

The Role and Relationship of Betatrophin with Exercise Among Obese Patients in Gym Centers in Mosul City

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ABSTRACT

Obesity is characterized by an increase in body fat and adipose tissue and is a cause of metabolic syndrome. therefore, the study contributed to studying the effect of exercise on the hormone betatrophin and its relationship to biochemical parameters related to obesity, the results showed that there was a significant increase in the level of betatrophin, which was $(586.2 \pm 112.6 \text{ pg/ml})$ compared to healthy individuals, which was $(253.6 \pm 37.2 \text{ pg/ml})$ pre-exercise, while its level was $(436.3 \pm 95.5 \text{ pg/ml})$ compared to the control group, which was $(200.8 \pm 23.5 \text{ pg/ml})$ post-exercise. also, a significant increase in the level of betatrophin was observed in females more than males, and its level increased with increasing age and BMI, While the level of betatrophin decreased with increasing duration of exercise, the results showed that pre-exercise there was a significant increase in insulin resistance, glucose, HbA1c, urea, creatinine, cholesterol, triglycerides, LDL-C, VLDL, atherogenic index, peroxidase enzyme, and a decrease in their levels post exercise. while there was a significant decrease in insulin, HDL-C, ARE enzymes, catalase, uric acid pre-exercise and an increase in their levels post-exercise, the research aimed to study the effect of exercise and its duration on the level of betatrophin and some clinical parameters related to obesity, and to study the relationship of betatrophin to antibiotics and oxidative stress..

KEYWORDS: Betatrophin, Obesity, exercise, oxidative stress

INTRODUCTION

Obesity is an excessive of fat in adipose tissue and causes many diseases such as stroke, cardiovascular disease, metabolic disease and cancer[1]. Obesity and overweight are among the most common health problems in the world[2] The exercise is as one of the important means of treating obesity, the incidence of obesity and overweight has increased during the past forty-five years, leading to 50% of adults being obese[3]. The physical activity is a beneficial health indicator for memory, sleep, anxiety, brain health, and cognition in obese women.[4] It is important to state that betatrophin is a hormone consisting of 198 amino acids. It plays a significant role in regulating fat and glucose metabolism. It is called angiopoietin-like protein ANGPTL8. It is produced in the adipose and liver tissue, it has a role in the proliferation of pancreatic beta cells in different stages of insulin resistance. [5,6] betatrophin is regulated by nutrition and inhibits the activity of lipoprotein lipase(LPL)[7]. The studies also indicate strong association between atherosclerotic lipoproteins and beta-trophin levels in type 2 diabetes patients.[8] It plays an outstanding part in regulating the metabolism of fats inside and outside cells and in inflammatory pathways, ANGPTL8 regulates plasma triglycerides through its interaction with ANGPTL3. It increases with high blood triglycerides. Exercise training is considered as a regulator and treatment for insulin resistance and diabetes caused by obesity. [9]

SUBJECTS AND METHODS

Sample collection

The samples have been collected from obese patients from gym centers in the city of Mosul by used (BMI) (kg/m^2), which is the internationally recognized body measurements to distinguish between normal weight, overweight, and obese patients. a control group has been taken from those with normal weight and a group of overweight and obese as patients. the study includes collecting (60) blood samples from normal individuals whose ages ranged between $(35-46 \leq)$ and $(25 \text{ males}, 35 \text{ females})$, also collection (75) samples from patients $(32 \text{ males}, 43 \text{ females})$ with the same ages as healthy people for the period from 15/10/2024 to 15/12/2024, the Samples were collected from obese patients at the gym, and then after a month, samples were collected from them again.

Preparation of Serum

(5) ml of blood is drawn from both patients and healthy individuals after sterilizing the area with alcohol. Then, the samples have been placed in gel tubes and left for 10 min. at 37°C in a water bath they have also been placed for 10 min. at a speed of 3000 xg in a centrifuge. Serum was separated from the samples. and then used to measure some biochemical parameters in it, post-month of exercise, blood is drawn from the obese patients and the same biochemical parameters are measured .

Procedure

Human betatrophin is estimated by using Biotech Kit-China, based on the Sandwich ELISA principle, the anti-hormone antibodies present in the plate wells compete with the anti-biotin antibodies linked to the enzyme Horseradish peroxidase (HRP) for the binding sites on the betatrophin antigens present in the added blood serum. [10] Insulin hormone is measured by using a kit -German by using the Cobas e411 device, based on the test principle that depends upon measuring the optical spectrum absorbed by the molecules of the substances to be measured in the blood.[11] Insulin resistance has been calculated from insulin conc. and fasting glucose by using the equation[12,13]

$$\text{HOMA-IR} = [\text{Fasting glucose (mg/dL)} \times \text{Insulin } (\mu\text{U/ml})] / 405$$

