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Original Scientific Article

THYROID HORMONES LEVELS AND MORPHOMETRIC SPECIFICS OF THYROID GLAND IN ApoE DEFICIENT (ApoE KO) MICE

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Summary: The specificity of biological features in strains used as animal models is of particular importance of the interpretation of experimental data. In this paper we examine thyroid hormones levels as well as diameter of thyroid follicles and the follicular epithelium height in ApoE-knockout (ApoE KO) mice, compared to the wild type (WT) - C57BL/6 strain as a control group. ApoE lipoprotein deficiency in this strain leads to development of severe hypercholesterolemia and atherosclerotic lesions in the blood vessels, even when mice are fed regular mouse chow. The results of our study revealed difference in FT_3 plasma level between the two strains of mice with no significant difference in FT_4 level. The morphometric analysis showed a significantly higher follicular epithelium in ApoE KO mice compared to the wild strain, which is an indication of greater TSH stimulation in ApoE KO strain. Regarding the diameter of thyroid follicles, our results corroborate no significant differences between the genetically modified animal strains, which are consistent with previous data from other studies that found no correlation of the above mentioned parameter with thyroid status of animals. The difference in thyroid function between the two strains of experimental animals could be due to altered peripheral metabolism of thyroid hormones, a consequence of the altered liver physiology in ApoE KO mice.

Key words: ApoE KO mice; thyroid gland; morphometry

Introduction

ApoE-knockout mice (apoE KO) have been extensively used to study the relation of hypercholesterolemia and lipoprotein oxidation to atherogenesis (1, 2, 3). Apolipoprotein E exerts several functions regarding lipid and cholesterol transport and metabolism: 1) apoE functions as an important carrier protein in the redistribution of lipids among cells (by incorporation into HDL (as HDL-E); 2) it plays a prominent role in the transport of cholesterol (by incorporating into intestinally synthesized chylomicrons); 3) it takes part in metabolism of plasma cholesterol and triglyceride (by interaction with the LDLR and the receptor binding of apoE lipoproteins (4, 5).

The lack of ApoE lipoprotein caused by the targeted deletion of the Apo E gene in this strain leads to the development of severe hypercholesterolemia and atherosclerotic lesions in the blood vessels, even when mice are fed regular mouse chow (6, 7). The development and phenotype appearance of the lesions are quite

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similar to those in human atherosclerosis (8).

Thyroid hormones have important role in the lipid metabolism and in the pathophysiology of atherosclerosis. There are many publications that report elevated levels of cholesterol and other serum lipids in hypothyroidism, which increase the risk of development of atherosclerosis (9, 10). On the other hand it has been reported that ApoE lipoprotein has a role in the intracellular entry of thyroid hormones in peripheral tissues (11, 12). Having in mind these interactions between the thyroid hormones and lipids, it is very important to establish the connection between thyroid function and the thyroid morphometric indices in the strains used as animal model in the research on atherosclerosis.

The goal of this study was to examine the thyroid function and morphometric specifics of the thyroid tissue in ApoE KO mice compared to the wild type (WT) - C57BL/6 mice.

Materials and methods

For the present study, 16 weeks old apoE KO mice (B6.129P2-apoE^{tm1} N11) in comparison to C57BL/6 background mice (i.e. wild type, WT), ten animals per group, were used. After overnight fast of 12 hours, animals were anesthetized under ketamine/xylazine anesthesia (90 mg/kg i.p. and 10 mg/kg, i.p. respectively). Blood samples were obtained by cardiac puncture, collected into tubes and centrifuged at 1450 × g at 4°C for 10 minutes. Lipoprotein levels in circulating blood were determined by enzyme-colorimetric test (Human diagnostics, Germany), and analyzed by Olympus, AU 400 – system. The serum levels of free T_4 (FT_4) and free T_3 (FT_3) were determinated using immunoradiometric assay according to the manufacturer's protocol (DYNO test; Brahms Diagnostics GmbH, Henningsdorf/Berlin, Germany) using the gamma counter (PC-RIA MAS STRATEC). Formalin-fixed and paraffin embedded thyroid glands were used for histomorphometric analysis. Histological sections were stained with hematoxylin/eosin (H&E) technique and morphometric features were analyzed by light microscope connected to a video camera (Nicon-Eclipse E600, Program Lucia 4.21). The measurements were made in thirty follicles, at five random different points. Follicular epithelium height and follicular diameter from peripheral and from the central parts of the thyroid lobes were measured with the Weibel's multipurpose test system M_{42} (Wild, Switzerland) (13).

