JMS 2023; Vol 6(3):99-108
UDC:616.45:616.441-008.6]:599.323.4.084
https://doi.org/10.55302/JMS2363099t

ADRENAL GLAND TLR EXPRESSION IN ApoE DEFICIENT HOMOZYGOUS (ApoE KO$^{-/-}$) MICE WITH IMPAIRED THYROID FUNCTION

Toni Tripunoski[1], Sasho Panov[2], Jasmina Dimitrova Shumkovska[2], Slavica Josifovska[2], Irena Kostadinova Petrova[4], Ana Ugrinska[1,3], Sinisha Stojanoski[1], Nevena Manevska[1], Verdi Stanojevic[5], Bojana Stoilovska Rizova[1], Daniela PopGjorceva[1]
[1]Institute of Pathophysiology and Nuclear Medicine, Medical Faculty, Ss. Cyril and Methodius University in Skopje, North Macedonia,
[2]Institute of Biology, Faculty of Natural Sciences and Mathematic, Ss. Cyril and Methodius University in Skopje, North Macedonia ,
[3]Department of Radioisotopes and Radiopharmaceuticals Production, University Institute of Positron Emission Tomography, Skopje, North Macedonia,
[4]Institute of Histology and Embryology, Medical Faculty, Ss. Cyril and Methodius University in Skopje, North Macedonia
[5]University Clinic of Gynecology and Obstetrics, Faculty of Medicine, Ss. Cyril and Methodius University in Skopje, North Macedonia

Abstract

The main aim of our study was to investigate the expression of TLR receptors in the adrenal gland of ApoE knockout mice in context of ApoE deficiency as well as impaired thyroid function (hypo- and hyperthyroidism). The study was conducted on two following experimental animal groups: hypothyroid homozygous mice (*ApoE KO*$^{-/-}$) treated with PTU; hyperthyroid homozygous mice (*ApoE KO*$^{-/-}$) treated with L-thyroxine (T_4) and two control groups: euthyroid homozygous mice (*ApoE KO*$^{-/-}$) and euthyroid wild-type C57BL/6 mice (Bb). Determination of TLR gene expression in the adrenal gland was performed by RT-PCR reaction with semi-quantitative detection of populations of mRNA molecules transcribed from the TLR2, TLR4 and TLR9 genes in the adrenal gland in the treated experimental groups and the two untreated control groups. The results of our study are in support of the hypothesis that both conditions of impaired thyroid function produce TLR agonists that increase the expression of TLR in the adrenal gland ($p<0,05$). Also, in the untreated/control group of mice (*ApoE KO*$^{-/-}$), an increased expression of TLR was found compared to the wild strain C57BL/6 mice (Bb) as a control group ($p<0.001$).

Keywords: TLR receptors, adrenal gland, ApoE deficiency, Hypothyroid, Hyperthyroid

Introduction

Tool-like receptors (TLRs) belong to the family of transmembrane receptors with a crucial role in the activation of the immune system during various types of inflammation (sterile or non-sterile nature), playing the role of signaling receptor molecules that regulate the production of various chemokines and cytokines [1].

Ligands that activate TLR can be of endogenous or exogenous origin.

The known exogenous ligands which tend to activate TLRs are mainly LPS derived from bacterial cell wall, bacterial DNA fragment with cytosine-phosphate-guanine (CpG) motif, and viral RNA fragments. Endogenous ligands for TLR activation are the endogenous LPS, fibrinogen, mm-LDL and oxy-LDL, heat-shock proteins (HSPs), oxygen radicals as well as other products of oxidative stress.

Although TLRs are mainly involved in the inflammations of an infectious origin, they are also increasingly attributed to the recognition of endogenous ligands, the products of tissue damage of various origins [2]. It has also been shown that the major atherogenic process of lipid accumulation in the vascular wall occurs through TLR activation by various endogenous and exogenous ligands. Increased expression of TLR-1, TLR-2, and TLR-3 has been found in atherosclerotic plaques [1].

In addition to previously mentioned local effects in atherosclerotic lesions, some studies suggest systemic effects of TLR, primarily through their activation and enhanced expression in the adrenal gland that correlate with increased levels of inflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10 and IL-12), both in vitro and in vivo [3].

Conditions of impaired thyroid function (hypo- and hyperthyroidism) are characterized by complex symptomatology as a consequence of the endocrine and metabolic disorders. Both of them, mainly

hypothyroidism, increase the risk of developing atherosclerosis. Some of the products of these disorders are endogenous TLR agonists and ligands, which can lead to increased expression of TLR genes [4-6].

The main goal of this study was to investigate the influence of apolipoprotein E deficiency, as well as thyroid status on TLR gene expression in adrenal glands in ApoE deficient homozygous (ApoE $KO^{-/-}$) mice as animal models.