Glucose is estimated by using RANDOX kit-USA based on the enzymatic method [14] while estimating glycated hemoglobin by using the (On Call A1c) kit-USA, based on the boronate affinity measurement principle, to determine the percentage of glycated hemoglobin, using the (On Call A1c) device.[15], Using BioSystems-kit-Spanish, urea is determined and based on the enzymatic method in which urea is decomposed by the enzyme urease into ammonium and carbon dioxide.[16] depending on the colorimetric method of Jaffa, creatinine is determined using BIOLABO-Kit- by reacting creatinine with picric acid in a basic medium then measuring the absorbance at wavelength 490[17]

As for the Cholesterol, it is determined by using BIOLABO kit-French by the colorimetric method using the enzymes cholesterol esterase, cholesterol oxidase, and peroxidase to form a pink quinoneimin complex.[18] Triglycerides have also been estimated using BIOLABO kit-French based on the colorimetric method in which lipase, glycerokinase, and peroxidase enzymes work to form a pink solution of the quinoneamine compound.[19] (HDL-C) is measured by using BIOLABO kit - French by using an enzymatic method this method precipitated (VLDL, LDL), and chylomicrons by adding magnesium chloride and phosphotungstic acid then the HDL remained alone in the filtrate after centrifugation ,HDL-C is then measured by using the cholesterol detection method[20] LDL-C is also measured by using the following equation[21]

$$\text{LDL conc. (mg/dl)} = \text{Conc. Of Cholesterol} - \text{HDL} - \text{VLDL}$$

While VLDL-C has been measured by using the equation[22]

$$\text{VLDL conc. (mg/dl)} = \text{TG} / 5$$

The Atherogenic Index(AI) is estimated by the equation[23]

$$\text{AI} = \log (\text{TG} / \text{HDL-C})$$

The spectrophotometric is also used to estimate the catalase enzyme, which depends on the decomposition of hydrogen peroxide by the catalase enzyme and stopping the reaction by using ammonium molybdate to form a yellow product.[24] while the activity of the peroxidase enzyme is estimated based on Peroxidase oxidation of hydrogen peroxide .[25] the activity of aryl esterase enzyme has also been estimated based on the analysis of phenyl acetate into phenol and acetic acid.[26] Uric acid is estimated based on the enzymatic method in which the enzyme uricase oxidizes uric acid[27].

Statistical analysis

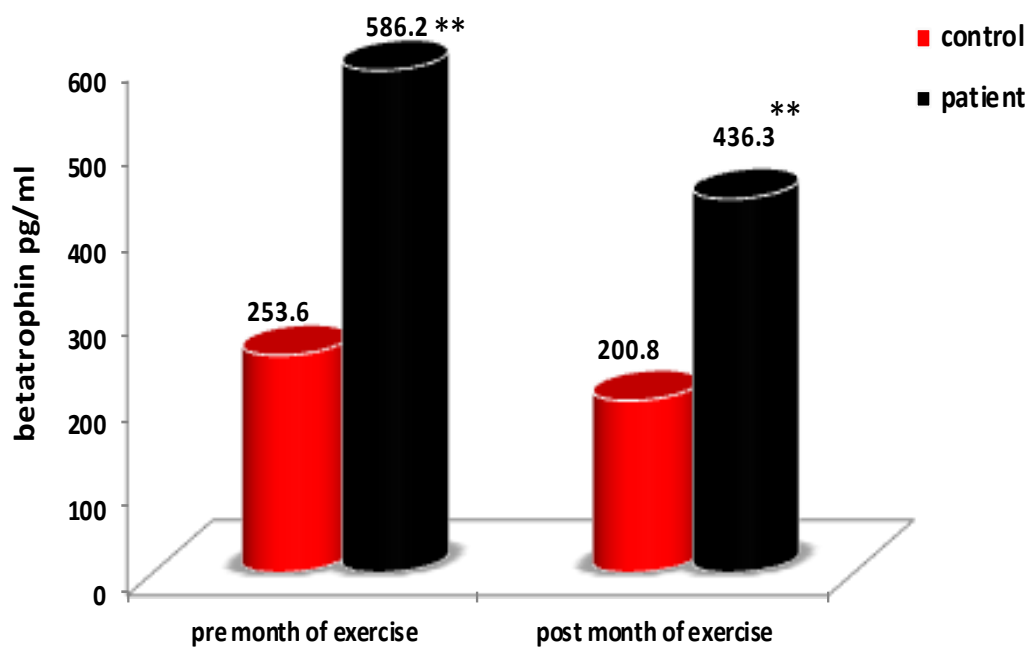
The results have been analyzed by using SPSS 25 to find the standard deviation and mean using analysis of variance. The t-test is used to compare two variables at a probability level of (0.05, 0.01, 0.001) ,and the Duncan's multiple range test is used to compare more than two variables and determine differences between groups, find the linear correlation coefficient between betatrophin and the measured clinical parameters to find the r-value.[28]

