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Autonomic and neuroendocrine responses to stress in patients with functional hypothalamic secondary amenorrhea

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Objective: To evaluate the ability of women affected by functional hypothalamic secondary amenorrhea (FHSA) or polycystic ovary syndrome (PCOS) to adapt to stress.

Design: Controlled clinical study.

Setting: University hospital.

Patient(s): Thirty-one patients affected by FHSA, 29 patients with PCOS, and 30 eumenorrheic women.

Intervention(s): The subjects took the Stroop Color Word (Stroop CW) test and underwent blood sampling.

Main Outcome Measure(s): Heart rate (HR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and serum cortisol levels.

Result(s): The healthy controls had better Stroop CW scores than patients with FHSA. Serum cortisol levels significantly increased during Stroop CW with respect to the baseline in patients with FHSA or PCOS but not in the healthy controls. The SBP, DBP, and HR of the controls as well as SBP and DBP of patients with PCOS were significantly higher than those measured in patients with FHSA both at the baseline and during Stroop CW.

Conclusion(s): Patients with FHSA do not cope as well as healthy patients, and their autonomic response to stress is worse than both controls and patients with PCOS. (Fertil Steril® 2000;73:812–6. ©2000 by American Society for Reproductive Medicine.)

Key Words: Functional hypothalamic secondary amenorrhea, stress, Stroop Color Word, heart inotropic parameters, cortisol

Secondary amenorrhea, the spontaneous cessation of the menstrual cycle for ≥6 months after a menstrual pattern has been established, can be explained by a variety of detectable pathological processes that involve disorders of the hypothalamus, pituitary, adrenal cortex, and ovary (1, 2). The so-called functional hypothalamic secondary amenorrhea (FHSA), or psychogenic amenorrhea, is identified with a stress-related hypogonadotropic hypothalamic amenorrhea (3), which is often the result of a wide interplay of endocrine, psychogenic, and social derangements (4).

Indeed, stressful life changes probably cause a wide spectrum of somatic dysfunctions; secondary amenorrhea could be included among them (4). Changes in the central neurotransmitter activity, induced by psychosocial stressors, can cause dramatic changes in the hypothalamic-pituitary-adrenal-ovarian axes, thus explaining amenorrhea and other reproductive failures (5).

A significant inhibition of LH secretory pattern was found in patients with FHSA compared with amenorrheic patients without psychic stress (6). Recent studies reported an altered cortisol response to corticotropin-releasing factor (CRF) stimulation in patients with hypothalamic amenorrhea (7) and showed a lower pregnancy rate in patients who had high anticipatory state anxiety and cortisol levels when they underwent IVF-ET (8).

Similarly, our group recently demonstrated a cause and effect relationship between stressful personal life events and the onset of secondary amenorrhea of the hypogonadotropic subtype (9) as well as a significant correlation between poor outcome IVF-ET and cardiovas-

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cular vulnerability to stress (10). These observations strongly support the hypothesis that hypothalamic amenorrhea is one of the first and most sensitive signs of stressful events in patients unable to cope with additional stress and that hypothalamic amenorrhea induces significant sympathoadrenal or mechanical changes (e.g., inotropic activity in the heart).

Questionnaires are the only tools that objectively evaluate coping ability. The Stroop CW test was developed in the 1980s to measure attention, and the form itself is also used to analyze coping ability in people with cognitive disorders. The changes in variables such as heart inotropic parameters that also occurs in healthy patients confirms that Stroop CW must be considered a considerable source of stress (11).

Therefore, the aim of the present study was to [1] analyze the effect of FHSA on the ability to adapt to stress; [2] identify possible correlations between coping ability and parameters such as systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR); and [3] compare the adaptative ability with the blood changes of a well-known stress-related marker such as cortisol in eumenorrheic women vs. in women with FHSA and vs. amenorrheic subjects with polycystic ovary syndrome (PCOS). This last group of patients (women with PCOS) was included in the study to introduce a third variable in which a condition of amenorrhea, but not FHSA, was present. Indeed, the pathogenesis of PCOS, despite ongoing debate, seems to be strictly hormone related rather than stress dependent.

