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Circulating thyrotropin is upregulated by estradiol

Salvatore Benvenga[a,b,c], Flavia Di Bari[a,*], Roberta Granese[d], Irene Borrielli[d], Grazia Giorgianni[e], Loredana Grasso[e], Maria Le Donne[d], Roberto Vita[a], Alessandro Antonelli[f]

[a] Department of Clinical and Experimental Medicine, Section of Endocrinology, University of Messina, 98125 Messina, Italy
[b] Master Program on Childhood, Adolescent and Women's Endocrine Health, University of Messina, 98125 Messina, Italy
[c] Interdepartmental Program on Molecular & Clinical Endocrinology, and Women's Endocrine Health, University hospital, A.O.U. Policlinico Gaetano Martino, 98125 Messina, Italy
[d] Department of Obstetrics and Gynecology, University Hospital G. Martino, 98125 Messina, Italy
[e] Service of Immunometry and Laboratory Diagnosis, University Hospital G. Martino, 98125 Messina, Italy
[f] Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy

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ABSTRACT

After encountering two women with serum thyrotropin (TSH) levels greater in periovulatory phase than in other days of the menstrual cycle, we hypothesized that TSH levels could be sensitive to changes in circulating estrogens in women.

The objective of this study was to evaluate whether serum TSH increases after an induced acute increase of serum estradiol, and compare serum TSH increase with that of prolactin (PRL) which is a classic estradiol-upregulated pituitary hormone.

In this retrospective study, we resorted to stored frozen sera from 55 women who had undergone the GnRH agonist (buserelin)-acute stimulation test of ovarian steroidogenesis. This test, that is preceded by dexamethasone administration to suppress adrenal steroidogenesis, had been performed to show an increased buserelin-stimulated response of 17-hydroxyprogesterone, a response that is frequent in polycystic ovary syndrome. Fifty-five women had enough serum volume at pertinent times (first observation early in the follicular phase and all times of the test) to permit assay of serum estradiol, TSH and PRL.

Before dexamethasone administration, estradiol averaged 26.4 ± 15.5 pg/ml (reference range 23–139, follicular phase), TSH 1.78 ± 0.86 mU/L (reference range 0.3–4.2) and PRL 409.4 ± 356 mU/L (reference range 70.8–556) (mean ± SD).

Serum estradiol, TSH and PRL averaged 47.2 ± 27 pg/ml, 0.77 ± 0.48 mU/L and 246.4 ± 206.8 mU/L just prior to the buserelin injection, but they peaked at 253.4 ± 113.5 pg/ml (nv 83–495, midcycle), 3.30 ± 1.65 mU/L and 540.3 ± 695.2 mU/L after injection. The responses to buserelin of estradiol, TSH and PRL were of wide magnitude. There was a significant correlation between TSH peak and serum estradiol peak, betweeen AUC0-24 h-TSH and AUC0-24 h-estradiol, or between PRL peak and estradiol peak and AUC0-24 h -PRL and AUC0-24 h-estradiol in only a subgroup of women.

Therefore, women with estradiol-dependent increase in serum TSH do exist. Reference bands of serum TSH dependent on the phases of the menstrual cycle should be available.

Introduction

When evaluated in the premenstrual phase of the cycle, the response of thyrotropin (TSH) to TSH-releasing hormone (TRH) in women is greater than in men [1]. Indeed, the TSH response to TRH is enhanced by estrogens, both in women on oral contraceptives and in men being treated with estrogens [1]. In contrast, the TSH response to TRH is reduced by thyroid hormones, corticosteroids, levodopa, dopamine, propranolol, and it falls with age [1]. As reviewed by Vuong et al. [2], TSH regulation by opioids is complex. Indeed, opioids suppress TSH secretion in rodents, but stimulate it in humans. In humans, the effects of the opioids and endogenous opioid peptides are more significant during the physiological nocturnal TSH surge [2]. In patients with opioid-dependence, serum TSH was lower in the acute abstinence

Abbreviations: AUC, area under the curve; E2, estradiol; FSH, follicle-stimulating hormone; FT3, free T3; GnRH, gonadotropin-releasing hormone; 17-OHPg, 17-hydroxyprogesterone; LH, luteinizing hormone; PCOS, polycystic ovary syndrome; PRL, prolactin; T3, triiodothyronine; T4, thyroxine; TRH, TSH-releasing hormone; TSH, thyrotropin

* Corresponding author at: Dept. of Clinical and Experimental Medicine, University of Messina, Policlinico Universitario "G. Martino", Padiglione H, 4 piano, 98125 Messina, Italy.
E-mail address: flaviadb1983@libero.it (F. Di Bari).

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period and after 30 days of abstinence compared with age- and sex-matched controls [3].