Material and Methods

Animal models

Our study was performed on 16 week old male homozygous $ApoE^{-/-}$ mice with C57BL/6 genetic basis and wild type (WT) - C57BL/6 male mice as a control group [7].

For the purposes of our study, the following experimental groups were established:

- Hypothyroid ApoE $KO^{-/-}$ mice (n = 30) where hypothyroidism was induced by propylthiouracil (PTU) 1mg/ml dissolved in drinking water. [8, 9].
- Hyperthyroid ApoE $KO^{-/-}$ mice (n = 30) where hyperthyroidism was induced by L-thyroxine 2g/ml dissolved in drinking water [8, 10].
- Euthyroid ApoE $KO^{-/-}$ mice (n = 30) as a control group.
- Euthyroid isogenic ApoE KO-/-) with C57BL/6 genetic basis (n = 30) as a control group.

Each of the experimental groups consisted of 5 male individuals in a cage. The treatment of the animals lasted 12 weeks, during which the animals were acclimated to room temperature (18-23°C), with light regime of 12 hours light and 12 hours dark. The daily dose of PTU and L-thyroxine was determined based on the daily drinking water of each group, divided by the number of animals in each cage.

After overnight 12-hours fasting, the animals were sacrificed by exsanguination under ketamine/xylazine anesthesia (90 mg/kg i.p. and 10 mg/kg, i.p. respectively).

Formalin-fixed and paraffin embedded thyroid glands, and immediately frozen, measured and stored at -80ºC adrenal glands were used for histomorphometric analysis and TLR gene expression, respectively.

The thyroid status of the experimental groups was determined by morphometric histological analysis of experimental animals' thyroid glands with hematoxylin/eosin (H&E) technique, while 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 at microscope field of view. Follicular epithelium height and follicular diameter from the peripheral and central parts of the thyroid lobes were measured. Ocular for morphometric analysis and the Weibel's multipurpose test system M_{42} (Wild, Switzerland) with 100 x and 40 x magnification for epithelium height and follicular diameter were used respectively [11].

Determination of TLR gene expression in the adrenal gland by RT-PCR analysis

The RT-PCR reaction in this study was used for semi-quantitative detection of populations of mRNA molecules transcribed from the TLR2, TLR4 and TLR9 genes in the adrenal gland of the treated experimental groups, untreated ApoE $KO^{-/-}$ control group, as well as wild-type (Bb) control group.

The cellular RNA was isolated from each sample by the guanidine-phenol-chloroform extraction method [12]. Each isolate was treated with 20 units of *RNasin* ribonuclease inhibitor (Sigma-Aldrich) and stored at -75 to -80° C.

RT-PCR analysis was performed with the *Enhanced Avian HS RT-PCR (Sigma-Aldrich)* reverse transcription system. Briefly, the following reaction mixture was assembled for first-strand cDNA synthesis:

about 0.5 µg RNA isolate, 3.5 M oligotimidylate primer (oligo-dT), 1 unit of *Enhanced AMV* reverse transcriptase, 1 unit of RN-aze inhibitor, 3 mM magnesium chloride and 10 mM of each dNTP.

The tubes were incubated at 25ºC for 15 minutes and then at 42ºC for 50 minutes.,

Region-specific PCR amplification of the corresponding genes was performed from the synthesized cDNA. 2µL of the synthesized cDNA, 1U/reaction of thermostable polymerase (JumpStart AccuTaq, Sigma-Aldrich), 200 mM of each dNTP, 3 mM of magnesium chloride and 0.4 mM of the following oligonucleotide were applied to the reaction mixture:

mB-actin*F* 5′- G T G GGG C G C CCC A G G C A C C A -3′
mB-actin R 5′- C T C C T T A A T G T C A C G C A C G A T -3′
TLR2 *F* 5′- A G C T C T TT G G C T C T T C T G -3′
TLR2 R 5′- A G A A C T G GGGG A T A T G C -3′;
TLR4 *F* 5′- G C A T G G C T T A C A C C A C C T C T -3′
TLR4 R 5′- G T G C T G A AAA T C C A G G T G C T -3′;
TLR9 *F* 5′- T C CC T G T A T A G A A T G T G -3′
TLR9 R 5′- T G G A G G C G T G A G A G -3′;

PCR amplifications were performed with the following program: initial denaturation at 94ºC for 3 minutes and followed by 33 cycles, each consisting of 3 steps, denaturation at 94ºC for 1 minute, annealing at 60ºC for 1 minute and extension at 68ºC for 1 minute and 45 seconds. The terminal extension is performed at 68ºC for 10 minutes [13].

PCR products were separated by gel electrophoresis in 2% agarose.

