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The Expression of Thymic AQP7 and Perilipin 1 (PLIN1) in Rats Fed a High-Fructose Diet is Modified by Voluntary Physical Activity

Jülide Tozkır[1], Nihayet Fırat[2], Ebru Göncü[3], Onur Ersoy[1], Pınar Tayfur[4], Orkide Palabıyık[1]

[1]Trakya University Vocational School of Health Services, Edirne, Turkey
[2]Trakya University Institute of Health Sciences, Department of Physiology, Edirne, Turkey
[3]Trakya University Medical Research and Application Centre, Department of Medical Genetics, Edirne, Turkey
[4]Bahçeşehir University Faculty of Health Science, İstanbul, Turkey

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Corresponding Author: Jülide Tozkır, Trakya University Vocational School of Health Services, Edirne, Turkey
E-mail: jduymaz@gmail.com **ORCID:** orcid.org/0000-0002-8436-8309

Abstract

Objective: Aquaporins (AQPs) are a large family of proteins that help transport water and small molecules. AQP7 is an AQP responsible for the extracellular transport of glycerol produced by lipolysis in adipocytes. Perilipin 1 (PLIN1) regulates lipolysis on the surface of lipid droplets in adipocytes and promotes the transport of glycerol out of the cell via AQP7. It is not known exactly how AQP are regulated in thymic tissue. The goal of this study was to evaluate the expression status of AQP7 and PLIN1 in thymic tissue during thymic involution and to determine how these expression patterns are affected by high-fructose diet and voluntary physical activity.

Materials and Methods: In this study, 18 adult female Sprague-Dawley rats were assigned to three groups: control (C), fructose (F), and fructose + physical activity (FA). Fructose was added at 20% to the drinking water of the F and FA groups. At the end of 8 weeks, rats were euthanized under appropriate conditions, and tissue and blood samples were collected. Histological evaluation of the thymus was performed by hematoxylin-eosin staining. The expression levels of AQP7 and PLIN1 were determined real-time reverse transcription polymerase chain reaction using appropriate primers.

Results: The weight of the thymus tissue decreased in the FA group compared with that in the F group due to exercise ($p=0.015$), but the cortex and medulla structure were histologically preserved. AQP7 was significantly decreased in the F group compared with the C and FA groups ($p=0.0079$ and $p=0.0127$, respectively).

Conclusion: AQPs and their associated molecules can be strategic targets for slowing or reversing thymic involution.

Keywords: Thymus, aquaglyceroporin 7, perilipin 1, fructose, voluntary physical activity

Introduction

Modern Western diets and the extensive use of added sugars in the food industry have significantly increased the intake of fructose. In the past, the daily intake of fructose from natural sources was approximately 16-20 g/day, whereas the increased consumption of industrial foods over the last fifty years has increased this amount to 80-150 g/day. The widespread consumption of ready-to-eat foods and beverages high in fructose has been linked to the risk of several diseases (1-3). The impact of fructose on elements of the immune system. This effect may help to understand the potential mechanisms underlying immune dysfunction related to disorders observed in association with high fructose consumption (4,5). The thymus gland is an important organ of the immune system (6). Thymus provides the maturation of T-cells. The thymus was previously ignored because it was thought to be involuted and not to function. However, the number of studies demonstrated the importance of thymus function in adulthood despite involution has been (7). Function and mass of thymus, and celularity decrease with age. Under conditions of aging and chronic inflammation, T-cell

ORCID: J. Tozkır: 0000-0002-8436-8309, N. Fırat: 0000-0002-2331-1691, E. Göncü: 0000-0002-6413-5209, O. Ersoy: 0000-0001-9829-7903, P. Tayfur: 0000-0002-2825-0760, O. Palabıyık: 0000-0002-3488-3740

production decreases, and fat and connective tissue increase with accelerated thymic involution (8). Thymic involution has effects on immune function, such as reduced resistance to pathogens, increased susceptibility to autoimmunity, and impaired tumor surveillance (8). Understanding the impact of lifestyle factors, such as exercise and diet on the functions of involuted thymus tissue is crucial. Studies have suggested that thymic involution can be slowed and that various features of immune senescence can be reversed by exercise (9,10). Any physical activity that leads to energy expenditure is defined as physical activity (11). Voluntary wheel running, which is not directly required for survival or homeostasis and is not affected by external factors, is widely used in mice and rats. Voluntary wheel running has been successfully used to investigate adaptive responses to exercise in cardiovascular, metabolic, neuromuscular, neurological, and immunological studies (12).

