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Original Research

Effects of menopause and diabetes on the rat thyroid gland: A histopathological and stereological examining

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ABSTRACT

Objective: Menopause is described as the arrest of the menstruation cycle and ending of reproductive potential. Diabetes mellitus (DM) occurs with Type 1 diabetes that is originated from the absence of insulin and Type 2 diabetes depending on insulin resistance. It is one of the most common endocrine disorders encountered in clinical practice that can cause serious health complications. Therefore, the objective of the present thesis was to investigate the effects of menopause and diabetes upon the thyroid using a rat model. **Materials and Methods:** 24, 12 weeks old female Sprague-Dawley rats were divided randomly into; non-diabetic healthy control group (Group I, $n = 6$), diabetic group (Group II, $n = 6$), ovariectomy group (Group III, $n = 6$), and ovariectomy plus diabetic group (Group IV, $n = 6$), respectively. **Results:** In histopathological examinations, the thyroid gland of the diabetic group had large follicles with cuboidal or almost squamous epithelial lining surrounding wide lumen. There were areas of disorganized follicles with decreased colloid. In the ovariectomized (OVX) rats, there was hyperplasia of the thyroid follicles and disorganized follicles with complete obstruction of their lumina. Mitotic cells were available. Some parafollicular cells had lack of cytoplasm. Post ovariectomy diabetes-induced group (Group IV), there were some species between follicles and remarkable reduction of colloid. Hyperplasia of the thyroid follicles, solid cell nests, and mitotic cells were also seen increasingly in this group. Follicular lumen area of ovariectomy group is closer to the control group. The increase of the lumen area in the DM group was the largest, diabetes+ovariectomy group also had an increase in the follicular lumen area. **Conclusion:** Finally; postmenopausal aging and diabetes in rats, may cause thyroid degeneration. DM and menopause both cause oxidative stress. But their damages on thyroid tissue are different. It means they cause oxidative stress via different ways. DM + OVX group compared to other groups has the greatest damage.

KEY WORDS: Aging, diabetes, menopause, rat, thyroid

INTRODUCTION

Menopause, represents the end of woman's reproductive life, is defined as the changeless stoppage of ovarian follicular activity and the menstrual cycle. This physiological process is characterized by alterations related with sex hormones and stepwise decrease of estrogen secretion. Thus, estradiol ranging from 5 to 25 pg/mL, while rising titers of gonadotrophins, so the levels of follicle stimulating hormone change between 40 and 250 mU/mL and luteinizing hormone changes from 30 to 150 mU/Ml [1,2]. Estrogen is an important regulatory hormone for oxidative stress [3]. Estrogen is known to increase producing thyroxine-binding globulin (TBG) that responsible for carrying the thyroid hormones in circulation. In premenopausal period, the changes in E2 levels affect TBG levels, this would also lead to changes in thyroxine and triiodothyronine levels. This changes in premenopausal term generally be short-term fluctuations in free thyroxine and free triiodothyronine levels cause may be. However, these changes do not reach the level of clinical importance. Among postmenopausal women, the age-related increasing of thyroid-stimulating hormone is remarkable. This average is higher than men in the same age profile [2,4,5]. In addition, the physiological effects of estrogen and progesterone on thyroid parafollicular cells enhance secretion of calcitonin [6,7].

Diabetes mellitus (DM) originates from increment of the blood glucose level as a result of decreasing insulin secretion or effect of insulin. Free radicals are formed in the wake of oxidative and nonenzymatic protein glycosylation resulting oxidative

stress in the organism. Diabetes and thyroid disorders affect each other mutually. On one side, thyroid hormones regulate carbohydrate metabolism and affect the function of the pancreas and the other side diabetes affects function of the thyroid gland [8-11]. The reduced liver glucose production is seen in hypothyroidism, and this is the description of reduced insulin requirements in diabetic patients with hypothyroidism [12]. Recurring hypoglycemic conditions can point the way of the development of hypothyroidism in patients with Type 1 diabetes. Hyperthyroidism has been proven to improve glucose intolerance and hyperglycemia. Uncontrolled blood glucose levels of diabetic patients with hyperthyroidism and thyrotoxicosis caused diabetic ketoacidosis have been shown in clinical studies [13,14].

