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Original Research Article

The Role of Transferrin, Iron and Some Biochemical Variables in Obese Males

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Abstract: The condition of obesity is a persistent ailment characterized by the atypical or excessive buildup of adipose tissue, resulting in compromised well-being and an elevated likelihood of morbidity and chronic health issues. The global incidence of individuals with obesity and overweight conditions has been on the rise for numerous decades. The goal of the study is to identify the relationship between body mass index (BMI), insulin, transferrin, iron, and lipid profile in obese males. Ninety males were recruited into the study; forty participants were assigned to the obese group were have BMI>30 kg/m^2, twenty to overweight group were have BMI between 25-30 kg/m^2, and another thirty to the control group were have slow BMI<25 kg/m^2. The serum Transferrin, serum fasting insulin, serum iron, lipid profile and fasting glucose was evaluated in each of studied groups. The Obese group showed significant increase of the serum insulin concentration compared with the control group (16.77±7.68 vs. 11.7 ±2.79, p <0.001). As well as in transferrin and lipid profile significant change was detected. No prominent differences were detected in the three groups in iron and glucose concentration. Investigation of serum transferrin can be participated in future as iron deficiency marker in obese subject in conjugation with serum iron and serum ferritin.

Keywords: Obesity, Transferrin, Insulin resistance, Iron deficiency.

INTRODUCTION

The growing prevalence of obesity is a persistent worldwide health crisis, particularly in low- and middle-income nations [1]. According to a recent projection by the World Obesity Federation (WOF), it is anticipated that the global prevalence of obesity will reach one billion individuals by 2030. This includes an estimated one in seven men [2].

The prevalence of obesity and iron deficiency is a significant public health concern with a global impact affecting a substantial proportion of the world's population [3]. Although numerous chronic diseases, including cardiovascular diseases, diabetes, and specific cancers, are primarily caused by being overweight or obese, iron deficiency or hypoferremia is the most widespread micronutrient deficiency worldwide [4].

Iron is a vital micronutrient present in various dietary sources, predominantly in red meats. As per previous research [5], the optimal physical and cognitive development of the human body necessitates the intake of a crucial nutrient. The iron content in the adult human body ranges from two to four grams, with the majority being stored in haemoglobin, while the rest (30-40%) is present in iron-binding proteins like ferritin and transferrin [6]. The utilization of iron primarily occurs within the bone marrow, where it is involved in the synthesis of heme, a component of hemoglobin. This process, known as erythropoiesis, is responsible for the production of red blood cells [7]. Transferrin, the iron-binding glycoprotein, plays a pivotal role in the regulation of iron transport within the body. Transferrin is known to deliver iron to cells through the endosomal cycle by binding to its universal receptor TFR1 [8]. The aforementioned function plays a crucial role not just in erythropoiesis, but also in muscle as well as B- and T-lymphocytes. This has been emphasized by the presence of coupled immunodeficiency with only mild anaemia in

individuals with a TFR1 homozygous mutation. The role of TFR1 extends beyond its function as an iron importer, as it is crucial for maintaining epithelial homeostasis in the gut. While TFR1 is not necessary for fundamental iron uptake in hepatocytes, it is essential in the process of iron loading to finely regulate the increase of hepcidin [9]. The condition of being obese is positively correlated with an increased risk of developing insulin resistance. Several inflammatory markers have been associated with a higher risk of insulin resistance in individuals who are obese. There are multiple contributing factors that are known to elicit insulin resistance, including but not limited to oxidative stress, endoplasmic reticulum stress, genetic predisposition, hypoxia, and lipodystrophy. These factors have been associated with the development of various pathological conditions [10, 11] Individuals diagnosed with type 2 diabetes mellitus and insulin resistance often exhibit indications of impaired metabolic function, as well as the accumulation and localization of lipids within the skeletal muscle and bloodstream [12]. Elevated levels of free fatty acids in the plasma have been observed to impede insulin-mediated glucose metabolism. Conversely, a decrease in plasma lipid levels has been found to augment insulin function in adipocytes, liver and skeletal muscle cells. The inclusion of plasma fatty acids has been observed to decrease the activation of insulin on the insulin receptor substrate-1 (IRS-1) linked phosphoinositide-3-kinase (PI-3K) pathway in skeletal muscle. There is evidence to suggest that the presence of lipids in conjunction with insulin resistance is associated with abnormalities in the translocation of glucose transporter 4 (GLUT4) [13]. The disorder state in question involves the participation of various colorful adipokines, such as adiponectin, hepcidin, TNF-α, resistin, and Inter Leukin. Hence, elevation in adiponectin levels results in improved insulin sensitivity, whereas resistin demonstrates adverse insulin effects. In vitro experiments have revealed that resistin has the ability to induce insulin resistance by inhibiting glucose transport. Moreover, in vivo studies have demonstrated that resistin can elevate hepatic glucose production and fasting blood glucose levels [13]. In a recent study, it was found that trelagliptin succinate has the potential to decrease insulin resistance in fat cells by reducing the content of resistance buried by them [14].