More recently, the expression and effects of aquaporin (AQPs) in thymus tissue, which provide a new perspective on adipose tissue homeostasis, have been investigated (13). AQPs are integral membrane proteins that function as channels that primarily help water to pass through the cell membrane. To date, 13 AQP isoforms (AQP0-12) have been described in mammals. As a subgroup of the AQP family, aquaglyceroporin (AQP3, AQP7, AQP9, and AQP10) allow the passage of both water and small molecules such as glycerol (14). Glycerol, a small 3-carbon alcohol stored as the backbone of triglycerides (TGs) in adipose tissue, is one of the most important metabolites for meeting energy needs. AQP7 is a specific glycerol channel that plays an important role in regulating glycerol efflux from adipose tissue and maintaining lipid and energy balance. Under lipogenic conditions of increased insulin stimulation, such as feeding, the translocation of AQP7 to the plasma membrane is prevented by the binding of perilipin 1 (PLIN1) to AQP7 on the surface of lipid droplets. Under lipolytic conditions of increased energy demand, such as fasting and exercise, TG in adipose tissue is hydrolyzed to free fatty acids and glycerol. To release glycerol into the circulation, the AQP7-PLIN1 bond is reduced by protein kinase A phosphorylation, allowing AQP7 to translocate to the plasma membrane (14,15). How *AQP7* and *PLIN1* gene expression is altered in thymic tissue during thymic has yet to be known involution. This study aimed to investigate the patterns of AQP7 and PLIN1 expression in thymic tissue during thymic involution and to determine how these patterns are affected by high-fructose diet and voluntary physical activity.

Materials and Methods

Experimental Protocol

The study was designed using 15-week-old female Sprague-Dawley rats. Ethical approval was obtained from Trakya University, Local Ethics Committee of Animal Experiments (decision no: 2023.04.03, date: 26.04.2024). All animal experiments were performed in conformity with the ARRIVE guidelines and in compliance with the European Union Directive 2010/63/EU for animal experimentation. The experimental groups of our study were the control (C), high-fructose diet (F), high-fructose diet, and voluntary physical activity (FA) groups, with n=6 animals per group (16). The mean weight of the animals at the start of the experiment was 206.4 ± 7.3 g. The animals were kept in polycarbonate cages under conditions in which temperature (22 ± 1°C) and humidity (50-60%) were controlled, with 12-h light and 12-h dark cycle. According to the study protocol, rats in the C group received normal drinking water and standard feed, whereas rats in the F and FA groups received drinking water supplemented with 20% fructose (200 g/L) and standard feed for 8 weeks. The animals were granted free access to food and water throughout the study period. Each of the rats in the FA group was placed in a cage with rotating wheels for voluntary exercise. A rotating wheel (31.5×10×108.1 cm) was attached to the cage to allow the rats to perform voluntary physical activity, and a recording device was used to record the frequency of rotation (17). One rat was kept in each cage throughout the experiment. Prior to the start of the experimental protocol, rats in the FA group underwent a 2-week adaptation period. Following the experimental phase, rats from all groups were euthanized with 10 mg/kg Rompun and 50 mg/kg ketamine anesthesia, after which blood, thymus, and visceral adipose tissue samples were harvested and stored at -80°C until further analysis.

