



# Changes in apoptosis and adipokine biomarkers in the heart tissue of rats with experimental hyperthyroidism

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## Abstract

**Background and purpose:** This study aimed to investigate the effects of hyperthyroidism on heart tissue through adipokines and apoptotic signaling pathways.

**Materials and Methods:** A total of 14 Sprague-Dawley male rats were assigned to 2 groups, a control and a hyperthyroid group. The control group received 0.9% NaCl, while the hyperthyroid group received 1 mg/kg levothyroxine dissolved in 0.9% NaCl throughout the study. Thyroid-stimulating hormone (TSH) and free thyroxine (FT<sub>4</sub>) levels in serum samples, whereas fibronectin type III domain-containing protein 5 (FNDC5), adiponectin, B-cell lymphoma-2 (Bcl-2) and tumour protein 53 (p53) levels in heart tissue were determined by ELISA method.

**Results:** Serum TSH level decreased ( $p < 0.01$ ) while the level of FT<sub>4</sub> significantly ( $p < 0.01$ ) increased in the hyperthyroid group compared to the control group. The level of adiponectin in the cardiac tissue of levothyroxine-treated rats was found to be significantly higher than in the control group ( $p < 0.01$ ). Additionally, compared to the control group, the antiapoptotic Bcl-2 level increased ( $p < 0.05$ ) while the proapoptotic p53 level decreased in the hyperthyroid group ( $p < 0.01$ ).

**Conclusions:** In this study, the effects of hyperthyroidism on hormone levels, which are important regulators of energy and metabolic homeostasis, and changes in apoptosis markers were revealed in heart tissue. We consider that apoptosis was potentially prevented by activating mechanisms in order to protect cardiac functions in the beginning, but this scene may be reversed in progressive hyperthyroidism cases. Therefore, their pathways need to be supported by more detailed and time-compared studies.

## INTRODUCTION

Hyperthyroidism is a pathological syndrome in which tissues are exposed to excessive amounts of circulating thyroid hormones, as a consequence of excessive production of thyroxine (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>), and is one of the most common endocrine diseases (1). Although hyperthyroidism affects many organs and systems, it shows its effect especially on the cardiac system and causes serious cardiac complications (1,2). It creates a hyperdynamic cardiovascular state as it leads to conditions such as increased left ventricular function, increased heart rate, and a marked reduction in systemic vascular resistance (3). Thyroid hormones (TH) regulate the basal metabolism and energy ho-

meostasis of tissues, but in this process, an increase in the formation of free oxygen radicals may occur as a result of the reduction of oxygen in the mitochondria. This causes the oxidant/antioxidant balance to be disrupted and oxidative stress to occur in hyperthyroidism, which is characterized by high TH levels, and as a result, DNA damage may occur and apoptosis mechanisms may be induced (4,5). However, the mechanisms involved in the pathogenesis of cardiac dysfunction mediated by thyroid dysfunction are quite complex and not fully elucidated.

Adipose tissue is not only a tissue that is effective in the regulation of energy homeostasis, but also a metabolically dynamic endocrine tissue that can synthesize many biologically active substances (6). Adipokines secreted from this tissue have key functions in many physiological processes (7). Irregularities in the secretion of adipokines are associated with oxidative stress and inflammation and play a key role in the initiation and progression of cardiovascular complications (8). Considering that adipose tissue is also under the influence of thyroid hormones (9), the interaction between hyperthyroidism and adipokines is very likely to contribute to cardiac dysfunction. Although it is known that hyperthyroidism is associated with cardiovascular changes, as noted above, little is known about the role of adipokines in this process.

