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An evidence for the transcriptional regulation of iodothyronine deiodinase 2 by progesterone in ovarectomized rats

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Abstract Recent literature lacks studies on the effects of progesterone withdrawal on peripheral conversion of thyroxin (T4) into triiodothyronine (T3) by iodothyronine deiodinase 2 (D2) in different body tissues. The present study aimed to assess the possible relation of progesterone to T4, T3, and D2 in ovarectomized rats. Thirty female Wistar rats were included into a sham-operated control group and an ovarectomized group. Four months following the surgical procedures, measurements of estradiol, progesterone, free T4, free T3, and thyroid-stimulating hormone (TSH) were done. Also, estradiol/progesterone and T4/T3 ratios were calculated. Tissue homogenates from the kidney, liver, brain, thyroid, mandible, and femur were used to assess expression of D2 mRNA. The estradiol/progesterone ratio showed a significant increase in ovarectomized rats. T4 showed a significant increase in contrast to T3 which showed a highly significant decrease following ovariectomy. The T4/T3 ratio was significantly increased in ovarectomized rats. In addition, D2 expression was significantly attenuated in all tissue homogenates of the ovarectomized group. The present work showed a significant positive correlation between T4 and T3 in the sham-operated control rats, which was abolished in ovarectomized rats. A negative significant correlation between progesterone and T4 was revealed in ovarectomized rats. There was also a significant positive correlation between progesterone and D2 expression in the ovarectomized group. The results of the present study hypothesize that progesterone withdrawal may underlie the decrement in D2 expression, with consequent reduction in the peripheral conversion of T4 into T3 leading to a hypothyroid state.

Keywords Deiodinase 2 · Ovariectomy · Progesterone · Thyroid hormones

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Introduction

Postmenopausal women are vulnerable to social and health problems. Pearcn stated that the prevalence of thyroid dysfunction increases among women over the age of 50 [11]. In addition, Schindler classified the incidence of thyroid disease in a population of postmenopausal women as clinical thyroid disease, about 2.4 %, and subclinical thyroid disease, about 23.2 %. Among the group with subclinical thyroid disease, 73.8 % were hypothyroid and 26.2 % were hyperthyroid [14].

Formerly, Hernández Valencia et al. postulated a higher frequency of hypothyroidism in aged women and questioned why they do have greater susceptibility [6].

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Understanding the mechanisms underlying the impairment of thyroid function in postmenopausal women is crucial for its management. It is of importance that even mild thyroid failure can have a number of clinical effects such as depression, memory loss, and cognitive impairment [14] besides increased cardiovascular risk [7].

Prereceptor mechanisms involved in thyroid hormone signaling are mediated by a family of selenoproteins named iodothyronine deiodinases [13]. Eighty to ninety percent of the circulating triiodothyronine (T3) are derived from the prohormone thyroxin (T4). The conversion of T4 to T3 is catalyzed by type 1 (D1) and type 2 (D2) deiodinases via outer-ring deiodination. In contrast, type 3 deiodinase (D3) catalyzes the inactivation of both T4 and T3 [16].

Studies with D1 knockout mice indicated that D1, although capable of T4 to T3 deiodination, plays a scavenger role, preferentially deiodinating sulfated forms of iodothyronines in the process of being eliminated in the bile and urine .[15].

D2-mediated T3 production happens intracellularly. Subsequently, T3 exits the cells and enters the plasma compartment, being responsible for most of extrathyroidal T3 production in healthy humans. [2].

The mechanisms underlying the pathophysiology of altered thyroid function in postmenopausal women still need further investigations specially concerning the peripheral conversion of T4 into active T3 via deiodinase 2.

Aim

The present study aimed to assess the possible effect of progesterone withdrawal on thyroid function and D2 expression in different tissues in ovarectomized rats.

Methods

Experimental design

Thirty adult female Wistar rats (21–22 weeks, weighing 200–250 g) were included in the present study. They were obtained from King Fahad Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were fed normal rat chow and allowed free access to water. They were kept at 20–22 °C temperature, under a 12-h light/dark cycle.

All experimental procedures were carried out in accordance with the guidelines of animal care and approved by the Research Ethics Committee, Faculty of Medicine, King Abdulaziz University.

