Decrease of Free Thyroxine Levels after Controlled Ovarian Hyperstimulation*

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ABSTRACT

Controlled ovarian hyperstimulation could lead to opposing effects on thyroid function. Therefore, in a prospective study of 65 women undergoing controlled ovarian hyperstimulation, thyroid hormones, T_4-binding globulin, TPO antibodies, gonadotropins, estradiol, and PRL were measured before and after controlled ovarian hyperstimulation.

After ovarian stimulation (mean ± SE of mean): free T_4 decreased, 14.4 ± 0.2 *vs.* 12.9 ± 0.2 pmol/L ($P < 0.0001$); thyroid-stimulating hormone increased, 2.3 ± 0.3 *vs.* 3.0 ± 0.4 mU/L ($P < 0.0001$); T_4-binding globulin increased, 25.2 ± 0.7 *vs.* 33.9 ± 0.9 mg/L ($P < 0.0001$); total T_4 increased, 98.1 ± 2.3 *vs.* 114.6 ± 2.5 nmol/L ($P < 0.0001$); total T_3 increased, 2.0 ± 0.04 *vs.* 2.3 ± 0.07 nmol/L ($P < 0.0001$); TPO antibodies decreased, 370 ± 233 U/mL *vs.* 355 ± 224 U/mL ($P < 0.0001$); LH decreased, 8.1 ± 1.1 *vs.* 0.4 ± 0.1 U/L ($P < 0.0001$); FSH did not change, 6.5 ± 0.6 *vs.* 7.9 ± 0.9 U/L ($P = 0.08$); human CG increased, <2 ± 0.0 *vs.* 195 ± 16 U/L ($P < 0.0001$); estradiol increased, 359.3 ± 25.9 pmol/L *vs.* 3491.8 ± 298.3 pmol/L ($P < 0.0001$); and PRL increased, 0.23 ± 0.02 *vs.* 0.95 ± 0.06 U/L ($P < 0.0001$).

Because low maternal free T_4 and elevated maternal thyroid-stimulating hormone levels during early gestation have been reported to be associated with impaired psychomotor development in the off-spring, our findings indicate the need for additional studies in the children of women who where exposed to high levels of estrogens around the time of conception. (*J Clin Endocrinol Metab* **85:** 545–548, 2000)

L OW MATERNAL free T_4 (fT_4) levels during early gestation are associated with impaired psychomotor development in infancy, persisting into childhood (1, 2).

During pregnancy, high estradiol (E_2) levels lead, through a rise in T_4-binding globulin (TBG), to a transient drop in fT_4 levels (3–5). Human CG (hCG) is known to exert a thyrotrophic action both *in vitro* and *in vivo*, best explained by the structural homology of thyroid-stimulating hormone (TSH) and hCG and their respective receptors (6). This explains why serum TSH does not rise during the first trimester but decreases reciprocally with the increase in hCG (5). Controlled ovarian hyperstimulation—for conventional *in vitro* fertilization (IVF) or intracytoplasmic sperm injection (ICSI)—could, therefore, lead to opposing effects on thyroid function: the thyrotrophic action of hCG could result in elevated fT_4 levels; alternatively, the induced rise in E_2 could lead to a lowering of fT_4.

The aim of the present study was to determine the acute effects of controlled ovarian hyperstimulation on thyroid function. We, therefore, designed a prospective study of women undergoing controlled ovarian hyperstimulation.

Materials and Methods

We studied 65 of 177 women who previously participated in a prospective study on thyroid autoimmunity and abortion (7). The study was

approved by the hospital ethics committee, and all subjects gave informed consent at initial presentation.