MATERIALS AND METHODS

Subjects

We studied 95 women with secondary amenorrhea (age between 18 and 35 years). The period of amenorrhea ranged between 6 and 28 months. All patients were referred to our division because of their menstrual disorders. They did not undergo any pharmacological treatment during the last 6 months before the study. The following criteria were used for inclusion in the study: [1] occurrence of secondary amenorrhea for ≥6 months before the beginning of the protocol; [2] absence of hyperprolactinemia, premature ovarian failure, or premature menopause; and [3] normal adrenal and thyroid functions.

Before admission to the study, every woman underwent clinical and psychological examination and transvaginal ultrasonography. Moreover, hormonal evaluation included: [1] determination of baseline levels of estradiol, androstenedione, progesterone, testosterone, and cortisol; [2] an LH pulsatility study as previously described (12); [3] determination of LH release after GnRH stimulation (13); and [4] determination of androgen plasma levels after dexamethasone suppression (13).

According to the above hormonal evaluations, subjects were classified into three groups: [1] 31 patients with FHSA (LH <3 mIU/mL); [2] 29 patients with PCOS (LH >10 mIU/mL and/or LH-FSH ratio of >2.5). The diagnosis of PCOS was always supported by the typical ultrasonographic pattern; and [3] 34 patients with secondary amenorrhea not resulting from conditions 1 and 2. These last 34 patients were not enrolled in the study because they did not meet the criteria for inclusion as reported above. Thirty eumenorrheic women (age between 18 and 36 years) recruited among students and staff (nurses and midwives) were included in the study as control group. In this last group, the criteria for inclusion were respected, and the subjects did not have clinical abnormalities. The study protocol was approved by the institutional review board of the University of Modena.

Protocol

In the present study, Stroop CW was performed as previously reported (10). Briefly, after a 5-minute resting period while sitting, subjects were asked to read three consecutive sheets of paper, allowing 45 seconds for each one. The first sheet had the words "red," "blue," "yellow," and "green" repeated 100 times. All these words were written in black ink that should be read, giving the score word (W). The second table had 100 spots of the four colors mentioned above that must be read, giving the score color (C). On the last sheet, the same four colors are printed in incongruent colors; the goal is to ignore the word itself and to name as much as possible the colors, resulting in the score CW.

To evaluate the interference caused by the words being printed in incongruent colors, an expected CW score was calculated according to the formula $C \times W/C + W$ and then subtracted from the experimental CW score as previously reported (10, 14). During Stroop CW, three blood samples for each patient were collected: the first was collected at baseline, the second 15 minutes from basic sampling, and the third 30 minutes from the first one.

To avoid any stress related to the blood-sampling procedure, a plastic catheter was inserted in the antecubital vein of the patient's arm at least 15 minutes before starting Stroop CW. At the end of the session, subjects remained seated for 15 minutes (10). Systolic blood pressure (SBP), diastolic blood pressure; (DBP), and heart rate (HR) were measured three times before testing, immediately after reading each sheet, and, finally, three times again, every 5 minutes, at the end of Stroop CW (10). All the measurements were conducted with use of an automatic blood pressure device (Dinamap-Critikon; Ethicon S.p.A. Italia, Rome, Italy). Both patients and controls were interviewed by the same investigator (M.L.M.), who was unaware of the endocrine status of patients (9, 10).

Comparison and Statistical Analysis

The χ^2 test with Yates' correction and the *t*-test for samples with different variances were used for the comparisons of the groups. Multivariate analysis of variance (MANOVA) and the t-test were used for computing continuous, within-subject variables (9). In particular, cardiovascular and corti-

TABLE 1

Word (W) score appeared to be significantly higher in the control group than in patients with FHSA.