Irisin is an important adipomyokine that is formed by the breakdown of fibronectin type III domain-containing protein 5 (FNDC5) molecules. The main effect of irisin is a conversion of white adipose tissue to brown adipose tissue by expending energy (10). Irisin, which is synthesized in many tissues such as skeletal muscle and adipose tissue, is intensely expressed in myocytes and plays an important role in the pathophysiology of cardiovascular diseases such as hypertension, coronary artery disease, and myocardial infarction (11). Changes in irisin level lead to mitochondrial dysfunction and accordingly, an increase in reactive oxygen species in cardiomyocytes and induce apoptosis (12). In addition, irisin shows a protective effect by supporting the regeneration of myocardial cells and myocardial repair originating from cardiac progenitor cells (11,12). Although adiponectin, which is associated with cardiovascular risk factors, is mostly known as an active peptide derived from adipose tissue, it is also intensely expressed in cardiomyocytes. It is also known that it has antiatherogenic and anti-inflammatory effects, increases endothelial vasodilation, and plays a role in myocardial remodelling after ischemic injury (13,14). At the same time, cardiovascular diseases can be triggered by the emergence of a hypermetabolic state due to hyperthyroidism, and at the same time, significant changes can occur in cardiac tissue energy metabolism. For this reason, there is an increasing interest in investigating the changes of irisin and adiponectin, which play a very active role in the regulation of energy and metabolic homeostasis, in heart tissue exposed to hyperthyroidism.

It is accepted that the Bcl-2 family can control the permeability of the mitochondrial outer membrane with mediators such as cytochrome C, and as a result, it plays an important role in the control and regulation of the mitochondrial pathway of apoptosis (15,16). Cardiomyocyte apoptosis is one of the major pathological changes that occurs in cardiac dysfunction. Growing evidence has emphasized a significant role for Bcl-2 through the p53 pathway in apoptosis and phases of cardiac diseases (17). Regulation by p53 has a critical function in the homeostasis of adult cardiomyocytes under physiological conditions (18). Previous investigations revealed a significant role of abnormal Bcl2 expression in cardiomyocyte apoptosis modulation in myocardial ischemia, heart failure, and myocardial infarction, etc. (16,19,20). It has been also suggested that dysfunction of the p53/Bcl-2 apoptosis signalling pathway act a role in cardiac hypertrophy in hyperthyroidism (21). Moreover, there is increasing evidence that disturbances in the regulation of expression of Bcl-2 family members may play a role not only in tumour formation but also in the pathogenesis of other diseases such as autoimmune diseases, infectious and neurodegenerative damage (22,23). However, in the literature reviews, there are conflicting results regarding the action pathways and mechanisms of Bcl-2 family members, and it is clear that more complicated studies are needed.

Although there has been an increasing interest in the effect of hyperthyroidism on the cardiovascular system in recent years, a limited number of studies have been found in the literature. This study aimed to evaluate the relationship between the changes in irisin and adiponectin levels and apoptosis markers in the heart tissues of rats with experimental hyperthyroidism.

## MATERIAL AND METHOD

### Experimental design

In the study, 14 Sprague-Dawley male rats, 1-2 months old, obtained from the Experimental Animals Application and Research Center of Kafkas University, were divided into groups as follows:

- Control group (n=7) - saline was applied intraperitoneally for 14 days;
- Hyperthyroid group (n=7) - levothyroxine 1 mg/kg daily was administered intraperitoneally for 14 days to induce hyperthyroidism.

During this period, animals were fed ad-libitum in accordance with standard heat, ventilation, and lighting conditions. All experimental procedures performed on experimental animals were carried out in accordance with national guidelines, and the necessary permissions were obtained from the Animal Experiments Local Ethics Committee of Kafkas University (2019/156).

### Preparation of samples and analyses

At the end of the study, blood samples were taken from animals under anaesthesia with 0.4 mL/kg sodium pentobarbital into tubes without anticoagulant. Then the heart tissues of the sacrificed animals were removed.

### Sample collection

Serum samples were allowed to clot for overnight at 2-8 °C before centrifugation for 20 min at 1000×g at 2-8 °C and isolation of supernatant for assays.

The heart tissues taken were washed in cold phosphate buffer (PBS; 0.01 M, pH=7.4) to remove blood that could remain in them. Then, pieces of similar size were cut and weighed, and 9 times their weight of PBS was added to the heart tissues and homogenized with a glass homogenizer on ice. The homogenates formed were centrifuged at 5000×g at 2-8 °C for 10 minutes in a refrigerated centrifuge, and heart tissue homogenates were obtained by collecting the supernatants.

The samples were kept at -80 °C until analysis. TSH (Elabscience, USA) and FT4 (Elabscience, USA) hormone levels in serum samples, and FNDC5 (Elabscience, USA), adiponectin (Elabscience, USA), Bcl-2 (Elabscience, USA) and p53 (Elabscience, USA) levels in heart tissue homogenates were determined with commercial ELISA kits (Elabscience, USA).

