Original Article

Adropin and Preptin Levels in β-Thalassemia Major Complications in Babylon Province

Zinah Abbass Ali, Samar Hasan Shammar
Department of Biochemistry, College of Medicine, University of Babylon, Hilla, Iraq

Abstract

Background: Thalassemia refers to a group of diseases in which one or more globin chains are produced less or not at all, causing a wide range of phenotypes from anemia to clinically asymptomatic individuals. Disturbances in the homeostasis of carbohydrates, serum lipids, and oxidative stress are the most common diseases brought on by iron overload in beta-thalassemia major (β-TM). **Objective:** To examine the association of biochemical parameters with β-TM complications in Babylon Province. **Materials and Methods:** This study involved 100 individuals, separated into two groups: 50 subjects and 50 controls. Commercial enzyme-linked immunosorbent assay kits were used to measure cystatin-C, ferritin, insulin, preptin, and adropin, while serum blood urea (B. urea), serum creatinine (S.Cr), and lipid profile along with fasting glucose concentration were calculated by colorimetric kit using the spectrophotometer method. **Results:** Serum samples were used to measure biochemical parameters of S.Cr, urea, cystatin-C, ferritin, fasting insulin, fasting glucose, adropin, preptin, and lipid profile. Adropin in the patient group of thalassemia was significantly decreased compared to the healthy controls ($P < 0.001$). However, preptin, cystatin-C, ferritin, B. urea, and S.Cr were elevated in the patient groups compared to the control group ($P < 0.005$). Also, the lipid profile, including triglycerides, cholesterol, high-density lipoprotein, low-density lipoprotein, and very low-density lipoprotein, showed significant differences between patient group of thalassemia and the control group. **Conclusion:** Adropin and preptin were considerable predictive markers for the development of complications in patients with β-TM.

Keywords: Adropin, β-thalassemia major, creatinine, preptin, urea

Introduction

Patients with thalassemia were found to have carbohydrate dysfunction, including glucose intolerance, hyperglycemia, reduced beta cell activity, and insulin resistance.

Reduced production of one or more globin chains causes thalassemia. The most significant categories are those that influence either alpha or beta-chain synthesis. Beta-thalassemia is caused by not creating adequate beta globin chain production, which results in more alpha globin chains than normal. These extra alpha globin chains are not soluble and build up inside the red cell.[1] This can cause a number of clinical symptoms. A person is diagnosed with beta-thalassemia major (β-TM), usually known as Cooley anemia, if the synthesis from both genes is significantly impaired or absent altogether. Because fetal hemoglobin is present at birth, people who have β-TM usually do not have any symptoms when they are first born. However, by the age of 6 months, symptoms typically start to appear. In untreated patients, this illness is marked by transfusion-dependent anemia, large splenomegaly, bone abnormalities, growth retardation, and a distinctive facial appearance.[2] Because of the accumulation of iron in the spleen, liver, heart, and endocrine organs, iron overload causes significant cellular damage and malfunction in these organs. In addition, disturbances in the homeostasis of serum lipids, carbohydrates, and oxidative stress are the most

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Address for correspondence: Dr. Zinah Abbass Ali, Department of Biochemistry, College of Medicine, University of Babylon, Hilla, Iraq.
E-mail: Zenaabbass1980@gmail.com

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common diseases brought on by iron overload in β-TM. These include carbohydrate dysfunction, such as glucose intolerance, hyperglycemia, reduced beta cell activity, and insulin sensitivity.[3-5]

Growth in serum level total cholesterol, low-density lipoprotein cholesterol (LDL-C), and a decrease in high-density lipoprotein cholesterol (HDL-C) are the well-known causative factors and predictors of coronary heart disease enlargement in patients with β-TM.[6]

In β-thalassemia patients, the emergent renal complications have elevated the universal conversation of views. Despite healthier survival caused by transfusion of blood and the therapy by iron chelation, the previously unrecognized renal complications persist as a burden of disease affecting these people.[6] There is less information about the effects of thalassemia on the kidney. Irregularities in kidney function, like elevated renal plasma flow, reduced urine concentrating ability, renal tubular acidosis, anemia, and iron-mediated toxicity, are considered risk factors for these abnormalities.[7] Oxidative stress and lipid peroxidation are produced by hypoxia and chronic anemia, which decrease tubular cell function. In thalassemia individuals, excess iron plays a significant role in the etiology of kidney damage, and glomerular dysfunction can be a side effect of iron chelator poisoning; in addition, renal impairment may be brought on by iron overload-induced hepatic and cardiac dysfunction.[8]