As described in more detail under Discussion, we have recently encountered two regularly menstruating women whose serum TSH fluctuated during their menstrual cycle [4]. Particularly, the highest TSH levels were observed when blood had been drawn at mid-cycle, coinciding with peak levels of serum estradiol (E2). Because TSH threshold is crucial for the diagnosis and differentiation of degree of thyroid failure (*viz.* subclinical or initial hypothyroidism *vs* overt hypothyroidism), and because TSH is also the biochemical index for gauging thyroid hormone replacement therapy, there would be clinically important consequences for the correct interpretation of serum TSH in women across their reproductive age. In the United States, 5% of the population aged 12 years or more (that is, approximately 15 million) have either subclinical or overt hypothyroidism [5]. A similar rate resulted from a meta-analysis of seven studies on the European population aged 18 years or more (*viz*, approximately 40 million) [6]. This translates into hundreds of millions TSH assays performed in the US or Europe for the diagnosis and periodic follow-up of hypothyroidism.

In brief, we tested the hypothesis that those two women [4] were the classic tip of the iceberg, namely we hypothesized that a number of women exist whose increase in circulating estrogens is accompanied by an increase in circulating TSH.

Materials and methods

Patients

To test our hypothesis with an unfunded study, we made the following reasoning. In lieu of enrolling a large cohort of women and measuring both serum E2 and TSH repeatedly throughout their menstrual cycle, in order to quickly and conveniently maximize finding of those women we resorted to stored frozen sera from women in whom ovarian steroidogenesis had been stimulated. These young adult women, who were free of past and current history of alcohol/illicit drug abuse, had undergone the gonadotropin-releasing hormone (GnRH) agonist (buserelin)-acute stimulation test of ovarian steroidogenesis for the purpose of showing an increased buserelin-stimulated response of 17-hydroxyprogesterone (17-OHPg). This increased response of 17-OHPg is frequent in polycystic ovary syndrome (PCOS). This test [7], that had some diffusion in the '90s, needs to be performed in the follicular phase under treatment with dexamethasone for suppressing adrenal steroidogenesis which, otherwise, would confound interpretation. However, dexamethasone also inhibits TSH secretion [8], a fact that decreases the chances of our hypothesis being correct.

GnRH agonist stimulation test of ovarian steroidogenesis

Dexamethasone was given orally in a dose of 0.5 mg four times daily for five consecutive days, the first day of dexamethasone administration coinciding with the second or third day of the menstrual cycle. On the morning of the 5th day, starting between 8:00 and 8:30, two blood samples were drawn 30 min apart. These two samples, which are referred to as times −30 and 0 h of the buserelin test, serve for measuring baseline hormone levels. Immediately after this second sample, 0.5 mg of the GnRH agonist buserelin, (Superfact®; Hoechst Marion Roussel SpA, Milano, Italy) were injected subcutaneously. Blood samples for measurement of gonadotropins and steroid hormones were taken at 1, 4, 20 and 24 h post-injection. In line with the cut-off point for normality (17-OHPg peak < 250 ng/dl) established in the original study on 13 normal women [7], our cut-off point established on 20 normal women was also < 250 ng/dl.

As said above, for the purposes of this particular study, we resorted to serum samples of women who had been subjected to the buserelin test and that had been stored at −20 °C. The women selected for the present study were those with enough volume of all relevant serum samples (first observation early in the follicular phase, and all six samples of the buserelin test [−30, 0, 1, 4, 20 and 24 h]) to permit the assay, in duplicate, of E2, PRL, TSH, triiodothyronine (T3) and free T3 (FT3). Assay of both T3 and FT3 was necessary to prove that the increase in serum TSH consisted in the pituitary release of biologically active TSH. In normal subjects, serum T3 concentrations increase from 30% to 100% above baseline at 120–180 min after the intravenous injection of 200 μg TRH, with the peak of serum TSH occurring between 15 and 30 min after TRH injection [9]. Because the GnRH-agonist test has to be performed under suppression of the adrenal steroidogenesis by dexamethasone [7] and because glucocorticoids inhibit both TSH secretion and conversion of thyroxine (T4) to T3 [8], we preferred to rely on measurement of both serum T3 and FT3, instead of measurement of either T3 or FT3, as evidence of TSH bioactivity.

Hormone assays

All hormones were measured using the corresponding chemiluminescent assays by Beckman Coulter. The local intra-assay and inter-assay coefficients of variations are 5.5 and 9.3% (E2), 1.5 and 4.2% (PRL), 2.5 and 3.8% (TSH), 2.8 and 4.1% (total T3), 2.6 and 5.1% (FT3).

Statistics

Data are reported as mean ± SD, median and range. The overall response of given hormones to the GnRH stimulation test is summarized by the area under the curve (AUC_{0-24h}), which was calculated by the classic trapezoidal method. Because of the nongaussian distribution, differences between continuous variables were evaluated by the Wilcoxon signed rank test. Differences between categorical variables were evaluated by the Fisher's exact test or chi-square (χ^2) test, as appropriate. Simple correlation analysis was performed to relate a given hormone index (baseline, peak, AUC) with a given E2 index (baseline, peak, AUC). In all statistical comparisons, a P value of < 0.05 was considered statistically significant, while a P value between 0.10 and 0.05 was considered borderline significant.