The gels were stained with ethidium bromide, visualized on a UV transluminator and digitally photographed (Canon PowerShot A70).

The fluorescence intensities of the gene-specific electrophoretic strip and the control of β-actin from each sample were digitally quantified by the gel analysis function of ImageJ software, version 1.33u [Rasband, W.S., ImageJ, National Institutes of Health, Bethesda, Maryland, USA, http://rsb.info.nih.gov/ij/, 1997-2004].,

The fluorescence intensities of the gene transcript bands were compared with the corresponding internal gene transcript bands of the β-actin gene in semi-quantitative measurements.

Results

Morphometric analysis of the thyroid gland confirmed the corresponding thyroid status of the respective experimental groups, as shown in Figure 1, and Tables 1 and 2 respectively.

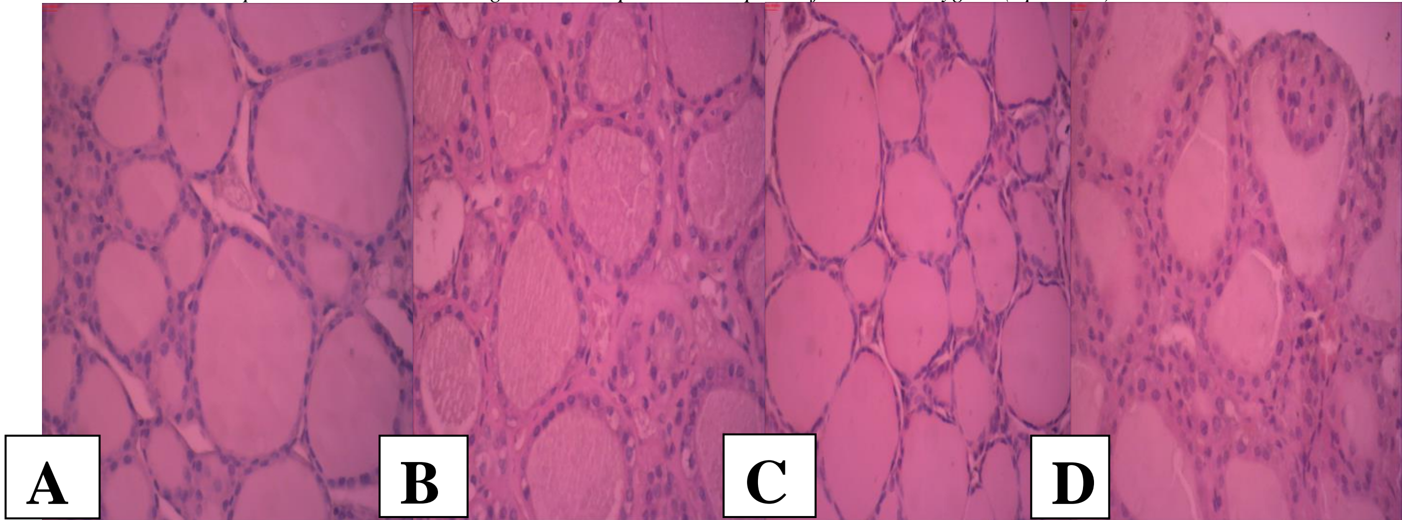

Figure 1. Micrographs of the thyroid gland (H&E staining, X 40). (A) Wild strain-Bb; (B) ApoE$^{-/-}$ control; (C) ApoE$^{-/-}$ T_4; (D) ApoE$^{-/-}$ PTU;

Table 1. The average diameter of the thyroid follicles and the height of the follicular epithelium in the treated experimental groups compared with ApoE$^{-/-}$ control group.

There are significant differences between treated experimental groups and the ApoE$^{-/-}$ control group: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Values are represented as mean ± SD.

	Bb control	ApoE$^{-/-}$ control
peripheral follicles (µm)	**110,30**±27,3	**108,95**±20,5

	ApoE$^{-/-}$ control	ApoE$^{-/-}$ T_4	ApoE$^{-/-}$ PTU
peripheral follicles (µm)	**108,95** ± 20,5	**124,05** ± 44,5**	**150,30** ± 21,9***
peripheral follicular epithelium (µm)	**9,90** ± 2,1	**4,95** ± 1,1***	**13,40** ± 1,75***
central follicles (µm)	**68,45** ± 17,5	**52,20** ± 8,8**	**80,90** ± 17,3*
central follicular epithelium (µm)	**10,30** ± 1,90	**4,10** ± 1,30***	**11,50** ± 1,50**

	Bb control	ApoE$^{-/-}$ control
peripheral follicular epithelium (µm)	**6,50**±1,9	**9,90**±2,1***
central follicles (µm)	**64,70**±16,0	**68,45**±17,5
central follicular epithelium (µm)	**6,85**±1,30	**10,30**±1,90***

There are significant differences between ApoE$^{-/-}$ and Bb control groups: *** $p < 0.001$.
Bb = wild-type mice (Bb) with C57BL/6 genetic basis

Our study determined the adrenal expression of TLR2, TLR4, and TLR9 in the two treated experimental groups, untreated ApoE$^{-/-}$ control group, as well as in the wild-type Bb control group. The results of the statistical analysis showed significantly higher expression of the three TLRs in the two treated experimental groups than in the ApoE$^{-/-}$ control group, and in the ApoE$^{-/-}$ control group compared to the Bb control group. These results are shown in Tables 3 and 4.