Rats were randomly divided into two groups (N=14 each): group I, control animals, which underwent a sham ovariectomy and group II, ovarectomized animals. Rats were anesthetized using ketamine (100 mg/kg, i.m.). Following surgical procedures, rats were housed individually for 7 days to allow for recovery and then regrouped in their home cages. Animals of both groups received no medications throughout the experiment (16 weeks).

At the end of the experiment after an overnight fast, blood samples were collected through a retro-orbital route, left to coagulate at room temperature, and centrifuged at 3,000 rpm for 30 min. The supernatant sera were separated and kept at −20 °C until used for assessment of estradiol, progesterone, free T3, free T4, and thyroid-stimulating hormone (TSH). Then animals were sacrificed under diethyl ether anesthesia; tissue samples from the kidney, liver, brain, thyroid, mandible, and femur were dissected and kept at −80 °C until the measurement of D2 mRNA.

Biochemical measurements

Electrochemiluminescence assay technique was used for measurement of serum estradiol and progesterone (Boehringer Mannheim Corporation, Germany) and free T4 and free T3 (Immuno Diagnostics Inc., USA), respectively. TSH was measured using a TSH ELISA kit (Shibayagi Co., Ltd., Japan).

Detection of D2 gene by real-time RT-PCR

The D2 gene was detected as follows:

1. Ribonucleic acid (RNA) extraction and complementary deoxyribonucleic acid (cDNA) synthesis

   Total RNA was isolated from different tissue homogenates using TRIzol Reagent™ (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA sample was dissolved in RNase-free water and quantified spectrophotometrically. The integrity of the RNA was studied by gel electrophoresis on a 1 % agarose gel, containing ethidium bromide. First-strand cDNA synthesis was performed with the SuperScript Choice System (Life

Table 1 Mean±SD of serum levels of estradiol, progesterone, estrogen/progesterone ratio, and D2 mRNA in control and ovarectomized rats (*t* test)

Parameters	Estrogen (pmol/L)	Progesterone (nmol/L)	Estrogen/progesterone ratio	Deiodinase 2 mRNA
Sham-operated control rats (*N*=14)	135.98±47.55	32.95±13.22	4.54±2.13	0.85±0.19
Ovarectomized rats (*N*=14)	45.66±12.27	6.91±3	7.99±4.3	0.14±0.05
P	<0.001	<0.001	<0.05	<0.001

Technologies, Breda, the Netherlands) by mixing 2 μg of total RNA with 0.5 μg of Oligo(dT)$_{12–18}$ Primer in a total volume of 12 μL. After the mixture was heated at 70 °C for 10 min, a solution containing 50 mmol/L Tris–HCl (pH 8.3), 75 mmol/L KCl, 3 mmol/L $MgCl_2$, 10 mmol/L DTT, 0.5 mmol/L dNTPs, 0.5 μL RNase inhibitor, and 200 U Superscript Reverse Transcriptase was added, resulting in a total volume of 20.5 μL. This mixture was incubated at 42 °C for 1 h and then stored at −80 °C until further use.

2. Real-time quantitative PCR

For real-time quantitative PCR, 1 μL of first-strand cDNA diluted 1:10 in RNase-free water was used in a total volume of 25 μL, containing 12.5 μL 2× SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer.

Primers were designed with the Primer Express software package (Applied Biosystems) for type 2 iodothyronine deiodinase gene (forward 5′-GGCT GACTTCCTGTTG-3′, reverse 5′-CACATCGGTC CTCTTGGTTCC-3′) and β-actin (forward 5′-TGTTGTCCCTGTATGCCTCT-3′, reverse 5′-TAATGTCACGCACGATTTCC-3′). PCR reactions, consisting of 95 °C for 10 min (1 cycle), 94 °C for 15 s, and 60 °C for 1 min (40 cycles), were performed on an ABI Prism 7900 HT Fast Real Time PCR system (Applied Biosystems). Data were analyzed with the ABI Prism 7900 sequence detection system software (version 2.2) and quantified with the comparative threshold cycle method with beta-actin as a housekeeping gene reference [12].

Statistical analysis

Statistical analysis of the data was done using SPSS for Windows package version 20 (SPSS Inc., Chicago, IL, USA). Independent *t* test was used to compare between means of the variables in the control and ovarectomized groups. Pearson's correlation coefficient was used to assess the association between variables. A *P* value <0.05 was considered significant.