RESULTS AND DISCUSSION

Study of the effect of betatrophin pre and post month of exercise in the blood serum of obese patients compared with the control group:

Figure 1. shows a significant decrease in the conc. rate of betatrophin in obese patients pre and post month of performing exercise, where it is $(586.2 \pm 122.6 \text{ pg/ml})$ and $(436.3 \pm 95.5 \text{ pg/ml})$, compared to the control group, where it was $(253.6 \pm 37.2 \text{ pg/ml})$ and $(200.8 \pm 23.5 \text{ pg/ml})$ respectively because it is possible to

stimulate an increase in triglycerides in the level of betatrophin in obese people, while exercise reduces its level in the blood serum and improves the biological clock and heart activity as a result of betatrophin's association with the basic function of regulating triglycerides and cholesterol in the blood by inhibiting lipoprotein lipase enzyme (LPL). [29,30]

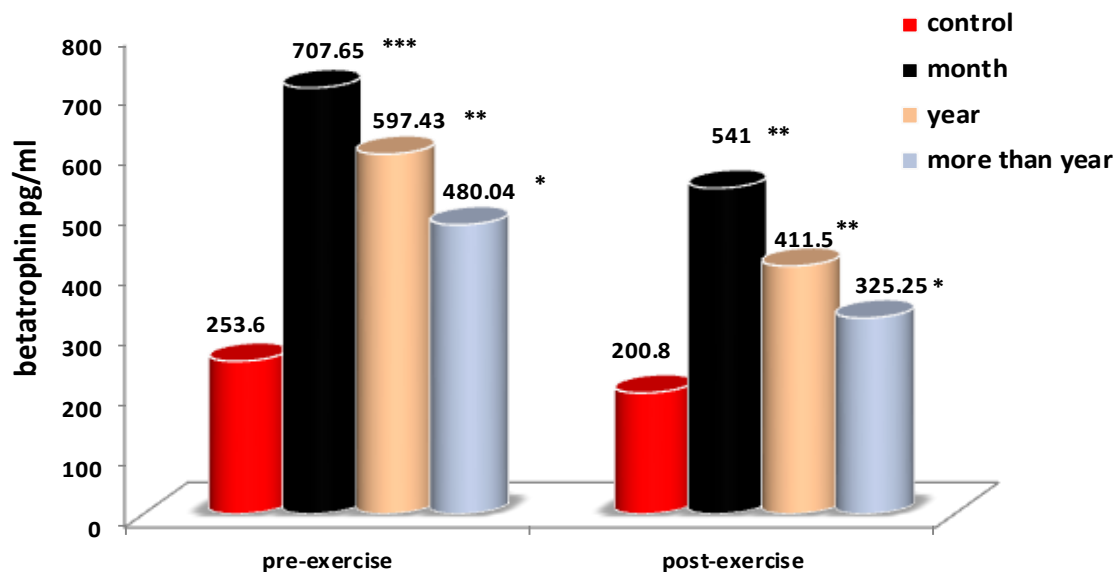


** significant difference at $p \leq 0.01$

Figure (1) : betatrophin level pre - post month of exercise in obese patients in comparison with the control

A study of the effect of exercise training duration on the concentration of betatrophin in the blood serum of obese patients compared to the control group:

Moreover, the results in figure 2. shows a significant decrease in betatrophin concentration with increasing exercise training duration this is consistent with [31] about exercise being used as an important therapeutic method in an intensity, frequency and duration for those suffering from diseases such as obesity metabolic syndrome and diabetes , and it has positive effects in reducing fatty tissue.



* significant difference at $p \leq 0.05$
 ** significant difference at $p \leq 0.01$
 *** significant difference at $p \leq 0.001$

Figure (2) : Study the effect of exercise duration on betatrophin level in obese patients pre-post month of exercise in comparison with the control

The effect of age and gender on betatrophin levels in obese patients compared to the control group:

Table 1. shows that the level of betatrophin increases with age, which is consistent with [32] that betatrophin is positively correlation with increasing age, while the level of betatrophin in females proves higher than in males, the reason is due to the increase in estrogen in females, which is responsible for the difference in lipoprotein metabolism, such as increase in HDL-C and the decrease in LDL-C ,lipoprotein (a), also increases in the activity of the lipoprotein lipase enzyme LPL.[33]

Table (1) : The effect of age and gender on betatrophin levels in obese patients pre - post month of exercise compared with control group