Statistical data processing was performed using the software system STATISTICA for Windows XP Professional. Group results are presented as means ± standard deviation (SD). The statistical significance of the differences between the means of the experimental groups was tested by the Student's *t*-test for unpaired samples. The differences were considered as statistically significant when $p<0.05$.

All procedures with the animals were in accordance with Local Animal Care Committee in conformity with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (ETS No. 123, Appendix A). The mice were housed in polycarbonate cages in a pathogen – free facility set on a 12h light-dark cycle and given *ad libitum* access to water and standard laboratory feed. Prior to the experimental procedures, the rats were fed a commercial standard pellet feed (Filpaso, 52.11, Skopje, Republic of Macedonia), named "standard feed" hereafter.

Results

The plasma levels of cholesterol, HDL and triacylglycerol (TAG) in ApoE KO mice were significantly higher compared to WT strain (Table 1). The 16 weeks old Apo E KO mice had increased cholesterol levels for 5 fold higher vs their wild counterparts (67.7 ± 23.3 mg/dL vs 383.7 ± 47.3 mg/dL, $p<0.001$).

In the present study, the level of FT_4 was in the range 19.78 ± 0.99 pmol/L in ApoE KO mice and 19.74 ± 0.75 pmol/L in WT. There was no significant difference between the two strains (table 2). The range of FT_3 in ApoE KO mice was 2.03 ± 0.38 pmol/L and at WT the range was 2.51 ± 0.31 pmol/L. Regarding FT3, we observed significantly reduced difference in ApoE KO mice vs WT mice, $p< 0.05$ (Table 1).

According to the morphometric analysis, the follicular epithelium height in ApoE KO mice was higher compared to those of the wild strain, but there was no difference with regard to the follicular diameter between the two strains. The increased height of the follicular epithelium in ApoE mice is shown in Figure 1.

Table 1: Plasma lipoprotein and thyroid hormone levels in ApoE KO mice and their WT counterparts

Strain	Plasma lipoprotein levels (mg/dL)			Thyroid hormone level (pmol/L)	
	Chol	TAG	HDL	FT_4	FT_3
ApoE KO	383.7+47.3***	117.7+24.5*	67.0+16.3*	19.78 ± 0.99	2.03 ± 0.38*
WT	67.7+23.3	71.0+18.4	33.2+5.1	19.74 ± 0.75	2.51 ± 0.31