Our IVF program has been described in detail previously (8). Ovarian stimulation consisted of a 1-mg GnRH-analog sc or intranasally from cycle day 1 to days 10–12, 225 IU human menopausal gonadotrophin (hMG) im from day 3 to days 9–11, and 10,000 IU hCG im 35 h before follicle puncture. Follicle growth was assessed by ultrasound from day 10. Follicle puncture was done on day 14 in the majority of women. There was no difference in controlled ovarian hyperstimulation in those receiving ICSI and conventional IVF. Blood was drawn at random during the menstrual cycle from all women at their initial visit and after ovulation induction immediately before or after transvaginal follicle puncture. After the initial visit, TSH was determined immediately, whereas all other assays were done from frozen samples (−70 C).

To investigate assay cross-reactivity, one ampoule hMG (75,000 U/L) was diluted in saline to 75 U/L. In this solution TSH and hCG were measured.

Assays

TSH, total T_3, fT_4, FSH, LH, hCG (total and β), and PRL were determined with an immunoluminometric assay (Chiron Corp., East Walpole, MA). Reference ranges were: TSH, 0.2–4.5 mU/L; total T_3, 1.3–2.8 nmol/L; fT_4, 9–22 pmol/L; and PRL, 0.03–0.63 U/L. Total T_4 was determined with a in-house RIA with a reference range of 64–132 nmol/L. TBG was determined with a RIA (Brahms, Berlin, Germany). Estradiol was determined with a RIA (Diagnostic Product Corporation, Los Angeles, CA). TPO antibodies were determined with a RIA (Henning Berlin GmbH & Co., Berlin, Germany).

Statistics

Qualitative data between groups were tested using the χ^2 test. Data on hormones and antibodies were analyzed by the Wilcoxon matched-pairs signed-ranks test; correlations are given as Spearman's rank correlation coefficient (all P values are two-sided). P values of less than 0.05 were considered significant. Data are presented as mean ± SE of mean.

Results

General data

There were no differences in age, cause of infertility, thyroid function, family history of thyroid disease, smoking behavior (Table 1), number of previous pregnancies and deliveries or spontaneous abortions between study subjects and those who did not consent to repeated blood sampling (nonparticipants). None of the study subjects used thyroid medication.

Thyroid function and TPO antibodies

After ovarian stimulation: fT_4 decreased, (before *vs.* after) 14.4 ± 0.2 pmol/L *vs.* 12.9 ± 0.2 pmol/L ($P < 0.0001$) (Fig. 1); total T_4 increased, 98.1 ± 2.3 nmol/L vs. 114.6 ± 2.5 nmol/L ($P < 0.0001$); total T_3 increased, 2.0 ± 0.05 nmol/L *vs.* 2.3 ± 0.07 nmol/L ($P < 0.0001$); TBG increased, 25.2 ± 0.7 mg/L *vs.* 33.9 ± 0.9 mg/L ($P < 0.0001$); TSH increased, 2.3 ± 0.3 mU/L *vs.* 3.0 ± 0.4 mU/L ($P < 0.0001$) (Fig. 2); and TPO antibodies decreased, 370 ± 233 U/mL *vs.* 355 ± 224 U/mL (median, 57 U/mL and 44 U/mL, respectively) ($P < 0.0001$).

In those women receiving ICSI, fT_4 was slightly, but significantly, higher after ovarian hyperstimulation compared with women receiving conventional IVF: 13.7 ± 0.4 pmol/L *vs.* 12.6 ± 0.3 pmol/L ($P < 0.05$).

Gonadotropins, E_2, and PRL

After ovarian stimulation: LH decreased, 8.1 ± 1.1 U/L *vs.* 0.4 ± 0.1 U/L ($P < 0.0001$); FSH did not change, 6.5 ± 0.6 U/L *vs.* 7.9 ± 0.9 U/L ($P = 0.08$); E_2 increased, 359.3 ± 25.9 pmol/L *vs.* 3491.8 ± 298.3 pmol/L ($P < 0.0001$); hCG increased, less than 2 ± 0.0 U/L *vs.* 195 ± 16 U/L ($P < 0.0001$); and PRL increased: 0.23 ± 0.02 U/L *vs.* 0.95 ± 0.06 U/L ($P < 0.0001$).