Results

Data are summarized in Tables 1–3, and graphically displayed in Figs. 1 and 2.

At first observation, early in the follicular phase, serum TSH averaged 1.78 ± 0.86 mU/L. On day 7–9 of the menstrual cycle, at the end of the short-term administration of dexamethasone (that is, time 0 of the acute stimulation test of ovarian steroidogenesis), serum TSH fell to 0.77 ± 0.48 mU/L ($P = 1.2 \times 10^{-11}$) (Table 1). However, between the 20th and 24th hour after the GnRH analog injection, serum TSH increased 4-fold to 3.30 ± 1.65 mU/L ($P = 1.1 \times 10^{-10}$ *vs* time 0, and $P = 3.9 \times 10^{-7}$ *vs* first observation) (Table 1). GnRH-stimulated levels of TSH above 4.0 mU/L were recorded in 17/55 women (30.9%), significantly more frequently than at first observation (1/55 = 1.8%; $P < 0.0001$, OR = 24.2 [95%CI24.2to189]). This single value of serum TSH > 4.0 mU/L was 5.1 mU/L. In contrast, 8 women had peak TSH levels above 5.0 mU/L (14.5%, $P = 0.032$ *vs* 1/55) and 3 women had TSH peak levels in the range of 6.0–7.6 mU/L (5.4%. $P = 0.24$ *vs* 0/55). In those 8 women, serum TSH at first observation ranged 1.3–5.1 mU/L, this last value belonging to the woman with the highest TSH peak (7.6 mU/L). It should be noted that peak TSH levels would have been significantly greater if the test were performed in the absence of the TSH-suppressive dexamethasone administration.

Serum PRL increased by 2-fold (Table 1). The increase of serum PRL and TSH coincided with an increase of the luteinizing hormone (LH)-driven increase in serum E2 (Fig. 1). Unlike E2, the post-injection levels of both TSH and PRL (and corresponding AUCs) were greater in the high 17-OHPg response group compared to the normal 17-OHPg

Table 1
Serum levels of estradiol (E2), thyrotropin (TSH) and prolactin (PRL) at first evaluation and during the test of acute stimulation of the LH-driven ovarian steroidogenesis by the subcutaneous injection of buserelin, a GnRH agonist.*

	E2, pg/ml	TSH, mU/L	PRL, mU/L
Baseline (first observation)	26.4 ± 15.1 [24] 12.4–87 (n = 51)	1.78 ± 0.86 [1.50] 0.45–5.1 (n = 45)	409.4 ± 356 [289] 36.3–1869 (n = 49)
Buserelin test			
0 h levels (pre-injection)	47.2 ± 27.0 [41.0] 11.3–140 (n = 55) *vs* first observation **P = 5.2 × 10^{-7}**	0.77 ± 0.48 [0.68] 0.04–2.7 (n = 55) *vs* first observation P = **1.2 × 10^{-11}**	246.4 ± 206.8 [198] 32.6–1215 (n = 55) *vs* first observation P = **0.0003**
Peak levels	253.4 ± 113.5 [232] 54–561 (n = 55) *vs* first observation P = **9.6 × 10^{-19}** *vs* 0 h P = **1.1 × 10^{-10}**	3.30 ± 1.65 [2.9] 0.3–7.6 (n = 55) *vs* first observation P = **3.9 × 10^{-7}** *vs* 0 h P = **1.12 × 10^{-10}**	540.3 ± 695.2 [400] 50.8–5050 (n = 55) *vs* first observation P = **0.025** *vs* 0 h P = **1.2 × 10^{-10}**
% change (peak over baseline)	526.8 ± 368.7 [397.4] 12.1–1612	397.9 ± 316.3 [337.3] 26.3–1543	142.3 ± 156.6 [93.8] (1.3–1003)
AUC 0–24 h	3516 ± 1533 (n = 56)	232 ± 977 (n = 56)	8904 ± 9505 (n = 56)

Peak for E2 and TSH was always detected at 20 or 24 h after injection of the GnRH agonist. Peak for PRL was detected at 4, 20 or 24 h.

* Data are reported as m ± SD [median] and range. Blood sampling at first observation occurred in the follicular phase. The buserelin test was performed in the menstrual cycle immediately following the menstrual cycle of the first observation. The buserelin test must be performed under dexamethasone administration to suppress any adrenal contribution to steroidogenesis (see Patients and Methods). AUC = area under the curve.

