RESEARCH ARTICLE

Effect of Hyperthyroidism on Lipid Metabolism and Evaluation of the Protective Role of Pomegranate Juice against the Risk of Oxidative Stress in Albino Rats

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Received: 11th October, 2021; Revised: 09th December, 2021; Accepted: 27th January, 2022; Available Online: 25th March, 2022

ABSTRACT

The study aimed to demonstrate the effect of hyperthyroidism on lipid metabolism and the importance of the protective role of pomegranate juice in reducing oxidative stress caused by hyperthyroidism. Thirty albino male rats (weights 125 ± 5 and age 4–6 weeks) were raised in the animal house of the College of Veterinary Medicine at Tikrit University for a period of 60 days. It was divided into six groups, the first included the control group treated with (1 mL of distilled water), the hyperthyroid group treated with thyroxine (TH) (300 µg/kg), the group treated with pomegranate juice (PG) (1 mL/100 g), and the group treated with thyroxine and pomegranate juice (TH +). PG), and the group treated with thyroxine and carbimazole (TH + CM) (0.5 mg/kg) and finally the synergistic group treated with thyroxine with pomegranate juice and carbimazole (TH + PG + CM). After the end of the experiment, blood samples were taken for biochemical tests. The results showed that (TH) treatment led to a significant increase in the level of T3 and T4 hormones and the level of malondialdehyde MDA and a significant decrease in the concentration of TSH hormone, lipid profile and antioxidants compared to the control group. The (PG) treatment led to a significant decrease in the level of total cholesterol TC, while the other studied treatments did not show significant differences compared with control group. The study showed that the treatment (TH + PG) led to a significant decrease in the level of T3 and T4 hormones and the level of MDA and a significant increase in the level of TSH, the level of TC cholesterol, triglycerides TG, high-density lipoproteins (HDL), the level of antioxidants in the blood serum and liver extract in compared with the treated group (TH). The treatment (TH + CM) showed a significant decrease in the level of thyroid hormones and a significant increase in the concentration of TSH and the level of lipid profile, while there were no significant differences in the level of antioxidants in the blood serum and liver extract except for SOD in the blood serum compared with (TH) group. The treatment (TH + PG + CM) also showed a significant decrease in the level of thyroid hormones and MDA in the blood serum and a significant increase in the concentration of TSH, while a significant increase in the level of TC, HDL and LDL and an increase in the level of antioxidants in the blood serum, while TG and LDL did not appear. VLDL showed significant differences in compared with the (TH) group. We conclude from the current study the specific biological role of thyroid hormones in the metabolism and growth of white rats and the protective role of pomegranate juice in improving the disorders caused by hyperthyroidism.

Keywords: Carbimazole, Hyperthyroidism, Oxidative stress, Pomegranate juice.

International Journal of Drug Delivery Technology (2022); DOI: 10.25258/ijddt.12.1.67

How to cite this article: Mahmood MS, Ahmed MA. Effect of Hyperthyroidism on Lipid Metabolism and Evaluation of the Protective Role of Pomegranate Juice against the Risk of Oxidative Stress in Albino Rats. International Journal of Drug Delivery Technology. 2022;12(1):373-378.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

The thyroid gland is one of the most important endocrine glands in the body. Its hormones thyroxine (T4) and triiodide (T3) are of great importance in regulating vital activities and metabolic processes, as these hormones contribute to regulating the function of almost all body tissues.[1] The increase in the secretion of thyroid hormones is one of the pathological conditions that affect the functioning of this gland. These cases are thyroid disorders, which may be caused by tumors affecting the gland, and be either malignant or benign, resulting in a condition known as hyperthyroidism.[2] In several studies, the relationship between excessive levels of thyroid hormones and serum lipids has been observed, as a decrease in the level of TG, TC and LDL-C was observed on the one hand, and the stimulation of LDL-C oxidation process on the other hand, which in turn leads to an increase in the incidence of