Both menopause and diabetes are effective in the formation of oxidative stress in tissues. However, menopause and diabetes are independent of each other about causing oxidative stress [15,16]. Although there are many clinical studies on the effects of DM or menopause on the function of the thyroid gland, the information about the damage of these disorders to the thyroid tissue are not available in the literature. Therefore, in this study, the effects of the experimental model of diabetes and menopause on the thyroid tissue were aimed to investigate by histopathological and stereological techniques.

MATERIALS AND METHODS

Animals and Experimental Protocol

Animals were kept in facilities attributed by international guidelines, and the experiment was performed and directed in unity with the Institutional Animal Care and Use Committee of Ataturk University. The animals were provided and housed in Ataturk University Experimental Animal Laboratory (ATADEM). In this study, 24 Sprague-Dawley female rats, 24 weeks of age, divided into 4 groups:

Group I: Non-diabetic healthy group (control, $n = 6$) Group II: Diabetic group (DM; $n = 6$)
Group III: Ovariectomized group, (OVX, $n = 6$)
Group IV: Post ovariectomy diabetes-induced group, (DM +OVX, $n = 6$)

Experimental procedure is summarized in Table 1.

Experimental Models

Ovariectomy procedure

Bilateral ovariectomy was performed by making incision (2-3 cm) in a longitudinal manner on the lower abdomen midline area, taking out the ovaries and closing the incision[17-19]. As an analgesic for 25 mg/kg metamizole sodium was given rats for 2 days after ovariectomy. OVX rats were held alive for 12 weeks.

Alloxan-induced Diabetes Procedure

The rats were induced diabetes by intraperitoneal injection administration of a single dose of aqueous alloxan monohydrate

Table 1: Experimental procedure

Groups	1 days	90 days	91 days	123 days
12 weeks of age 24 Sprague-Dawley female rats				
Control (n=6)	-	Normal saline was injected	-	Rats were sacrificed
DM (n=6)	-	Diabetes induction	Blood glucose level measured	Rats were sacrificed
OVX (n=6)	Ovariectomy operation	Normal saline was injected	-	Rats were sacrificed
DM+OVX (n=6)	Ovariectomy operation	Diabetes induction	Blood glucose level measured	Rats were sacrificed

DM: Diabetes mellitus, OVX: Ovariectomized

(120 mg/kg for body weight, Sigma-Aldrich Co, Germany) [20]. Alloxan was prepared in solution of 0.9% NaCl and intraperitoneally injected to rats that were not fed for one night. 4-6 h after application of alloxan, depending on at high level insulin secretion from the pancreas, which can cause fatal hypoglycemia, intra-peritoneally 5 ml glucose solution (20%) was injected to eliminate this adverse effect and for 24 h a solution of 5% glucose was put into the drinking water and also intake of food was allowed. After 72 h than alloxan administration, to control fasting blood glucose levels blood samples obtained from the rats' tail vein by a blood glucose monitor (Accu-Chek Active). A diabetic rat was determined as having at least 200 mg/dl of serum glucose level, and diabetic rats were held alive for 8 weeks.

Research Methods

Hematoxylin and eosin (H and E) staining

For histological staining, all rats were sacrificed under high dose of ether anesthesia, and then each thyroid gland was removed and fixed in 10% neutral formalin solution for 72 h. In short, thyroid glands were dehydrated with graded concentrations of alcohol, cleared with xylene series and immersed in liquid paraffin series for embedding in paraffin. Thereafter, paraffin-embedded tissues were sectioned on a microtome (Leica RM2125RT) into 5-µm sections. The sections were stained with H and E according to the general protocol.

Quantitative Analyses

Stereological estimation

Stereo-investigator software version 9 (Microbrightfield, CA, USA) was used for stereological examination. The apparatus was collected of a personal computer, a charge-coupled device digital camera (Optronics MicroFire), a light microscope (Leica DM4000 B) and motorized computer-controlled specimen stage (Bio- Precision MAC 5000 controller system). The tissue sections were put onto a motor-driven stage connected to the microscope and were reflected, via the camera at 40 objective, onto the monitor. Each serous acinus was determined randomly by systematic sample method, moving the microscope stage left to right, in a stepwise way. Using the

"cavalieri method" belonging to the software Stereo Investigator (Microbrightfield) system, the mean serous acinus volume was examined according to described by Gundersen [21].