The objective of this investigation was to evaluate and contrast the impact of insulin on Transferrin and iron levels in males of varying weight categories, including obese, overweight, and normal weight individuals. Our hypothesis posited that there would be an increase in Transferrin levels in individuals with obesity as compared to those who are overweight or within a healthy weight range. Moreover, the present investigation is anticipated to contribute to the body of scientific literature that proposes the utilization of Transferrin as a biological indicator in the identification of iron deficiency among males who are obese.

PATIENTS AND METHODS

Subjects: A cross-sectional study was conducted from the beginning of November 2022 until the end of May 2023 among 60 overweight and obese healthy male (mean age 39 ± 16.1 years ;) For the comparison, a total of 30 apparently healthy control subjects (mean age 38.1±15.4 years ;) without a personal history of any metabolic abnormalities were recruited to the study. The controls were taken from the same geographic areas as the patients. All individuals were randomly recruited from Kirkuk Governorates. Specifically, we recruited Participant into three category according to BMI obese BMI> 30 kg/m^2 (n=40), overweight BMI 25-30 kg/m^2 (n=20) and control BMI<25 kg/m^2 (n=30).

In this study, all participants were performed anthropometric and biophysical tests. The anthropometric and biophysical tests were carried out at the in laboratory and outside the laboratory building, respectively. Height and weight were measured for participants by trained nurses. Weight and height were measured in kilogram (kg) and centimeter (cm) respectively. Body weight was measured by a balance scale to the nearest half-kilogram with the individuals in light clothing and without shoes. The body mass index (BMI) was taken from the ratio of the body mass (kilograms) formula divided by body height (meters) squared. Waist circumference was measured in centimeters (cm) at the end of normal expiration half way between the lowest rib and the iliac crest with the investigator standing at the side to ensure that the measuring tape is horizontal across the back and the front of the participant. After anthropometric measurements, after that for all participants' 5-mL samples of venous blood were collected from the forearm vein.

The blood samples were centrifuged at 4000 g for 10 min. Serum plasma was analyzed using ELISA analyzer, Genotik USA with sandwich Elisa method for the determination of serum insulin. For analyses, *Biolabo* diagnostic kits used for determination of serum Transferrin, iron and lipid profile levels.

Compliance with ethical standards: Before the initiation of the study, all participants received an explanation of the procedure and the risks that would later be faced in their participation, and they provided informed consent to participate in this study. The study was approved by the ethics committee of the Director of health Kirkuk, and all procedures were in accordance with the Declaration of Helsinki.

Statistical Analysis

Computerized statistical analysis was performed using SPSS statistic program v29 and Prism Graphpad v9. Comparison was carried out using one way ANOVA T-Test probability (P value). The P value < 0.05 was considered

statistically significant and P value > 0.05 considered non-significant statistically. Correlation coefficient used to find the correlation between studied markers by using Pearson correlation.

RESULTS

The anthropometric and biophysical characteristics of participant from all of the groups are shown in Table 1. Significant differences were found among three groups regarding anthropometric and biophysical variables.