Adropin is one of the biomarkers that can be impacted by TM. Both animals and humans have the hormone adropin in their circulatory systems. It consists of 43 amino acids and is formed through the proteolytic cleavage of precursors with 76 amino acids. Adropin, hypothesized to be a hormone encoded for the energy homeostasis-associated (*Enho*) gene, is encoded through this gene. It is primarily formed by the brain and liver, with peripheral tissues such as the lungs, heart, digestive tract, renal medulla, muscles, and breast cancer cells. In addition, the significant function of adropin controls the physiological processes of fatty acid oxidation and glucose metabolism.[9] It is a peptide hormone mostly associated with energy homeostasis and vascular protection, but it could also be linked with inflammation through its network of pathways and interactions. Different pathophysiological conditions can contribute to changing adropin levels in the human body. Recent studies have shown that decreased concentration of adropin level is associated with many diseases such as rheumatoid arthritis, type 2 diabetes mellitus, coronavirus disease-19, coronary artery disease, and inflammatory bowel diseases.[10]

Preptin, a peptide first identified in 2001, is frequently secreted by beta cells in response to postprandial glucose levels in the blood. It is responsible for the physiological insulin secretion caused by glucose concentration. Preptin contributes to carbohydrate regulation, protein, and lipid metabolism. Preptin is mostly produced in the salivary glands, pancreas, kidneys, and mammary glands. Preptin has an anabolic effect on the bone that works in synergy with insulin activities. In addition, preptin is exposed to increase the secretion of insulin.[11] Cystatin-C is likely a strong and reliable endogenous biomarker for glomerular filtration rate, as it is produced at a constant rate in nucleated cells, with a certain amount bound to protein. A large quantity filtered through the glomerulus is then metabolized and reabsorbed in the proximal tubule.[12-14]

This study aimed to investigate the association of biochemical parameters with β-TM complications in Babylon Province, Iraq.

Materials and Methods

Patients and study design

In this case-control study, 100 individuals were separated into two groups: 50 patients and 50 apparently healthy volunteers. The age ranged between 17 and 35 years for patients and control. Samples were collected from Imam Al-Sadiq Hospital and Al-Hilla Teaching Hospital in Hilla City during the period from September 2022 to January 2023.

Venous blood samples were collected in gel tubes and centrifuged at 3000 rpm for 20 min. The samples were then stored in a deep freezer of the central blood bank at −60°C. Serum levels of cystatin-C, ferritin, insulin, preptin, and adropin were assessed by sandwich enzyme-linked immunosorbent assay. Serum blood urea (B. urea), serum creatinine (S.Cr), lipid profile, and fasting glucose concentration were measured using colorimetric kits (Biolabo, France) and the spectrophotometer method.

Inclusion criteria and exclusion criteria

Inclusion criteria included patients with β-TM, and exclusion criteria included any patient with diabetes mellitus, patients with other hemoglobinopathies, pregnant patients, and any patient with chronic liver disease.

Ethical approval

All participants in this study were informed before collecting samples, and a verbal agreement was obtained from each of them. The subject data, permission form, and the study protocol were examined and approved by a local ethics committee, according to document number 18 on July 7, 2022.

Statistical analysis

Using SPSS version 23 (SPSS, IBM Company, Chicago, IL, USA), the data analysis was carried out. *P* values of <0.05 are regarded as significant. *P* values of >0.05 were reflected as non-significant.

Results

Demographical and clinical characteristics of the studied groups

The distribution of patients *with β*-TM according to age is shown in Table 1. A total of 50 patients with thalassemia were included in this study, with an age range of 17–35 years. The control group 50 was apparently healthy subjects with an age range of 17–35 years. The present investigation established no statistically significant age difference among thalassemia patients and the control group ($P > 0.05$). In addition, the result of the body mass index test revealed no significant difference ($P > 0.05$) when compared between patients with chief thalassemia and healthy subjects.