Table 2. The average diameter of the thyroid follicles and the height of the follicular epithelium in the control groups.

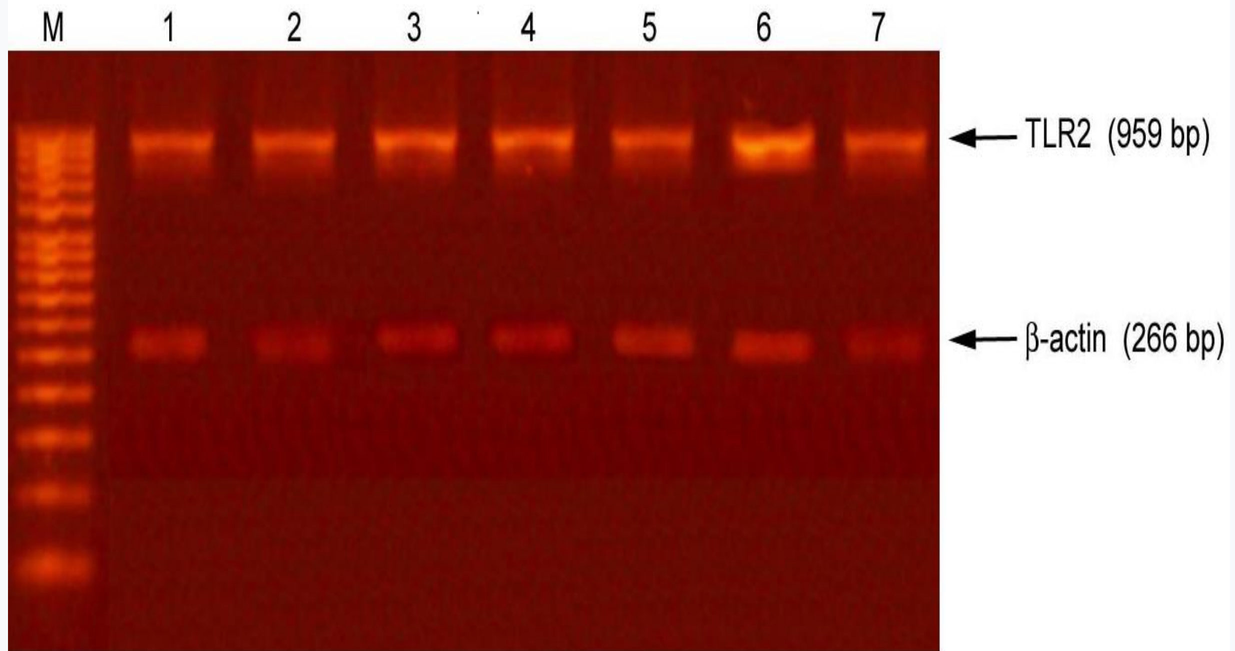

Figure 2. Representative image from TLR2 RT-PCR.

Table 3. Adrenal TLR expression in the treated experimental groups compared with the $ApoE^{-/-}$ control group.

	$ApoE^{-/-}$ control	$ApoE^{-/-}$T4	$ApoE^{-/-}$PTU
TLR 2	**3,65**±1,72	**5,59**±1,57*	**6,01**±2,05*
TLR 4	**5,77**±2,97	**8,58**±2,38*	**9,85**±3,38*
TLR 9	**5,74**±1,90	**9,99**±4,23*	**10,23**±4,71*

There are significant differences between the treated experimental groups and the $ApoE^{-/-}$ control group: * $p < 0.05$.

Table 4. Adrenal TLR expression in $ApoE^{-/-}$ and Bb control groups

	Bb control	$ApoE^{-/-}$control
TLR 2	**2,25**±0,50	**3,65**±1,72***
TLR 4	**3,09**±1,13	**5,77**±2,97***
TLR 9	**3,28**±1,32	**5,74**±1,90***

There are significant differences between $ApoE^{-/-}$ and Bb control groups: *** $p < 0.001$.