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heart diseases.[3] It was found that hyperthyroid patients suffer from symptoms that indicate changes in the hemodynamic dynamism and movement of the cardiovascular system, and these changes are the opposite of what happens in the case of hypothyroidism.[4] Several studies have shown a complex relationship between the thyroid gland and the lirer,[5-6] where there is a relative relationship between thyroid diseases and the damage in the liver.[7] The thyroid gland influences the growth and development of body organs and the regulation of energy metabolism in a regular way, all of which depends on the natural relationship between the functions of the thyroid gland and the liver.[6,8] Given the current development in studies on using herbs and plants in medical treatments instead of the chemicals contained in manufactured drugs, God almighty has blessed us with many of these beneficial plants that can be used as food or medicine. Many studies have proven that the extracts of these plants and herbs give a better result than the chemically manufactured substance itself, which may be associated with negative side effects, but when extracting the substance from the plant, the presence of secondary substances with the active substance enhances the role of the plant substance in treatment. Pomegranate is a food rich in antioxidants that maintain the body cells to resist diseases. The multiple phenolic compounds present in pomegranate are three times their presence in green tea and eight times that in grapes, apple juice and oranges.[9-10] Pomegranate juice has a preventive and chemotherapeutic activity for many diseases, including cancerous diseases, as it is one of the rich sources of Ellagic acid, limiting genetic mutations that may turn into cancerous cells. It inhibits its activity in thyroid tissue.[11] The study aims to know the effect of hyperthyroidism in laboratory animals on lipid metabolism and the protective role of pomegranate juice in reducing the harmful effects of hyperthyroidism.

MATERIALS AND METHODS

Collecting Pomegranate Fruits and Preparing Juice

Pomegranate fruits (*Punica granatum*) of the Khoshi type were collected from a farm in Salah al-Din Governorate in the month of October (2020). The fruits were washed, then the seeds (Aril) were smashed manually, then the seeds were pressed using an electric squeezing machine (DMS), and the pomegranate juice was prepared. The juice was stored frozen in the refrigerator until use.

Measuring the Effectiveness of Free Radical Scavenging

The ability of pomegranate juice to scavenge free radicals was measured using the method of Liu *et al.* (1997) and modified by Xu and Chang (2007) as follows:

The Method

For every 1-mL of juice dilution, add 3 mL of a solution (0.1mM) of 2.2-Diphenyl-1-picrylhydrazyl scavenging activity (DPPH) and mix the solution vigorously and then leave in the dark for (30) minutes to complete the reaction (The color turned violet to pale yellow), then it was measured at the wavelength (517) nm versus the solvent-only efficient (Shimamura *et al.*, 2014; Genwali *et al.*, 2013; Otohinoyi *et al.*, 2014). Percentage of inhibition according to the method attached to the test kit.

Preparing Study Animals

In this study, 30 albino male rats, their ages ranged from (4–6) weeks, and their weights ranged between (125 ± 5) g, obtained from the animal house of the College of Veterinary Medicine, Tikrit University, were used in this study. They were placed in plastic cages with dimensions (40 x 30 x 40) cm. Rats were reared in the animal house unit, College of Veterinary Medicine, Tikrit University, where these rats were subjected to appropriate laboratory conditions in terms of ventilation and temperature of 25 ± 1°C, and lighting was at an average of 12 hours of light and 12 hours of darkness. The rats were fed a standard diet consisting of (maize 35%, wheat 35%, soybean 20%, concentrated protein 10%, and dry milk 1%) in addition to preservatives.

Distribute the Study Animals

Rats were divided into six groups, the first included the control group treated with (1 mL of distilled water), the hyperthyroid group treated with thyroxine (300 µg/kg), the group treated with pomegranate juice (1 mL/100 g), the group treated with thyroxine and pomegranate juice together, and the group treated with thyroxine and treatment with carbimazole (0.5 mg/kg) and finally the synergistic group treated with thyroxine with pomegranate juice and carbimazole together. After the end of the experiment, blood samples were taken for biochemical tests.

Blood Sample Collection

The animals were starved for 12 hours before the start of the autopsy, the animals were weighed and anesthetized using chloroform prepared from (Fluka AG) company, blood samples were collected by cutting the jugular vein, and the blood was placed in gel tubes, then the samples were discarded using a centrifuge at a speed of 3000 One cycle per minute for 15 minutes, separating the serum from the other components using micropipettes, then keeping the serum in the freezer at -80°C until the required tests are performed, which is the measurement of the lipid profile, which includes cholesterol and triglycerides. High-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), hormone levels (T4, T3, TSH) and levels of antioxidants glutathione (GSH), super-oxide dismutase (SOD) and catalase), and analysis of the level of lipid peroxide Malondialdehyde (MDA) in the blood serum.

