RESEARCH

High concentration of estradiol has a negative correlation with free thyroxine during the second trimester of pregnancy

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Abstract

Objective: To explore the relationship between estradiol (E2) and thyroid function during the second trimester of pregnancy and the effect of E2 on sodium iodide transporter (NIS) expression in cultured thyroid cells.
Materials and methods: We analyzed relationships between E2 and thyroid function in 196 pregnant women during the second trimester. Multiple linear regression analysis was performed between E2 and thyroid function. The human thyroid Nthy-ori3-1 cells were cultured in different E2 concentrations, and the mRNA levels of NIS, estrogen receptor (ER)-α, and ER-β were measured by quantitative real-time PCR. Their protein levels were assessed by western blot.
Results: E2 was positively correlated with thyroid-stimulating hormone (TSH) and negatively correlated with free thyroxine (FT4) ($P < 0.05$). When we corrected for age, BMI, alanine aminotransferase, and serum creatinine, E2 was still negatively correlated with FT4 ($P < 0.5$) during the second trimester. In Nthy-ori3-1 cells treated with 10 nM E2, NIS and ER-β mRNA levels were significantly reduced, while ER-α mRNA level was not altered ($P > 0.5$). Moreover, 10 nM E2 significantly decreased protein levels of ER-β, phosphorylated versions of protein kinase A (p-PKA), phosphorylated versions of cAMP response element-binding protein (p-CREB), and NIS, while treatment with the ER-β inhibitor restored the expression of p-PKA, p-CREB, and NIS ($P < 0.05$).
Conclusion: High concentration of E2 has a negative correlation with FT4. High concentration of E2 can inhibit the NIS expression through the ER-β-mediated pathway, which may cause thyroid hormone fluctuations during pregnancy.

Key Words

- estradiol (E2)
- sodium iodide transporter (NIS)
- pregnancy
- thyroid function

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Introduction

Pregnancy is a unique period of female life when a series of hormone changes jointly regulate thyroid function. Maternal human chorionic gonadotropin (hCG) levels peak at 30,000–100,000 U/L in the eighth to tenth week of gestation and stabilize at 5000–20,000 U/L in the second trimester of pregnancy (1). Because the subunit of hCG is similar to the α subunit of thyroid-stimulating hormone (TSH) (2), it can directly stimulate the TSH receptor and increase thyroid hormone secretion (3, 4). Therefore, with the increase of hCG in early pregnancy, free thyroxine (FT4) also shows an increasing trend. Conversely, compared with normal pregnant women, FT4 decreased by 13% in the

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second trimester of pregnancy, while hCG level was at a stable high level. FT4 increases with a dramatic increase in hCG and slightly higher estradiol (E2) in the first trimester of pregnancy. FT4 declines concomitantly with even higher hCG and E2 during the second trimester. What causes FT4 to decrease in the second trimester? Furlanetto and colleagues found that E2 inhibited the stimulating effect of TSH on sodium iodide transporter (NIS) expression (5). Notably, E2 increases slightly in the first trimester and dramatically in the second trimester; indeed, E2 levels in the second trimester are 10–100 times higher than in nonpregnant women (6). Does higher E2 during the second trimester inhibit thyroid hormone synthesis?

Thyroid hormone biosynthesis requires the involvement of thyroperoxidase (TPO), thyroglobulin (TG), NIS, and TSH. NIS mediates the uptake and concentration of iodide ion, which is the first step of thyroid hormone synthesis (7), and this transporter is found in the human thyroid follicle substrate membrane (8). The NIS expression is influenced by a variety of factors, such as TSH playing a key role (9). Thyroid transcription factor-1 (TTF-1), thyroid transcription factor-2 (TTF-2), and paired box 8 (Pax-8) can also stimulate thyroid hormone synthesis by regulating the expression of NIS, TPO, TG, and TSH receptors in the thyroid (10, 11). Previous studies found that cytokines including TNFα and TNFβ, interferons (IFNα, IFNβ, and IFNγ), interleukins (IL1α, IL1β, and IL6), and TGFβ can also inhibit the mRNA expression and iodine uptake rate of NIS in rat thyroid follicular FRTL-5 and human primary thyroid gland cells, but the mechanism is unclear (12, 13). Furlanetto *et al*. (14) demonstrated that E2 can directly decrease iodide uptake by FRTL-5 cells. Estrogen plays its role mainly through the estrogen receptor (ER), which includes ER-α and ER-β; it plays a regulatory role by directly acting on the genome by binding to the ERE of the target gene (15). Building on these findings, we aimed to explore the relationship between E2 and thyroid function in the second trimester of pregnancy and examine how E2 impacts NIS in thyroid cells *in vitro*. Our results provide a theoretical basis for thyroid function changes caused by the significant increase in E2 levels during pregnancy.