Statistical Analysis

The statistical analysis was completed using SPSS (IBM SPSS 20). Differences among the means were statistically analyzed by one-way ANOVA test followed by least Standard deviation (SD) test ($P < 0.05$ was determined as significant). The values were stated as means ± SD.

RESULTS

Histopathological Results

In histopathological examinations, when the morphological structures of thyroid were observed as intact in the control group, in experimental groups many abnormalities were observed distinctively.

In the control group, the thyroid tissue was found to be consisted of thyroid follicles which appeared normally rounded lined with a single cuboidal cell layer. The central follicles seemed smaller in size than peripheral ones. The follicles were lined with cuboidal cells with rounded nuclei. The connective tissue capsule was thin and the connective tissue septa were hardly identified dividing the gland into numerous small ill-defined lobules [Figure 1a].

The thyroid gland of the diabetic group had large follicles with cuboidal or almost squamous epithelial lining surrounding wide lumen. There were areas of disorganized follicles with decreased colloid. Connective tissue was minimal between the follicles. There were large species between some follicles. Some follicular cells demonstrated little cytoplasmic vacuolization. The peripheral follicles were larger [Figure 1b].

In the OVX rats, there were areas of disorganized follicles. Furthermore, the thyroid gland of this group was distinguished by dense areas of follicular cells. It is remarkable that hyperplasia of the thyroid follicles was available. Solid cell nests were located outside the basal lamina and in between the thyroid follicles. Mitotic cells can be seen clearly, and there were a lot of cells with hyperchromatic nucleus. Some parafollicular cells had lack of cytoplasm. All follicles were full of colloid [Figure 1c].

Post ovariectomy diabetes-induced group [Group IV], there were some species between follicles, remarkable reduction of colloid and disorganized follicles. Hyperplasia of the thyroid follicles, solid cell nests, and mitotic cells were also seen increasingly in this group. Some follicles contained peripheral vacuoles. The connective tissue capsule was thick. The follicles are variable in size. Parafollicular cell number compared with the control group was greater. Besides degenerative parafollicular cells were observed [Figure 1d].

The histopathological results are summarized in Table 2.

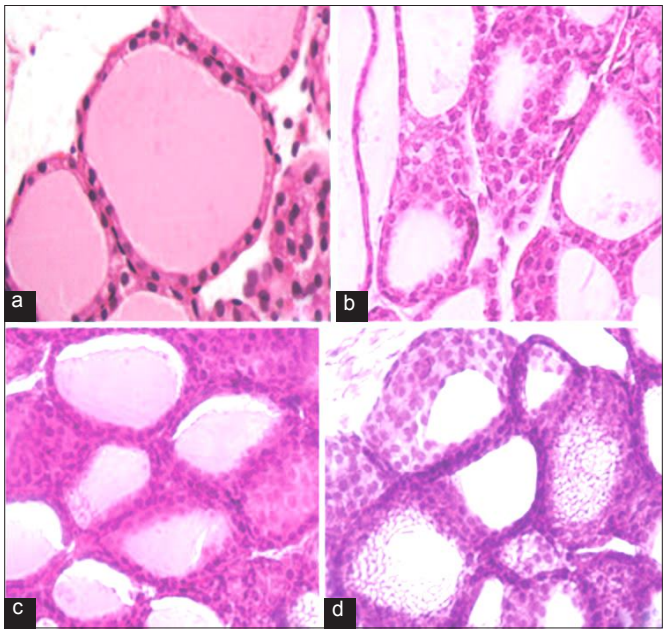


Figure 1: Photomicrographs of hematoxylin and eosin stained sections: [a] Thyroid gland of the control group. [b] The thyroid gland of the diabetic group with large follicles with reduced colloid, minimal connective tissue between the follicles and large spaces between some follicles. [c] Ovariectomized group's thyroid gland has disorganized follicles with complete obstruction of their lumina showing hyperplasia of the thyroid follicles. [d] Thyroid gland of postovariectomy diabetes-induced group has disorganized follicles, some species between follicles and reduction of colloid are available (H&E, x400)