Age (year)	Pre-month of exercise		Post-month of exercise	
	Control	Patients	Control	Patients
35-45	233.1 ± 50.5	560.6 ± 113**	192.2 ± 14.8	436.2 ± 97.7**
≥46	269 ± 18.1	597 ± 117.2**	212.2 ± 31.5	436.5 ± 105.4**
Gender	Betatrophin level pg/ml			
	Pre-month of exercise		Post-month of exercise	
	Control	Patients	Control	Patients
Male	246.6 ± 49.3	557.3 ± 94.9**	189.4 ± 16.9	414.4 ± 103.9**
Female	262.9 ± 16.4	611 ± 127.6**	209.3 ± 26.3	455.1 ± 91.6**

** significant difference between obese patients pre – post month of exercise in comparison with control at $p \leq 0.01$

Study of the effect of body mass index(BMI) on the concentration of betatrophin in the blood serum of obese patients compared to the control group:

Our results in the Table 2. shows that there is a significant decrease in betatrophin post- month of exercise training in obese people in comparison with healthy people, and the decrease increases with increasing (BMI) this is consistent with [34], that the level of betatrophin in the serum shows a close correlation with the body mass index BMI and the body span rate WHR, which are indicators of the risk of obesity, exercise can reduce the level of betatrophin and convert triglycerides into energy, [35]

Table (2) : The effect of body mass index on betatrophin levels in obese patients pre - post month of exercise compared with control group

BMI kg/m2	Betatrophin level pg/ml			
	Pre-month of exercise		Post-month of exercise	
	Control	Patients	Control	Patients
Over weight 25-29.9	253.6 ± 39.4	465 ± 149.8**	200.8 ± 23.5	370.9 ± 50.1**
Obese ≥30	253.6 ± 39.4	628.5 ± 125**	200.8 ± 23.5	541 ± 27.7**

** significant difference at p<0.01

Study some biochemical parameters among obese patients Pre and post month of exercise training compared to the control group:

Table 3. also shows an increase in insulin concentration and a decrease in his resistance post-month of exercise training, the reason is that betatrophin enhances insulin resistance by increasing the proliferation of beta cells, and exercise is considered as a regulator and treatment for insulin resistance and diabetes caused by obesity.[36,37]the table also shows a decrease in both fasting and random glucose levels and in HbA1c post-month of exercise, this is because exercise increases the absorption of glucose and converts it into energy (ATP) from carbohydrates, fats and proteins, thus reducing blood glucose levels.[35] A decrease in the concentration of both urea and creatinine has clearly been observed post-month of exercise compared to pre-month of exercise, this is consistent with[38,39]that the positive correlation of betatrophin with urea, creatinine, fasting blood sugar, triglycerides, cholesterol, and inversely with glomerular filtration rate, albumin, HDL-C in serum, while the results show a significant increase in both cholesterol and LDL-C Pre-month of exercise compared to control and a decrease in their concentration post-month of exercise training, this is because betatrophin regulates the process of fat metabolism, which includes the activity of lipoprotein lipase LPL and is closely linked to total cholesterol and LDL-C ³⁴a significant decrease in triglyceride and (VLDL-C) conc. has also been observed Post-month of exercise compared to healthy controls, betatrophin hydrolyzes triglycerides (TG) in chylomicrons and VLDL, generating free fatty acids, which are then absorbed by tissues, including heart , muscle, and fat , then Angptl4 and Angptl must be cleaved to release their amino termini to inhibit lipoprotein lipase (LPL)[40] a decrease in the atherogenic factor has also been observed post-month of exercise compared to control, this is because this index consists of cholesterol, triglycerides, and high-density lipoprotein HDL-C, which are markers for assessing the risk of cardiovascular and atherosclerosis diseases in which HDL-C is low and both cholesterol and triglycerides are high.[41]

Table (3) : Study some biochemical parameters pre - post month of exercise in obese patients compared with control group

Biochemical parameters	Mean ± S.D.			
	Pre-month of exercise		Post-month of exercise	
	Control	Patients	control	Patients
Insulin µU/ml	13.6 ± 0.8	9.5 ± 1.7**	15.1 ± 1	12.1 ± 3*

Insulin resistance	2.2 ± 0.9	2.5 ± 1.1	1.9 ± 0.9	2 ± 0.7
Random glucose mg/dl	138.3 ± 15.3	160.8 ± 12.1**	130.9 ± 12.6	133.6 ± 10.4
Fasting glucose mg/dl	86.6 ± 7.8	85.8 ± 6.7*	77.6 ± 6.8	77 ± 7.3
HbA1c %	5.1 ± 0.6	5.1 ± 0.7	4.8 ± 0.6	4.5 ± 0.4
Urea mg/dl	30.5 ± 6	33.3 ± 7.2	25.5 ± 3.7	29.5 ± 6.9
Creatinine mg/dl	0.8 ± 0.2	0.9 ± 0.1	0.6 ± 0.2	0.8 ± 0.1
Cholesterol mg/dl	162.7 ± 26.1	236.5 ± 39.8**	143.6 ± 16	205.1 ± 28.2**
TG mg/dl	132.4 ± 7.6	201.5 ± 43**	117.4 ± 17.3	176.2 ± 30.1**
LDL-C mg/dl	82.9 ± 29.3	154.6 ± 38.8**	68.9 ± 19.2	121 ± 31.4**
VLDL-C mg/dl	26.5 ± 1.5	39.5 ± 7.9**	22.6 ± 1.7	35.2 ± 6**
HDL-C mg/dl	53.3 ± 4.3	42.4 ± 4.6**	56 ± 4.4	48.9 ± 5.3**
Atherogenic index	4.2 ± 1.2	5.6 ± 1**	3.4 ± 0.9	4.2 ± 0.8*