* $p < 0.05$; *** $p < 0.001$

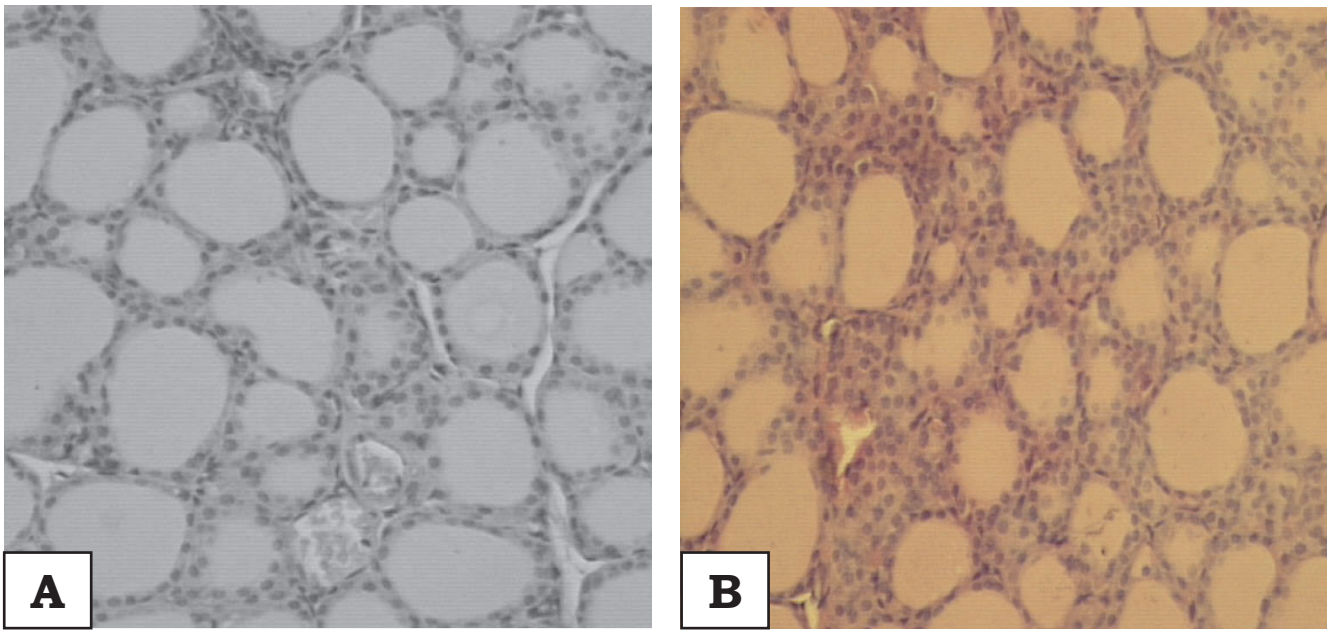

Figure 1: Thyroid gland morphology in WT (A) and ApoE KO (B) mice (H&E x 20). Histological section of thyroid tissue characterized by the difference in height of the thyroid epithelium between the two groups with no significant difference in the size and shape of the thyroid follicles

Table 2: Follicles diameter and epithelial cells height in ApoE KO mice and their WT counterparts

Follicles diameter and epithelial cells height (μm)		
	WT	ApoE KO
Peripheral epithelial cells height	6.5 ± 1.91 (N=9)	9.9 ± 2.1*** (N=8)
Peripheral follicles diameter	110.3 ± 27.4 (N=8)	108.95 ± 20.47 (N=8)
Central epithelial cells height	6.85 ± 1.31 (N=8)	10.3 ± 1.95*** (N=10)
Central follicles diameter	64.7 ± 16.0 (N=9	68.45 ± 17.57 (N=7)

*** $p < 0.001$

The mean height of the peripheral epithelium in ApoE KO was 9.9 ± 2.5 μm compared to 6.5 ± 1.9 μm in WT mice (Table 3). The difference was even more noticeable in the central epithelial cells in which the mean height in ApoE KO was 10.3 ± 1.9μm compared to 6.8 ± 1.3 μm in WT mice (Table 2).

Discussion

We compared some morphometric characteristics of the thyroid gland between ApoE KO and wild type - C57BL/6 mice, as an indicator of thyroid status for the both strains (14, 15). The results of biochemical analysis showed significantly higher plasma concentrations of cholesterol and TAG in the ApoE KO strain (Table 1) mainly due to the impaired reverse cholesterol transport (16). According to the statistical analysis, the existence of significant difference in the height of the follicular epithelium compared to vehicle control was very intriguing. Namely, the values for the height of thyroid epithelium were significantly higher in ApoE KO compared to wild strain mice, both in peripheral and in central follicles of the thyroid lobes (Table 2). Regarding the diameter of thyroid follicles, no significant difference between the two experimental groups was found (Table 2). These features, greater than usual, are a typical characteristic of TSH stimulation of the thyroid tissue. In experimental hypothyroidism, there is always a negative correlation between the level of thyroid hormones (T_4 and T_3) and the follicular epithelium height, while no correlation with the diameter of thyroid follicles is found (17, 18).

Our results also showed lower FT_3 levels in ApoE KO mice, indicating a lower peripheral conversion rate of FT_4 to FT_3. According to our knowledge, no data concerning this issue are published in the literature up to date. On the other hand the morphometric indices in our study suggest increased TSH stimulation that is due to the lower levels of FT_3 in ApoE KO mice.