### Statistical analysis

As a result of Shapiro-Wilk tests (SPSS 18.0 package program), the data showed normal distribution, it was decided that parametric tests would be appropriate. Then, an independent sample t-test was performed to evaluate the variables between groups. The data obtained in the study are given as mean ± standard deviation; the statistical analysis results obtained were considered significant when  $p < 0.05$ .

### RESULTS

The results showed that the TSH levels was significantly lower in the hyperthyroid group compared to the controls ( $p < 0.01$ ), whereas free T4 (FT4) levels were statistically significantly increased ( $p < 0.01$ ) (Figure 1).

Adiponectin levels was significantly increased in heart tissues homogenates obtained from the group with hyperthyroidism compared to the control group ( $p < 0.01$ ). Although we observed the increased levels of the transmembrane protein FNDC5, which is the precursor of the irisin hormone, in the hyperthyroid group, the changes were not statistically significant ( $p > 0.05$ ) (Figure 2).

The levels of p53, one of the important apoptosis markers, decreased significantly in heart tissue homogenates compared to the control group ( $p < 0.01$ ). On the other

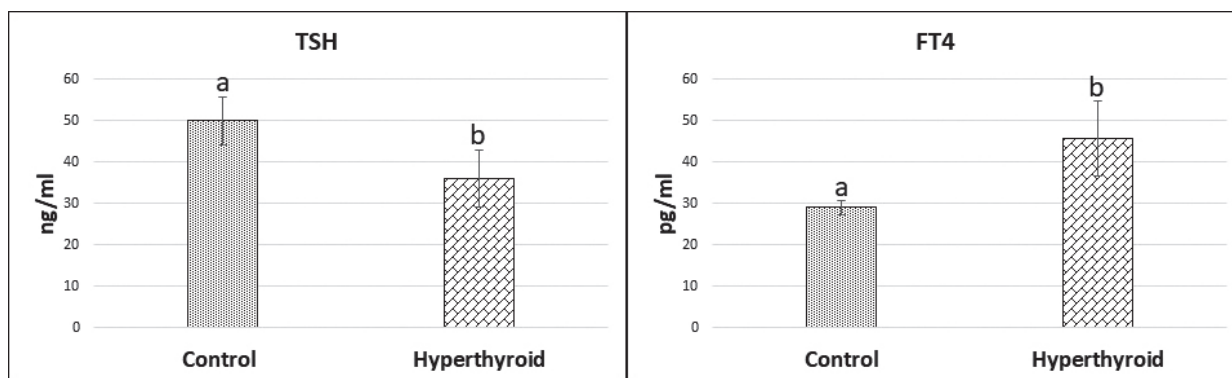


Figure 1. Serum TSH and FT4 levels of control and hyperthyroid group animals. a-b:  $p < 0.01$