* significant difference at p≤0.05

** significant difference at p≤0.01

Study of the effect of some antioxidants and oxidants on obese patients pre and post month of exercise training compared to the control group:

The results in table 4. show a significant increase in the antioxidant enzymes of catalase and aryl esterase post-month of exercise, this is because these enzymes work to reduce oxidative stress resulting from free radicals, eliminate them, and maintain the balance of cellular oxidation and reduction[42] the aryl esterase enzyme is also linked to high-density lipoprotein HDL and can decompose lipid peroxides, thus having a protective effect against atherosclerosis and heart disease[34] a non significant increase in uric acid concentration is observed post-month of exercise, this is because uric acid increases with metabolic syndrome, obesity, and insulin resistance, it is an antioxidant, so it increases in response to oxidative stress in patients.[44] While there is an increase in the activity of the peroxidase enzyme pre-month of exercise and a decrease in its activity post-month of exercise, this is due to the increase in oxidative stress and active oxygen species in obese individuals, as the enzyme works to convert hydrogen peroxide to water to eliminate its effects.[45]

Table (4) : Concentration of oxidants and antioxidants in obese patients pre-post month of exercise compared with control group

Antioxidants and oxidants	Mean ± S.D.			
	Pre-month of exercise		Post month of -exercise	
	Control	Patients	control	Patients
Aryl esterase U/ml	82.1 ± 5.1	46.5 ± 4.4**	97.9 ± 3.6	59.3 ± 1.5**
Catalase KU/L	5.8 ± 0.2	3.7 ± 0.4**	6.2 ± 0.3	4.4 ± 0.4**
Peroxidase U/L	58 ± 8.3	149.2 ± 4.1**	56.6 ± 3.3	113.5 ± 16.7**
Uric acid mg/dl	5.6 ± 0.8	5.4 ± 1.1	6.1 ± 1	6.3 ± 1.1

** significant difference at p≤0.01

Correlation between betatrophin and some clinical parameters in obese patients Pre and post month of exercise and control group:

The results in table 5. show is an important negative correlation between betatrophin with insulin and a significant positive correlation between it with glucose. this is consistent with [46] that betatrophin is inversely related to the increase in the conc. of insulin C-peptide and increases in the increase in glucose in obesity and T2DM. the results also show a significant positive relationship between betatrophin and TG, VLDL-C, while a significant negative relationship between it and HDL-C, due to the positive correlation between betatrophin with the (BMI) and oxygen consumption in obese patients, which improves post exercise[30,47] while the relationship is significantly negative

between betatrophin and the peroxidase enzyme due to the increase in oxidative stress in obesity and inflammation leading to a decrease in oxidative defense[48].

Table (5) : Correlation between betatrophin with some biochemical parameters in obese patients pre - post month of exercise and control

Biochemical parameters	Pre-month of exercise		Post month of -exercise	
	r-value	r-value	r-value	r-value
	Control	Patients	control	patients
Insulin μ U/ml	-762*	0.212	0.526	-0.313
Insulin resistance	0.246	0.206	0.354	0.116
Random glucose mg/dl	0.783*	0.032	-0.615	0.170
Fasting glucose mg/dl	0.286	0.293	0.656	0.606*
HbA1c %	0.570	-0.509	0.455	-0.204
Urea mg/dl	0.144	-0.288	-0.235	-0.317
Creatinine mg/dl	0.189	-0.163	-0.243	-0.143
Cholesterol mg/dl	0.401	0.060	-0.317	-0.204
TG mg/dl	-0.028	0.558*	-0.188	0.481
LDL-C mg/dl	0.350	-0.046	-0.437	-0.377
VLDL-C mg/dl	-0.028	0.546	0.799*	0.481
HDL-C mg/dl	0.050	-0.028	-0.904**	-0.588*
Atherogenic index	0.277	0.072	-0.490	-0.485
ARE U/ml	0.286	0.287	0.365	0.466
Catalase KU/L	-0.438	0.226	-0.129	0.039
Peroxidase U/L	-0.911**	-0.452	0.232	-0.294
Uric acid mg/dl	-0.239	0.108	0.177	0.154