	Controls	FHSA	PCOS
No. of words	121.16 ± 11.67*	114.33 ± 12.91*	117.16 ± 11.52
No. of colors	83.73 ± 10.20	81.93 ± 9.08	80.13 ± 10.36
No. of colors-words	55.93 ± 9.37	56.26 ± 9.42	54.36 ± 6.97

Note: Values are means ± SD.
*$P<.02$.

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sol data were analyzed by MANOVA, and the changes during Stroop CW and the groups (healthy controls and patients) were considered sources of variation. $P<.05$ was considered statistically significant.

RESULTS

Table 1 shows that the performances of patients with FHSA or PCOS at the reading of the last two sheets of the Stroop CW test are similar to those of the control group, whereas the number of colors read in the first sheet (score W) is significantly lower in patients with FHSA than in controls (114.33 ± 12.91 vs. 121.16 ± 11.67, $P<.02$).

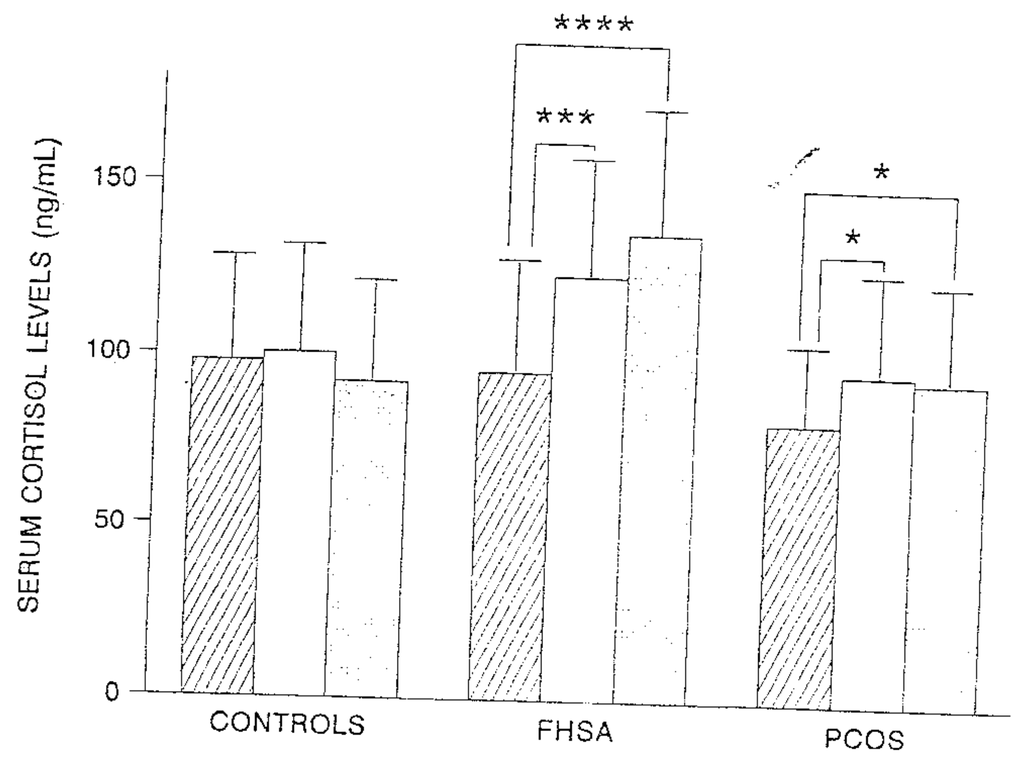
Serum cortisol concentrations were also stimulated by Stroop CW and showed different patterns in the three different groups of patients. No significant differences were observed in the control group at the three times of sampling, whereas cortisol levels significantly increased in the patients with FHSA and PCOS both at 15 minutes and at 30 minutes compared with the specific baselines (FHSA at 15 minutes: 123.65 ± 28.68 ng/mL vs. 95.54 ± 31.95 ng/mL; FHSA at 30 minutes: 135.98 ± 37.63 vs. 95.54 ± 31.95 ng/mL; $P=.0003$ and $P=.00002$, respectively; PCOS at 15 minutes: 96.21 ± 28.78 ng/mL vs. 81.44 ± 20.09 ng/mL; PCOS at 30 minutes: 94.39 ± 26.48 ng/mL vs. 81.44 ± 20.09 ng/mL; $P=.02$ and $P=.04$, respectively) (Fig. 1).