Light Microscopy

After euthanasia, thymus tissue samples from all groups were fixed in 10% neutral buffered formalin (Cat. No: HT501128, Sigma Aldrich, Taufkirchen, Germany) for 24 hours. After fixation, the samples were washed in tap water and dehydrated with an increasing ethanol series (70%, 90%, 96%, 100, 100) (Cat. No.: 100983, Merck Millipore, Darmstadt, Germany). After dehydration, the tissues were treated with toluene (Cat. No: 108325, Merck Millipore, Darmstadt, Germany) for 1 h for transparency. Paraffin blocks were then obtained by first placing the tissues in low-melting paraffin (42-44°C, Cat. No: 107150, Merck Millipore, Darmstadt, Germany) and then in paraffin with a melting temperature of 56-58°C (Cat. No: 107160, Merck Millipore, Darmstadt, Germany). Tissue sections, 5 μm in thickness, were prepared from paraffin blocks using a microtome for histological analysis and subsequently stained with hematoxylin (Cat. No: MHS16, Sigma Aldrich, Taufkirchen, Germany) and eosin (Cat. No: 230251, Merck Millipore, Darmstadt, Germany) (H-E).

RNA Isolation and cDNA Synthesis

In the present study, total RNA was purified from thymus and visceral adipose tissues stored under optimal conditions to determine the expression of the *AQP7* and *PLIN1* genes. This was achieved using a commercially available kit, following the instructions provided by the manufacturer (Cat. No: BS584-#Q616BR0V, BIO BASIC EZ-10 Spin Column Total RNA Mini-Preps Super Kit, Canada). The total RNA concentration and quality were determined using a NanoDrop spectrophotometer (NanoDrop ND-1000 UV/VIS Spectrophotometer, USA). Sterile (free of RNase, DNase, pyrogens, PCR inhibitors, endotoxins and DNA) were transferred to sterile polypropylene tubes using filtered pipette tips and stored in portions. The total RNA concentration was adjusted to 200 ng/µL, and the RNA was reverse transcribed using high-capacity cDNA reverse transcription kits (Cat. No: G236, OneScript® Plus cDNA Synthesis Kit Applied Biological Materials Inc., Canada) according to the manufacturer's instructions. All cDNAs were stored at -20°C until further analysis. Expression of AQP7 (F: 5' ATCCTTGTTTGCGTTCTTGG-3', R: 5'- GCGTGAATTAAGCCCAGGTA-3') (18), PLIN1 (F: 5'-ACAGCACCAAAGAAGCCCAC-3', R: 5'-TCTTTTGCCGTCCTGAAGA-3') (19), and beta-actin (housekeeping) (F:5' CCTCTGAACCCTAAGGCCAAC-3', R:5'- TGCCACAGGATTCCATACCC-3') was analyzed by RTPCR using SYBR Green (Applied Biosystems, 7500 Fast Real-Time PCR, USA) with appropriate primers. The experiments were performed in three replicates. For data analysis, data normalization was performed by calculating the ΔΔCT and $2^{-\Delta\Delta CT}$ values. Comparisons were performed between adipose tissues known to contain target molecules and experimental tissues.

Biochemical Assessment and Glucose and Glycerol Determination

Glucose levels in serum obtained from blood samples were analyzed at the Central Laboratory of Trakya University Faculty of Medicine (Roche Cobas 8000 c702, Switzerland). Glycerol levels were determined as mmol/L by ELISA using a kit (Cat No: E-BC-K340-M, Elabscience® Glycerol Colorimetric Assay Kit, USA) according to the manufacturer's instructions. Washing steps were performed using a BioTek Instruments ELx50 instrument (USA) according to the manufacturer's instructions, and data analysis was performed using a BioTek Instruments ELx800, USA. The experiments were performed in three replicates.

Statistical Analysis

Data are expressed as the mean ± standard error of the mean. Statistical analyses were conducted using SPSS for Windows, version 21.0 (IBM Corp., Armonk, NY, USA). To evaluate the normality of the data distribution, the one-sample Kolmogorov-Smirnov test was applied. Gene expression differences between adipose and thymus tissues in the control and experimental groups (C, F, and FA) were compared using one-way ANOVA with Dunn's post-hoc test. The effects of fructose administration and voluntary exercise were examined via two-way ANOVA, with Bonferroni's post hoc correction applied for group. GraphPad Prism for Windows v6.03 (GraphPad Software, Inc., La Jolla, CA, USA) was used for graph creation, and statistical significance was determined at $p<0.05$.