Results

Estradiol and progesterone levels were significantly decreased in ovarectomized rats compared to the sham-operated control, while the estradiol/progesterone ratio showed a significant increase in the ovarectomized group compared to the control group ($P<0.05$; Table 1).

The serum level of free T4 showed a significant increase in the ovarectomized group, in contrast to free T3 which showed a highly significant decrease when compared to the control group. Meanwhile, the obtained level of TSH in ovarectomized rats showed a nonsignificant decrease. The significant increase in the serum level of T4 with the highly significant decrease in the level of T3 resulted in a highly significant increase ($P<0.001$) in the T4/T3 ratio in the ovarectomized rats (Table 2).

Table 2 Mean±SD of free T4, free T3, T4/T3 ratio, and TSH in control and ovarectomized rats (*t* test)

Parameters	T4 (pmol/L)	T3 (pmol/L)	T4/T3 ratio	TSH (μIU/mL)
Sham-operated control rats (*N*=14)	24.89±4.36	4.79±0.93	5.29±0.93	204±111.4
Ovarectomized rats (*N*=14)	28.66±4.82	3.25±0.48	8.88±1.3	184.19±128.6
P	<0.05	<0.001	<0.001	NS

NS not significant

Table 3 Pearson's correlation results of T4 versus T3 and progesterone versus T4 and D2 in the sham-operated control and ovarectomized groups

Variables		Sham-operated control group	Ovarectomized group
T4 vs T3	*r*	0.577	0.385
	P	<0.05	NS
Progesterone vs T4	*r*	−0.628	−0.635
	P	<0.05	<0.05
Progesterone vs D2	*r*	−0.363	0.753
	P	NS	<0.01

NS not significant

In the ovarectomized rats, the D2 expression revealed a significant attenuation in all tissue homogenates when compared to the control group ($P<0.001$; Table 1). Means of the individual values obtained from each animal were calculated and used for comparison between both groups. Comparing results of both groups regarding D2 expression in each individual organ revealed a significant reduction in the ovarectomized group.

The correlation studies of the present work showed a significant positive correlation between free T4 and free T3 in the sham-operated control rats ($r=0.577$, $P<0.05$). This significant correlation was abolished in ovarectomized rats. A negative significant correlation between progesterone and T4 was revealed in the ovarectomized rats, meaning that the decrease in the level of progesterone was associated with an increase in the level of T4. Furthermore, the correlation between progesterone and D2 mRNA in the ovarectomized group revealed a significant positive correlation (Table 3 and Figs. 1, 2, 3, 4, 5, and 6).

Discussion

In the present study, ovariectomy resulted in a significant increase in the estradiol/progesterone ratio together with a significant increase in free T4 and a significant decrease in free T3. Also, a significant attenuation in D2 expression and a positive correlation between progesterone and mRNA level of D2 were obtained. These findings point to the possible effect of progesterone