Materials and methods

Study subjects

This was a cross-sectional study of 196 pregnant women in the second trimester (12 weeks < gestational week < 28 weeks) who visited the obstetrics outpatient department of our hospital between October 2017 and May 2018 (Fig. 1). We collected basic clinical data including age, systolic blood pressure (SBP), diastolic blood pressure (DBP), and BMI from patients who met the following inclusion criteria: (i) Chinese nationality, (ii) >18 years old, (iii) singleton pregnancy during the second trimester, and (iv) normal titers of anti-thyroglobulin antibodies (TgAb) (<115 IU/mL) and thyroperoxidase antibodies (TPOAb) (<34 IU/mL). Subjects were excluded if they met any of the

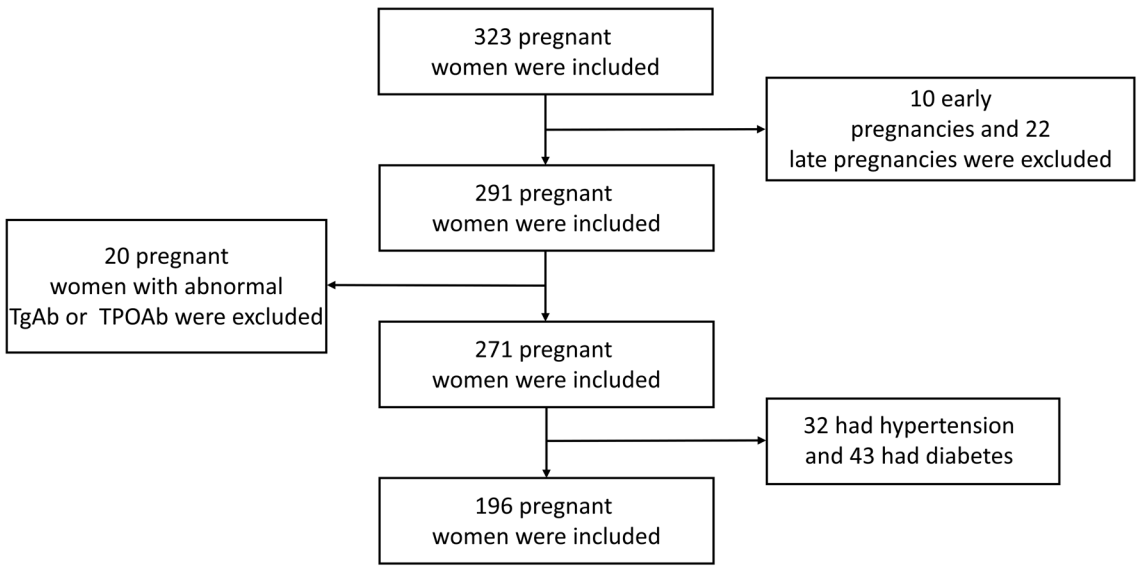

Figure 1
Population selection process.

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following criteria: (i) miscarriage risk, (ii) iron deficiency, (iii) history of thyroid disease or other chronic diseases (e.g. autoimmune diseases, hypertension, cancer, and diabetes), and (iv) use of oral drugs affecting thyroid function (e.g. traditional Chinese medicine, nutrient supplement, glucocorticoids, dopamine, antiepileptic drugs, and progesterone). Patients who did not comply with the test procedures were also excluded. All participants signed informed consent forms, and the study was approved by the Medical Ethics Committee of the Fifth People's Hospital of Shanghai, Fudan University (Ethical batch number: (2016) LSD (081)).

Clinical test index

All venous blood samples were collected in the morning (approximately 08:00 h) and tested by the hospital laboratory. Levels of alanine aminotransferase (ALT) and creatinine (Cr) were measured with an automatic chemical analyzer (Automatic Modular P800, Roche). TSH, FT4, TPOAb, and TgAb were detected by electrochemiluminescence immunoassays (cobas 8000, Roche). The intra-assay coefficient of variation (CVs) values of TSH, FT4, TPOAb, and TgAb were 1.74–2.43%, 1.72–2.50%, 4.09–7.11%, and 4.19–6.40%, respectively. The corresponding inter-assay CVs were 2.03–4.10%, 2.12–2.54%, 5.02–8.50%, and 5.33–7.21%, respectively.

To assess high E2 levels during pregnancy, serum samples were diluted at 1:10 according to the Diluent MultiAssay instructions, and the specific E2 concentrations were measured as suggested by the manufacturer (Roche Diagnostics). The intra- and inter-assay CVs were 1.35–2.97% and 2.71–4.12%, respectively.

