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Effect of Erythropoietin on Some aspects of Carbohydrate and Lipid metabolism in Obese and diabetic rats

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Keywords

- Erythropoietin
- Diabetes
- Obesity
- HOMA-I/R
- HOMA S %

Abstract

Objectives: To study effect of erythropoietin (EPO) on some aspects of carbohydrate and lipid metabolism in obese and diabetic rats. Material and methods. This study was carried on (50) albino female rats; divided into 5 groups, each consisted of 10 rats: Control, obese, diabetic, erythropoietin treated obese group (after induction of obesity it was injected by erythropoietin 600u/kg I.P. once daily every other day for 5 weeks) and erythropoietin treated diabetic group (after induction of diabetes it was treated as previous group). At the end of experimental period serum samples were collected for estimation of: fasting serum glucose, serum insulin, insulin resistance (HOMA IR), insulin sensitivity (HOMA S%), serum LDL, serum HDL, serum triglyceride (TG), serum cholesterol, glycosylated Hb% and measurement of body mass Index (BMI) **Results:** Erythropoietin treated obese group showed significant decrease in fasting glucose, insulin, HOMA IR, serum LDL, TG, cholesterol and BMI. While, serum HDL and HOMA S% were significantly increased when these results were compared to obese group. Erythropoietin treated diabetic group produced insignificant change in all studied parameters with exception of significant decrease in fasting glucose, TG and glycosylated Hb% when these results compared to diabetic group. **Conclusion:** EPO can be considered as adjunct anti-diabetic/obesity drug to reduce blood glucose, hypolipidemic effect and attenuate weight gain.

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INTRODUCTION

In the last few decades, the prevalence of obesity and diabetes has been increasing rapidly (1). There is strong association between obesity , cardiovascular disease, type 2diabetes and other chronic diseases, this trend suggests a substantial increase in obesity –related morbidity and mortality for the future (2) ,as regarding diabetes mellitus its effect include long term damage, dysfunction and failure of various organs (3) . The increased risk of several co-morbidities associated with obesity and diabetes mellitus increase the need for developing a new applicable treatment.

Erythropoietin (EPO) is a glycoprotein predominantly produced in the kidney and important for production of red blood cell also it acts on several non-erythropoietic tissues. The presence of erythropoietin receptors in cells other than erythroid progenitors suggests that erythropoietin has other biological function in addition to erythropoiesis (4). It has been proved that EPO signaling has a role in regulation of body weight, blood glucose and fat mass (5) in patient with chronic renal disease. EPO administration has shown significant improvement in several metabolic parameters including fasting glucose level and insulin sensitivity (6). Also the findings that pancreatic β-cells harbor functional EPO-Rs and that EPO acts directly on them raise the possibility that EPO treatment may also affect insulin secretion by the pancreatic cells(7).

Previous studies clearly documented the effect of EPO on lipid profile in patient with end stage renal disease by decreasing serum levels of triglycerides, total cholesterol and LDL-cholesterol(8). Therefore, the aim of the present study was to analyze the effect of EPO on some aspects of carbohydrate and lipid metabolism in obese and diabetic rats.

MATERIALS AND METHODS

Animals

The present work was carried on 50 female albino rats ranging in weight between 160-180g, 12-16 weeks old. The rats were housed in isolated animal cages, in a standard animal laboratory room and had free access to water and food all over the period of the work, and kept at room temperature.

The animals were divided into five groups (10 rats in each group):

Group 1 (control group): The animals of this group which were fed standard ad libitum (free access of animal to food and water) commercial chow with tap water injected i.p.by 1ml saline daily for 5 weeks.

Group 2 (Obese group): Obesity induced in these rats by feeding a high fat diet composed of 70% fat, 20% carbohydrates and 10% protein. It consists of cooked caw fat, casein, bread and green vegetables for 4 weeks (9). At the end of feeding period their body weights ranged between 220-260g. Rats in this group were injected by 1 ml saline i.p. daily for 5 weeks after induction of obesity.