Method of Preparation Liver Tissues Extraction

Liver samples of 1 g were taken and placed in the crushed homogenization medium (HM), which consisted of tris-HCL buffer (mM20), EDTA (mM1), sucrose (0.25M), with a pH of 7.4. Where each sample of liver (1 g) was placed in 10 mL of this medium and placed in refrigerated test tubes and placed in deep freeze, the liver pieces were crushed by a tissue crushing device Homogenizer type (SHM1) at a speed of 16,000 rpm for 30 seconds, Then the extract obtained from the crushing was

transferred to a refrigerated centrifuge at a speed of 3000 rpm in 15 minutes. After the centrifugation process was completed, the filtrate was taken and kept in the freezer at a temperature of (-80) (Al-Qaraghuli *et al.*, 2014). Biochemical represented by SOD, GSH, CAT and MDA.

Statistical Analysis

Significant differences were extracted using ANOVA-one way test, and these differences were confirmed by standard error, and significant differences were determined according to Duncan's multiple ranges test at a level of significance ($p \leq 0.05$) (Bruning and Kintz, 1977).

RESULTS

The study results showed the effectiveness of pomegranate juice on curbing free radicals, as the shape indicates that the juice has the ability to scavenge free radicals, and this is due to the effective components of pomegranate juice as antioxidant activity.

The results of the current study (Table 1) showed a significant increase in the concentration of T4 and T3 and a decrease in the concentration of TSH in the blood serum of group treated with thyroxine and no significant difference in the concentration of T4, T3 and TSH in the blood serum of group treated with thyroxine juice. Pomegranate compared with the healthy control group, and a significant decrease in the concentration of T4 and T3 hormones and an increase in the TSH concentration in the blood serum were found in the groups treated with thyroxine, pomegranate juice, thyroxine, carbimazole, thyroxine, pomegranate juice and carbimazole compared to the group treated with thyroxine only.

Table 1: Concentration of T4, T3 and TSH in blood serum

	T4 (mIU/mL)	*T3 (mIU/mL)*	*TSH (mIU/mL)*
Control	82^{b}	88.25^{c}	2.03^{b}
TH	115.5^{a}	123.33^{a}	0.923^{d}
PG	84.2^{b}	88.67^{c}	2.01^{b}
TH+ PG	95.75^{b}	102^{b}	1.64^{c}
TH+ CM	67.5^{c}	66^{d}	2.89^{a}
TH+PG+CM	84^{b}	73^{d}	2.14^{b}

-different letters mean a significant differences in value $p \leq 0.05\%$.
-TH=Thyroxine (300 µg/kg), PG=pomegranate juice (1-mL/100g), CM= carbimazole (0.5 mg/kg).
-TH and PG compared versus to control, other groups versus to TH.

The results showed, as in Table 2, a significant decrease in the concentration of total cholesterol (TC), triglycerides (TG), high-density lipoproteins (HDL), low-density lipoproteins (LDL), and very-low-density lipoproteins (VLDL).) in the blood serum of the group treated with thyroxine compared to the healthy control group, while the group treated with pomegranate juice showed a significant decrease in the concentration of TC and no significant difference in the concentration of TG, LDL, VLDL and HDL in the blood serum compared with the healthy control group, while the treatment with thyroxine and pomegranate juice showed a significant increase in the concentration of TC, TG and HDL and no significant difference in the concentration of LDL and VLDL in the blood serum compared with the group treated with thyroxine, while the results of the group treated with thyroxine and carbimazole showed a significant increase in the concentration of TC, TG and HDL, LDL, and VLDL in serum compared with the thyroxine-treated group, while the group treated with thyroxine, pomegranate juice and carbimazole showed a significant increase in the concentration of TC,

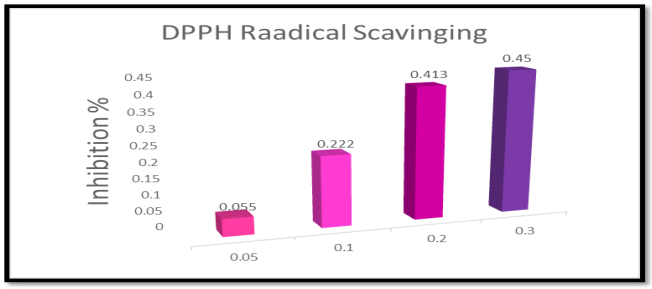

Figure 1: Shows the inhibitory ability of pomegranate juice to inhibit free radicals