Chemicals, drugs, antibodies, and reagents

E2 was purchased from Sigma. The ER-α inhibitor (Fulvestrant, ICI 182780) and ER-β inhibitor (R,R)-tetrahydrochrysene (THC) were obtained from APExBIO (Houston, TX, USA). Stock solutions of E2 were prepared in ethanol at 0.1, 1, 10, and 100 nM and stored at –20°C until use. The antibodies against phosphorylated versions of protein kinase A (p-PKA) and phosphorylated versions of cAMP response element-binding protein (p-CREB) were purchased from Cell Signaling Technology. The antibodies against NIS were purchased from Proteintech (Chicago, IL, USA). Reverse transcription, reverse transcriptase PCR, and amplification kits were purchased from TaKaRa.

Cell line and cell culture

Human thyroid Nthy-ori3-1 cells were purchased from Guangzhou Jennio Biotech Co, Ltd (Guangzhou, China) and maintained in RP-1640 medium (Sigma) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% nonessential amino acids, and 1% penicillin and streptomycin. Cells were maintained in a humidified incubator at 37°C in 5% CO_2, with the medium changed every 3–4 days.

RNA extraction and quantitative real-time PCR

Nthy-ori3-1 cells were treated with 0.1, 1, 10, or 100 nM E2 and 10 nM E2 + 100 nM (R,R)-THC for 48 h. Total RNA was extracted with TRIzol Reagent (TaKaRa) according to the manufacturer's instructions and quantified using UV spectrophotometry (Applied Biosystems). cDNAs were synthesized using 1 g total RNA in a 10 mL reaction mixture with Oligo-(dT) 18 primer and 2× Brilliant II SYBR Green QPCR Master Mix (TaKaRa) (5). qRT-PCR reactions were performed on a Cycle iQ system using 1 uM primers (Table 1). The mRNA levels were measured using the SYBR Premix Ex Taq™ system (TaKaRa). The reactions were performed for 30 s at 95°C, followed by 40 cycles of 95°C for 5 s and 60°C for 30 s. Product specificity was verified by melting curve analysis. NIS, ER-α, and ER-β mRNA levels were quantified and calculated using the relative quantitative method (2-ΔΔCt) and normalized to that of β-actin.

Table 1 Primers for RT-PCR analyses.

Genes	Forward	Reverse
NIS	CCTATCGCTATGGCCTCAAGT	CGTGGCTACAATGTACTGCAAA
TPO	CTGTCACGCTGGTTATGGC	GCTAGAGACACGAGACTCCTCA
TSHR	GGAATGGGGTGTTCGTCTCC	GCGTTGAATATCCTTGCAGGT
TG	AGACACCTCCTACCTCCCTCA	GCTAGAGACACGAGACTCCTCA
ER-α	GAGGAGGGAGAATGTTG	CTGAAGGGTCTGGTAGG
ER-β	AACACCTGGGCACCTTT	GAGCATCCCTCTTTGAA

ER, estrogen receptor; NIS, sodium iodide transporter; TG, thyroglobulin; TPO, thyroperoxidase; TSHR, thyroid stimulating hormone receptor.

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Western blot analysis

Nthy-ori3-1 cells were treated with 50 mU/L TSH, 10 nM E2, and 10 nM E2 + 100 nM (R,R)-THC. Western blotting was performed as described previously (5). At 48 h, the cellular proteins were extracted using radioimmunoprecipitation assay lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1% Nonidet P 40 (NP-40), 0.5% deoxycholic acid, and 0.1% SDS. The concentrations of total protein were quantified using the bicinchoninic acid assay (Byuntian, Wuhan, China). Next, 30 g of total protein from each sample were mixed with 5× SDS-PAGE sample buffer at a 4:1 ratio, and proteins were denatured at 99°C for 10 min. Proteins were separated by 10% SDS-PAGE and transferred onto PVDF membranes (Millipore). After incubation with 5% skim milk for 2 h, the membranes were incubated with primary antibodies directed toward ER-β, p-PKA, p-CREB, or NIS for 18 h at 4°C. The membranes were incubated with horseradish peroxidase-conjugated secondary antibody (1:10,000) for 1 h at room temperature with agitation. Proteins of interest were visualized using an ECL reagent (Millipore) and detected using the ImageQuant LAS4000 system (Alpha Innotech). Densitometry was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

One-hundred and ninety-six pregnant women were divided into three groups based on tertiles of E2 levels: lowest group (below 4442.51 nmol/L), middle group (4442.51–7919.08) and highest group (above 7919.08 nmol/L). All data were tested for normality. Normally and non-normally distributed data are expressed as mean ± S.D. ($\chi \pm s$) and median (interquartile interval) (M (25%, 75%)), respectively. Student's *t*-test was used to identify the difference in mean between groups. ANOVA was used to identify differences among three groups. Non-Gaussian parameters were directly compared with the Kruskal–Wallis H test. Spearman correlation and stepwise linear regression analysis were used to establish the relationship between E2 and other material parameters. The stepwise linear regression analysis was used to establish the relationship between E2 and thyroid function parameters. Statistical analysis was performed using the SPSS software (version 22.0). Differences were considered significant at $P < 0.05$.