* Correlation significant at 0.05 level

** Correlation significant at 0.01 level

CONCLUSION

The study shows the effect of exercise on the level of betatrophin pre and post month of exercise training, as a decrease in its concentration finds, and this decrease increases with the increase in the duration of exercise training, its level is higher in females than males and it is positively correlated with BMI and age also it is noted ,a decrease in biochemical parameters related to obesity, such as cholesterol ,TG,LDL-C,VLDL-C,and oxidants,while an increase in antioxidants HDL-C post month of exercise therefore, betatrophin is an indicator of oxidative stress and a therapeutic target for patients with obesity and T2DM post exercise due to its association with dyslipidemia and insulin resistance. .\

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REFERENCES

1. Asghar,A., Sheikh,N.,(2017) Role of immune cells in obesity induced low grade inflammation and insulin resistance .Cell Immunol, 315:18-26.
- 2.Ogden,CL.,Carroll,MD.,Kit,BK.,Flegal,KM.,(2014)Prevalence of Childhood and adult obesity in the United States ,2011-2012.JAMA,311(8):806-814.
3. Jayed ,A., Soltani ,S., Emadi ,A., Zargar ,MS., Najafi ,A.,(2024) Aerobic Excercise and weight loss in Adults Asystematic Review and Dose-Response Meta-Analysis. JAMA Net Open, 7(12):e2452185 .