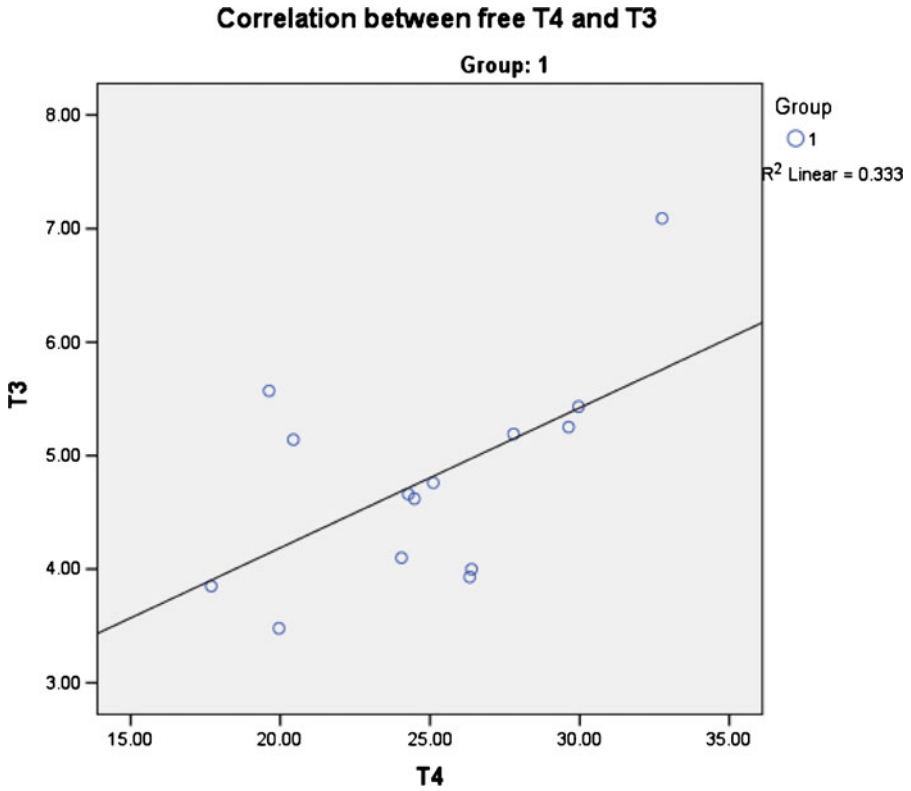

Fig. 1 Correlation between T4 and T3 in the sham-operated control group

deficiency on transcription of D2 and the conversion of T4 intoT3 at the cellular level.

The significant increase in estradiol/progesterone ratio in the ovarectomized rats compared to the sham-operated control rats in the present study proves that progesterone hormone gets much compromised by ovariectomy than estrogen hormone which still could be formed by the adrenal cortex and adipose tissue.

An interesting finding in the present study is the one concerning plasma levels of thyroid hormones. In the ovarectomized rats, there was a significant increase in free T4 and a significant decrease in the free T3 level with a highly significant increase of the T4/T3 ratio. These results indicate suppressed conversion of T4 intoT3 at the cellular level. This is confirmed by an important finding in the present work which is the significant decrease in D2 mRNA in all examined tissues in ovarectomized rats compared to sham-operated control rats.

Further support to our hypothesis is offered by the correlation results of the present work. It revealed a significant positive correlation between T4 and T3 in the sham-operated control rats which became insignificant 4 months following ovariectomy.

Previous works regarding thyroid status in postmenopausal women and ovarectomized animals showed conflicting results. Pantaleao et al. [10] showed insignificant changes in T4, T3, and TSH levels in rats, 21 days following bilateral ovariectomy. Their insignificant results could be explained by the very short duration of the experiment compared to ours.

Earlier, Nagata et al. [9] demonstrated in their study group that 33 among 210 postmenopausal women had an elevated serum TSH concentration with a normal serum free T4 concentration, suggesting a subclinical hypothyroid state.

Another study which comprised 350 women with different menopausal symptoms showed that 21 women had hypothyroidism and 18 had hyperthyroidism [1]. The different duration of menopausal symptoms in the previous studies could explain the inconsistency of results.

Regarding the rat's life, 4 months following ovariectomy is a long duration, which means that our findings could be observed in elderly women who had been menopausal for a long time.