Table 2
Serum levels of estradiol (E2), thyrotropin (TSH) and prolactin (PRL) at first evaluation and during the test of acute stimulation of the LH-driven ovarian steroidogenesis by the subcutaneous injection of buserelin. The 55 women were stratified dychotomically based on the high response (HR) or normal response (NR) of serum 17-hydroxyprogesterone (17-OHPg) to buserelin.*

	HR 17-OHPg (n = 26)	NR 17-OHPg (n = 29)	Statistics, P value
Baseline			
E2, pg/ml	24.35 ± 16.1 [20.6]	28.4 ± 14.1 [25.1]	0.17
TSH, mU/L	1.83 ± 0.91 [1.70]	1.73 ± 0.73 [1.4]	0.45
PRL, pg/ml	404.5 ± 268.2 [3 4 5]	414.0 ± 430 [2 8 3]	0.31
Buserelin test			
E2, 0 h	42.0 ± 21.4 [4 0]	51.8 ± 30.8 [41.1]	0.36
TSH, 0 h	0.80 ± 0.53 [0.63]	0.76 ± 0.46 [0.61]	0.83
PRL, 0 h	254.2 ± 191.3 [198]	239.4 ± 222.0 [198]	0.58
E2 peak	303.2 140.9 ± [280]	206 ± 75.8 [204]	**0.0015**
TSH peak	3.62 ± 1.87 [3.35]	3.12 ± 1.66 [2.6]	0.36
PRL peak	506.2 ± 362.8 [414]	570.9 ± 901.3 [341]	0.17
E2, % change	711.2 ± 361.3 [732.7]	361.3 ± 292.8 [263]	**4.0 × 10^{-5}**
TSH, % change	472.4 ± 408.8 [374.7]	328.7 ± 176. 8 [316.7]	0.36
PRL, % change	158.2 ± 194.3 [105.3]	128.2 ± 114.7 [93.6]	0.61
E2, AUC 0–24 h	4227 ± 1615 [3854]	3128 ± 1138 [2761]	**0.005**
TSH, AUC 0–24 h	48.5 ± 22.7 [46.1]	43.3 ± 20.8 [38.1]	0.48
PRL, AUC 0–24 h	9523 ± 6826 [7766]	9319 ± 11608 [6807]	0.15

* Data are reported as m ± SD [median].

response group, but differences were statistically insignificant (Table 2).

That the TSH released after the GnRH injection is biologically active was proven by the increase in both serum T3 [+44%, not shown] and FT3 (+31%) (Fig. 1).

Table 3
Summary of the correlation between the specified indices for serum E2 and TSH or PRL, as measured before (time 0) or after the subcutaneous injection of buserelin, in the whole cohort or in subgroups of women with a high degree of relationship between variables based upon simple visual inspection of data.

	Variable X (TSH), variable Y (E2)	Variable X (PRL), variable Y (E2)
X (0), Y (0)	r = −0.019 (P = 0.891) N = 17 r = 0.06 [−0.43 to 0.53], P = 0.81	r = 0.053 (P = 0.70) N = 10 r = 0.320 [−0.39 to 0.79], P = 0.37
X (0), Y (peak)	r = −0.093 (P = 0.500) N = 17 r = 0.390 [−0.11 to 0,73], P = 0.12	r = 0.006 (P = 0.96) N = 10 r = 0.864 [0.51–0.97], **P = 0.0013**
X (peak), Y (peak)	r = −0.149 (P = 0.277) N = 17 r = 0.79 [0.50–0.92], **P = 0.00016**	r = 0.009 (P = 0.95) N = 10 r = 0.902 [0.63–0.98], **P = 0.00036**
X (% change), Y (% change)*	r = −0.113 (P = 0.41) N = 17 r = −0.10 [−0.55 to 0.40], P = 0.70	r = 0.024 (P = 0.86) N = 10 r = 0.12 [−0.55 to 0.70], P = 0.74
X (AUC 0–24 h), Y (AUC 0–24 h)§	r = −0.196 (P = 0.152) N = 17 r = 0.851 [0.62–0.94], **P = 1.5 × 10^{-5}**	r = 0.058 (P = 0.68) N = 10 r = 0.866 [0.52–0.97], **P = 0.0012**

In the 55 women, E2 peak was significantly correlated to E2 time 0 (r = 0.394 [95% CI 0.144 to 0.597], P = 0.0029). TSH peak was also significantly correlated to TSH time 0 (r = 0.537 [95% CI = 0.32 to 0.70], P = 2.3 × 10^{-5}). PRL peak was significanltly correlated to PRL time 0 (r = 0.842 [95% CI = 0.74 to 0.90], P = 2 × 10^{-16}).

* % change is peak over baseline (time 0 of the test). Linear correlation analyzed after $\log_{10}$ transformation of E2, TSH and PRL, due to their nongaussian distribution.

§ The correlation for the 17 women (TSH) or the 10 women (PRL) is shown in the insets of Fig. 2.

Correlation between the GnRH agonist-stimulated responses of E2 and TSH or PRL

The individual pairs (TSH and E2, or PRL and E2) of various indices are summarized in Table 3, and presented graphically in Fig. 2.