There are several possible explanations for the differences in thyroid morphology and function between the two strains. The altered thyroid function in ApoE KO mice could be due to accumulation of oxidative damage and functional disorder of the liver, typical for an ApoE KO strain, fed a standard animal diet (19). The liver has a very important role in the thyroid hormone metabolism. The impaired conversion of T_4 to T_3, a consequence of lower activity of hepatic *5' deiodinase*, leads to reduction of the physiologically active T_3 and consequently increases the physiologically inert reverse triiodothyronine (rT_3). Patients with chronic liver disease have marked elevation in rT_3 accompanied by reduced T_3, as well as increasing TSH levels (20). This type of altered thyroid function could be a serious basis for an explanation of our findings.

The absence of ApoE lipoprotein in the ApoE KO strain also might play a role in the altered thyroid metabolism in these mice, considering the proposed role of this lipoprotein in the intracellular transport of thyroxin. Such claims are based on previous studies that showed the existence of a thyroid hormone binding domain in the ApoE molecule, which could be involved in the intracellular transport of thyroxin (21). The authors of the study assumed that this could facilitate the entry of thyroid hormones into cells through ApoB/ApoE receptors, widely distributed in tissues (22).

The lower intracellular internalization of the thyroxin that leads to mental retardation due to ApoE polymorphism was also proposed by Wang et al. (12). This study has shown link between Apoe polymorphism and the incidence of mental retardation in the population of iodine deficient regions of China. The mental retardation was more frequent between individuals with ApoE4 isoform (12). Based on these facts, the complete absence of ApoE lipoprotein in ApoE KO mice should have significant implications on intracellular entry of thyroid hormones in peripheral tissues. The setting resembles the syndrome of thyroid hormone peripheral resistance with higher levels of FT_4 and normal or slightly elevated TSH (23). This kind of changes could induce morphometric changes similar to the ones reported in our study.

Conclusion

The results of our study revealed difference in the height of the thyrocytes and levels of FT_3 between the two strains of mice. The possible explanation of these results is the altered liver function in ApoE KO mice and subsequently altered peripheral metabolism of thyroid hormones or the role of the ApoE lipoprotein in the intracellular internalization of the thyroid hormones. Having in mind the role of thyroid hormones in the genesis of atherosclerosis, the establishment of normal thyroid hormone range in ApoE KO mice is very important when developing animal models for the research on atherosclerosis. This would be particularly important for studying concomitant conditions with impaired thyroid function, when ApoE KO strains are used as animal models.

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RAVEN ŠČITNIČNIH HORMONOV IN MORFOLOŠKE POSEBNOSTI ŠČITNICE PRI MIŠIH BREZ GENA ZA APOE (ApoE KO) TER KONTROLNIH MIŠIH DIVJEGA TIPA

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Povzetek: Poznavanje specifičnih bioloških lastnostih pri sevih, ki se uporabljajo kot živalski modeli, je pomembno za pravilno interpretacijo rezultatov raziskav. V članku so opisane raziskave delovanja ščitnice (raven ščitničnih hormonov v krvi, premer ščitničnih foliklov ter višina folikularnega epitelija) pri miših z izbitim genom ApoE in primerjava teh vrednosti z vrednostmi pri miših divjega tipa seva C57BL/6. Pomanjkanje lipoproteina ApoE pri tem sevu miši povzroči močno hiperholesterolemijo in aterosklerotičnih poškodbe v krvnih žilah, tudi kadar se miši hranijo z navadno krmo. Rezultati raziskave so pokazali razlike v ravni prostega T_3 v krvni plazmi med obema skupinama miši, ni pa bilo statistično značilnih razlik v ravni prostega T_4. Morfometrične analize so pokazale statistično značilno višji folikularni epitelij pri miših z izbitim genom ApoE v primerjavi z divjim tipom miši, kar kaže na večje spodbujanje ščitnice s TSH pri miših z izbitim genom ApoE v primerjavi z divjim tipom miši. Pri premeru ščitničnih foliklov niso bile ugotovljene statistično značilne razlike med skupinama miši, kar je skladno s prejšnjimi podatki iz drugih študij, pri katerih niso našli povezanosti med omenjenim parametrom in statusom ščitnice pri živalih. Razlika v delovanju ščitnice pri obeh skupinah miši je morda posledica sprememb v periferni presnovi ščitničnih hormonov zaradi spremenjenega delovanja jeter pri miših brez gena ApoE.

Ključne besede: ApoE KO miši; ščitnica; morfometrija