4. Pojednic ,R., Arpino ,ED., Halliday ,L., Bantham ,A.(2022)The Benefits of Physical Activity for people with obesity, Independent of weight loss:Asystematic Review.Int.J. Environ.Res.Public Health ,19(9);4981.
5. Zheng ,J., Liu, J., Hong,BS., Ke ,W., Huang ,M.,et al.(2020) Circulating betatrophin /ANGPTL8 levels correlate with body fat distribution in individuals with normal glucose tolerance but not those with glucose disorders.BMC Endocr.disord,20;51 .
6. Siddiqa ,A., Ahmad J., Ali ,A., Paracha ,RZ., Bibi ,Z., Babar ,A.,(2016) Structural characterization of ANGPTL8(betatrophin)with its interacting partner lipoprotein lipase. Computational Biology and Chemistry ,61;210-220.
7. Leisherer ,A., Ebner,J., Muendlein ,A., Brandtner ,EM., Zach ,C., Geiger ,K., Fraunberger ,P.,H Drexel ,H.(2020) High betatrophin in Coronary patients protects from Cardiovascular events.Atherosclerosis, 293;62-68 .
8. Lorincz ,H., Csiha ,S., Batku ,B., Somodi ,S., Sztanek,F., Seres ,L., Parsgh ,G., Harangi ,M.(2023) Gender-Dependent Associations between Serum Betatrophin levels and Lipoprotein Subfractions in Diabetic and Nondiabetic Obese patients.Int.J.Mol.Sci,24(22):16504.
9. Abu-Farha ,M., Sriraman ,D., Cherian ,P., Alkhairi ,I., Elkum ,N., Behbehani ,K., Abubaker ,J.(2016)Circulating ANGPTL8/Betatrophin is increased in obesity and reduced after Exercise Training.PLOS One,11(1);e0147367.
10. Abou-Samara ,AB., Zhang ,R.(2014)An explanation for recent discrepancies in levels of human circulating betatrophin .Diabetologia,57(10):2232-2234.
11. Kurniawan ,LB., Adnan ,E., Windarwati Mulyono ,B.(2020) Insulin resistance and testosterone level in Indonesian young adult males. *Romanian Journal of Internal Medicine*,85(2):93-98.
12. Koo ,DJ., Lee ,MY., Jung ,I., Moon ,SJ., Kwon ,H., Park ,SE., Lee ,WY.(2021) Baseline homeostasis model assessment of insulin resistance associated with fibrosis progression in patients with nonalcoholic fatty liver disease without diabetes: A cohort study. *PLoS,one*, 16 (8): e0255535.
13. Mohmoud,ES.,AL-Hayali,MA., Abdulmawjood,SA.(2024)Effect of quercetin on some biochemicalparameters in adult rats treated with nitrite .*Journal of Applied and Natural Science*,16(1):221-225.
14. Ronzhin,N., Baron ,A., Puzyr ,A., Baron ,I., Burov ,A., Bondar ,V.(2018) Modified Nanodiamonds as a New Carrier for Developing Reusable Enzymatic Test-Systems for Determination of Physiologically Important Substances. *Modern Clinical Medicine. Research*, 2; 7-17.
15. Vaswani ,S., Mashon ,RS., & Kakkar ,N.(2019) Elevated HbF Labelled as LA1C/cHb1 on BioRad D10 HPLC: Missed Diagnosis of Homozygous Beta Thalassemia. *Indian Journal of Hematology and Blood Transfusion*, 35: 201-202.
16. KVerma ,A., Dubey ,GP., Agrawal ,A.(2012) Biochemical Studies on Serum Hb, sugar ,Urea , Lipid Profile under Influence of Ocimum sanctum L in Aged patients.*Research J. Pharm. And Tech*,5(6):791-794.
17. Parmar ,AK., Valand ,NN., Solanki ,B., Menon ,SK.(2016) Picric acid capped silver nanoparticles as a probe for colorimetric sensing of creatinine in human blood and cerebrospinal fluid samples.*Analyst*,141;1488-1498.
18. Burtis ,CA., Ashwood,ER., Bruns,DE.(2015)" Tietz Textbook of Clinical Chemistry and Molecular Diagnostics.By Saunders, an imprint of Elsevier Inc, USA,356-363.
19. Menezes,FG., Neves ,ACO., Delima ,DF., Lourenco ,Sh., de Silva ,LC., de Lima ,KMG(2015) Bioorganic Concepts is involved in the determination of glucose, cholesterol and triglycerides in plasma. Using the enzymatic colorimetric method. *Quim. Nova*, 38(4): 588-594.
20. Silva ,VRA., Albuquerque ,EC., dos Santos ,APC., Santos ,JCA., Juntor ,LSS., Presada ,WA., Vinagre ,CGC., Couto ,RD.(2019)HDL-Particles Separation employing different Precipitation agents:Functional properties of the impact of chemical precipitation on Lipoprotein Particle-Size and paraoxonase -1-activity.*Quim.Nova*,2(8):859-865.
21. Piani ,F., Cicero ,AF., Borghi ,C., &Addato ,SD.(2021) Is the 2020 Sampson equation the best formula for LDL-Cestimation?. *European Journal of Internal Medicine*,83: 99-101.
22. Hwany ,S., Gwon ,C., Seo ,MD., Cho ,J., Uh ,Y.,(2021)Adeep Network for Estimating Low Density Lipoprotein Cholesterol from Electronic Health Records :Real-Time Routine Clinical Application.*JMIR.Med.Inform*,9(8):e29331.
23. Bo ,MS., Cheah ,WL., Lwin ,S., New ,T., Win ,TT., Aung ,M.(2018) Understanding the relationship between atherogenicindex of plasma and cardiovascular disease risk factors amongstaff of an University in Malaysia. *Journal of Nutrition and Metabolism*,3:1-6.
24. Hadwan ,MH., Abed ,HN.(2016) Data supporting the spectrophotometric method for the estimation of catalase activity. *Data in brief*,6:194-1999.
25. Nelson, JL., Kulkarn ,AP.(1990) Partial purification and characterization of peroxidase activity from human placenta. *BiochemJ*, 268:79-747.
26. Jasim ,RF., Allwsh ,TA.(2008)Study of Arylesterase and its Relationship with Some Clinical Variables in Atherosclerotic Patients in Mosul (partI). *Rafidain,Journal ofScience*,19(2);157-143.
27. Bidie,ADP., Koffi ,E., Yapi ,FH., Yemie ,AA., Djaman ,JA., Gueede-Guina ,F.(2010)Evaluation of the toxicity of a methanolic total extract of *Mitragyna ciliata* a natural anti-malaric.*International Journal of Biological and Chemical Sciences*,4(5):1509-1518.
28. Schober ,P.,TR Vetter ,TR.(2020) Non Parametric Statistical Methods in Medical Research. *Anesthesia and Analgesia*,131 (6):1862-1863 .
29. Siddiqa ,A., Ahmad J., Ali ,A., Paracha ,RZ., Bibi ,Z. (2016)Aslam B.Structural Characreization of ANGPTL8(betatrophin)with interacting partner Lipoprotein Lipase.*Computational Biology and Chemistry*,61:210-220.
30. Susanto ,H.,Sugiharto., Taufiq ,A., Pranoto ,A., Purnomo ,JDT.(2023)Dynamic alteration of plasma levels of betatrophin in younger female onset obesity post acute moderate -intensity exercise training.*Saudi Journal of Biological Sciences*,30(2):193546.