Among 50 patients with β-thalassemia and the healthy group who contributed to this study, there were 20 (60%) males and 30 (40%) females.

The findings demonstrated a significant difference in blood creatinine and urea, ferritin, cystatin-C, glucose homeostatic model assessment for insulin resistance (HOMA-IR), and adropin levels between patients and their healthy group ($P < 0.05$), as presented in Table 2.

The findings demonstrated a significant difference in blood triglycerides, cholesterol, HDL-C, LDL-C, very low-density lipoprotein, and preptin levels between the patients and the healthy control group ($P < 0.05$), as illustrated in Table 3.

Results of receiver operating curve (ROC) and area under the curve (AUC) analysis for preptin and adropin levels as likely diagnostic parameters for prediction of thalassemia. Patients are indicated that preptin was revealed additional suitable optimal diagnostic marker for predicting thalassemia cases compared to the control group (sensitivity = 82%, specificity = 72%) at a cutoff point (130.15) and *P* value (0.0001), as confirmed by Figure 1. Meanwhile, adropin showed an AUC = 1, sensitivity = 100%, specificity = 100% cutoff value = 0.395, and $P = 0.001$, as confirmed by Figure 2.

Table 1: Age distribution in patients and control

Parameters	Mean ± SD		*P* value
	Patients group ($n = 50$)	Control group ($n = 50$)	
Age (17–35 years)	27.52 ± 8.17	26.06 ± 9.56	0.904

SD: standard deviation, $P < 0.05$: significant

Discussion

The present study showed that serum urea and creatinine levels do not control the primary stages of renal injury. Creatinine and serum urea were significantly elevated in the *β*-TM group compared to the control group. By the study of Demosthenous *et al.*[15] in the United States, the level of S.Cr in patients receiving deferasirox was greater than that in healthy individuals. Cystatin-C is the primary marker with high specificity and sensitivity for monitoring glomerular and tubular dysfunction. In β-TM, the complication of glomerular dysfunction is not rare, and periodic assessment of renal function in these patients is required as they may have hidden renal dysfunction. Slight differences in cystatin-C levels after treatment with deferasirox were not an indicator of kidney damage.[16] S. ferritin levels provide an additional choice as a biomarker to predict a variety of prognostic and clinical outcomes in patients with β-thalassemia. We evaluated the frequency, pattern, and associations of renal iron accumulation in β-thalassemia. Conversely, assessment of serum ferritin

Table 2: General features of the enrolled patients

General features	Group	*N*	Mean ± SD	*P* value
B. urea mg/dL	Patients	50	38.2 ± 8.3	<0.05
	Control	50	18.3 ± 6.7	
Creatinine (mg/dL)	Patients	50	0.91 ± 0.12	<0.05
	Control	50	0.82 ± 0.22	
Ferritin (ng/mL)	Patients	50	3521 ± 1324	<0.05
	Control	50	189 ± 85	
Cystatin-C (ng/mL)	Patients	50	1181.2 ± 454.8	<0.05
	Control	50	270.6 ± 55.9	
Glucose (mg/dL)	Patients	50	125.25 ± 15.8	<0.05
	Control	50	76.6 ± 8.71	
Insulin (μIU/mL)	Patients	50	10.9 ± 2.1	<0.05
	Control	50	6.91 ± 1.63	
HOMA-IR	Patients	50	3.21 ± 1.34	<0.05
	Control	50	1.51 ± 0.31	
Adropin (ng/mL)	Patients	50	0.62 ± 0.21	<0.05
	Control	50	1.19 ± 0.21	

Table 3: General characteristics of the enrolled patients

Characteristics	Group	*N*	Mean ± SD	*P* value
TG (mg/dL)	Patients	50	83.09 ± 5.4	<0.05
	Control	50	80.03 ± 7.7	
Cholesterol (mg/dL)	Patients	50	120.9 ± 10.3	<0.05
	Control	50	133.1 ± 17.5	
HDL-C (mg/dL)	Patients	50	23.53 ± 2.5	<0.05
	Control	50	42.6 ± 3.9	
LDL-C (mg/dL)	Patients	50	79.7 ± 10.9	<0.05
	Control	50	89.6 ± 15.2	
VLDLC	Patients	50	15.8 ± 4.1	<0.05
	Control	50	18.3 ± 2.6	
Preptin (pg/mL)	Patients	50	128.9 ± 17.6	<0.05
	Control	50	124.2 ± 14.2	