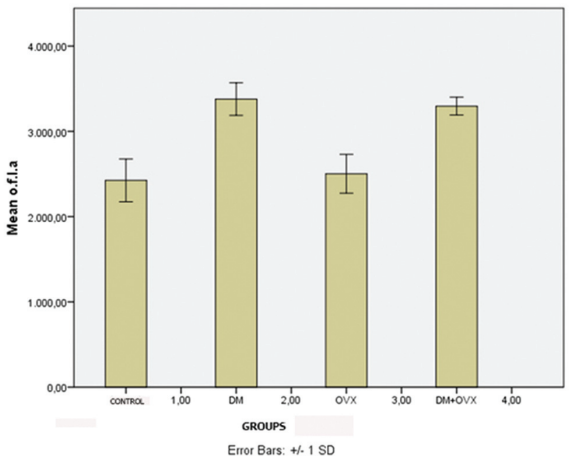
Table 2: Histopathological changes on thyroid glands of all groups

Groups	Amount of colloid	Follicles with hyperplasia	Disorganized follicles	Flattening in the follicular epithelium	Solid cell nest
Group I	++				+
Group II	+		+++	++	++
Group III	+++	+++	++		+++
Group IV	+	+++	+++	+	+++

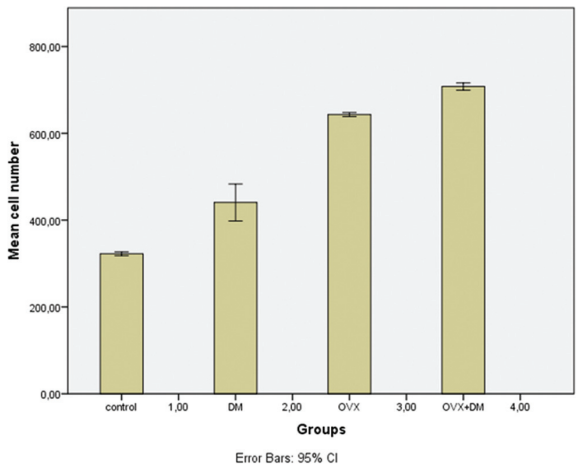
Stereologic Results

According to our tissue stereological findings, there were statistically significant differences between all groups. The thyroid gland follicular lumen area was measured by the method of Cavalieri in chart analysis performed with IBM SPSS 20 statistics significant differences between all groups was observed. Increasing in lumen area of other experimental groups compared with the control group follicles was significant. Follicular lumen area of ovariectomy group is closer to the control group, the increase of the lumen area in the DM group was the largest, DM +OVX group also had increase in the follicular lumen area [Graphic 1 and Table 3].

The average follicular cell number in the sampling grid area (~ 50000 μm²) was the greatest in the DM+OVX group because of hyperplasia. OVX group was closer to the DM +OVX group [Graphic 2].



Graphic 1: Thyroid follicular lumen area (f.l.a.) measured by the method of Cavalieri



Graphic 2: The average follicular cell number in the sampling grid area (~50000 μm²)

DISCUSSION

DM, one of the most important endocrine disorders, and menopause, the cessation of a woman's reproductive ability, are important cases. Both menopause and DM cause oxidative stress. Reactive oxygen species are chemically reactive molecules containing oxygen. Amounts of reactive oxygen species and antioxidants are in balance normally. Some studies showed that in rats postmenopausal aging and experimental diabetes cause oxidative stress in the brain, liver, and lung tissues [22-27].