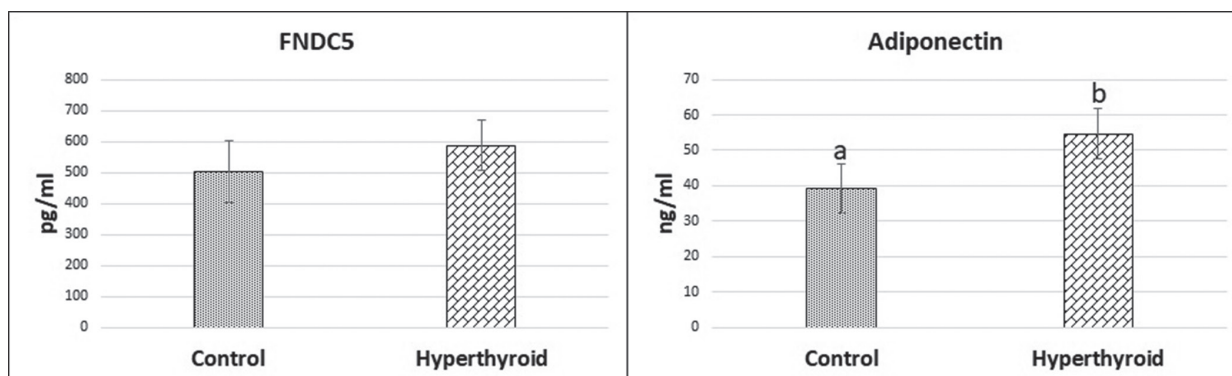
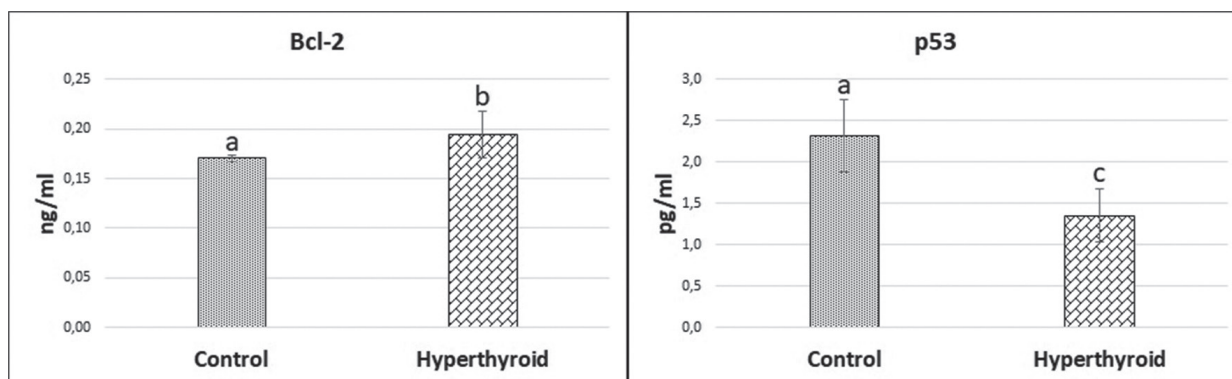


Figure 2. FNDC5 and adiponectin hormone levels in the heart tissue of control and hyperthyroid animals. a-b:  $p < 0.01$



**Figure 3.** Heart tissue Bcl-2 and p53 levels of control and hyperthyroid group animals. a-b:  $p < 0.05$ , a-c:  $p < 0.01$

hand, it was found that levels of Bcl-2, one of the most important antiapoptotic markers, increased significantly in the heart tissue of the hyperthyroid group compared to the controls ( $p < 0.05$ ) (Figure 3).

## DISCUSSION

Thyroid hormones have very important functions in regulating body temperature, providing the balance between the energy spent in the body and the energy production needed. For this reason, when thyroid dysfunctions occur, disruptions occur in the regulation of metabolism (24). Thyroid gland diseases are one of the most common endocrinological abnormalities that physicians encounter, and thyroiditis, hyperthyroidism, hypothyroidism and thyroid cancer are the leading ones. Hyperthyroidism is one of the most frequently encountered thyroid dysfunctions, and classical or primary hyperthyroidism is defined as a pathological condition characterized by increased synthesis and secretion of thyroid hormones (T3 and T4) and suppressed TSH levels (25,26). Although thyroid dysfunctions affect many organs and systems in the body, one of the most affected systems is the cardiovascular system. Although hyperthyroidism causes an increase in resting heart rate, blood volume, stroke volume, myocardial contractility and ejection fraction, and changes in energy homeostasis in the heart, it basically leads to the formation of a hypermetabolic state (27). Cardiac muscle is a highly oxidative tissue that produces more than 90% of its energy from mitochondrial respiration. The energy requirement of the heart tissue is regulated by strong energy signalling pathways between oxygen consumption and energy use (28,29). Irisin, a product of proteolytical cleavage of FNDC5, is not only an important regulator of energy metabolism, but it acts a myokine that is abundantly expressed in muscle, heart, and adipose tissues. The interest in investigating the role of irisin in cardiac physiology has increased with each passing day, with the detection of high levels of irisin production in the heart muscles even when exercise is not performed (30). Although little is known about the efficacy of irisin on cardiac tissue and its