Results

Clinical characteristics of the second trimester of pregnancy with different E2 levels

A total of 196 women in the second trimester of pregnancy were recruited for this study. On the basis of serum E2 concentrations, the sample characteristics and laboratory data of 196 pregnant women in the second trimester are listed in Table 2. No significant differences in BMI, SBP, DBP, ALT, Cr, TSH, TPOAb, and TgAb were found among the different E2 concentration groups. On the other hand, with the increase in estrogen concentrations, the age becomes significantly different ($P < 0.05$). Interestingly, significant differences were also observed in FT4 levels among different E2 concentration groups ($P < 0.05$). Subjects were divided into three groups according to tertiles of E2 levels: lowest

Table 2 Clinical characteristics of pregnant women with different E2 levels during the second trimester.

Parameters	Lowest group (E2 < 4442.51 nmol/L)	Middle group (4442.51 ≤ E2 ≤ 7919.08 nmol/L)	Highest group (E2 ≥ 7919.08 nmol/L)
n (%)	64.00 (32.65)	67.00 (34.19)	65.00 (33.16)
Age (years)	29.00 (26.00–31.00)	29.00 (24.75–34.25)	26.00 (22.5–26.00)
Metabolic factors			
BMI (kg/m^2)	21.87 (20.02–23.34)	21.02 (19.45–23.26)	21.97 (19.95–24.30)
SBP (mmHg)	115.60 ± 13.00	116.27 ± 14.12	113.79 ± 11.30
DBP (mmHg)	70.45 ± 8.83	69.25 ± 9.69	69.39 ± 9.20
ALT (U/L)	11.00 (7.90–15.50)	16.00 (8.50–25.25)	10.90 (9.05–19.00)
Cr (μmol/L)	46.72 ± 7.32	45.46 ± 5.57	44.12 ± 5.01
Thyroid hormones			
TSH (mIU/L)	3.79 (2.21–4.76)	3.40 (1.48–4.66)	3.49 (2.64–4.46)
FT4 (pmol/L)	15.20 ± 2.16	15.00 ± 2.27	14.29 ± 1.95
TPOAb (IU/mL)	13.20 (10.75–28.70)	12.70 (11.00–19.55)	16.00 (12.00–21.90)
TgAb (IU/mL)	21.00 (12.40–129.10)	13.85 (11.78–82.35)	14.10 (11.35–34.90)

[a]$P < 0.05$, lowest group vs middle group vs highest group.
ALT, alanine aminotransferase; Cr, creatinine; DBP, diastolic blood pressure; FT4, free thyroxine; SBP, systolic blood pressure; TSH, thyroid-stimulating hormone.

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group (below 4442.51 nmol/L), middle group (4442.51–7919.08 nmol/L), and highest group (above 7919.08 nmol/L). There was a stepwise decrease in the level of FT4 (15.20 ± 2.16 pmol/L vs 15.00 ± 2.27 vs 14.29 ± 1.95, $P < 0.05$) across the lowest, middle and highest estrogen groups.

E2 levels were significantly correlated with thyroid function in the second trimester of pregnancy

We analyzed the correlation between E2 and thyroid function (TSH and FT4) and found that E2 was positively correlated with TSH (r = 0.13, $P < 0.05$) and negatively correlated with FT4 (r = –0.27, $P < 0.05$) (Fig. 2). Linear regression analysis showed that E2 levels were correlated with FT4 when the covariate only included E2. Similar results were also obtained after stepwise addition of further potential risk factors such as age, BMI, ALT, and serum creatinine (SCR) to the model. It should be noted that after the parameters such as age, BMI, ALT, and SCR were added to the model, the relationship between E2 and FT4 could be also obtained. However, no consistent relation between the E2 and TSH levels was observed (β = 0.035, $P = 0.622$) (Table 3).