In DM, insulin deficiency brings hyperglycemia so non-enzymatic glycosylation of proteins occurs. This case first begins in blood proteins, later in interstitial tissues and collagen in the vessel wall and other proteins. Non-enzymatic glycosylation of proteins are called Advanced Glycation End-Products (AGE). AGE-Products connects receptors which exist in a lot of cells like endothelial cells, lymphocytes, monocytes, etc. Thus, cytokines are released so inflammation, fibroblast proliferation, increased endothelial permeability, an increase in extracellular matrix occur [28]. In our study, oxidative stress is found to be significantly increased in diabetic rats compared to healthy subjects. In the DM group, we observed cellular and follicular changes, such as changes in connective tissue findings were consistent with findings in the literature. It can be helpful to view clinical researches to understand the effects of DM on the thyroid gland. Radaideh and colleagues in a study recommended that diabetic patients must be examined for asymptomatic thyroid dysfunction [29]. Alvarez-Marfany and friends indicate in postpartum women with Type 1 diabetes are at high risk for thyroid dysfunction [30]. Moreover, the potential life-threatening ketoacidosis because of hypoglycemia in cases of hypothyroidism and thyrotoxicosis are shown by Vondra *et al*. [31].

The dramatic decrease of estrogen is one of the important events in the development of menopause. Estrogen has an important role about cell growth, embryonic development, and the continuity of life [32]. This direct physiological effects of estrogen at the cellular level is known to perform with the latter three mechanisms including, genomics indirect genomic and non-genomic [33]. With this mechanism, various gene expressions took place and produced some proteins which are responsible for some both extracellular and intracellular functions.

Studies showed that in post-menopausal rats oxidative stress occurs [22]. Estrogen, particularly has been reported that by reducing the oxidized lipids by its antioxidant properties [34]. The reason of histopathological changes in thyroid tissue of the OVX rat is lack of estrogen probably. Some studies showed that the lack of estrogen that caused by ovariectomy decrease the release and synthesis of calcitonin in parafollicular cells [35-37].

Iodine taken by thyroids follicular cells from blood is oxidized by thyroid peroxidase and connects thyroglobulin molecule [38]. Thyroid follicular cells accumulate their own secretion called colloids in the lumen. Thyroid hormones are secreted from these cells by the influence of thyroid stimulating hormone [39]. Lack of iodine in the diet makes the thyroid gland hypoactive, so follicular cells turn into squamous epithelium and amount of collagen reduces. When the thyroid gland gets active, the epithelial cells get prismatic [40].

This study shows for the first time the impact of DM and ovariectomy on thyroid tissue in rats. Our results showed that DM +OVX group compared to other groups has the greatest damage. In this group, flattening of follicular cubic cells, gaps between follicles, decreasing number of solid cell nests and decrease the amount of colloid were observed.

Table 3: Follicular cell number in about 67530 μm² sampling area

Groups	Control	DM	OVX	DM+OVX
Mean±SD	322.66±1.66	440.66±16.60	643.33±16.60	707.83±3.21
P value	0.005	0.005	0.005	0.005

DM: Diabetes mellitus, OVX: Ovariectomized

Diabetes has been found to disrupt the structure of the thyroid. Following the procedure of ovariectomy, the structure of the thyroid was disrupted. We show that how DM and ovariectomy affect thyroid tissue. DM and menopause both cause oxidative stress. But their damages on thyroid tissue are different. It means they cause oxidative stress via different ways. When diabetes occurs at the time of lack of estrogen protection, the structure of the thyroid dramatically deteriorates compared to the other groups. In addition to studies of diabetes and menopause models applied to assess histopathological studies of oxidative stress parameters has to be studied.