role in cardiac hypertrophy and dysfunction, recent studies have shown that irisin increases the expression of some genes that protect cardiac myocytes and cardiac function and inhibits some genes that damage cardiac physiology (31) and it has been described as a new cardioprotective myokine (32). It has been suggested that irisin has various effects on mitochondrial dysfunction, oxidative stress, metabolic imbalance, energy expenditure, and heart failure prognosis, that it controls hypertension by modulating vasodilation, and can increase vasoconstriction through the hypothalamus (33). Considering these dual effects of irisin, it is thought that it may be a critical therapeutic target and one of the important physiological markers in cardiovascular diseases. In the study, it was determined that FNDC5 levels in the heart tissue were increased in the hyperthyroid group compared to the control group, but the changes were not statistically significant, and we consider that this increase may be due to the ability of the irisin to balance cardiac functions. Şahin *et al.* (34) stated that there is a significant relationship between serum irisin levels and thyroid hormones in hyperthyroidism, and that irisin levels may change in response to metabolic disorders such as increased thermogenesis and basal metabolic rate in hyperthyroidism. At the same time, changes in body mass, changes related to fat-glucose metabolism, and thermogenesis may occur in thyroid dysfunctions. Therefore, it has begun to arouse interest in whether changes in adiponectin levels accompany thyroid dysfunctions (35). Although adiponectin is mainly synthesized in adipose tissue, Piñeiro *et al.* (14) found that adiponectin was also synthesized and secreted by isolated murine and human cardiomyocytes. They also suggested that local production of this hormone by cardiomyocytes may play a role in the regulation of cardiac metabolism and function. It has been stated in previous studies that adiponectin protects the heart from events such as I/R damage, hypertrophy, and mediates inflammation, apoptosis, oxidative/nitrative stress, modulation of fibrosis and hypertrophy, and regulation of cardiac metabolism (36,37). In this study, it was determined that in experimentally induced hyperthyroidism, adiponectin levels in heart tissue increased signifi-

cantly compared to the control groups. In another study, it has been suggested that excessive thyroid hormones may cause an increase in circulating adiponectin, and that thyroid hormones may be one of the *in vivo* regulators of adiponectin secretion (38). However, some researchers stated that there was no change in serum adiponectin levels between hyperthyroid and control subjects. From this point of view, there are still contradictory results on the subject (39).

Upadhyay *et al.* (40) reported that the hypermetabolic state in hyperthyroidism causes oxidative damage in some tissues, including the liver, heart and muscles, and hyperthyroidism induces apoptosis in rat liver via the mitochondria-mediated pathway. Teixeira *et al.* (21) suggested that hyperthyroidism may lead to the activation of proteins associated with inflammation, apoptosis, hypertrophy, and heart failure. In another hyperthyroidism study, it was reported that there was no significant difference when the changes in serum p53, Fas-L, and Bcl-2 levels were evaluated in newly diagnosed, treated and control group subjects (41). In this study, it was found that there was a significant increase in Bcl-2 levels and a decrease in p53 levels in the heart tissue in the hyperthyroid group. It is thought that this may be related to the effect of thyroid hormones increasing the expression of protein Bcl-2 and decreasing the Bax:Bcl-2 ratio. This effect of thyroid hormones has been also mentioned by De Castro *et al.* (42). However, it has been demonstrated by various studies that this situation is reversed in cases of progressive hyperthyroidism, especially when hypertrophy occurs (43). Albrahim and Robert (44) stated that the kidney sections of mice with hyperthyroidism showed a mild positive reaction for Bcl-2 expression. In another study, they declined Bcl-2 expression was also decreased in the liver of groups treated with L-thyroxine (45). In the early stages of hyperthyroidism, mechanisms to protect cardiac functions can be activated to prevent apoptosis. However, the role of p53/Bcl-2 signaling and adipokines in cardiac dysfunction in hyperthyroidism is still not fully elucidated. In addition, the duration of hyperthyroidism, and its pathogenesis, the presence of another underlying disease, whether hypertrophy develops or not can cause differences in this scene. Little is known about hyperthyroidism and the efficacy of thyroid hormones on cardiac tissue apoptosis mechanisms and pathways, and more studies are needed.

As a result, in this study, the effects of hyperthyroidism on the levels of hormones, which are important regulators of energy and metabolic homeostasis in heart tissue, as well as apoptosis markers levels were revealed. Additional studies are needed to elucidate apoptosis mechanisms and pathways in more detailed.

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