Correlations

A significant correlation exists between the increase in E_2 and the increase in TBG (Fig. 3) and total T_4; $r = 0.50$, $P <$ 0.0001 and $r = 0.73$, $P < 0.0001$, respectively. The increase in TBG was significantly correlated with the decrease in fT_4; $r = -0.26$, $P < 0.05$ (Fig. 4).

Assay cross-reactivity

In the saline containing 75 U/L FSH and LH (hMG), TSH and hCG levels were, respectively, 0.02 mU/L and 12 U/L, indicating that the gonadotropins did not contribute significantly to the levels of TSH and hCG estimated after ovulation induction.

Discussion

In this prospective study, we found controlled ovarian hyperstimulation to lead to a lowering of fT_4 levels concom-
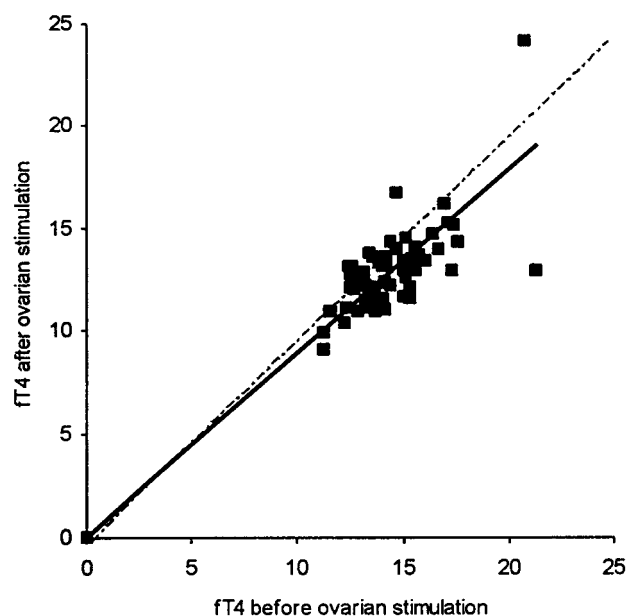

FIG. 1. fT_4 levels before and after ovarian stimulation.

TABLE 1. General data

Variable	Study subjects (n = 65)	Nonparticipants (n = 112)	P value
Mean ($\pm$SEM) TSH level (mU/L)[a]	2.3 ± 0.3	1.8 ± 0.1	NS
Median TSH level (mU/L)[a]	1.65	1.65	NS
No. of patients (%) with indicated TSH level			
<0.20	2 (3)	4 (4)	NS
≥ 0.2–≤ 4.5	59 (91)	102 (91)	NS
>4.5	4 (6)	4 (4)	NS
Mean ($\pm$SD) age (yr)	32.7 ± 4.0	32.1 ± 4.4	NS
No. of patients with indicated cause of infertility (%)[b]			
Male infertility or fallopian tube obstruction	44 (68)	72 (64)	NS
Endometriosis	4 (6)	7 (6)	NS
PCOS	4 (6)	7 (6)	NS
Idiopathic	13 (20)	24 (21)	NS
No. of patients with a family history of thyroid disease (%)[c]	7 (11)	13 (12)	NS
No. of patients who smoke (%)[d]	16 (25)	44 (39)	NS
No. of patients who underwent conventional IVF	49 (75)	77 (69)	NS
No. of patients who underwent ICSI	16 (25)	35 (31)	NS