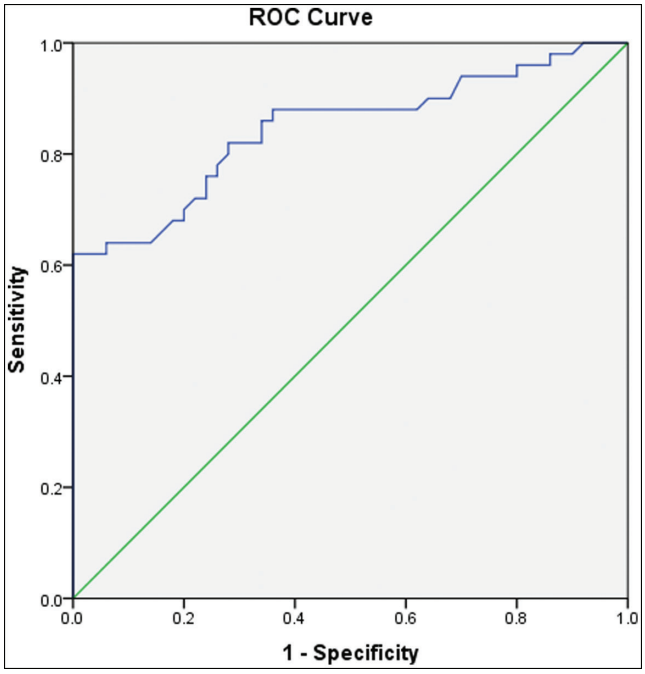

Figure 1: ROC curves for preptin in thalassemia patients to analyze the optimal diagnostic points for predicting cases as compared to the control group

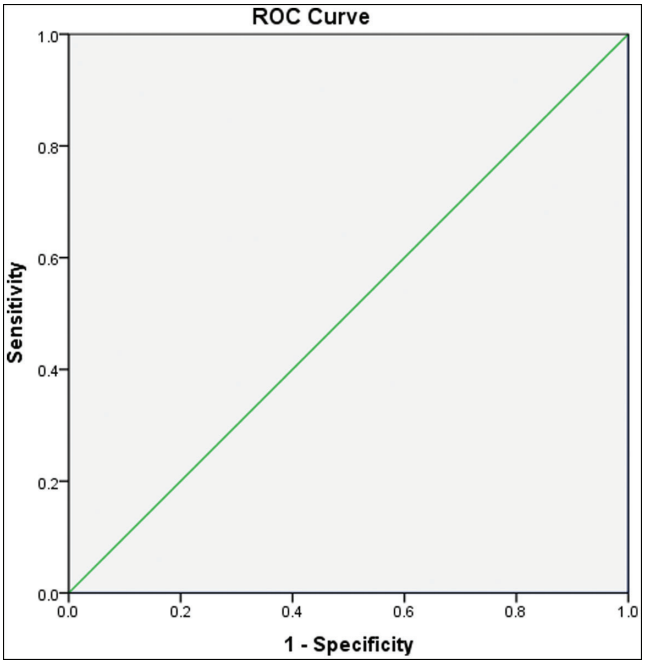

Figure 2: ROC curves for adropin in thalassemia patients to analyze the optimal diagnostic points for predicting cases as compared to the control group

is a routine and cost-effective marker to measure iron overload and the effect of treatment with iron chelation. The elevation of ferritin in the current study was found, which was compatible with the findings obtained by Taher and Saliba.[17]

Our research has shown that individuals with β-TM have significantly higher serum levels of ferritin, glucose, insulin, and HOMA-IR than healthy controls, whereas adropin is significantly decreased in β-TM as compared with healthy control subjects. In addition, serum adropin level revealed a significant negative correlation with ferritin, serum glucose, insulin, and HOMA-IR. The findings revealed that individuals with β-TM had a greater likelihood of having impaired glucose (diabetes or pre-diabetes), fasting insulin levels, and HOMA-IR than healthy individuals.