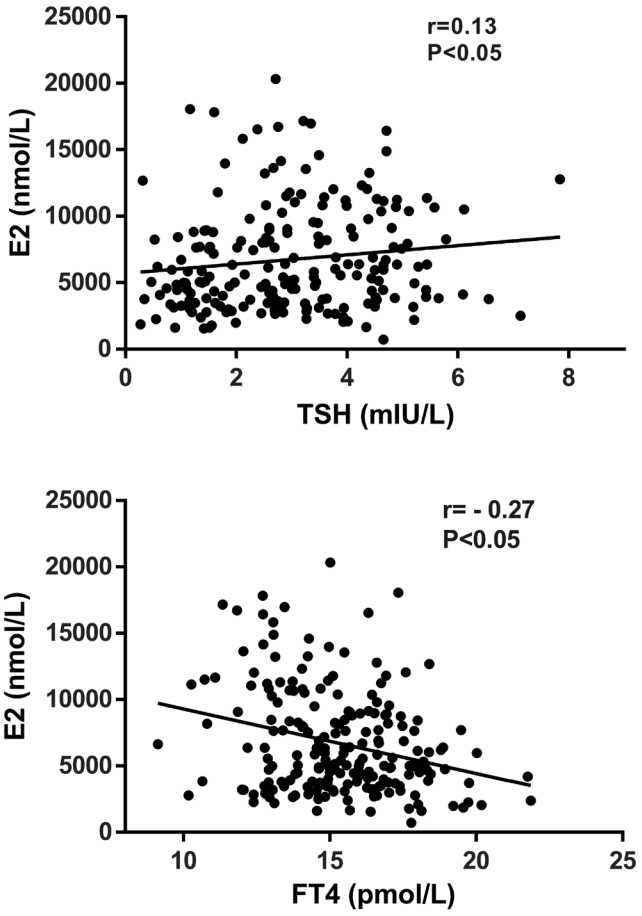

Figure 2
Relationship between E2 and thyroid hormone levels (TSH and FT4) in the second trimester of pregnancy.

Table 3 Correlation between TSH or FT4 with E2 levels in adjusted linear regression models.

	FT4			TSH		
	β	S.E.	*P*	β	S.E.	*P*
Model 1 (E2)	–0.177	0.000	0.012	0.035	0.000	0.622
Model 2 (E2, age)	–0.198	0.006	0.016	0.012	0.000	0.867
Model 3 (E2, age, BMI)	–0.193	0.000	0.007	0.016	0.000	0.829
Model 4 (E2, age, BMI, ALT, SCR)	–0.190	0.000	0.008	0.016	0.000	0.824

ALT, alanine aminotransferase; E2, estradiol; FT4, free thyroxine; SCR, serum creatinine; TSH, thyroid-stimulating hormone.

Effects of high concentrations of E2 on the mRNA level of thyroxine synthesis-related gene

Human Nthy-ori3-1 thyroid cells were cocultured with different concentrations of E2 (0.1, 1, 10, or 100 nM) for 48 h. Compared with the control, the TSHR, TPO, and TG mRNA expression did not alter after stimulation of different E2 concentrations ($P > 0.05$). When stimulated by 0.1 nM E2, the mRNA expression of NIS increased, but the difference was not statistically significant ($P > 0.05$). After 1, 10, and 100 nM E2 stimulation, the mRNA expression of NIS decreased significantly ($P < 0.05$), and when E2 concentration was 10 nM, the inhibition effect on NIS was most dramatic (Fig. 3).

High E2 concentrations downregulate NIS expression through ER-β mediated pathway

Human Nthy-ori3-1 thyroid cells were cocultured with different concentrations of E2 (0.1, 1, 10, or 100 nM) for 48 h. ER-α mRNA expression was significantly stimulated by 0.1, 1, and 100 nM E2 ($P < 0.05$), while no alteration was observed by 10 nM E2 ($P > 0.05$). On the other hand, 0.1 nM E2 slightly increased the ER-β mRNA expression ($P > 0.05$), while 1, 10, and 100 nM E2 significantly decreased ER-β mRNA levels ($P < 0.05$). The inhibitory effect was the most obvious when the concentration of E2 was 10 nM, which is similar to the level of E2 in the second trimester of pregnancy ($P < 0.05$, Fig. 4).

To clarify how 10 nM E2 affected the NIS expression, we incubated Nthy-ori3-1 cells with 50 mU/L TSH, 10 nM E2, or 10 nM E2 combined with 100 nM (R,R)-THC for