Table 2: Lipid profile concentration in blood serum

	T C (mg/dL)	*TG (mg/dL)*	*HDL (mg/dL)*	*HDL (mg/dL)*	*LDL (mg/dL)*	*VLDL (mg/dL)*
Control	79.25 ± 6.75 b	63.75 ± 3.86 b	17.0 ± 2.65 a	17.0 ± 2.65 A	52.4 ± 1.89 b	13.5 ± 0.52 b
TH	43.50 ± 9.33 d	45.67 ± 7.57 c	6.67 ± 1.53 c	6.67 ± 1.53 C	28.53 ± 4.7 d	9.13 ± 1.51 c
PG	69.25 ± 4.57 c	57.0 ± 5.57 bc	19.0 ± 2.0 a	19.0 ± 2.0 a	34.67 ± 6.54 c	10.2 ± 2.4 bc
TH+PG	64.33 ± 5.69c	62.0 ± 2.65 b	13.75 ± 3.02 b	13.75 ± 3.02 b	36.8 ± 3.68 c	10.1 ± 2.3 bc
TH+CM	91.33 ± 7.37 a	86.67 ± 3.79 a	15.0 ± 4.24 a	15.0 ± 4.24 a	61.3 ± 4.48 a	15.4 ± 2.6 a
TH+PG+CM	65.67 ± 4.73 c	52.33 ± 4.16 c	16.5 ± 3.54 a	16.5 ± 3.54 a	54.8 ± 5.1 ab	9.33 ± 1.17 c

-different letters mean a significant differences in value $p \leq 0.05\%$.
-TH=Thyroxine (300 µg/kg), PG = pomegranate juice (1-mL/100g), CM= carbimazole (0.5 mg/kg).
-TH and PG, compared versus to control, other groups versus to TH.

Table 3: Oxidant-antioxidant statues in blood serum

	GSH µmol/mL	*CAT kU/mL*	*SOD µmol/mL*	*MDA nmol/mL*
Control	0.466 ± 0.02^{ab}	1.45 ± 0.03^{a}	0.812 ± 0.02^{a}	1.17 ± 0.04^{c}
TH	0.318 ± 0.01^{d}	0.966 ± 0.03^{d}	0.463 ± 0.01^{d}	2.19 ± 0.05^{a}
PG	0.488 ± 0.03^{a}	1.57 ± 0.02^{a}	0.833 ± 0.02^{ab}	1.106 ± 0.04^{c}
TH+PG	0.419 ± 0.03^{bc}	1.23 ± 0.01^{b}	0.622 ± 0.01^{b}	1.41 ± 0.02^{b}
TH+ CM	0.355 ± 0.01^{cd}	1.03 ± 0.02^{cd}	0.504 ± 0.02^{c}	2.27 ± 0.05^{a}
TH+PG+CM	0.407 ± 0.01^{bc}	1.19 ± 0.03^{bc}	0.636 ± 0.01^{b}	1.51 ± 0.04^{b}

-different letters mean a significant differences in value $p \leq 0.05\%$.
-TH=Thyroxine (300 µg/kg),PG=pomegranate juice(1 mL/100g), CM= carbimazole (0.5 mg/kg).
-TH and PG compared versus to control, other groups versus to TH.

Table 4: Oxidant-antioxidant status in liver homogenized tissue

	GSH µmol/g	*CAT kU/g*	*SOD µmol/g*	*MDA nmol/g*
Control	0.422 ± 0.06^{ab}	1.26 ± 0.2^{ab}	0.644 ± 0.2^{ab}	1.21 ± 0.2^{c}
TH	0.314 ± 0.02^{d}	0.67 ± 0.01^{d}	0.374 ± 0.04^{d}	1.99 ± 0.2^{a}
PG	0.471 ± 0.01^{a}	1.48 ± 0.3^{a}	0.776 ± 0.1^{a}	1.08 ± 0.3^{c}
TH+PG	0.385 ± 0.03^{b}	1.1 ± 0.1^{bc}	0.554 ± 0.2^{b}	1.51 ± 0.2^{b}
TH+ CM	0.326 ± 0.03^{cd}	0.84 ± 0.04^{cd}	0.401 ± 0.1^{c}	1.75 ± 0.2^{ab}
TH+PG+CM	0.374 ± 0.02^{c}	1.06 ± 0.04^{c}	0.469 ± 0.2^{bc}	1.89 ± 0.2^{a}