It is evident how responses were widely scattered (Fig. 2). However, some women (n = 17 for the relationship concerning E2 and TSH, and

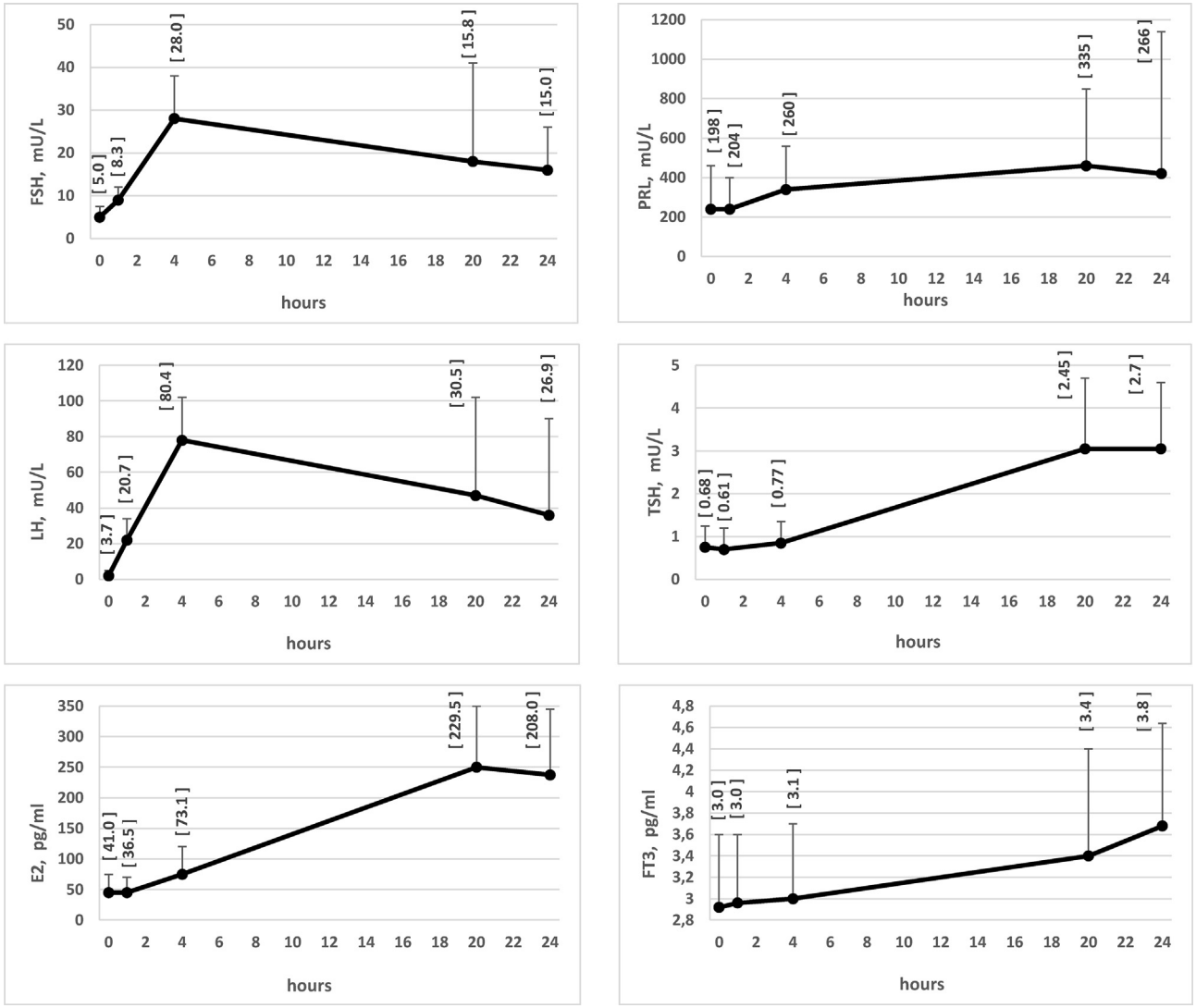

Fig. 1. Changes in serum levels of gonadotrotropins [follicle-stimulating hormone (FSH), luteinizing hormone (LH)], estradiol (E2), prolactin (PRL), thyrotropin (TSH) and free triiodothyronine (FT3) prior to and up to 24 h after the subcutaneous injection of 0.5 mg buserelin, a gonadotropin-releasing hormone agonist. Data are mean ± SD [and median].

10 for the relationship concerning E2 and PRL [Table 3 and Fig. 2, insets]) had a statistically significant positive correlation between peak levels of TSH or PRL and peak levels of E2, and between $AUC_{0\text{-}24h}$ of TSH or PRL and $AUC_{0\text{-}24h}$ of E2. Other women had a scanty change of the pituitary hormone in the face of great change of E2, while still others had the opposite pattern (*viz,* great change of the pituitary hormone in the face of a scanty change of E2), suggesting hypersensitivy of TSH or PRL to estrogens. For the purposes of our work, this last group of women and the 17 with a statistically significant correlation between E2 and TSH were those that fitted our hypothesis.