Group 3 (Diabetic group): Diabetes induced by multiple low doses of streptozotocin injected i.p. in a dose of 40mg/kg for 5 consecutive days, after a period of two weeks blood sample analysis of rats showed marked hyperglycemia with blood glucose level up to 200 mg/dl (10). Rats in this

group were injected by 1 ml saline i.p. daily for 5 weeks after induction of diabetes.

Group 4: (Erythropoietin treated obese group) rats were injected i.p. with erythropoietin in a dose of 600u/kg once daily every other day for 5 weeks after induction of obesity (11).

Group 5: Erythropoietin treated diabetic group: rats were injected i.p. with erythropoietin two weeks after the start of streptozotocin injection in a dose of 50µg/kg (600u/kg) once daily every other day for 5 weeks (11). Normal and high fat diet constituents were purchased from El-Gomhoria Company, Cairo, Egypt. High fat diet was preserved at 4°C until used All protocols were approved by Tanta Faculty of medicine ethical Committee e Diets of all groups are equal in amount but different only in there constituent

Biochemical assay:

At the end of the experimental period, the animals were fasted overnight, rats were anesthetized by i.p. injection of pentobarbital sodium (50mg/Kg body weight) (12). Then, body weight and body length (nose –anus length) (13) were measured for all groups, blood sample were collected by decapitation of rats and centrifuged at 3000 rpm for 10 minutes and the separated serum was then transferred into clean storage tubes for estimation of the following parameters except for estimation of glycosylated hemoglobin% and whole blood sample used for determination of fasting glucose level according to method of Tietz (14). Serum insulin level according to method of Kao et al., (15) where intra-assay and inter-assay coefficients of variation<10% and ELISA reader capable of reading absorbance at 450nm. HOMA IR (HOMA-I/R = fasting insulin (µIU/ml) ×fasting glucose (mg/dl)/405) and HOMA S %(1/log insulin (µIU/ml) +log glucose (mg/dl) was measured using homeostasis model of assessment (HOMA) analysis (16). Serum LDL cholesterol was measured according to method of Assmann et al, (17). Serum HDL cholesterol was measured according to method of Grove (18). Serum triglycerides level was measured by GPO enzymatic method Mcgowan et al., (19). Serum total cholesterol level was measured by BioMed-cholesterol-LS kits according to Tietz, (20). Glycosylated hemoglobin%: was measured according to method of Bissé and Abraham (21). Body mass index (BMI) was measured using the formula of Novelli et al, (13).The kits and biochemical used in the study obtained from Sigma chemical Co.

Statistical analysis:

The data were expressed as the mean ± standard deviation. Data from our study were analyzed using the unpaired student's t-test to assess significant difference between two groups. P-values <0.05 were considered statistically significant. All the analyses were performed using Graph Pad Instat, 32 bit for win 95/NT (Version 3.05).

RESULTS

Our results are shown in tables 1 and 2 and figures 1-4. In the obese group, fasting glucose is insignificantly changed when compared to control group, while serum insulin, HOMA IR, serum LDL, serum triglyceride, serum cholesterol and BMI showed significant increase when compared with the control group. On the other hand HOMA S% and serum HDL in the obese group showed

significant decrease when compared with control group (table 1 and figures 1 and 2).

In the erythropoietin treated obese group, fasting glucose, serum insulin ,HOMA IR,serum LDL, serum TG, cholesterol and BMI (g/cm2) showed significant decrease when compared with the control obese group. A significant increase in HOMA S% serum level of HDL in the erythropoietin treated obese group when compared with the obese group. However, glycosylated Hb results in obese group and in erythropoietin treated obese cannot be statistically analyzed as SD is zero (table 1 and figures 3 and 4).