Table 1: Biophysical and anthropometric variables among all groups

Variable	Control n=30 Mean ±SD	Overweight n=20 Mean ±SD	P-value	Obese n=40 Mean ±SD	P-value
Age (years)	38.1±15.4	37.5± 14.55	0.8909	39 ± 16.1	0.8143
Systolic blood pressure	125 ± 8.8	135 ± 10.5	0.0007*	140 ± 11.7	<0.0001*
Diastolic blood pressure	75 ± 9.1	85 ± 11.4	0.0012*	90 ± 11.6	<0.0001*
Body Mass Index (kg/m2)	23.37±1.53	27.22±0.61	< 0.001*	32.29±4.1	< 0.001*
Waist Circumference (cm)	83.99±7.77	90.32±7.24	0.0056*	99.12±10.45	< 0.001*
SD is the standard deviation. *One-way ANOVA. Statistically significant differences between Obese, overweight and control ($p < 0.05$).					

Chemical Tests: As shown in table 2, Figure 1, and Figure 2. ANOVA showed significant increased level of Transferrin in obese group when compared to control group (2.67 g/l±0.37 vs. 2.29 g/l ±025 p<0.001), indicating transferrin level increase in obesity. However, ANOVA revealed no significant for iron level and glucose level in obese group (p = 0.068, *p=0.94, respectively*) and (p=0.99, p=*0.93*) in overweight group. Significant differences were detected in insulin level in obese and overweight group when compared to control group (16.77±7.68, 14.98±4.78 vs. 11.7 ±2.79 and p= 0.003, p= <0.001, respectively).

Table 2: Biochemical variables among all groups

Variable	Control (n=30)	Overweight (n=20)	P-value	Obese (n=40)	P-value
Fasting glucose (m\dl)	102.1±8.15	101.9±9.22	0.9360	102.3±13.4	0.9426
Fasting insulin (µu\ml)	11.7 ±2.79	14.98± 4.78	0.0036*	16.77±7.68	<0.001*
Serum Iron (ug\dl)	122.9 ± 28.2	124.3 ±35.8	0.9969	157.6 ±92.5	0.0681
Serum transferrin (g\l)	2.29±0.25	2.50 ± 0.22	0.007*	2.67 ± 0.37	<0.001*
SD is the standard deviation. *One-way ANOVA. Statistically significant differences between Obese, overweight and control ($p < 0.05$).					

Lipid Profile: As shown in table 3 there are significant increase in cholesterol, triglyceride, LDL and VLDL level in obese group when compared to normal group while significant lower level of HDL present in obese males in comparison with controls.

Table 3: Lipid profile biochemical variable among all groups

Variable	Control n=30 Mean ±SD	Overweight n=20 Mean ±SD	P-value	Obese n=40 Mean ±SD	P-value
Total Cholestrol	178.5 ± 19	196.8 ±21.9	0.002*	207.1 ± 32.4	<0.001*
triglyceride	119.8 ±31.4	130.9 ± 42.8	0.2953	175.6 ± 85.1	0.001*
HDL	40.3 ±1.97	37 ± 4.05	0.004*	36 ± 5.69	0.002*
LDL	114.2 ±17	133.6 ± 17.9	0.003*	135.9 ± 20.6	<0.001*
VLDL	23.9±6.29	26.1±8.5	0.2982	35.1±17	0.001*
SD is the standard deviation. *One-way ANOVA. Statistically significant differences between Obese, overweight and control ($p < 0.05$).					

Pearson Correlation: As shown in table 4 and figure 2, correlation coefficient (r) reveals. Significant positive association between insulin and transferrin in obese groups (r =0.51, p = 0.006). However, a significant negative correlation between insulin and Iron found in obese group (r= -0.36, p <0.02). Specifically, ferritin level decrease in increase of insulin level (Figure 3).