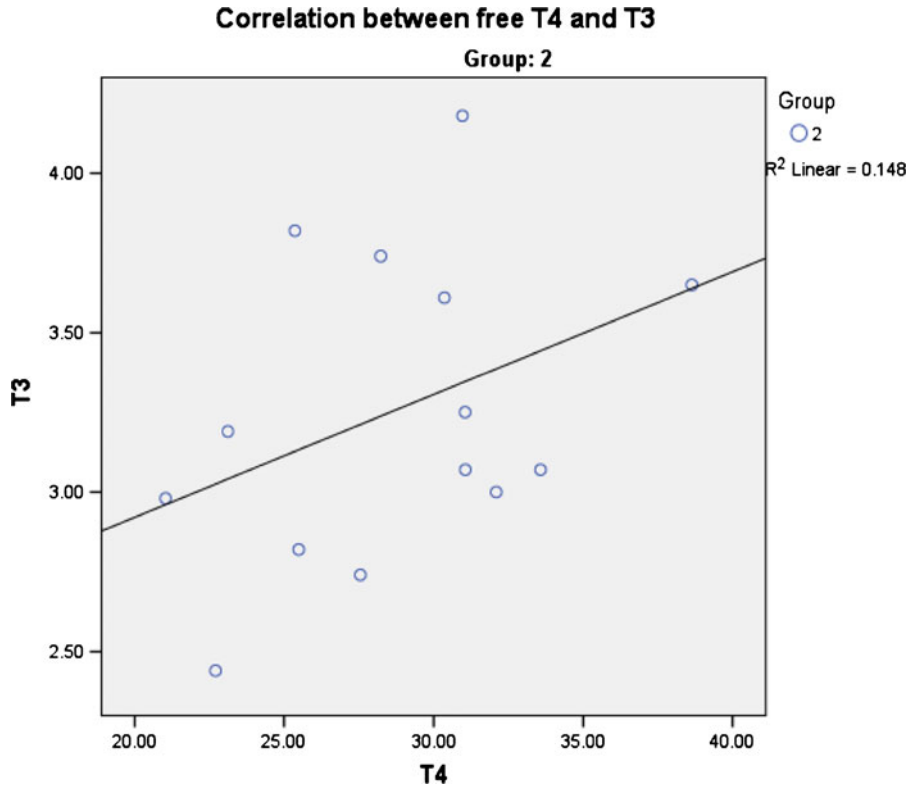

Fig. 2 Correlation between T4 and T3 in the ovarectomized group

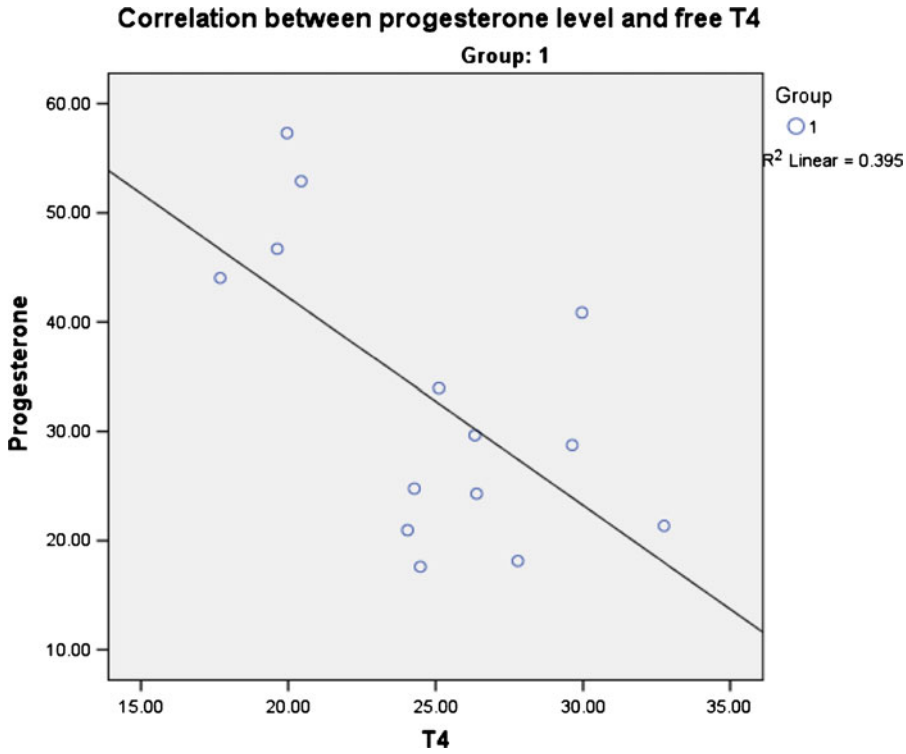

Fig. 3 Correlation between progesterone and T4 in the sham-operated control group