Table (1): Comparison between all parameters studied in control normal, control obese and control diabetic groups

Parameters	Control group (n=10)	Obese group (n=10)	Diabetic group (n=10)	P_1 value	P_2 value
Fasting glucose (mg/dl)	85.48±7.38	91.94±7.70	227.19±15.40*	0.071	0.0001
Serum insulin (µIU/ml)	13.98±2.04	21.76±4.85*	9.98±1.81*	0.0002	0.0001
HOMA IR	1.77±0.25	2.76±0.63*	1.60±0.31	0.0002	0.187
HOMA S%	57.73±8.74	37.83±8.42*	64.37±12.3	0.0001	0.1812
Serum LDL (mg/dl)	49.75±6.48	75.54±12.13*	77.87±7.87*	0.0001	0.0001
Serum HDL (mg/dl)	49.27±3.07	35.29±5.57*	33.38±5.92*	0.0001	0.0001
Serum TG (mg/dl)	144.69±9.19	162.64±8.97*	158.16±9.14*	0.0003	0.0041
Serum cholesterol (mg/dl)	123.34±6.67	150.46±13.41*	128.90±11.40	0.0001	0.1998
BMI (g/cm)	0.56±0.08	0.78±0.07*	0.44±0.05*	0.0001	0.0007

Data are given as mean ± SD. (P_1) Obese group vs control group & (P_2) Diabetic group vs control.
*Denote statistical significance vs control group.

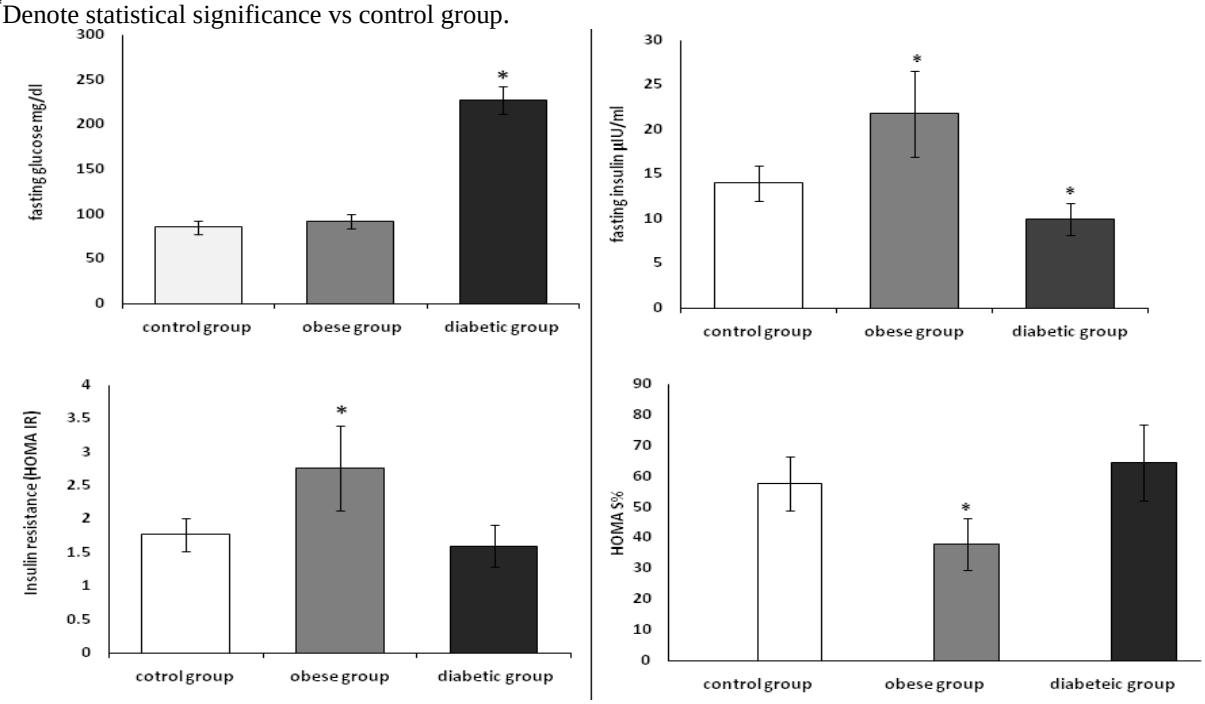

Fig. (1): Fasting glucose, fasting insulin, HOMA IR, HOMA S% in control normal, control obese and control diabetic groups. *Denote statistical significance vs control group.