Table 4: Pearson Correlation Results between Insulin and Transferrin, and Iron in obese group

Combination	R	95.00% CI	n	P
Insulin – Transferrin	0.51	[.24-.71]	40	.0.006*
Insulin - iron	-0.36	[-.6_ -.05]	40	0.02
R=correlation coefficient, CI= confidence intervals, n= number. * Significant at p<0.05				

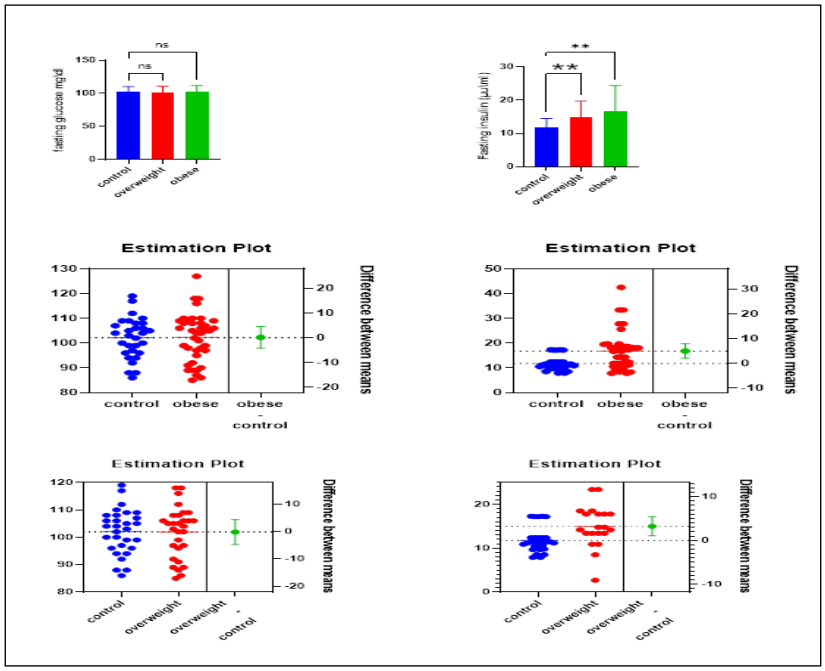

Figure 1: Bar plot of mean values with 95% CI error bars accompanied by Estimation plot with 95% Confidence intervals of mean differences between the groups for Glucose (left) and Insulin (Right). ** Highly Significant at (p<0.001), NS (non-significant)

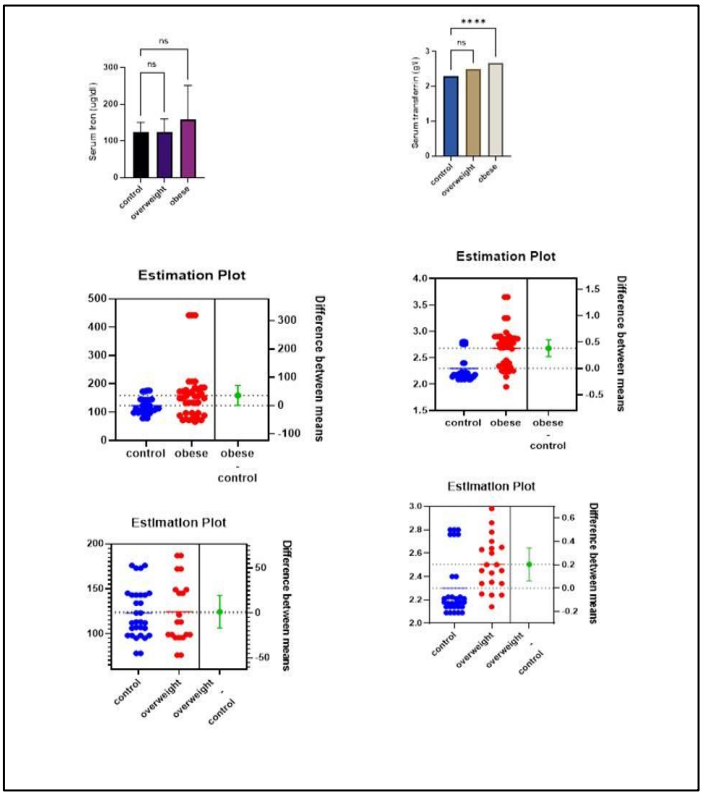

Figure 2: Bar plot of mean values with 95% CI error bars accompanied by Estimation plot with 95% Confidence intervals of mean differences between the groups for Iron (left) and transferrin (Right). *****Highly Significant at (p<0.0001), NS (non-significant)