The response to Stroop CW in amenorrheic patients and controls as a function of inotropic findings in the heart is reported in Table 2; the values are means ± SD. At the baseline, SBP, DBP, and HR values of the control group and SBP and DBP values of PCOS were significantly higher than those in patients with FHSA, whereas no significant differences were observed between controls and PCOS at 15 minutes or at 30 minutes.

During the Stroop CW test, the inotropic responses in the heart changed: SBP values were significantly higher in patients with PCOS than in controls and in patients with FHSA at the reading of the first sheet (131.8 ± 12.84 vs. 121.26 ± 24.06 and 121.66 ± 15.11, respectively; $P=.02$ in both comparisons). During the reading of the second sheet, SBP became similar between patients with PCOS and controls,

FIGURE 1

Cortisol serum levels in the two groups of patients and controls. The striped bars, the solid bars, and the stippled bars represent the different sampling times: 0, 15, and 30 minutes, respectively. *$P<.05$; ***$P<.001$; ****$P<.0001$.



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and there was a statistically significant difference between these two groups and patients with FHSA (131.13 ± 15.99 and 127.73 ± 13.93 vs. 120.96 ± 14.22; $P=.006$ and $P=.03$, respectively). Similarly, DBP and HR were significantly higher in patients with PCOS than in women affected by FHSA at the reading of the first and third sheet, respectively (73.93 ± 10.35 vs. 68.83 ± 12.88 and 92.73 ± 14.62 vs. 86.6 ± 12.65, respectively; $P=.04$ in both comparisons).

DISCUSSION

The data reported in the present study show that both coping ability and cardiac response to stress decrease in patients with FHSA, whereas cortisol response to stress increases when FHSA is present.

The main goal of the Stroop CW test is to present automatic reading of the written word while naming the color in which the word is written, by using analogical processing. With the use of this test, we investigated how patients react when experiencing cognitive stressful events. As shown previously (11, 15), the response to the Stroop CW test is physiologically associated with increased SBP, HR, and cardiac output, i.e., the involvement of the sympathetic nervous system; for this reason, Stroop CW was used to investigate cerebral hemodynamic adjustments (16) and to evaluate the influence of personality traits on the peripheral cardiovascular response (17, 18).

At the same time, FHSA can be caused by a combination

TABLE 2

SBP, DBP, and HR changes during the Stroop CW test.

	Controls	FHSA	PCOS
Systolic blood pressure (mm Hg)			
Baseline SBP	116.76 ± 9.59*	110.03 ± 11.05*†	115.63 ± 15.32†
1st sheet	121.26 ± 24.06‡	121.66 ± 15.11	131.8 ± 12.84‡
2nd sheet	127.73 ± 13.93§	120.96 ± 14.22§‖	131.13 ± 15.99‖
3rd sheet	127.06 ± 12.07	122.16 ± 14.55	124.96 ± 11.48
Recovery	115.33 ± 10.83	111.26 ± 11.24	113.5 ± 13.09
Diastolic blood pressure (mm Hg)			
Baseline DBP	63.83 ± 6.23*	60.46 ± 7.41*†	64.86 ± 11.26†
1st sheet	71 ± 12.08‡	68.83 ± 12.88	73.93 ± 10.35‡
2nd sheet	71.9 ± 8.95	68.33 ± 10.35	70.76 ± 9.06
3rd sheet	70.86 ± 12.29	68.7 ± 10.44	67.1 ± 12.42
Recovery	59.9 ± 9.47	59.46 ± 11.39	60.43 ± 9.91
Heart rate (beats/min)			
Baseline HR	79.86 ± 11.94*	72.26 ± 14.53*	75.16 ± 11.58
1st sheet	92.1 ± 14.59	93.2 ± 22.83	95.56 ± 13.21
2nd sheet	90.16 ± 14.15	86.6 ± 21.39	90.36 ± 12.19
3rd sheet	92.73 ± 14.62‡	90.03 ± 21.70	86.6 ± 12.65‡
Recovery	77.5 ± 11.27	73.36 ± 15.77	76.4 ± 11.62