PCOS, Polycystic ovary syndrome.
[a] TSH level not determined in two nonparticipants.
[b] Cause of infertility unknown in two nonparticipants.
[c] Family history of thyroid disease unknown in two nonparticipants.
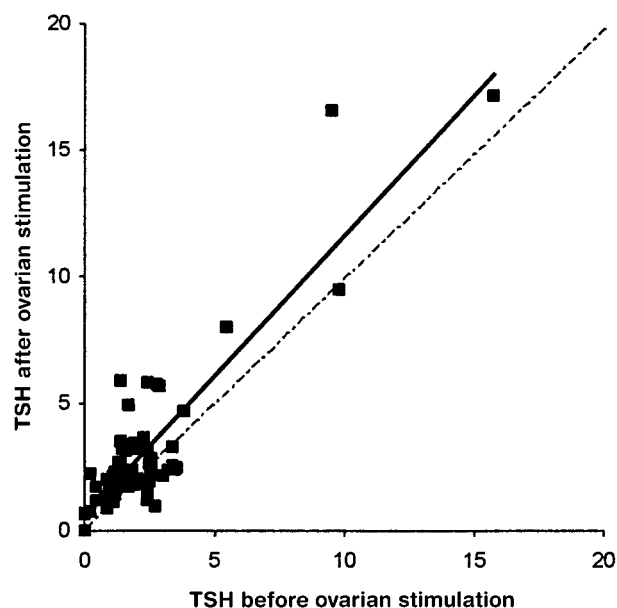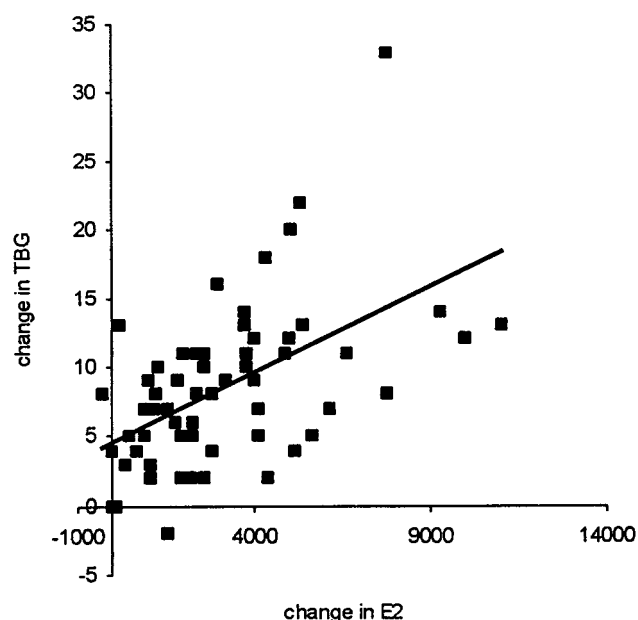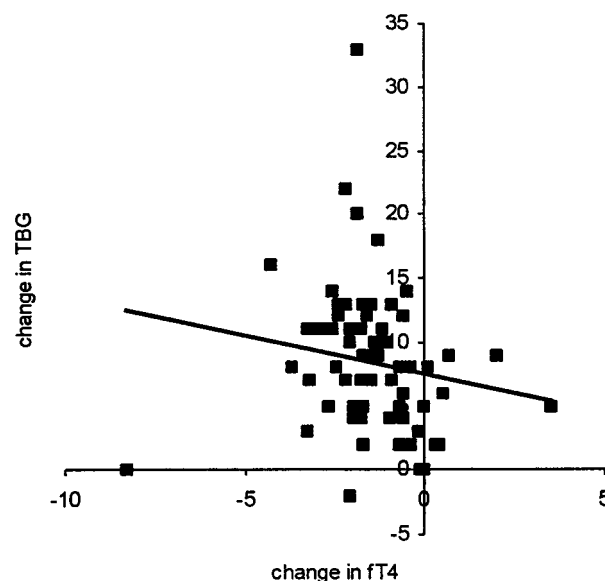[d] Smoking behavior unknown in three women, one of whom was a participant.

FIG. 2. TSH levels before and after ovarian stimulation.

FIG. 4. Change in fT$_4$ vs. change in TBG.

FIG. 3. Change in E$_2$ vs. change in TBG.

pregnancy have lower IQ scores and more school problems at 7–9 yr of age than children born from mothers who were euthyroid during pregnancy. These studies provide compelling evidence that even relatively mild disturbances in maternal thyroid function can lead to persistent and clinically relevant impairment in neuropsychological performance.