-different letters mean a significant differences in value $P \leq 0.05\%$.
-TH=Thyroxine(300 µg/kg),PG=pomegranate juice(1 mL/100g), CM= carbimazole (0.5 mg/kg).
-TH and PG compared versus to control, other groups versus to TH.

HDL, LDL and There was no significant difference in the concentration of TG and VLDL in the blood serum compared with the thyroxine-treated group.

The results of the current study, as in Tables 3 and 4, showed a significant decrease in the concentration of Glutathione (GSH), CAT and SOD. An increase in the level of MDA in the group treated with thyroxine, and no significant difference in the concentration of GSH, CAT and SOD in the serum of the group treated with juice Pomegranate compared with the healthy control group, the results also showed a significant increase in the concentration of GSH, CAT and SOD in the blood serum in the group treated with thyroxine and pomegranate juice and the group treated with thyroxine, pomegranate juice and carbimazole, as well as a significant increase in the concentration of SOD and no significant difference in The level of GSH and CAT in the group treated with thyroxine and carbimazole compared with the group treated with thyroxine.

DISCUSSION

Thyroxine treatment-induced hyperthyroidism in rats, as the level of T4 and T3 hormones increased in the blood, and in turn a significant decrease in TSH level occurred (Table 1). The decrease in TSH level resulting from T4 treatment is due to the negative feedback mechanism Back Mechanism An increase in T4 level will stimulate Thyrotrophs in the anterior lobe of the pituitary gland to lower TSH production.[12,13] Also, the increase in the level of T4 will affect the cells of the hypothalamus, reducing the secretion of Thyrotropin Releasing Hormone (TRH), which in turn inhibits the production of TSH.[14,15] It was also noted through the results of the study (Table 1) that pomegranate juice maintains the level of thyroid hormones when they are present in normal concentrations in healthy rats, while it was noted that it has a reducing effect on hormones when they are present in high concentrations in the blood. This may be because pomegranate juice contains flavonoids, including Rutin, which increases the expression of the TSH gene and thus increases its secretion from the pituitary gland. Iodide is taken by the thyroid gland to make its own hormones and stimulates the enzyme thyroperoxidase, which is the key to the synthesis of thyroid hormones.[16,17] The previous researches[18,19] mentioned in a study that the effect of pomegranate on T4, T3 and TSH hormones is due to the content of pomegranate juice on vitamins A, E, and C, and that vitamins E and C work to increase the secretion of hormones. Thyroid gland, as these vitamins stimulate the pituitary gland to secrete TSH and thus stimulate the secretion of T4 and T3 hormones from the thyroid gland, as noted by[20] that pomegranate contains Ellagic acid, which has an effect in increasing thyroid secretion of both T4 and T3 hormones. In addition, pomegranate juice contains anthocyanins that raise the concentration of adiponectin. This hormone has an important role in increasing the synthesis of thyroid hormones, especially T4, as a result of the interaction of the terminal C-globular of adiponectin with receptors located in the mitochondria of thyroid cells, and this indicates the contribution of adiponectin hormone with Thyroid hormones in some of their physiological functions such as reducing body fat, increasing fat oxidation and regulating temperature.[21] Pomegranate juice increases the level of insulin secretion, which increases the ability of cells to take glucose from the blood, thus stimulating metabolic activities in cells.[22]