Discussion

Taking advantage of the acute stimulation of ovarian steroidogenesis (including E2) that is caused by the single injection of a GnRH agonist, we have provided evidence for the acute estrogen-associated upregulation of circulating TSH and for its wide individual magnitude. The overall magnitude of the upregulation could have been even greater, considering that the GnRH agonist test is performed under administration of corticosteroids, which are well-known inhibitors of TSH secretion [8,10–12]. This inhibitory action of glucocorticoids occurs both in the hypothalamus (decreased TRH mRNA levels) and in the pituitary (decreased release of TSH from the thyrotrophs in a PKC-dependent manner through the protein annexin).

The direct, positive E2-depedency of TSH cannot be counteracted by the inverse, negative corticosteroid-dependency. In percent terms, the increase in serum TSH levels after the increase in serum E2 following one single s.c. injection of a GnRH agonist is greater than the increase in serum PRL levels. Similar acute increase in serum E2 levels occurs naturally every month after the GnRH-driven increase in serum gonadotropins and subsequent stimulation of the ovarian steroidogenesis. The pattern of the GnRH-stimulated increase in serum gonadotropins that we observed in this study [mean peak LH and follicle-stimulating hormone (FSH) = 78.6 and 27.7 mU/L] mirrors well the pattern observed naturally during the menstrual cycle. This pattern consists of serum LH concentrations prevailing over serum FSH concentrations at mid-cycle. In the follicular, midcycle and luteal phase, serum LH ranges 1.7–15, 21.9–56.6 and 0.6–16.3 mU/L, while serum FSH ranges 4–13, 5–22 and 2–13 mU/L, respectively [13]. The upper normal limit of serum LH at midcycle is reported at 76.3 mU/L by others [14]. In our laboratory, follicular, midcycle and luteal phase serum LH reference ranges are 2.1–10.9, 19.2–103 and 1.2–12.9 mU/L, while serum FSH reference ranges are 3.8–8.8, 4.5–22.5 and 1.8–5.1 mU/L, respectively. Also the magnitude of the increase in serum E2 following the GnRH agonist injection (54–561 pg/ml) is comparable to the 150–750 pg/ml [13] serum E2 levels measured at ovulation. Other authors report that