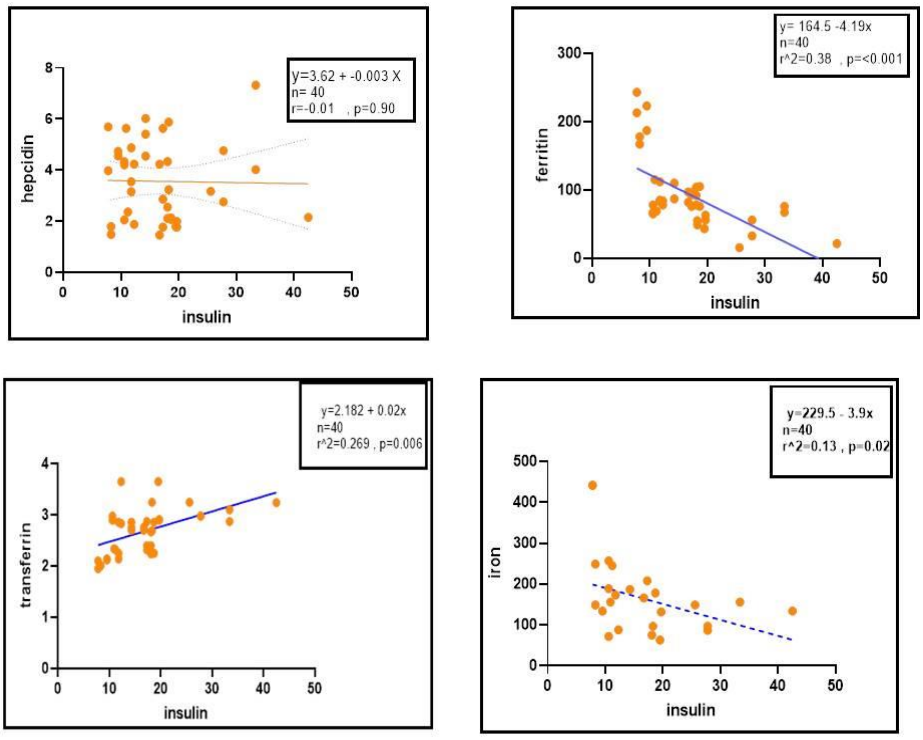

Figure 3: Scatter plot depicting the correlation between Insulin and each of Hepcidin, Ferritin, transferrin and Iron. Regression line was added to ease the interpretation (obese group)

DISCUSSION

Male obesity has become a global health issue, Research on the pathophysiological mechanisms behind obesity and their impact on iron metabolism disorders and the emergence of iron deficiency anemia was sparked by the discovery of the endocrine activity of adipose tissue and adipokines [15]. The main findings of this study are: (1) There were no prominent differences were detected in all three groups to Iron level (2) compared to control group, obesity significantly increased insulin concentration; (3) there were significant increase level of Transferrin in obese males when compared to normal weight males. (4) Strong negative association found between iron and insulin in obese. (5) Significant positive association found between transferrin and insulin in obese group.

This study is consistent with other previous studies [16-20] which also found that BMI is positively correlated with serum transferrin level. The results of this study suggest that there is growing evidence for the association between obesity and iron insufficiency this explained clearly by finding of McClain *et al.*, [21] study on adipose tissue transferrin expression which demonstrated The observed inverse regulation of transferrin receptor mRNA levels with Transferrin in adipocytes implies that adipocyte Transferrin expression may have significant implications for adipocyte iron homeostasis.