Results

Effects of Voluntary Exercise on Thymus Weight and Histology

Upon completion of the experimental protocol, the voluntary physical activity in the FA group was 9253 ± 3149 m/day. No significant differences were detected between the groups with regard to body weight (BW) (C=277 ± 10 g, F=219 ± 9 g and FA=22 ± 10 g, p=0.233). The thymus weight was calculated and compared with the relative weight corresponding to 100 g of BW. Although the thymus weight was higher in the F group than in the C and FA groups, the difference was not statistically significant. A significant reduction was observed in the FA group compared with the F group (p=0.015) (Figure 1).

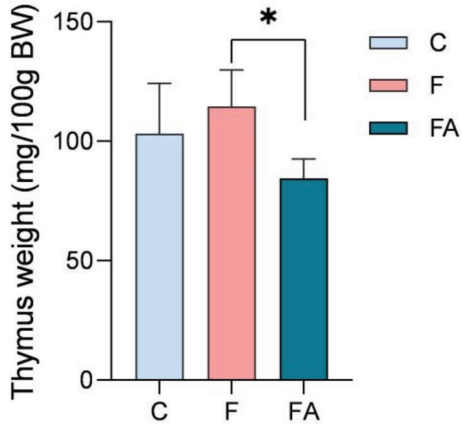

Figure 1. Comparison of thymus weights. Thymus weight (mg/g) was calculated as mg per 100 g body weight. (C) Control group, (F) high-fructose diet group, (FA) high- fructose diet, and voluntary physical activity group. n=6 per group. The data are presented as the means ± SEM (*p=0.022).

SEM: Standard error of mean

In the thymus of the C group, the border between the cortex and medulla was observed, and a normal histological structure was observed (Figure 2A). In the F group, the distinction between the cortex and medulla was not definitive, and a reduction in thymocyte density in the cortical region was observed (Figure 2B). In the FA group, the cortical thymocyte density was similar to that of the control group. The boundaries between the cortex and medulla were also distinguishable (Figure 2C).

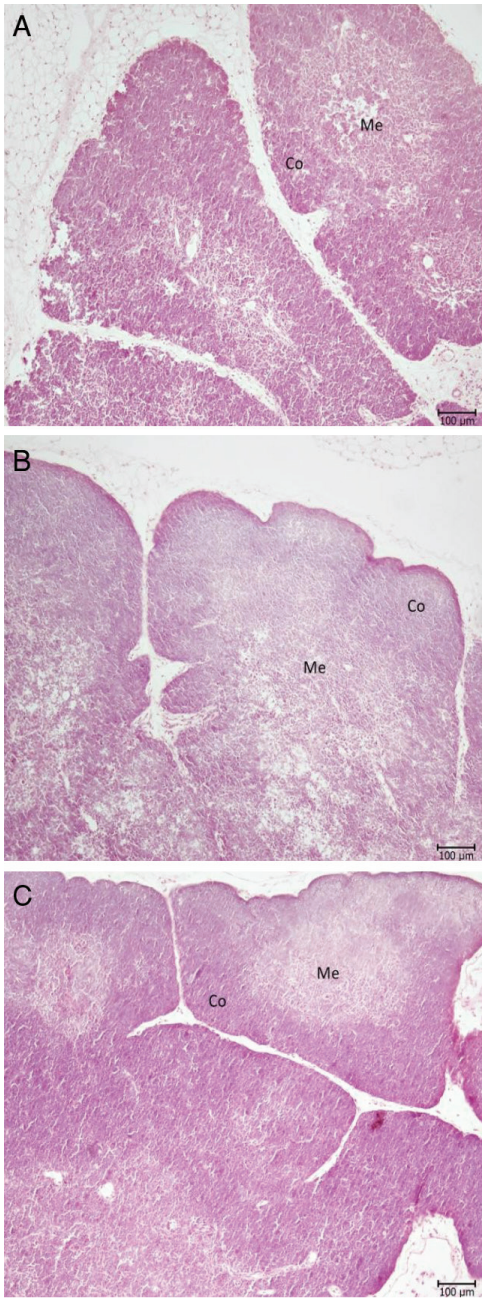

Figure 2. Rat thymus tissue was assessed by hematoxylin and eosin staining. A) C group. B) F group. C) FA group. (C) Control group, (F) high-fructose diet group, (FA) high-fructose diet and voluntary physical activity group. Co=Cortex, Me=Medulla. The light microscope magnification was 100x Scale bar, 100 µm.