The increase in impaired glucose and fasting insulin levels in β-TM as compared with healthy control groups was reported in other studies as well, which matched our findings.[18] The prevalence of impaired glucose for β-TM in the current study who need frequent blood transfusions is likely due to iron overload and accumulation of iron in pancreatic β cells and liver, causing more inflammation of these organs. Both insulin resistance and oxidative stress are brought on by the iron turnover brought on by the hemolysis of the microcytic erythrocytes in β-TM.[19] This study reported that patients with β-TM had lower serum levels of adropin, and we expect that adropin may

Table 4: Correlation between serum level of adropin with clinical biomarkers in patients with β-TM

Clinical biomarkers	*r*	*P* value
B. urea	−0.55	<0.05
Creatinine	−0.61	<0.05
Ferritin	−0.59	<0.05
Cystatin-C	−0.42	<0.05
Glucose	−0.67	<0.05
Insulin	−0.76	<0.05
HOMA-IR	−0.43	<0.05

Table 5: Correlation between serum level of preptin with clinical biomarkers in patients with β-TM

Parameters	*r*	*P* value
Adropin	−0.61	<0.05
B. urea	−0.4	<0.05
Creatinine	−0.7	<0.05
Ferritin	−0.67	<0.05
Cystatin-C	−0.51	<0.05
Glucose	−0.4	<0.05
Insulin	−0.7	<0.05
HOMA-IR	−0.63	<0.05
TG	−0.59	<0.05
Cholesterol	0.62	<0.05
HDL-C	0.53	<0.05
LDL-C	0.71	<0.05
VLDLC	0.48	<0.05

be a risk factor or potential biomarker for predicting the development and progression of many diseases.[20]

The endothelium plays an essential role in the maintenance of vascular homeostasis. The decreased concentration of adropin level in the human body is contributed to endothelial dysfunction, which causes the development and progression of cardiovascular disease. On the other hand, enhancement of adropin level can have a positive effect on endothelial dysfunction, since it contributes to regulating endothelial cell function by upregulating endothelial nitric oxide synthase.[21,22]

In this study, findings suggest that decreased circulating adropin levels may be associated with the risk of type 2 diabetes mellitus and atherosclerosis in patients with β-TM. Etiological variables may cause diabetes in thalassemia patients. Transfusion of additional iron may cause death to pancreatic β cells and diabetes. Patients with thalassemia who have poor chelation treatment and older patients when they begin treatment are at higher risk of having impaired glucose metabolism.[23]

Other factors that may cause alteration in β-cell insulin secretion and affect glucose metabolism include insulin resistance, autoimmunity, liver disease, and hepatitis C virus infection. So, low levels of preptin are responsible for this failure in the activities of β-cell.[24] Earlier studies found a relation between TM and blood preptin levels in newly diagnosed people; from now, preptin is probably involved in its pathogenesis.[25] The correlation between serum levels of preptin and adropin with clinical biomarkers in patients with β-TM is shown in Tables 4 and 5.

Conclusion

There is a relationship between carbohydrate disturbances, lipid profile, and renal dysfunction in β-TM complications. This relationship was represented or clarified by the change in the concentration of the biomarkers described above.

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Conflicts of interest

There are no conflicts of interest.