REFERENCES

1. te Velde ER, Scheffer GJ, Dorland M, Broekmans FJ, Fauser BC. Developmental and endocrine aspects of normal ovarian aging. Mol Cell Endocrinol 1998;145:67-73.
2. Zapantis G, Santoro N. The menopausal transition: Characteristics and management. Best Pract Res Clin Endocrinol Metab 2003;17:33-52.
3. Mancini A, Raimondo S, Persano M, Di Segni C, Cammarano M, Gadotti G, *et al*. Estrogens as antioxidant modulators in human fertility. Int J Endocrinol 2013;2013:607939.
4. Letonturier P. Thyroid function in women: Problems created by oral contraceptives, pregnancy and menopause. Contracept Fertil Sex (Paris) 1983;11:79-84.
5. Schindler AE. Thyroid function and postmenopause. Gynecol Endocrinol 2003;17:79-85.
6. Filipovic B, Sosic-Jurjevic B, Nestorovic N, Manojlovic Stojanoski M, Kostic N, Milosevic V, *et al*. The thyroid C cells of ovariectomized rats treated with estradiol. Histochem Cell Biol 2003;120:409-14.
7. Ito MB, Schraer H, Gay CV. The effects of calcitonin, parathyroid hormone and prostaglandin E2 on cyclic AMP levels of isolated osteoclasts. Comp Biochem Physiol A Comp Physiol 1985;81:653-7.
8. Canhoroz M. Serum Adiponektin Seviyesinin Glisemik Kontrol Üzerine Etkisinin Değerlendirilmesi. İstanbul: T.C. Sağlık Bakanlığı Haydarpaşa Numune Eğitim Ve Araştırma Hastanesi; 2005.
9. Gray RS, Irvine WJ, Clarke BF. Screening for thyroid dysfunction in diabetics. Br Med J 1979;2:1439.
10. Fritz MA, Speroff L. Clinical Gynecologic Endocrinology and Infertility. 9th edition, Lippincott Williams & Wilkins, Philadelphia, US, pp 583-649, 2011.
11. Kapdağlı D. Postmenopozal Kadınlarda Cinsel Yaşam Kalitesinin Değerlendirilmesi, in Aile Hekimliği Anabilim Dalı. Adana: Çukurova Üniversitesi; 2009.
12. Okajima F, Ui M. Metabolism of glucose in hyper-and hypo-thyroid rats *in vivo*. Glucose-turnover values and futile-cycle activities obtained with 14C and 3H-labelled glucose. Biochem J 1979;182:565-75.
13. Sestoft L, Christensen NJ, Saltin B. Responses of glucose and glucoregulatory hormones to exercise in thyrotoxic and myxoedematous patients before and after 3 months of treatment. Clin Sci (Lond) 1991;81:91-9.
14. Solá E, Morillas C, Garzón S, Gómez-Balaguer M, Hernández-Mijares A. Association between diabetic ketoacidosis and thyrotoxicosis. Acta Diabetol 2002;39:235-7.
15. Sanchez-Rodriguez MA, Zacarías-Flores M, Arronte-Rosales A, Correa-Muñoz E, Mendoza-Núñez VM. Menopause as risk factor for oxidative stress. Menopause 2012;19:361-7.
16. Seghrouchni I, Drai J, Bannier E, Rivière J, Calmard P, Garcia I, *et al*. Oxidative stress parameters in type I, type II and insulin-treated type 2 diabetes mellitus; Insulin treatment efficiency. Clin Chim Acta 2002I;321:89-96.
17. Castillo C, Salazar V, Ariznavarreta C, Vara E, Tresguerres JA. Effect of isoflavone administration on age-related hepatocyte changes in old ovariectomized femal wistar rats. Phytomedicine 2006;13:468-76.
18. Minami N, Mori N, Nagasaka M, Ito O, Ogawa M, Kurosawa H, *et al*. Exercise training fails to modify arterial baroreflex sensitivity in ovariectomized female rats. Tohoku J Exp Med 2007;211:339-45.
19. Zheng, W, Jimenez-Linan M, Rubin BS, Halvorson LM. Anterior pituitary gene expression with reproductive aging in the female rat. Biol Reprod 2007;76:1091-102.
20. Halici Z, Bilen H, Albayrak F, Uyanik A, Cetinkaya R, Suleyman H, *et al*. Does telmisartan prevent hepatic fibrosis in rats with alloxan-induced diabetes? Eur J Pharmacol 2009;614:146-52.
21. Bauer J, Gries W, Bahmer FA. Volume estimation of multicellular colon carcinoma spheroids using cavalieri's principle. Pathol Res Pract 1995;191:1192-7.