Effects of High Fructose Diet on Serum Glucose Levels

When the serum glucose levels of all groups were compared, the glucose levels of the F group were significantly greater than those of the C group (p=0.028, Figure 3).

***AQP7* and *PLIN1* Gene Expression in Thymic Tissue**

Thymus tissue. The *AQP7* and *PLIN1* genes were expressed in the thymus tissue of rats (Figure 4 A and B, respectively).

AQP7 gene expression was decreased in the F group compared with the C and FA groups (p=0.0079 and p=0.0127, respectively). The comparison between C and FA groups were not significant (Figure 5 A). The differences between the groups regarding *PLIN1* gene expression were not significant (Figure 5 B).

Effects of Voluntary Physical Activity on Glycerol Levels in the Thymus and Visceral Adipose Tissue

Glycerol content was determined in thymic and visceral adipose tissue isolates. No differences were observed between the groups (Figure 6).

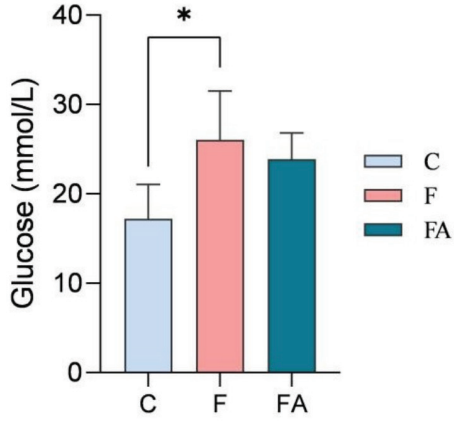

Figure 3. A comparison of the serum glucose levels between groups. (C) Control group, (F) high-fructose diet group, (FA) high-fructose diet, and voluntary physical activity group. n=6 per group. The data are presented as the means ± SEM (*p=0.028).

SEM: Standard error of mean

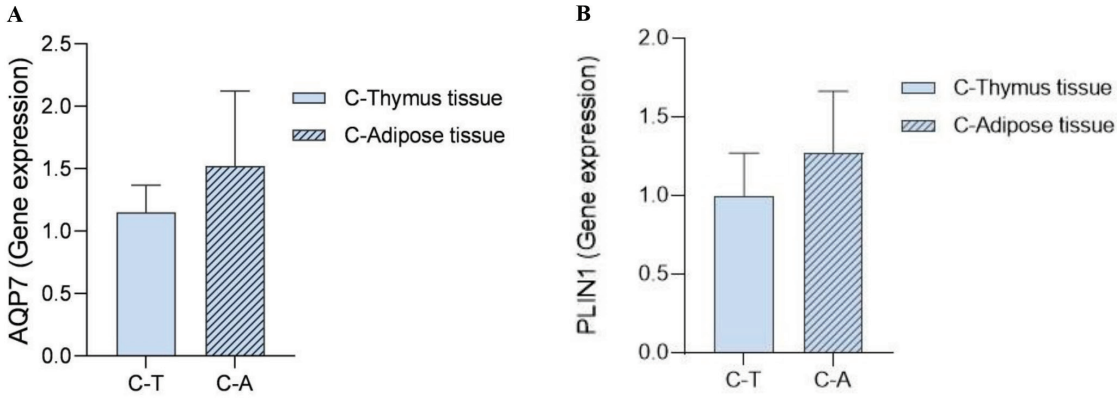

Figure 4. Demonstration of *AQP7* (A) and *PLIN1* (B) gene expression in thymus (C-T) and adipose tissue (C-A) in the control (C) group. For both AQP7 and PLIN1, n=6 per group, and the data are presented as the means ± SEM. (A) C-T vs. C-A; p=0.319, (B) C-T vs. C-A; p=0.937.