Discussion

Although the main role of TLR is to activate the immune response under the influence of ligands of microbial origin, many studies have shown that they can also be activated by endogenous ligands from a variety of cellular damage processes, such as those caused by oxidative stress [6,14-17].

According to this, as well as the wide tissue distribution of TLRs, their mediating role in the development of sterile inflammation in various tissues and organs (including atherogenesis) is emphasized [1,18-19].

In addition to participating in the local occurrence and development of atherosclerotic plaques [4], some authors believe that TLRs also have a systemic proaterogenic effect by stimulating the adrenal gland [20, 21]. The main motive in our study was to examine the expression of some TLR genes in the conditions of impaired thyroid function and concomitant ApoE lipoprotein deficiency.

The results obtained from this segment of the study showed increased expression of the examined TLR genes in all treated experimental groups, however, the data for constitutively increased expression in the untreated control $ApoE^{-/-}$ group was also interesting.

The major atheroprotective function of ApoE lipoprotein is its participation in plasma clearance of atherogenic lipoproteins. Numerous studies indicate its other properties that prevent the initiation and development of atherosclerosis, regardless of its involvement in lipoprotein metabolism.

This refers to the ability of ApoE lipoprotein to inhibit lipid oxidation and thus prevent oxidative cell damage [22], a role in preventing the expression of adhesive molecules on the surface of vascular endothelium [23], as well as inhibition of vascular smooth muscle activation [24,25].

Additional confirmation to the multifunctional character of ApoE lipoprotein is the possession of anti-inflammatory properties. Studies have shown that the ApoE lipoprotein, whether synthesized locally or from the circulation, inhibits macrophage secretion of proinflammatory cytokines, thus suppressing the inflammatory response of tissues induced by various agents [26].

A possible mechanism for the anti-inflammatory effect of ApoE lipoprotein is the perturbation of the membrane microenvironment that occurs during cholesterol efflux from the cells, which interrupts the signal for the production of cytokines mediated by the activation of TLR, and thus consequent secretion of IL-6,Il-1β and TNF-α [26,27].

These findings may be an addition to explaining the results of our study of the constitutive activity of the examined TLR genes in the untreated control $ApoE^{-/-}$ group.

The hyperthyroid condition is characterized not only by increased oxidative stress but also by increased lipolysis and consequent increase in free fatty acids [28].

Based on previous research, it has been established that oxidative stress products and free fatty acids such as endogenous TLR ligands play a role in the activation of proinflammatory signaling pathways and the pathogenesis of many diseases, such as peripheral insulin resistance [29,30,15,16,17].

This would be particularly important for the development of insulin resistance and the consequent complications of hyperthyroidism [31].

Based on the above, we hypothesized that the increased expression of TLR genes in the thyroxine-treated experimental group could be caused precisely by the endogenous oxidative products and free fatty acids generated during the hyperthyroid state.

Among the endogenous ligands that activate TLR are the oxidized forms of LDL (ox-LDL and mm-LDL), which have been shown to occur in significant quantities during the pathological conditions accompanied by oxidative stress and hyperlipidemia, such as hypothyroidism [32,33].

This finding was a starting point for explaining the results of our study regarding the greater expression of TLR in the hypothyroid group.

Oxidatively modified forms of LDL cholesterol, that play a central role in the formation of atherosclerotic plaques by activating TLR (primarily TLR2 and TLR4), affect local inflammatory processes in atherosclerosis.

The minimally modified form of LDL (mm-LDL) via TLR stimulates vascular endothelial and smooth muscle cells towards synthesis of proinflammatory cytokines, chemokines, and adhesive molecules. In this way, monocytes are attracted and retained, migrating to the subendothelial space, where under the influence of proinflammatory cytokines and growth factors they differentiate into macrophages [5].

Conditions characterized by chronic hyperlipidaemia and consequent oxidative modification of cholesterol inevitably lead to their excessive intracellular accumulation and foam cell formation.

The epilogue of such events is often cell apoptosis and the release of proinflammatory oxidized lipid derivates into plaque, leading to exacerbation of local inflammation. One of the consequences of oxidative stress in the process of atherogenesis is the damage to mitochondria, which usually results in their autophagy, as an evolutionarily conserved process in lysosomal recycling of cytoplasmic material [34].

Mitochondrial DNA (mtDNA), which avoids this process of autophagy and is released into the extracellular space, activates TLR9 and thus elicits an appropriate inflammatory response [35-36].

Namely, similar to bacterial DNA, mtDNA also contains an inflammatory non-methylated CpG motif, unlike nuclear DNA which is modified with additional methyl groups in some sequences, known as CpG motifs, which distinguishes the immune system from microbial DNA [37].