Note: Values are means ± SD.
SBP comparisons: *$P<.01$; †$P=.05$; ‡$P<.05$; §$P<.05$; ‖$P<.01$.
DBP comparisons: *$P<.05$; †$P<.05$; ‡$P<.05$.
HR comparisons: *$P=.01$; †$P<.05$.

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of endocrine and psychogenic dysfunctions and stressful life events (7, 9, 19). A number of studies demonstrated that stressful events significantly modify the production and release of specific stress-related hormones and neurotransmitters such as cortisol and corticotropin-releasing factor by inducing significant changes in the ovarian function and in the hypothalamic-pituitary-adrenal axis (2, 20). The evidence that the Stroop-induced cognitive process is physiologically associated with the activation of the hypothalamic-pituitary-adrenal axis (11, 15) suggests that cortisol may play a role not only as a hormonal marker of different psychological stress degrees but also as modulating central neurotransmitters.

In our study, the mental conflict induced by the Stroop CW was solved by every patient, but the healthy subjects read more colors than the amenorrheic patients in the first sheet "Word" of the test. Blood samples collected at baseline, 15 minutes from baseline sampling, and 30 minutes from the first one showed that serum cortisol levels significantly increased in patients with FHSA and PCOS both at 15 minutes and at 30 minutes compared with the baseline; in contrast, no significant changes were observed in the controls. On average, a significant decrease inotropic activity in the heart was observed in patients with FHSA compared with PCOS and controls.

The hypothesis that the healthy controls feel that the task is less important than the amenorrheic patients is not applicable because they did not know the comparative aim of the test. If the Stroop CW inhibits the automatic responses to favor analogical processing, the emotional implication of the Stroop CW test might be objectively different in the two groups of women and, in particular, it would be lower in patients with FHSA than in healthy subjects or in women with PCOS.

The reduction in the ability to cope observed in our study may be explained by the higher quantity of energy and mental effort needed to solve the Stroop CW test (21). This finding is supported by the recent observation by Pardo et al. (21) that the anterior cingulate cortex, an area of the central nervous system anatomically connected with the attentional area and the hypothalamus, is activated during the processing of stress response. This hypothesis could also explain the surprising absence of response in the heart inotropic parameters to the stressful condition induced by Stroop CW in FHSA. These data are similar to those observed in other patients such as women undergoing IVF (10), infertile women (19), and women with mental stress during pregnancy (11, 17).

We speculate that this type of overall response is a strong sign of the individual's reactivity to reproductive problems such as amenorrhea, whereas only the particular psychological conditions linked to FHSA (9) indicate that patients with FHSA may not be able to cope with stressful events as well as the control patients or patients with PCOS. A significant endocrine increase of a classic stress-related hormone such as cortisol (7) was confirmed by the present study. The

cortisol serum trend in FHSA is the opposite for that observed for cardiac inotropism and coping ability.

In conclusion, the findings of our research demonstrate a relation between susceptibility to stress and FHSA. Further studies to clarify the neuroendocrine mechanisms connected with this type of amenorrhea are needed.

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