutinizing hormone.

FSH = Follicle stimulating hormone.

* =p<0.05, **=p<0.01 compared with non-obese group.

Table 3: Pearson's correlation coefficient of DHEA to menstrual cycle hormones in obese (n=26) and non-obese (n=39) women.

Group	Progesterone at day 10	FSH at day 24	LH at day 24
Obese	0.945**	0.929**	0.707*
Non-obese	0.494**	0.353*	0.483**

DHEA = Dehydroepiandrosterone.

FSH = Follicle stimulating hormone.

LH = Leutinizing hormone.

*=$p < 0.05$, **=$p < 0.001$.