31. Karaman ,ME., Arslan ,C., Gursu ,MF.(2022)Effects of different exercise loads on Serum betatrophin (ANGPT-8/Lipasin)and Cartonectin (CTRP-3)levels in metabolic syndrome.Turkis Journal of Biochemistry,47(1):71-78.
32. Espes ,D., Martinell ,M., Carlsson ,P.(2014)Increased Circulating betatrophin Concentrations in patients with Type 2 abetes.Research Article,2014:1-6.
33. Lorincz ,H., Csiha ,S., Ratkn ,B., Somodi,S., Sztanek,F., Seres ,I., Paragh ,G., Harangi ,M.(2023)Gender Dependent Associations between Serum betatrophin levels and lipoprotein Subfractions in Diabetic and Nondiaetic Obese Patients.Int.J.Mol.Sci,24(22):16504.
34. Jin-Zhon ,Z., Chun-Xiao ,L.,D Yi-Ning ,D., De-Jian ,Z., Zhi-Yun ,F., Xing-Youg ,W., et.al,(2016)Serum betatrophin levelincreased in Subjects with metabolic Syndrome :acase –control study Aumento del nivel de betatrofina ensuero en sujetos con syndrome metabolico:studio de casosy controles .Nutr.Hosp,3(2):303-309.
35. Rejeki,PS., Baskara ,PG., Herawati ,L., Pranoto ,A., Setiawan ,HK., Lesmana ,R., Halim ,S.(2022)Moderate –intensity exercise decreases the circulating level of betatrophin and its correlation among markers of obesity in women.J Basic Clin Physiol,33(6):769-777.
36. El Ouaamari ,A., Kawamori ,D.,E Dirice ,E., Liew,CW., et. al,(2013)Liver-Derived Systemic Factors Drive BCell Hyperplasiain isulin-Resistant States.Cell Reports ,3:401-410.
37. Abu-Farha ,M., Sriraman ,D., Cherian ,P., Alkhairi ,I., Elkum ,N., Behbehani ,K.,J Abubaker ,J.(2016)Circulating ANGPTL8/Betatrophin is increased in obesity and reduced after Exercise Training",PLOS One,11(1):e0147367.
38. Mounir ,A., ElBendary ,AS., Ahmed ,M., Okda ,HI.(2024)Evaluation of Seru levels of Sestrin 2 and betatrophin in type 2 diabetic patients with diabetic nephropathy.BMC Nephrology,25(1):231.
39. Chen ,CC., Susanto ,H., Chuang ,WH., Liu ,TY., Wang ,CH.(2016)Higher Serum betatrophin level in type 2 diabetes subjects is associated with urinary albumin excretion and renal function.Cardiovasc Diabetol,15:3.
40. Zhang ,R., AB Abou-Samra ,AB. (2014)Adual role of lipasin (betatrophin)in lipid metabolism and glucose homeostasis :consensus and controversy.Cardiovasc .Diabetol,13:133.
41. Kim ,SH., Cho ,YK., Kim ,YJ.,CH Jung ,CH., et al.(2022)Association of the atherogenic index of plasma with cardiovascular risk beyond the traditional risk factor : anationwide population based cohort study .Cardiovasc Diabetol,1:81.
42. Asatiani ,N., Sapojnikova ,N.,T Kartvelishvili, T., Asanisvili ,L., Sichinava ,N., Chikovani ,Z.(2025)Blood Catalase ,Superoxide,Dismutase,and Glutathione peroxidase Activities in Alcohol-and Opiod-Addicted Patients.Medicina,6(2):204.
43. Ozdin ,M., Gursu ,MF.(2020)The relationship between Paraoxonase ,arylestearase, lipoprotein(a)and other lipid parameters in patients with Comary heart disease.CMJ,42:271-276.
44. Singh ,K., Kumar ,P., Joshi ,A., Shivhare ,DK., Mahto ,SK., Singh ,A., Lamba ,BMS.(2019)"Study of association of serum uric acid with albuminuria and carotid atherosclerosis in type 2 diabetes mellitus patients. Journal of Family Medicine and Primary. Care, 8(12): 4027.
45. Bdaiwi ,LF., Baker ,AL., Jalal Aldin ,ZS.(2022) Investigation of the Effect of Rhubarb stalks extracts on Mice Exposed to Oxidative Stress Archives of Razi Institute, 77(5); 1865-1871.
46. Tokumoto ,S., Hamamoto ,Y., Fujimoto ,K., Yamagnchi,E., Okamura,E,S Honjo,S., et al.(2015)Correlatio of circulating betatrophin Concentrations with insulin secretion Capacity,evaluated by glucagon Stimulation test.Diabetic.Medicine, 2015:653-656.
- 47 Yi ,M., Chen ,RP., Yang ,R., Guo ,XF., Zheng ,JC., Chen ,H.(2015)Betatrophin Acts as Diagnostic Biomarker in Type 2 diabetes Melitus and IS Negtively Associated With HDL-Cholesterol.Int.J.Endocrinol,2015:479157.
48. Ivascenko ,T., Voicehovskis ,VV., Voicehovska ,JG., Skesters ,A., Apsite ,K., Grigorjeva ,J., Kivite-Urtane ,A., Pahomova ,N., Kalesj,O.(2021)Oxidative Stress ,depression,and risk of recurrence of stable coronary heart disease.Cor Vasa,63;555-563.