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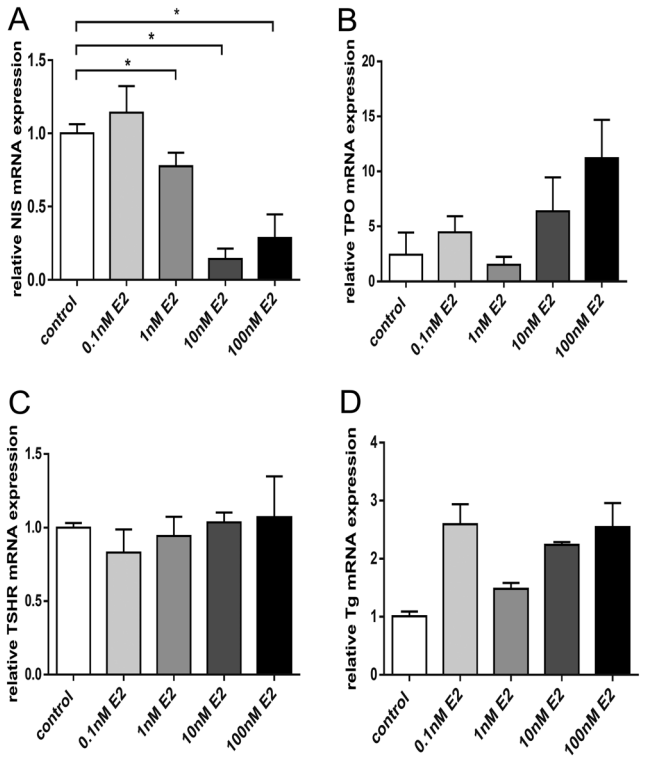

Figure 3
Different concentrations of E2 regulate thyroid-specific gene expression.

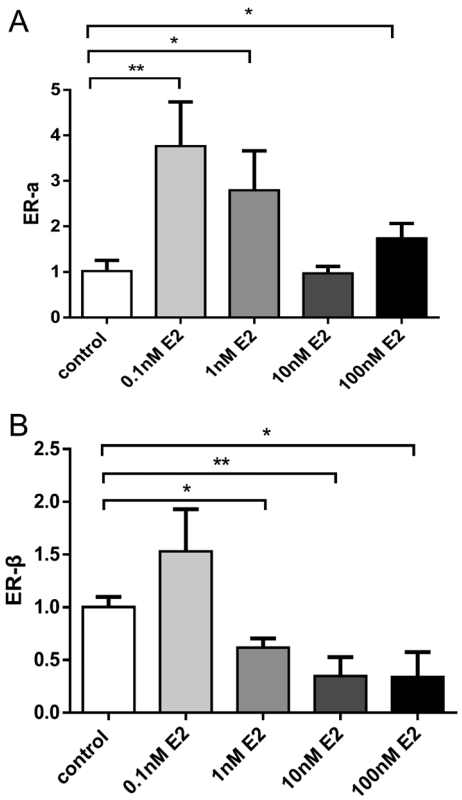

Figure 4
High E2 inhibited ER-β mRNA expression.

48 h. Although 50 mU/L TSH significantly increased the NIS mRNA expression ($P<0.05$), no significant effect was observed on ER-β mRNA expression ($P>0.05$). Treatment with 10 nM E2 significantly inhibited the ER-β and NIS mRNA expression ($P<0.05$). The addition of the (R,R)-THC rescued the mRNA expression of ER-β and NIS ($P<0.05$, Fig. 5A and B). In contrast, protein expression levels of ER-β, p-PKA, p-CREB, and NIS were significantly increased after 50 mU/L TSH treatment ($P<0.05$). Treatment with 10 nM E2 decreased ER-β, p-PKA, p-CREB, and NIS protein levels, which were rescued with the addition of (R,R)-THC ($P<0.05$, Fig. 5C).

Discussion

To clarify why FT4 declines with higher hCG levels in the second trimester of pregnancy, we investigated the relationship between E2 and thyroid function and found that E2 levels in dilution tests were positively correlated with TSH and negatively correlated with FT4 in samples taken from pregnant women. We found that different E2 concentrations directly inhibited the mRNA expression of NIS without obvious effects on mRNA levels of TG, TPO, or TSHR. However, a previous *in vivo* study reported that high concentrations of E2 (12.69 ± 5.84 nmol/L), equivalent to the concentration of E2 in the second trimester of pregnancy, can stimulate TPO activity in rats (16). The effects of E2 on other thyroid synthesis-related genes (e.g. TG and TSHR) have not been studied. In the initial analysis phase, we were concerned about the levels of biochemical indicators among different estrogen levels, and we can see that there were no significant differences in BMI, SBP, DBP, ALT, Cr, TPOAb, and TgAb among the different E2 concentration groups.