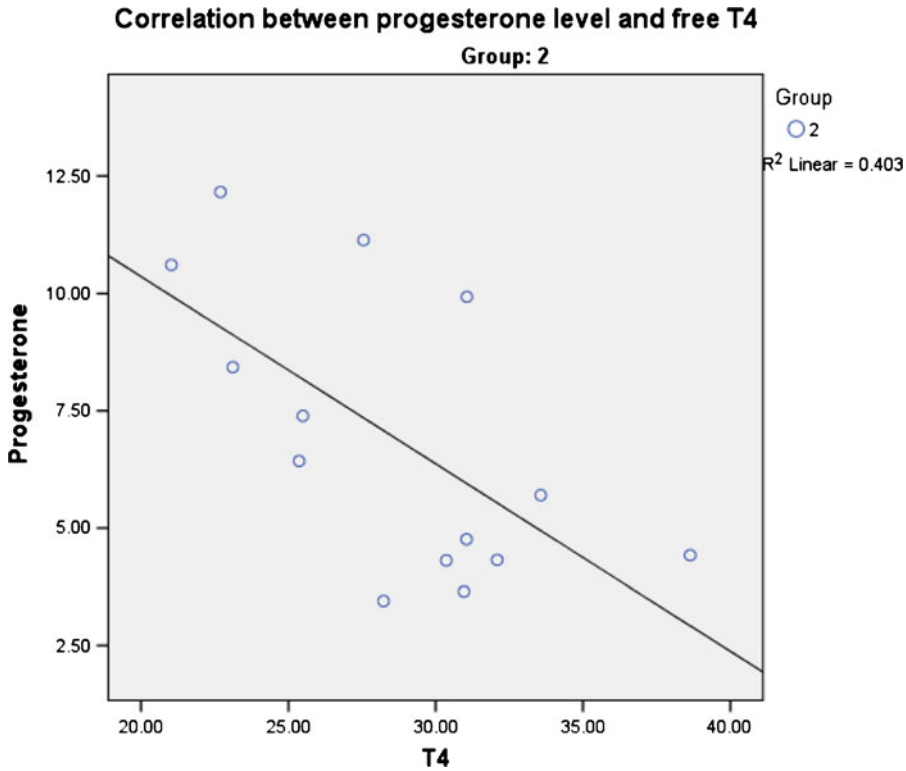

Fig. 4 Correlation between progesterone and T4 in the ovarectomized group

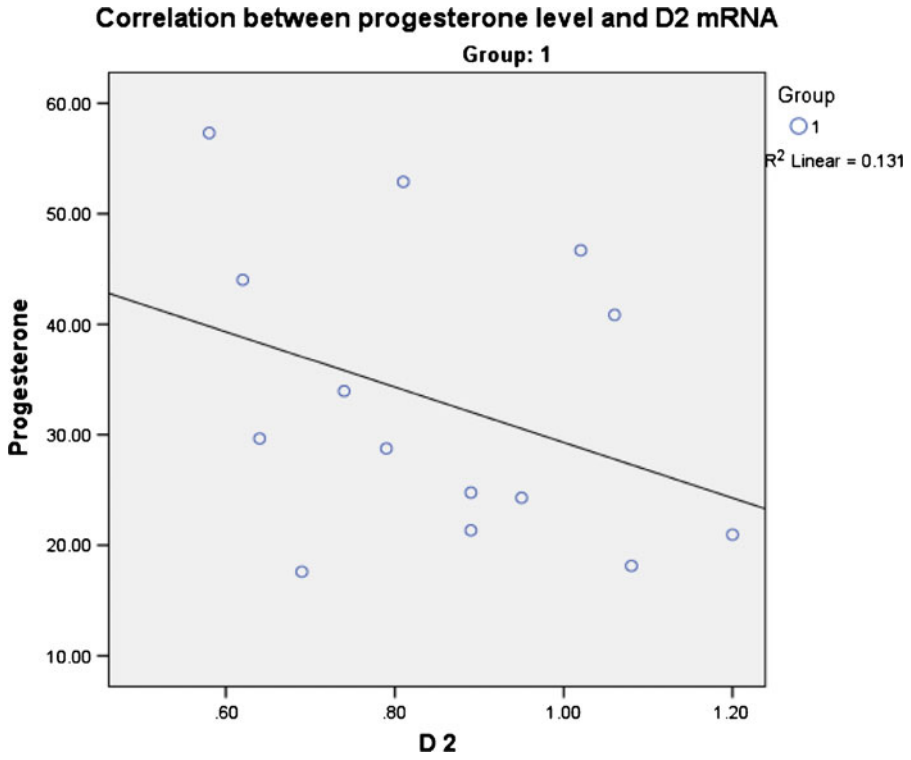

Fig. 5 Correlation between progesterone and D2 in the sham-operated control group

The present work postulates a new hypothesis. The marked reduction of progesterone in postmenopause might underlie the reduced activation of T4 into T3. In support to this hypothesis is the negative significant