SEM: Standard error of mean

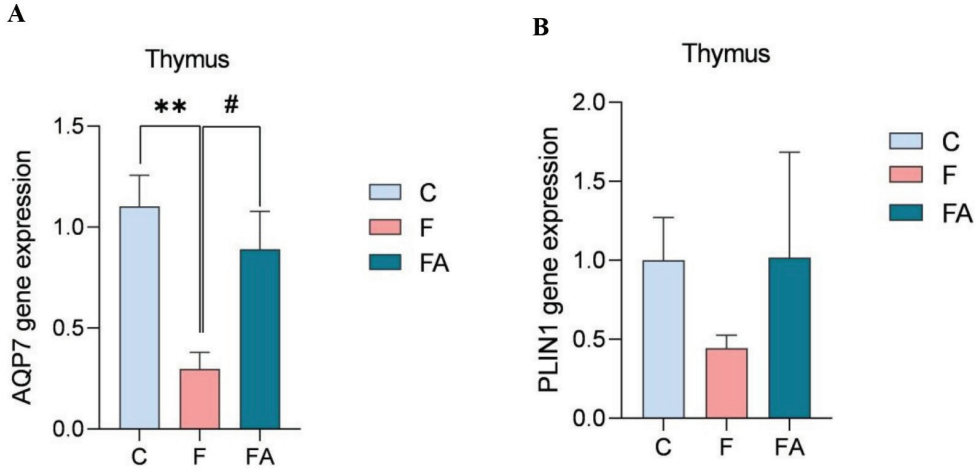

Figure 5. Comparison of AQP7 (A) and PLIN1 (B) expression in thymus tissue between groups. (C) Control group, (F) high-fructose diet group, (FA) high-fructose diet and voluntary physical activity group. n=6 per group. The data are presented as the means ± SEM (**p=0.0079, #p=0.0127). (A) C vs. FA; p=0.135, (B) C vs. F; p=0.699, C vs. FA; p=0.835, F vs. FA; p=0.999.

SEM: Standard error of mean

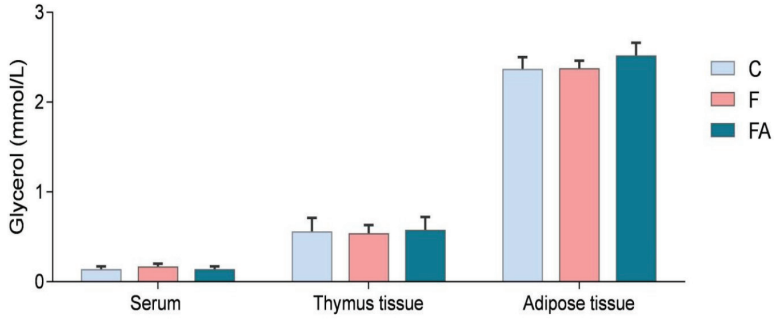

Figure 6. Comparison of glycerol levels in serum, thymus tissue and visceral adipose tissue between groups. (C) Control group, (F) high-fructose diet group, (FA) high- fructose diet, and voluntary physical activity group. n=6 per group. The data are presented as the means ± SEM. Serum: C vs. F; p=0.508, C vs. FA; p=0.992, F vs. FA; p=0.353. Thymus: C vs. F; p=0.999, C vs. FA; p=0.982, F vs. FA; p=0.405 Adipose: C vs. F; p=0.992, C vs. FA; p=0.110, F vs. FA; p=0.136.