References

1. Hokland P, Daar S, Khair W, Sheth S, Taher AT, Torti L, *et al*. Thalassaemia—A global view. Br J Haematol 2023;201:199-214.
2. Al-Thamir SN, Al-Hussainy SM. Effects of iron overload and treatment methods on serum levels of zinc (Zn) and copper (Cu) in beta thalassemia major (BTM) patients. Med J Babylon 2011;8:263-70.
3. Tantawy AA, El Bablawy N, Adly AA, Ebeid FS. Early predictors of renal dysfunction in Egyptian patients with β-thalassemia major and intermedia. Mediterr J Hematol Infect Dis 2014;6:e2014057.
4. Deveci B, Kurtoglu A, Kurtoglu E, Salim O, Toptas T. Documentation of renal glomerular and tubular impairment and glomerular hyperfiltration in multitransfused patients with beta thalassemia. Ann Hematol 2016;95:375-81.
5. Kawabata H. The mechanisms of systemic iron homeostasis and etiology, diagnosis, and treatment of hereditary hemochromatosis. Int J Hematol 2018;107:31-43.
6. Setoodeh S, Khorsand M, Takhshid MA. The effects of iron overload, insulin resistance, and oxidative stress on metabolic disorders in patients with β-thalassemia major. J Diabetes Metab Disord 2020;19:767-74.
7. Chen Y, Chang Z, Liu Y, Zhao Y, Fu J, Zhang Y, *et al*. Triglyceride to high-density lipoprotein cholesterol ratio and cardiovascular events in the general population: A systematic review and meta-analysis of cohort studies. Nutr Metab Cardiovasc Dis 2022;32:318-29.
8. Zou Y, Zhong L, Hu C, Zhong M, Peng N, Sheng G. LDL/HDL cholesterol ratio is associated with new-onset NAFLD in Chinese non-obese people with normal lipids: A 5 year longitudinal cohort study. Lipids Health Dis 2021;20:28.
9. Ali II, D'Souza C, Singh J, Adeghate E. Adropin's role in energy homeostasis and metabolic disorders. Int J Mol Sci 2022;23:8318.
10. Brnić D, Martinovic D, Zivkovic PM, Tokic D, Hadjina IT, Rusic D, *et al*. Serum adropin levels are reduced in patients with inflammatory bowel diseases. Sci Rep 2020;10:1-9.
11. De Sanctis V, Soliman A, Yassin M. Iron overload and glucose metabolism in subjects with *β*-thalassaemia major: An overview. Curr Diabetes Rev 2013;9:332-41.
12. Azami M, Sharifi A, Norozi S, Mansouri A, Sayehmiri K. Prevalence of diabetes, impaired fasting glucose and impaired glucose tolerance in patients with thalassemia major in Iran: A meta-analysis study. Caspian J Intern Med 2017;8:1-15.
13. Hasan BK, Saud WH. Zinc and copper in transfusion-dependent thalassemia patients on different iron chelators in Basrah: A case-control study. Med J Babylon 2023;20:124-9.

14. Badeli H, Baghersalimi A, Eslami S, Saadat F, Rad AH, Basavand R, *et al*. Early kidney damage markers after deferasirox treatment in patients with thalassemia major: A case-control study. Oxid Med Cell Longev 2019;2019:1-8.
15. Demosthenous C, Eleftheriou P, Apostolou C, Sarafidis P, Perifanis V, Vlachaki E. *β*-thalassemia and renal complications: A narrative review of pathophysiologic mechanisms. Integr Mol Med 2018;5:4.
16. Sadeghi-Bojd S, Hashemi M, Karimi M. Renal tubular function in patients with beta-thalassemia major in Zahedan, southeast Iran. Singapore Med J 2008;49:410-2.
17. Taher AT, Saliba AN. Iron overload in thalassemia: Different organs at different rates. Hematology Am Soc Hematol Educ Program 2017;2017:265-71.
18. Ansari AM, Bhat KG, Dsa SS. Study of insulin resistance in patients with B-thalassemia major patients and validity of TYG index. J Pediatr Hematol Oncol 2018;40:128-31.
19. Bhat KG, Periasamy PK. Effect of long-term transfusion therapy on the glycometabolic status and pancreatic beta cell function in patients with beta thalassemia major. J Fam Med Primary Care 2016;3:119-23.
20. Wei W, Liu H, Qiu X, Zhang J, Huang J, Chen H, *et al*. The association between serum adropin and carotid atherosclerosis in patients with type 2 diabetes mellitus: A cross-sectional study. Diabetol Metab Syndr 2022;14:27.
21. Mishra AK, Tiwari A. Iron overload in beta thalassemia major and intermedia patients. Maedica 2013;8:328-32.
22. Rasheed SMH, Eidan AJ, Al-Dujaili AH, Abada LH, Al-Charrakh AH. Different cytokines and lipid profile in suicidal and non-suicidal adults with major depression. Ann Trop Med Public Health 2019;22:S282.
23. Zheng J, Liu M, Chen L, Yin F, Zhu X, Gou J, *et al*. Association between serum adropin level and coronary artery disease: A systematic review and meta-analysis. Cardiovasc Diagn Ther 2019;9:1-7.
24. Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance. Mol Metab 2015;4:310-24.
25. Jasaszwili M, Billert M, Strowski MZ, Nowak KW, Skrzypski M. Adropin as a fat-burning hormone with multiple functions—Review of a decade of research. Molecules 2020;25:549.