We realize that there are still some limitations in this study. Firstly, we did not pay attention to the other obesity linked hormones like (leptin and adeponectin), which may have been the factors that could differentiate the results. Secondly, the lack of several inflammatory marker, such as c-reactive protein (CRP), interleukin-6 (IL-6). These marker are needed to explain the unanswered phenomena in this study[22], moreover, children should be studied carefully in the same manner[23], the effect of the type of diet [24, 25] need to be followed to exclude the effects of lifestyle changes on the level of transferrin.

CONCLUSION

We demonstrated that obese males are more efficient in developing iron deficiency compared with normal weight males. There were no prominent differences detected in all three groups to iron and glucose level. Obesity had crucial effect on insulin concentration. Transferrin can be used as biological marker for iron deficiency in obesity.

CONFLICT OF INTEREST

The authors state no conflict of interest with respect to the research, authorship, and/or publication of this article.

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REFERENCES

1. Shekar, M., & Popkin, B. (2020). Obesity: health and economic consequences of an impending global challenge: World Bank Publications.
2. Sarhat, E. R., Wadi, S. A., & Mahmood, A. R. (2018). Effect of ethanolic extraction of moringa oleifera on paraoxonase and arylesterase enzyme activity in high fat diet-induced obesity in rats. *Research Journal of Pharmacy and Technology*, *11*(10), 4601-4604.
3. Sarhat, E. R. (2018). Study the levels of Leptin, and Adiponectin with Paraoxonase in Obese Individuals (male & female). *Tikrit Journal of Pure Science*, *20*(2), 14-20. ISSN 2415-1726. http://tjps.tu.edu.iq/index.php/j/article/view/185/176.
4. Sarhat, K. G. W. E. R., & Jabir, T. H. (2019). Assessment of melatonin and oxidant-antioxidant markers in infertile men in Thi-Qar Province. *Indian J. Forensic Med. Toxicol*, *13*(4), 1500-1504.
5. Al-Fartusie, F. S., & Mohssan, S. N. (2017). Essential trace elements and their vital roles in human body. *Indian J Adv Chem Sci*, *5*(3), 127-136.
6. Anderson, G. J., & Frazer, D. M. (2017). Current understanding of iron homeostasis. *The American journal of clinical nutrition*, *106*(suppl_6), 1559S-1566S. doi: 10.3945/ajcn.117.155804.
7. Milto, I. V., Suhodolo, I. V., Prokopieva, V. D., & Klimenteva, T. K. (2016). Molecular and cellular bases of iron metabolism in humans. *Biochemistry (Moscow)*, *81*, 549-564. doi: 10.1134/S0006297916060018
8. Sarhat, E. R., Wadi, S. A., Sedeeq, B. I., Sarhat, T. R., & Jasim, N. A. (2019). Study of histopathological and biochemical effect of Punica granatum L. extract on streptozotocin-induced diabetes in rabbits. *Iraqi Journal of Veterinary Sciences*, *33*(2), 189-194.
9. Fillebeen, C., Charlebois, E., Wagner, J., Katsarou, A., Mui, J., Vali, H., ... & Pantopoulos, K. (2019). Transferrin receptor 1 controls systemic iron homeostasis by fine-tuning hepcidin expression to hepatocellular iron load. *Blood, The Journal of the American Society of Hematology*, *133*(4), 344-355.
10. Parrow, N. L., Li, Y., Feola, M., Guerra, A., Casu, C., Prasad, P., ... & Fleming, R. E. (2019). Lobe specificity of iron binding to transferrin modulates murine erythropoiesis and iron homeostasis. *Blood, The Journal of the American Society of Hematology*, *134*(17), 1373-1384.