SEM: Standard error of mean

Discussion

This study demonstrated that a high-fructose diet had a negative effect on thymus histology and decreased *AQP7* gene expression and that volunteer physical activity could reverse these effects. The differences in PLIN1 expression were not statistically significant. Although the thymus undergoes involution, its function and cellular capacity in adulthood are associated with many conditions, such as inflammation, infection control, vaccination success, autoimmunity development, and cancer surveillance (20). In this context, it is important to elucidate the effects of conditions that can be easily modified in modern life, such as exercise and diet, on thymus function. There is still some debate as to whether the increase in fructose consumption in recent years is beneficial or harmful to human health. Similar to other experimental studies in which fructose was administered in the drinking water or by adding fructose to the diet, in our study, a MetS model was created by adding 20% fructose to the animals' drinking water for 8 weeks (21,22). A high-fructose diet caused an increase in serum glucose levels. The increase in serum glucose levels due to fructose intake can be explained by the insulin-independent conversion of fructose to glycogen via metabolism in the liver and the release of liver glycogen into the blood as glucose (23). Low-grade chronic inflammatory diseases such as MetS and obesity, which can be caused by a high-fructose diet, accelerate thymic involution (24). A key feature of thymic involution is the deposition of adipose tissue within stromal compartments (7). Thymic involution starts after the 2nd month in rats (25). The study was conducted with 15-week-old rats. In the study conducted by Sengupta et al. (26), the human age equivalent of this group of rats was defined as 18 years of age. This age range includes young individuals in whom thymus involution has begun. Aquaglyceroporins, particularly AQP7, are key molecules that regulate the metabolic dynamics of adipose tissue (27). This study is one of the leading studies showing that AQP7 and PLIN1 are expressed in the rat thymus. AQP7 and PLIN1 expression differed among the C, F, and FA groups. AQP7 and PLIN1 were expressed at low levels in the thymus of sedentary rats fed a high- fructose diet. AQP7 expression was decreased in sedentary rats fed a high-fructose diet compared with the control group. Physical activity increased AQP7 expression in rats fed a high-fructose diet, bringing the expression levels closer to those of the control group. Physical activity reversed high fructose diet-induced changes in AQP7 expression in the thymus. Since there are no studies on AQP7 in the thymus, the functions of this molecule in the thymus tissue is not known. However, some findings indicate that AQP7 may play a role in antigen presentation processes that are important for thymus function. According to Hara-Chikuma M et al. (28), AQP7 in cutaneous dendritic cells is mainly responsible for antigen uptake and subsequent dendritic cell migration. Additionally, it has been suggested that it contributes to antigen presentation and the activation of downstream immune responses (28,29). Given that similar processes may occur in the thymus tissue, the roles of AQPs and AQP7 in thymic tissue function should be elucidated. The levels of glycerol, a molecule that mediates the exit of AQP7 from cells, were examined, and no difference was found in serum and tissue lysates. This result is likely related to the method used. A more sensitive investigation of intracellular/extracellular amounts may provide more meaningful results in functional terms.

A comparison of the weight of the thymus between the groups showed a significant reduction in the weight of the thymus in the FA group compared with the F group. Studies have demonstrated that physical activity has an effect on thymus weight. A study by Estruel-Amades et al. (30) supported our findings. In this study, it was reported that thymus weight was significantly decreased in exercise groups compared with sedentary groups when groups with different exercise intensities were compared (30). Another study by Droste et al. (31) showed that thymus weight decreased with voluntary physical activity and was associated with a decrease in serum glucocorticoid levels.

Further studies are required to elucidate the impact of high-fructose diet and voluntary physical activity on glucocorticoid levels. Although the weight of the thymus decreased in the FA group, the histological structure of the thymus was similar to that of group C in terms of thymocyte density in the cortex and the cortex-medulla junction. Another question that needs to be clarified is why the thymus weight decreases with exercise, despite the preservation of its histological structure. As the first study to demonstrate changes in AQP7 expression in rat thymus tissue in relation to high-fructose diet and voluntary physical activity, this study is important in terms of opening the door to issues that need to be clarified in this field.

Conclusions

The thymus is an organ that can be affected by conditions such as diet and exercise. More detailed studies are required to determine how the thymus is affected by these easily modifiable conditions. The contributions of these nonpharmacological treatment approaches to the preservation of thymic structure should be investigated. In this context, the potential of AQPs, which provide new perspectives on adipose tissue metabolism, as a strategic target to create conditions that can slow and/or reverse thymic involution should be understood.

Ethics

Ethics Committee Approval: Ethical approval was obtained from Trakya University, Local Ethics Committee of Animal Experiments (decision no: 2023.04.03, date: 26.04.2024).

Informed Consent: Not necessary.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: N.F., P.T., O.P., Concept: J.T., O.P., Design: J.T., O.P., Data Collection or Processing: J.T., N.F., E.G., O.E., P.T, O.P., Analysis or Interpretation: J.T., N.F., E.G., O.E., P.T, O.P., Literature Search: J.T., O.P., Writing: J.T., O.P.

Conflict of Interest: No conflict of interest was declared by the authors.

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