In the first trimester, maternal hCG directly stimulates the TSH receptor, increasing thyroid hormone production and resulting in a subsequent reduction in serum TSH concentration. The fraction of women with a suppressed TSH falls to about 10% in the second trimester and 5% in the third trimester. Serum TSH and its reference range gradually rise in the seconnd and third trimesters but remain lower than in nonpregnant women. FT4 does show the expected inverse relationship with serum TSH in pregnancy (1). In our study, although we found that E2 correlated negatively with FT4 ($r=-0.27$, $P<0.05$), at the same time it correlated positively with TSH ($r=0.13$, $P<0.05$), but the correlation between E2 and TSH is weak. It is worth noting that no significant difference in TSH was found among the different

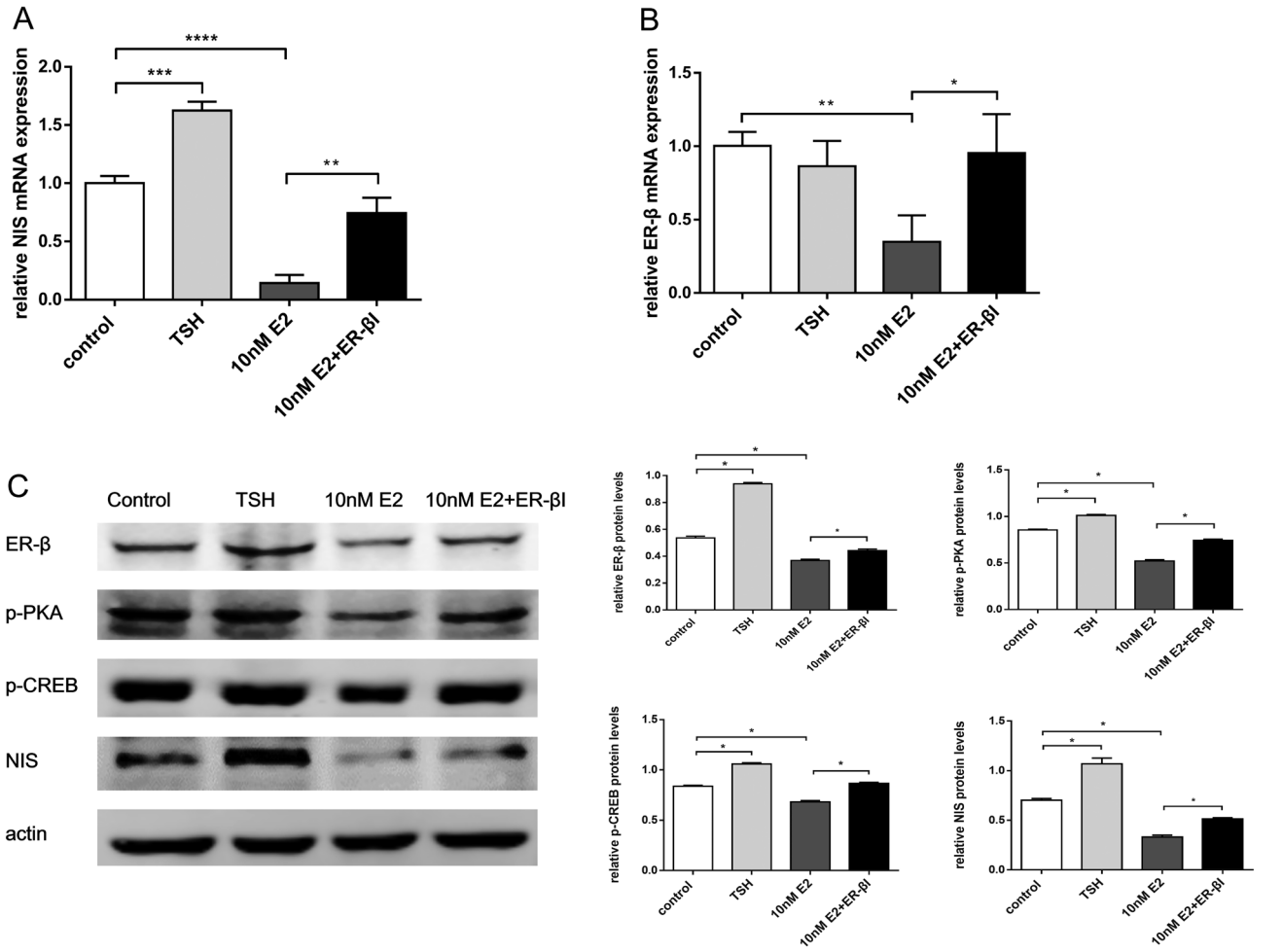

Figure 5
High concentration of E2 induces downregulation of NIS expression through ER-β/PKA/CREB pathway.