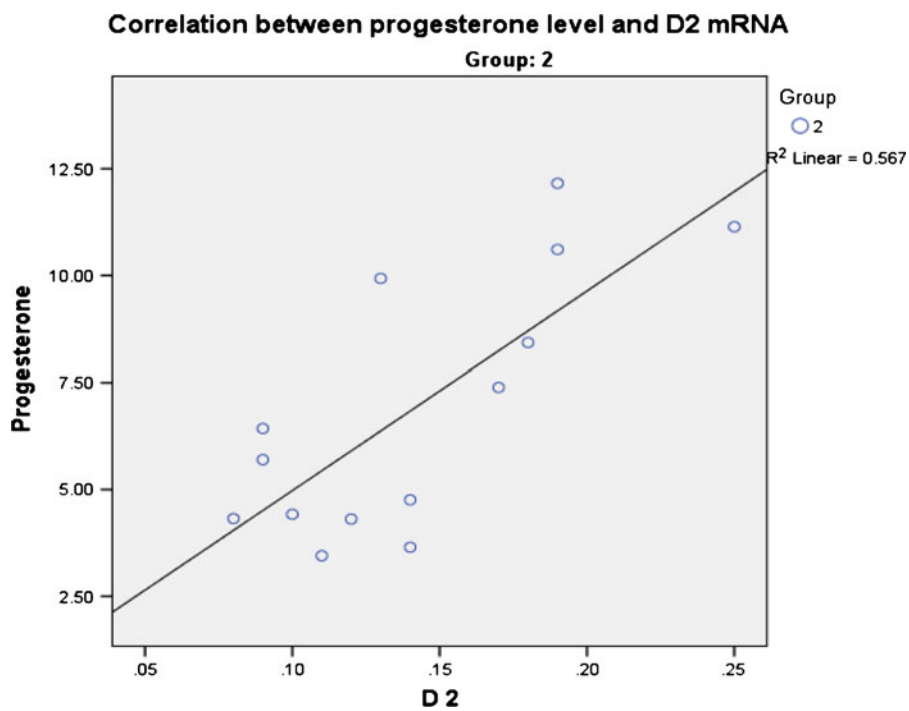

Fig. 6 Correlation between progesterone and D2 in the ovarectomized group

correlation between progesterone and free T4 and the significant positive correlation between progesterone and D2 mRNA in the ovarectomized rats.

Our data suggest that progesterone could induce or enhance D2 transcription which confers cells with the capacity to produce excess amounts of T3.

In support of our point of view, Emily et al. [4] observed that uterine D2 activity was increased ninefold by estrogen and fourfold by progesterone. On the other hand, treatment with estrogen resulted in a ninefold increase in uterine D3 activity, whereas no significant increase was seen with progesterone regarding D3 activity. Although this study demonstrated the effects of estrogen and progesterone in ovarectomized rats given either of the two hormones for only 4 days and only on uterine tissues, it is apparent that estrogen increased D2 and D3 so it ultimately would not enhance the T3 level, while progesterone alone did increase D2 without a comparable increase in D3 which could result in an unopposed enhancement of T4 to T3 conversion.

The increase in intracellular T3 concentration in the pituitary thyrotrophs shuts down TSH beta gene expression, decreasing its level, while the decrease in T3 results in an opposite effect on TSH beta gene expression [3].

In the present study, the significant decrease in free T3 was not accompanied by an increase in TSH level; instead, there was an insignificant decrease in TSH. The explanation could be based on the findings of Leonard et al. [8] who observed that D2 half-life could be prolonged by depletion of rat pituitary cellular ATP levels independently of changes in mRNA levels. This means that the decrease in the pituitary D2 might be compensated by the prolongation in its half-life.

Consequently, the T3 level in the pituitary will not be much decreased and T3 feedback will not significantly alter TSH release by the pituitary thyrotrophs. In addition, T4 could be the lead in inhibiting TSH at the pituitary level [5] which could also explain our finding regarding the TSH level.

It still needs further research to determine the changes in D2 mRNA and its activity at the cellular level, meaning not only in peripheral body tissues but also in the pituitary and hypothalamus, to get deeper insights into thyroid hormone signaling in the postmenopausal state.

Conclusion

Progesterone withdrawal during menopause could underlie the decrement in D2 expression, with consequent reduction in the peripheral conversion of T4 into T3 leading to a hypothyroid state.

Conflict of interest No conflict of interests.

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