Animal, as well as human, studies have shown that due to the inability of the fetal thyroid to produce thyroid hormone during early pregnancy the maturing brain is totally dependent on maternal supply of thyroid hormone (9–12), thus, offering an explanation for the association between low maternal fT$_4$ levels and impaired psychomotor development in the offspring.

Ovarian hyperstimulation leads to hyperestrogenism, which in turn leads to increased TBG levels (for review see Ref. 3) and, therefore, to a decrease in fT$_4$ (3). During early pregnancy, some degree of thyroid stimulation by hCG exists; in some women this can give rise to gestational hyperthyroidism (3, 6). In molar pregnancy or chorionic carcinoma severe hyperthyroidism may even be the presenting symptom (6, 13, 14). Apparently, the hCG administration as applied during ovarian hyperstimulation is too short and/or too low-dosed to counterbalance the opposing effect of increased T$_4$ binding.

It might be argued that thyroid function at baseline was influenced by the phase of the menstrual cycle. However, it has been shown previously that thyroid function is unchanged during the follicular and luteal phases (5). Another possible source of bias could be assay interference. However, we found only negligible cross-reactivity. Moreover, during ovarian hyperstimulation, PRL (a polypeptide hormone) increased significantly and this change was near significantly correlated with the rise in TSH (a glycoprotein hormone); r = 0.22, $P = 0.09$, indicating a pituitary source of TSH and not assay cross-reactivity. Finally, we only measured TBG, whereas albumin and transthyretin can bind T$_4$ as well. However, during pregnancy the fraction of T$_4$ bound by TBG increases to more than 75%, as compared to two thirds in a

itant with a rise in TSH, indicating decreased T$_4$ availability at the tissue level at the time of follicle puncture. This increase in TSH is in contrast with the generally observed decrease in TSH during the first trimester of pregnancy (3–5). The possible significance of these findings lies in the recently described association between maternal thyroid status and subsequent neuropsychological development of the child (1, 2). Pop *et al.* (1) found fT$_4$ levels within the lowest 10th percentile at 12 weeks gestation in apparently healthy women to be associated with impaired psychomotor development at 10 months of age in their offspring. That these differences persist into childhood has very recently been shown by Haddow *et al.* (2), who provide evidence that children born from mothers with hypothyroidism during the second trimester of

nonpregnant state (3). In addition, concomitantly with the rise in TBG, total T_4 levels—measuring all bound T_4—increased as well.

There is strong evidence that sex steroids can modulate Th1/Th2 cytokine balance in such a way that during pregnancy cell-mediated immune function and Th1 cytokine production are suppressed and humoral immunity and Th2 cytokine production are enhanced (15, 16). The significant decline in TPO titers argues against a role of thyroid autoimmunity in the lowering of fT_4 levels during controlled ovarian hyperstimulation.

In several studies (17–22), no difference in the developmental outcome in children conceived by conventional IVF compared with non-IVF controls was found. However, in none of these studies maternal fT_4 levels in early gestation were taken into consideration. When comparing children conceived by ICSI (25% of our study subjects underwent ICSI) with children conceived by conventional IVF, there is an increased risk of developmental delay at 1 yr of age in children conceived by ICSI (23). In our study, we found fT_4 levels after ovarian hyperstimulation to be slightly higher in women who subsequently underwent ICSI compared with those who underwent conventional IVF.

In conclusion, we have found that ovarian hyperstimulation leads to a lowering of fT_4 levels with a concomitant rise in TSH. These findings have potential implications for women whose fT_4 levels are already in the lower range of normal before the start of ovarian hyperstimulation, especially if living in areas of iodine deficiency, with its relative hypothyroxinemia during pregnancy (3, 4). We hypothesize that if low maternal fT_4 levels in early gestation lead to impaired psychomotor development further lowering, albeit only transiently, just before the period in which the developing brain is critically dependent on the maternal supply of T_4 may amplify this effect. These results indicate the need for further research to determine whether the observed changes in fT_4 levels will, indeed, lead to delayed psychomotor development in the offspring of women undergoing controlled ovarian hyperstimulation.

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