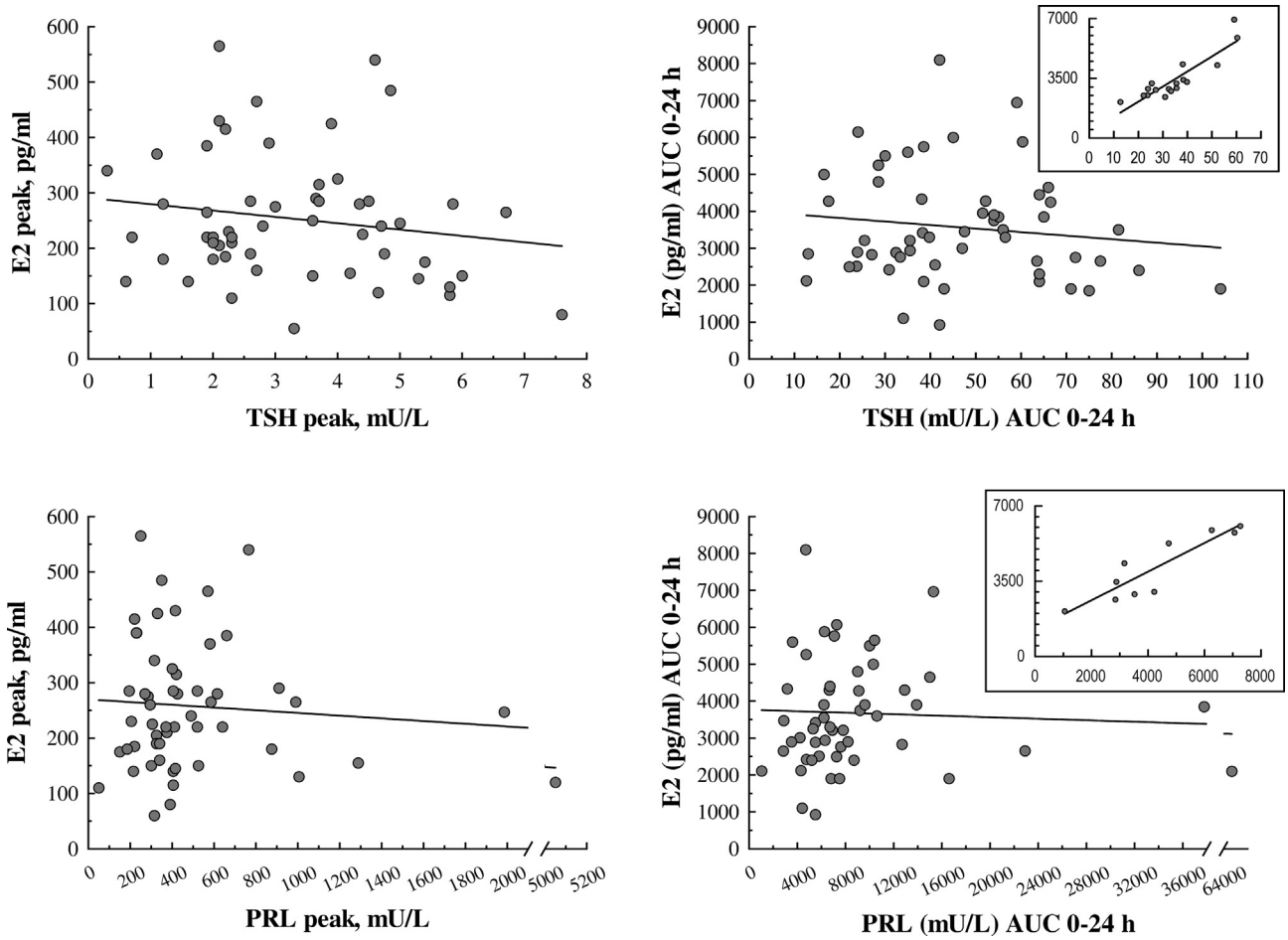

Fig. 2. Correlation between the buserelin-induced responses in serum E2 and buserelin-induced responses in serum TSH or PRL. Responses shown in the figure are peak levels or area under the curve (AUC0-24 h). For the insets in the two right panels, see Table 3.

serum E2 levels in the follicular phase and luteal phase range 20–150 and 30–450 pg/ml, respectively [14]. In our laboratory, follicular, midcycle and luteal phase serum E2 reference ranges are 23–139, 83–495 and 42–338 pg/ml. Thus, our observations are applicable to the clinical context.

Starting at 6–8 weeks of gestation, maternal serum E2 increases progressively until term, with individual values that, at the 36th week, vary between 6 and 40 ng/ml (6000 and 40,000 pg/ml) [15] and average approximately 15 ng/ml (15,000 pg/ml) or approximately 50-fold over maximal prepregnancy levels [16]. Serum PRL parallels such changes in serum E2, with PRL increase starting from about week 8 to peak levels of 200–400 ng/ml [4220–8440 mU/L], or approximately 10–40 times prepregnancy levels [15]. Serum TSH also increases progressively during gestation [17–21]. For instance, median (5th-95th percentiles) in the first, second and third trimester reported by Panesar et al. [18] are 0.8 (0.03–2.30), 1.10 (0.03–3.10) and 1.30 (0.13–3.50). The corresponding levels reported by Bocos-Terraz et al. [19] are 0.92 (0.03–2.65), 1.12 (0.12–2.64) and 1.29 (0.23–2.56), while those reported by Rajput et al. [20] are 1.40 (0.44–3.46), 1.74 (0.73–3.03) and 2.22 mU/L (0.86–4.38), and those reported as 2.5th-97.5th percentile by Moon et al. [21] are 1.15 (0.01–4.10), 1.55 (0.01–4.26) and 2.12 mU/L (0.15–4.57). Further to gestation in 20 pregnant women, serum TSH was measured during the menstrual cycle in 10 healthy women, and it was shown to peak a few days after ovulation [17].

The estrogen-dependency of serum TSH levels can also be appreciated in women on oral contraceptives [22]. Median serum TSH in 108 females on oral contraceptives was 1.56 mU/L, but it was 1.29 mU/L (−17.3%) in 66 females not using oral contraceptives [23]. A number of studies reported higher levels of serum TSH in females compared to males [24–26]. Particularly, in a well-characterized, disease-free population, median (2.5th-95th percentile) serum TSH levels of females aged 20–39 years were 2.49 mU/L (0.75–7.90), > 2.23 mU/L (0.70–6.50) of age-matched males [26]. Finally, a 10-year-old boy with congenital adrenal hyperplasia and associated hyperplastic testicular adrenal rests had high serum concentrations of 17-OHPg, E2, testosterone, basal and TRH-stimulated TSH and PRL [27]. Serum E2 correlated directly with PRL and TSH [27]. Upon dexamethasone therapy, steroid hormones, PRL and TSH returned progressively normal [27].

Our data agree with experimental studies in rats demonstrating that increasing serum estrogen levels within the physiological range increase both basal and TRH-stimulated release of TSH and PRL [28,29]. How can estrogens up-regulate serum levels of TSH, knowing that thyrotrophs have estrogen receptors, though less abundant than other adenohypophysis cell types [30]? A robust E2-driven TSH release at mid-cycle and consequent increment of serum TSH may result from one or both these possibilities: (i.) a physiologically high surge in circulating E2 with associated robust response of the E2 receptors in the thyrotrophs, (ii.) hypersensitivity of E2 receptors to less robust circulating E2 levels. However, additional mechanisms may operate, such as E2-driven inhibition of the negative feedback that thyroid hormones exert on both basal and TRH-stimulated TSH release [31], E2-induction of TRH receptors in the pituitary [32] or decreased TRH degradation [33].