E2 concentration groups. Furthermore, we did not find a correlation between E2 and TSH after regression analysis. We deduced that E2 may first affect thyroid hormone metabolism and then influenced TSH levels through the thyroid–pituitary negative feedback axis. So we designed the thyroid cell culture experiment and then observed the changes in the thyroid hormone synthesis gene after adding different concentrations of E2 *in vitro*. Our major observation is that E2 regulates thyroid-specific gene NIS expression. We found that 48 h was the best time to observe E2 effects on Nthy-ori3-1 cells, which was consistent with previous studies (5, 17). After 48 h, E2 significantly decreased the NIS expression, with the strongest effect at 10 nM, a concentration similar to that in the second trimester of human pregnancy. Another study showed that E2 blocked the TSH-induced NIS expression after 48 h in FRTL-5 cells (5). Other groups reported that E2 has direct physiological effects on thyroid tissue/cells through ER-dependent mechanisms (18, 19), and ER is abundantly expressed in normal thyroid gland tissues (20, 21). To clarify which ER type exerts these effects, we used RT-PCR to detect mRNA levels of classical ER-α and ER-β. We found that 10 nM E2 significantly inhibited ER-β but not ER-α mRNA levels. Previous evidence suggested that ER-α or its variants may be located on the plasma membrane and linked to nongenomic signaling (22). Conversely, ER-β was observed entirely within the nucleus, where it acts by binding the estrogen-responsive element in the promoter regions of target genes to reduce proliferation (17). The complex of E2 and ER-β binds with the canca1 promoter to regulate the activity of CREB, an essential gene that mediates cAMP response and tyrosine hydroxylase gene expression (23). p-CREB can also bind to the enhancer region of the NIS gene to increase NIS transcription and translation (24).

Under the hypothesis that TSH binds TSHR to stimulate the NIS expression through the cAMP-PKA-CREB

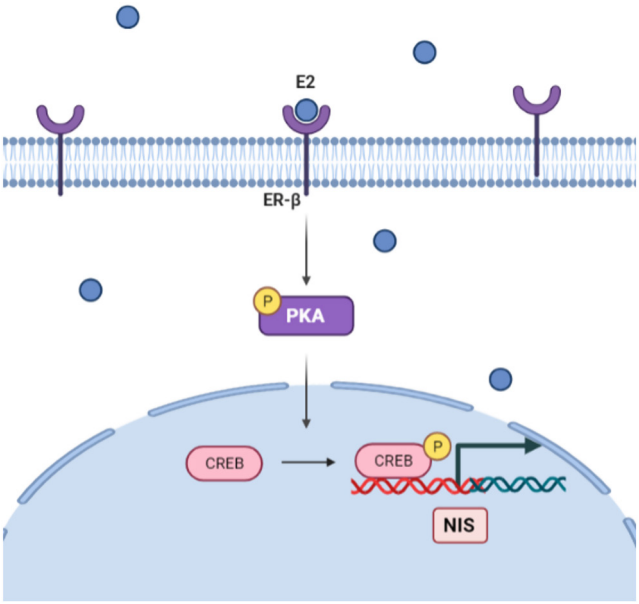

Figure 6
Hypothetical diagram: E2 inhibited the ER-β expression and further reduced the NIS expression through the PKA–CREB signaling pathway.

signaling pathway (25), we found that TSH increased NIS, p-PKA, and p-CREB protein levels. While protein levels of NIS along with downstream p-PKA and p-CREB were inhibited by 10 nM E2, these changes were reversed by adding (R,R)-THC. E2 can strongly repress PKA-mediated CREB phosphorylation and NIS expression through ER-β (Fig. 6). Previous studies found that (R,R)-THC mainly influences the space conformation of ER-β and thus affects the binding between E2 and ER-β (26, 27); so ER-β expression was increased after adding (R,R)-THC. We found that 10 nM E2 inhibited ER-β mRNA and protein expression and further reduced NIS levels through the PKA–CREB signaling pathway. More studies are needed to understand the functional interrelation of E2 and ER-β in thyroid follicular cells. Specifically, how E2 inhibited ER-β is still unclear, and this should be explored with *in vivo* and *in vitro* experiments.

Collectively, our findings demonstrate that 10 nM E2 inhibited p-PKA, p-CREB, and NIS levels by decreasing the expression of ER-β. One advantage of our study is that we used the dilution method to determine the specific E2 concentrations, so we were able to precisely assess the relationship between E2 concentrations and thyroid function. It also partly explains the continuous decline of FT4 in the second trimester and provides a novel explanation for the increase in thyroid hormone demand during pregnancy. E2 was found to suppress the NIS expression and may act as a potential endocrine disruptor. However, future studies should assess different E2 effects on thyroid hormone levels, evaluate the side effects, and look for the appropriate dosage of estrogen therapy in *in vivo* experiments. A shortcoming of this work is that we did not detect the iodine uptake rate of human Nthy-ori3-1 cells for functional analyses. We have not been able to eliminate the interference of iodine and only used ER-β inhibitors for negative regulation; thus, future studies should assess if ER-β agonists positively regulate these pathways.

Conclusion

High concentration of E2 has a negative correlation with FT4. High concentration of E2 can inhibit NIS expression through the ER-β-mediated pathway, which may cause thyroid hormone fluctuations during pregnancy.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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