11. Khalaf, S. J., Aljader, G. H., Sarhat, E. R., Sarhat, T. R., & ABASS, K. S. (2021). Antidiabetic effect of Aqueous Extract of Medicago Sativa with Enhanced Histopathology of Pancreas in Alloxan Induced Diabetic Rats. *PJMH S*, *15*(2), 492-496.
12. Hamad, M. S., Sarhat, E. R., Khalaf, S. J., Sarhat, T. R., & Abass, K. S. (2020). Characteristic Abnormalities In Serum Biochemistry In Patients With Breast Cancer. *Syst Rev Pharm*, *11*, 1967-1971.
13. Daniele, G., Eldor, R., Merovci, A., Clarke, G. D., Xiong, J., Tripathy, D., ... & DeFronzo, R. A. (2014). Chronic reduction of plasma free fatty acid improves mitochondrial function and whole-body insulin sensitivity in obese and type 2 diabetic individuals. *Diabetes*, *63*(8), 2812-2820.
14. Shimoda, Y., Okada, S., Yamada, E., Pessin, J. E., & Yamada, M. (2015). Tctex1d2 is a negative regulator of GLUT4 translocation and glucose uptake. *Endocrinology*, *156*(10), 3548-3558.
15. Alshwaiyat, N. M., Ahmad, A., Wan Hassan, W. M. R., & Al-Jamal, H. A. N. (2021). Association between obesity and iron deficiency. *Experimental and Therapeutic Medicine*, *22*(5), 1-7.
16. Tofano, R. J., Pescinni-Salzedas, L. M., Chagas, E. F. B., Detregiachi, C. R. P., Guiguer, E. L., Araujo, A. C., ... & Barbalho, S. M. (2020). Association of metabolic syndrome and hyperferritinemia in patients at cardiovascular risk. *Diabetes, Metabolic Syndrome and Obesity*, 13, 3239-3248.
17. Mörwald, K., Aigner, E., Bergsten, P., Brunner, S. M., Forslund, A., Kullberg, J., ... & Mangge, H. (2020). Serum ferritin correlates with liver fat in male adolescents with obesity. *Frontiers in Endocrinology*, *11*, 340.
18. Ming, X., Li, Z., Yang, X., Cai, W., Wang, G., Yang, M., ... & Chen, X. (2022). Serum transferrin level is associated with the severity of obstructive sleep apnea independently of obesity: A propensity score-match observational study. *Obesity Facts*, *15*(4), 487-497.
19. Khalaf, S. J., Zbaar, S. A., Abid, I. M., Sarhat, E. R., Hamad, M. S., & Abass, K. S. (2022). Glycosylated hemoglobin and its correlation with both vitamin D and Cortisol. *Archivos Venezolanos De Farmacología Y Terapéutica*, *41*(4), 222–225. https://doi. org/10.5281/zenodo.6944912
20. Abid, I. M., Khalaf, S. J., Zbaar, S. A., Sarhat, E. R., Hamad, M. S., & Abass, K. S. (2022). Dental caries and hormonal changes in postmenopausal women. *Archivos Venezolanos de Farmacologia y Terapeutica*, *41*(4), 216–221. https://doi. org/10.5281/zenodo.6944884.
21. McClain, D. A., Sharma, N. K., Jain, S., Harrison, A., Salaye, L. N., Comeau, M. E., ... & Das, S. K. (2018). Adipose tissue transferrin and insulin resistance. *The Journal of Clinical Endocrinology & Metabolism*, *103*(11), 4197-4208.

22. Fenech, A., Dimech, T., Grech, M. C., & Attard Montalto, S. (2021). Audit on doctors' documentation of overweight and obesity in children attending general children's outpatient's clinics in Malta. *Malta Medical Journal, 32*(1), 84-97.
23. Kovalova, Y. (2021). Irisin and interleukins-33 and-37 levels in patients with ischemic heart disease under obesity influence. *Medicinski časopis, 55*(3).
24. Svobodová, G., Šadibolová, M., Omwanghe, E. A., Ambrož, M., Maletínská, L., & Boušová, I. (2022). HIGH-FAT DIET INDUCES CHANGES IN ACTIVITY AND EXPRESSION OF DRUG-METABOLIZING ENZYMES IN MOUSE LIVER. *MMSL, 91*(Suppl.1), 81.
25. Maryam, T. T., & Entedhar, R. S. (2023). METFORMIN EFFECTS ON NEUREGULIN-1 IN POLYCYSTIC OVARIAN WOMEN. *GEORGIAN MEDICAL NEWS, 4*(337), 56-62.