In addition to the aforementioned local effects, the widespread distribution of TLR, especially in endocrine cells (thyrocytes, adipocytes, Langerhans islets, adrenal glands), imposes the concept of their systemic effects on the etiopathogenesis of multiple diseases.

Our assumptions about the indirect effects of hypo- and hyperthyroidism on atherogenesis, through the activation of TLR in the adrenal gland and the subsequent increased production of glucocorticoids, as well as the activation of the renin-angiotensin-aldosterone system are made in this context.

The conditions of hypo- and hyperthyroidism that were subject of our study are characterized by a disturbed hypothalamic-pituitary regulatory axis [38-39].

The results of our study are in support of the hypothesis that both conditions of impaired thyroid function produce TLR agonists that increase the expression of TLR in the adrenal gland. We based our assumptions on the results of studies showing that direct activation of TLR in the adrenal gland induces increased production of corticosteroids [20,21].

On the other hand, TLR deficiency or their dysfunction is accompanied by a reduction in the steroid adrenal response in experimentally induced endotoxemia [40].

Similar to local TLR activation [4], mineralocorticoids and glucocorticoids stimulate the formation of cholesterol esters as well as the expression of adipocyte fatty acid binding protein2 (aP2) in macrophages [41-43].

The aP2 protein is a member of the family of cytoplasmic fatty acid binding proteins (FABPs), which participates in the intracellular distribution of fatty acids across cell compartments, modulates intracellular lipid metabolism, and participates in the regulation of gene expression [44,45].

Its increased expression in macrophages enhances intracellular fatty acid deposition, thereby stimulating foam cell formation and development of atherosclerosis [4,46].

In addition to these local effects, glucocorticoids have systemic hemodynamic effects that indirectly increase the predisposition to initiate and develop atherosclerosis. The influence of sodium retention on the increase of plasma volume is minor, while the greatest effect is accomplished through the increased peripheral vascular resistance caused by the suppressive action of glucocorticoids on the kallikrein-kinin system, prostaglandins and nitric oxide and activation of the renin-angiotensin system, as well as stimulation of α1-adrenergic receptors and angiotensin type II receptors [47,48].

Conclusion

Under the experimental conditions of our study, an increased expression of the TLR genes was observed in all treated experimental groups, as well as a constitutively increased expression in the untreated control $ApoE^{-/-}$ group compared to the control Bb group.

These findings suggest that the two states of impaired thyroid function (hypo- and hyperthyroidism) through the altered metabolism and consequent metabolic products lead to increased expression of TLR in the adrenal gland in both treated experimental groups.

Also, the deficiency of ApoE lipoprotein itself leads to increased expression of TLR genes, through altered liver function and subsequently altered peripheral metabolism of thyroid hormones in

ApoE$^{-/-}$ mice, which can be a consequence of ApoE deficiency and consequently impaired cellular internalization of thyroid hormones.

References

1. Cole JE, Georgiou E, Monaco C. The expression and functions of toll-like receptors in atherosclerosis. Mediators Inflamm. 2010; 2010:393946. doi: 10.1155/2010/393946
2. Michelsen KS, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, Akira S, Rajavashisth TB, Arditi M. Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. Proc Natl Acad Sci U S A 2004; 101(29): 10679-84.
3. Tran N et al. Toll-Like Receptor 9 Expression in Murine and Human Adrenal Glands and Possible Implications during Inflammation. The Journal of Clinical Endocrinology & Metabolism 2007; 92(7):2773–2783.
4. KKazemi MR, McDonald CM, Shigenaga JK, Grunfeld C, Feingold KR. Adipocyte fatty acid-binding protein expression and lipid accumulation are increased during activation of murine macrophages by toll-like receptor agonists. Arterioscler Thromb Vasc Biol. 2005; 25(6): 1220-1224.
5. Miller YI, Viriyakosol S, Worrall DS, Boullier A, Butler S, Witztum JL. Toll-Like Receptor 4–Dependent and –Independent Cytokine Secretion Induced by Minimally Oxidized Low-Density Lipoprotein in Macrophages. Arterioscler Thromb Vasc Biol. 2005; 25: 1213-1219.
6. Erridge C. Endogenous ligands of TLR2 and TLR4: agonists or assistants? Journal of Leukocyte Biologyvol. 2010; 87(6): 989-999.
7. Watts GF and Chan DC. Of Mice and Men: Blowing Away the Cobwebs From the Mechanism of Action of Niacin on HDL Metabolism. Arterioscler Thromb Vasc Biol. 2008; 28:1892-1895.
8. Ferreira E, Silva AE, Serakides R, Gomes AES, Cassali GD. Model of induction of thyroid dysfunctions in adult female mice. Arq Bras Med Vet Zootec. 2007; 59(5): 1245-49.
9. Mysliwiec J et all. Role of interleukin-6 on RANKL-RANK/osteoprotegerin system in hypothyroid ovariectomized mice. Folia Histochem Cytobiol. 2010; 48(4):549-54.
10. Tamaru M et all. Dendritic cells produce interleukin-12 in hyperthyroid mice. European Journal of Endocrinology 1999; 141: 625–629.
11. Weibel, E.R. Stereological methods. Practical methods for biological morphometry. Academic Press, London. 1979.
12. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987; 162(1):156-9.
13. Applequist SE, Wallin RPA, Ljunggren HG.Variable expression of Toll-like receptor in murine innate and adaptive immune cell lines. Int Immunol. 2002; 14:1065-1074.
14. Frantz S, Kelly RA, Bourcier T. Role of TLR-2 in the activation of nuclear factor kappaB by oxidative stress in cardiac myocytes. J Biol Chem. 2001; 276(7):5197-203.
15. Paul-Clark MJ, McMaster SK, Sorrentino R, Sriskandan S, Bailey LK, Moreno L, Ryffel B, Quesniaux VF, Mitchell JA. Toll-like receptor 2 is essential for the sensing of oxidants during inflammation. Am J Respir Crit Care Med. 2009; 179(4):299-306.
16. Wang XY, Sarkar D, Fisher PB. Stress-sensing toll-like receptor as a driver of angiogenesis. Pigment Cell Melanoma Res. 2011; 24(1):7-9.
17. Malinin NL, West XZ, Byzova TV. Oxidation as "the stress of life". Aging (Albany NY) 2011; 3(9):906-10.
18. Sánchez E, Orozco G, and J. Martín J. Toll-like receptors and human pathology. Inmunología 2004; 23(4): 328-338.
19. Tsan MF, Gao B. Endogenous ligands of Toll-like receptors. J Leukoc Biol. 2004; 76(3):514-9.