22. Altunkaynak BZ, Unal D, Altunkaynak ME, Halici Z, Kalkan Y, Keles ON, *et al*. Effects of diabetes and ovariectomy on rat hippocampus a biochemical and stereological study. Gynecol Endocrinol 2012;28:228-33.
23. Bellanti F, Matteo M, Rollo T, De Rosario F, Greco P, Vendemiale G, *et al*. Sex hormones modulate circulating antioxidant enzymes: Impact of estrogen therapy. Redox Biol 2013;1:340-6.
24. Pozzi S, Benedusi V, Maggi A, Vegeto E. Estrogen action in neuroprotection and brain inflammation. Ann N Y Acad Sci 2006;1089: 302-23.
25. Tan TM, Caputo C, Medici F, Pambakian AL, Dornhorst A, Meeran K, *et al*. MELAS syndrome, diabetes and thyroid disease: The role of mitochondrial oxidative stress. Clin Endocrinol (Oxf) 2009;70:340-1.
26. Hunkar T, Aktan F, Ceylan A, Karasu C. Effects of cod liver oil on tissue antioxidant pathways in normal and streptozotocin-diabetic rats. Cell Biochem Funct 2002;20:297-302.
27. Ozansoy G, Akin B, Aktan F, Karasu C. Short-term gemfibrozil treatment reverses lipid profile and peroxidation but does not alter blood glucose and tissue antioxidant enzymes in chronically diabetic rats. Mol Cell Biochem 2001;216:59-63.
28. Hasegawa J, Hidaka H, Tateda M, Kudo T, Sagai S, Miyazaki M, *et al*. An analysis of clinical risk factors of deep neck infection. Auris Nasus Larynx 2011;38:101-7.
29. Radaideh AR, Nusier MK, Amari FL, Bateiha AE, El-Khateeb MS, Naser AS, *et al*. Thyroid dysfunction in patients with type 2 diabetes mellitus in jordan. Saudi Med J 2004;25:1046-50.
30. Alvarez-Marfany M, Roman SH, Drexler AJ, Robertson C, Stagnaro-Green A. Long-term prospective study of postpartum thyroid dysfunction in women with insulin dependent diabetes mellitus. J Clin Endocrinol Metab 1994;79:10-6.
31. Vondra K, Vrbikova J, Dvorakova K. Thyroid gland diseases in adult patients with diabetes mellitus. Minerva Endocrinol 2005;30:217-36.
32. Tomikawa J, Homma T, Tajima S, Shibata T, Inamoto Y, Takase K, *et al*. Molecular characterization and estrogen regulation of hypothalamic KISS1 gene in the pig. Biol Reprod 2010;82:313-9.
33. Zhao C, Dahlman-Wright K, Gustafsson JA. Estrogen receptor beta: An overview and update. Nucl Recept Signal 2008;6:e003.
34. Lamon-Fava S, Postfai B, Diffenderfer M, DeLuca C, O'Connor J Jr, Welty FK, *et al*. Role of the estrogen and progestin in hormonal replacement therapy on apolipoprotein A-I kinetics in postmenopausal women. Arterioscler Thromb Vasc Biol 2006;26:385-91.
35. Cressent M, Bouizar Z, Moukhtar MS, Milhaud G. Effect of ovariectomy on circulating calcitonin levels in the rat. Proc Soc Exp Biol Med 1981;166:92-5.
36. Devlin H, Lumb G, Ferguson MW. Serum parathyroid hormone and calcitonin levels following ovariectomy in the adult rat. Exp Clin Endocrinol 1992;99:84-6.
37. Gnudi S, Giardino R, Mongiorgi R, Zati A, Giavaresi GL, Bertocchi G, *et al*. Osteoporosis by ovariectomy: Calcitonin effect in an experimental model on the rat. Boll Soc Ital Biol Sper 1993;69:563-70.
38. Dimitriadis G, Baker B, Marsh H, Mandarino L, Rizza R, Bergman R, *et al*. Effect of thyroid hormone excess on action, secretion, and metabolism of insulin in humans. Am J Physiol 1985;248:E593-601.
39. Iversen, E, Laurberg P. Thyrotrophin-releasing hormone (TRH) and hormone secretion from the follicular and C-cells of perfused dog thyroid lobes. Acta Endocrinol (Copenh) 1985;109:499-504.
40. Nadler NJ, Leblond CP, Bogoroch R. The rate of iodine metabolism by the thyroid follicle as a function of its size. Endocrinology 1954;54:154-72.

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