Likewise, pomegranate juice regulates the concentration of thyroid hormones either through gamma-linoleic acid (GLA), which can control and regulate the secretion of thyroid hormones then reduce the severity of thyroxine action and inhibit its effect, or Thiocyanins, when iodine levels are excessive or thyroid hormone levels are high, to inhibit the union of iodide with thyroglobin by inhibiting the enzyme thyroid peroxidase and thus reduce the formation of thyroid hormones that destroys thiocyanins and thus stimulates thyroid cells to take up iodine and manufacture thyroid hormones.[23] Carbimazole may block the T3 and T4 formation pathways, leading to a decrease in blood serum level.[24] Some researchers[25] noted that carbimazole may inhibit the transformation of T4 to T3 in tissues. Also some researchers[26] found that giving propylthiouracil leads to a significant decrease in T3 and T4 levels. As well as the group treated with thyroxine, pomegranate juice and carbimazole together, the statistical analysis results showed a decrease in the concentration of T4 and T3 hormones and an increase in the concentration of TSH. It had been previously explained in terms of the effect of linoleic acid present in the pomegranate and the effect of carbimazole in increasing the secretion of TSH indirectly through Impeding the formation of thyroid hormones. In this case we can say that pomegranate juice and carbimazole have a synergistic role in lowering thyroid hormones and thus reducing the harmful effect of hyperthyroidism. The decrease in the level of total cholesterol is due to thyroxine. In a study conducted by some researchers.[27] The levels of total cholesterol in the blood of hyperthyroid patients were significantly lower than the control group and significantly different when compared with hypothyroid patients. Many studies have also been conducted on a group of hyperthyroid patients that the reason for the low level of cholesterol is due to the fact that the final effects of the high level of thyroid hormones are the decrease in the stores of most fats.[28] Hyperthyroid patients had lower total and LDL cholesterol levels and that fT3 and fT4 were inversely associated with cholesterol, LDL and HDL levels, while TSH was positively correlated with cholesterol, LDL and HDL, and in the case of low The level of TG in the blood serum.[29] It may be due to the fact that treatment with thyroxine stimulates the process of removing chylomicron residues from the blood, or it may stimulate the lipolytic enzyme, which in turn breaks down the TG-rich lipoproteins, and at the same time, thyroxine activates the protein that transports cholesterol ester. Transfer protein (CETP) transports TG to VLDL-C for degradation, thereby reducing serum TG levels.[30] The decrease in the level of HDL in the blood serum is due to the high level of oxidation and the activity of the enzyme Cholesterol ester transferase, which works to transfer cholesterol ester from HDL-C to VLDL-C, so that HDL becomes rich in triglycerides and reduces its affinity to Apo-A, so it remains free, which leads to its filtration from the kidneys.[31] Thyroid hormones stimulate hepatic lipase, which breaks down the HDL-C molecule and takes cholesterol from it inside the liver cells to be converted into bile acids.[32] It also stimulates CETP, which transfers cholesterol to HDL-C molecule and then Transfers it to the liver. Concerning the decrease in LDL concentration, studies conducted on a group of hyperthyroid patients have shown that a decrease in the level of lipid concentrations was observed, especially in the level of LDL-C concentration.[27] Hyperthyroid patients had significantly lower HDL and LDL levels than hypothyroid patients.[33] Decrease in LDL-C may be due to the ability of thyroxine to penetrate the cell membrane of cells due to its ability to dissolve in fats.[34] That thyroxine has a mechanism that facilitates its entry into cells using LDL-C receptors that are found on the surfaces of cells, especially Liver cells, where LDL-C receptors are used to facilitate the entry of cholesterol into the cells, and when T4 enters the target cells, it turns into the active form T3 by deiodination.[35] The T3 hormone controls the process of LDL-C regulation by the liver, which regulates the process of taking cholesterol from the blood with the process of manufacturing LDL and then destroying it in the same organ,[36] and thyroid hormones have an important role in controlling the metabolism process. Cholesterol through its own receptors, where the binding of T3 with its receptors leads to an increase in gene expression and the translation and transcription of special genes responsible for special enzymes, including: (HMG-CoA) the enzyme key to the steps of intracellular cholesterol synthesis, as well as the initiators of LDL receptor genes -C contains thyroid hormone response elements that allow T3 to regulate gene expression for the manufacture of LDL-C receptors and thus increase it on the surfaces of hepatocytes. In addition, T3 and T4 hormones reduce LDL levels due to their stimulation of LDL-C receptor gene expression.[37] As for the decrease in VLDL concentration, in a study, it was observed that an increase in VLDL molecule catabolism rate in the blood serum and therefore, its percentage in the blood decreased. The reason is due to the effect of T4 on the activities of TG-rich lipolytic enzymes.[30]

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