20. Bornstein SR, Schumann RR, Rettori V, McCann SM, Zacharowski K. Toll-like receptor 2 and Toll-like receptor 4 expression in human adrenals. Horm Metab Res. 2004a.; 36: 470–473.
21. Vakharia K and Hinson JP. Lipopolysaccharide Directly Stimulates Cortisol Secretion by Human Adrenal Cells by a Cyclooxygenase-Dependent Mechanism. Endocrinology 2005; 146(3):1398–1402.
22. Miyata M. and Smith JD.Apolipoprotein E allele-specific antioxidant activity and effects on cytotoxicity by oxidative insults and β-amyloid peptides. Nat. Genet.1996; 14: 55-61.
23. Stannard AK, Riddell DR, Sacre SM, Tagalakis AD, Langer C, von Eckardstein A, Cullen P, Athanasopoulo T, Dickson G and Owen JS. Cell-derived apolipoprotein E (ApoE) particles Inhibit vascular cell adhesion molecule-1 (VCAM-1) expression in human endothelial cells. J. Biol. Chem. 2001; 276: 46011-46016.
24. Ishigami M, Swertfege, DK, Hui MS, Granholm NA and Hui DY. Apolipoprotein E inhibition of vascular smooth muscle cell proliferation but not the inhibition of migration is mediated through activation of inducible nitric oxide synthase. Arterioscler. Thromb. Vasc. Biol. 2000; 20: 1020-1026.
25. Kawamura A, Baitsch D, Telgmann R, Feuerborn R, Weissen-Plenz G, Hagedorn C, Saku K, Brand-Herrmann SM, von Eckardstein A, Assmann G and Nofer JR. Apolipoprotein E interrupts interleukin-1β signaling in vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 2007; 27: 1610-1617.
26. Zhu Y, Kodvawala A, Hui DY. Apolipoprotein E inhibits toll-like receptor (TLR)-3- and TLR-4-mediated macrophage activation through distinct mechanisms. Biochem J. 2010; 428(1): 47-54.
27. Vedhachalam C, Narayanaswami V, Neto N, Forte TM, Phillips MC, Lund-Katz S and Bielicki JK. The C-terminal lipid-binding domain of apolipoprotein E is a highly efficient mediator of ABCA1-dependent cholesterol efflux that promotes the assembly of high-density lipoproteins. Biochemistry 2007; 46: 2583-2593.
28. Riis AL, Gravholt CH, Djurhuus CB, Norrelund H, Jprgensen JO, Moller N. Elevated regional lipolysis in hyperthyroidism. J Clin Endocrinol Metab. 2002: 87: 4747-4753.
29. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest. 2006; 116(11):3015-25.
30. Kim F, Pham M, Luttrell I, Bannerman DD, Tupper J, Thaler J, Hawn TR, Raines EW, Schwartz MW. Toll-like receptor-4 mediates vascular inflammation and insulin resistance in diet-induced obesity. Circ Res. 2007; 100(11):1589-96.
31. Brenta G. Why Can Insulin Resistance Be a Natural Consequence of Thyroid Dysfunction? J Thyroid Res. 2011; 2011:152850.
32. Costantini F, Pierdomenico SD, De Cesare D, De Remigis P, Bucciarelli T, Bittolo-Bon G, Cazzolato G, Nubile G, Guagnano MT, Sensi S, Cuccurullo F, Mezzetti A. Effect of thyroid function on LDL oxidation. Arterioscler Thromb Vasc Biol. 1998;18(5):732-7.
33. Geng H, Wang A, Rong G, Zhu B, Deng Y, Chen J, Zhong R. The effects of ox-LDL in human atherosclerosis may be mediated in part via the toll-like receptor 4 pathway. Mol Cell Biochem. 2010;342(1-2):201-6. doi: 10.1007/s11010-010-0484-8. Epub 2010 May 14. PMID: 20467793.
34. Konstantinidis K, Kitsis RN. Cardiovascular biology: Escaped DNA inflames the heart. Nature 2012; 485(7397):179-80.
35. Okabe Y, Kawane K, Akira S, Taniguchi T, Nagata S. Toll-like receptor-independent gene induction program activated by mammalian DNA escaped from apoptotic DNA degradation. J Exp Med. 2005; 21;202(10):1333-9. doi: 10.1084/jem.20051654. PMID: 16301743; PMCID: PMC2212973.
36. Ding Z, Liu S, Wang X, Khaidakov M, Dai Y, Mehta JL. Oxidant stress in mitochondrial DNA damage, autophagy and inflammation in atherosclerosis. Sci Rep. 2013; 3:1077. doi:10.1038/ srep 0107
37. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that