As mentioned in the Introduction, the present work was prompted by a clinical observation on two young adult women with regular menses [4]. In one woman on L-T4 replacement therapy, serum TSH

ranged between 2.0 and 2.6 mU/L within days 2 and 8 of the follicular phase, but 3.9–4.6 mU/L between days 12–16. Serum E2 was about 100 pg/ml on the 4th day, but 306 pg/ml on day 14. Prior to L-T4 replacement therapy, serum TSH was 13.2 mU/L in the follicular phase, but 19.7 mU/L at mid-cycle. This woman [4] resembles 3 of the 55 women in the present cohort. Indeed, one of these 3 women had TSH at observation early in the follicular phase = 2.2 mU/L and TSH post-buserelin TSH peak = 4.7 mU/L; the second woman had corresponding TSH levels at 2.4 and 4.6 mU/L, and the third at 2.3 and 4.2 mU/L. In the second patient reported previously [4], TSH levels were 2.6 and 2.8 mU/L on days 5 and 6, but 3.7–5.1 mU/L on days 13–16. This woman [4] is similar to another two of the 55 women, since TSH at observation early in the follicular phase and post-buserelin was 2.2 and 5.4 mU/L in one woman, and 3.2 and 5.8 mU/L in the other.

Based on our data, the following misleading scenarios can occur. In a reproductive-age woman, a serum TSH that was initially found above the normal reference range but entirely within the normal reference range at a subsequent check, might well be interpreted as reversible or transient subclinical hypothyroidism. However, the real situation is that this woman was and is fully euthyroid, because she had her initial serum TSH measured at or close to mid-cycle, while the subsequent serum TSH was measured far away from mid-cycle. At extreme, where TSH is higher than normal, the quick interpretation would be progression to subclinical hypothyroidism. However, this woman might well be euthyroid, because she had her initial TSH measured early in the follicular phase or just before menstruation, while her second TSH was measured at or close to mid-cycle. Furthermore, in a setting where thyroid function is evaluated by measuring TSH only (rather than by TSH plus free thyroid hormones), an initial serum TSH < 10 mU/L (e.g., 8 mU/L) and a repeat serum TSH > 10 mU/L (e.g., 13.5 mU/L) along the follow-up of a reproductive age woman with subclinical hypothyroidism would be interpreted as progression to overt hypothyroidism with subsequent unnecessary L-T4 replacement therapy.

When we had run the buserelin test in volunteers to obtain the 17-OHPg threshold for abnormality, one volunteer woman was on replacement therapy with L-T4. We retrieved her frozen serum and measured E2, TSH and FT3. These levels were 32 pg/ml, 0.71 mU/L and 3.3 pg/ml just before buserelin injection, and they peaked at 334 pg/ml (+944%), 1.3 mU/L (+91.5%) and 4.1 pg/ml (+24.2%) after injection. This observation goes along with the experimental observation that E2 inhibits the negative feedback exerted by thyroid hormones on both basal and TRH-stimulated TSH release [31]. Hence, the E2-stimulated secretion of TSH operates even when the thyrotrophes are under the negative feedback by thyroid hormones. Should the approximately 90% increase in serum TSH occur at midcycle in a woman on L-T4 therapy whose follicular phase TSH was 2.8 mU/L, the mid-cycle 5.3 mU/L would be interpreted as poor compliance or under-treated hypothyroidism possibly due to L-T4 malabsorption. Unnecessary diagnostic work-up would ensue. In approximately 10–15% of hypothyroid patients on L-T4 therapy, serum TSH is above target, and no known cause for this elevation can be found [34]. Indeed, at least in some of such female patients “the problem” could be TSH sensitivity to endogenous estrogens.

One strength of our study is that the magnitude of increase of gonadotropins and E2 during the buserelin test matched the corresponding increase that occurs naturally during the menstrual cycle. Such increase of gonadotropins and E2 is acutely GnRH-driven both in the buserelin test and naturally. Furthermore, the increase in serum TSH during the buserelin test parallels the increase of PRL, a well-know estrogen-dependent hormone. One limitation is the unavoidable use of dexamethasone for a few days in preparation of the buserelin test, this being a transient excess of corticosteroids that does not occur naturally before mid-cycle. However, this limitation goes against the results we wished to obtain to test our hypothesis, because glucocorticoids inhibit TSH secretion. One other limitation, which again is intrinsic in the buserelin test, is that we did not measure TSH for several days past the 24 h post-buserelin injection in order to see how long would it take for serum TSH to fall at levels comparable to the preinjection methods.

It appears reasonable to conclude that reference bands of serum TSH dependent on the phases of the menstrual cycle should be construed and rendered clinically available.

Conflict of interest statement

This research did not receive any specific grant: from funding agencies in the public, commercial or not-for-profit sectors.

Declaration of interest

None.

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