escapes from autophagy causes inflammation and heart failure. Nature 2012; 485(7397):251-255.

38. Johnson EO, Calogero AE, Konstandi M, Kamilaris TC, La Vignera S, Chrousos GP. Effects of short- and long-duration hypothyroidism on hypothalamic-pituitary-adrenal axis function in rats: in vitro and in situ studies. Endocrin. 2012; 42(3):684-93
39. Johnson EO, Kamilaris TC, Calogero AE, Gold PW, Chrousos GP. Experimentally-induced hyperthyroidism is associated with activation of the rat hypothalamic-pituitary-adrenal axis. Eur J Endocrinol. 2005; 153(1):177-85.
40. Bornstein SR, Zacharowski P, Schumann RR, Barthel A, Tran N, Papewalis C, Rettori V, McCann SM, Schulze-Osthoff K, Scherbaum WA, Tarnow J, Zacharowski K. Impaired adrenal stress response in Toll-like receptor 2-deficient mice. Proc Natl Acad Sci USA 2004b.; 101: 16695-16700.
41. Cheng W, Lau OD, Abumrad NA. Two antiatherogenic effects of progesterone on human macrophages; inhibition of cholesteryl ester synthesis and block of its enhancement by glucocorticoids. J Clin Endocrinol Metab. 1999; 84: 265–271.
42. Penfornis P, Viengchareun S, Le Menuet D, Cluzeaud F, Zennaro MC, Lombes M. The mineralocorticoid receptor mediates aldosterone-induced differentiation of T37i cells into brown adipocytes. Am J Physiol Endocrinol Metab. 2000; 279: E386–E394.
43. Feingold KR, Shigenaga JK, Kazemi MR, McDonald CM, Patzek SM, Cross AS, Moser A, Grunfeld C. Mechanisms of triglyceride accumulation in activated macrophages. J. Leukoc. Biol. 2012; 92:829-839
44. Boord JB, Fazio S, Linton MF. Cytoplasmic fatty acid-binding proteins: emerging roles in metabolism and atherosclerosis. Curr Opin Lipidol. 2002; 13(2):141-147.
45. Storch J, Thumser AE. Tissue-specific functions in the fatty acid-binding protein family. J Biol Chem. 2010; 285(43):32679-32683.
46. Peeters W, de Kleijn DP, Vink A, van de Weg S, Schoneveld AH, Sze SK, van der Spek PJ, de Vries JP, Moll FL, Pasterkamp G. Adipocyte fatty acid binding protein in atherosclerotic plaques is associated with local vulnerability and is predictive for the occurrence of adverse cardiovascular events. Eur Heart J. 2011; 32(14):1758-1768.
47 Saruta T. Mechanism of Glucocorticoid -Induced Hypertension. Hypertens Res. 1996; 19:1-8.
48. Ong SLH and Whitworth JA. Glucocorticoid-induced hypertension and the nitric oxide system. Expert Review of Endocrinology & Metabolism 2012; 7(3):273-280.