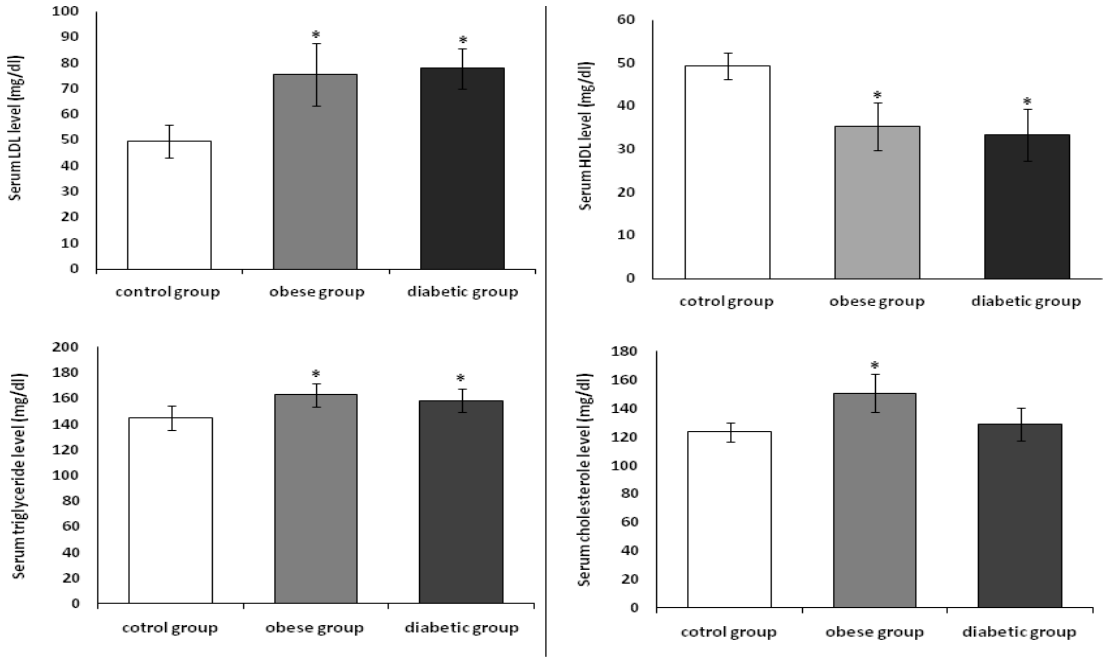

Fig. (2): Serum LDL, HDL, triglycerides and cholesterol levels in control normal, control obese and control diabetic groups**.** *Denote statistical significance vs control group.

In diabetic group the fasting glucose, serum LDL, serum triglyceride showed significant increase when compared to the control group. On the other hand serum insulin, serum HDL and BMI in the diabetic group showed significant decrease when compared to control group. However the HOMA IR, HOMA S% and cholesterol in the diabetic group are insignificant when compared to control group, in diabetes, the glycosylated Hb results cannot be statistically analyzed as SD is zero for control group (table 2).

In erythropoietin diabetic group treated group, The fasting glucose, serum level of TG and glycosylated Hb% showed significant decrease

when compared with the diabetic non treated group, serum insulin, HOMA S%, serum LDL level, serum HDL, cholesterol and BMI showed insignificant change when compared to the control diabetic group (table 2).

DISCUSSION

Induction of obesity in rats results in a picture of metabolic syndrome (22). EPO treated obese group showed significant decrease in fasting glucose, serum insulin, HOMA IR, LDL, triglyceride, total cholesterol and BMI .However HDL and HOMA S% were significantly increased, the reduction of the serum glucose level may be due to inhibition of gluconeogenesis by decreasing the expression of phosphoenol-pyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) which are two rate-limiting enzymes for hepatic glucose production(23).Furthermore, EPO administration increases vascular endothelial growth factor(VEGF) expression in the pancreatic islets which mediates angiogenesis in the islets via cell JAK2 pathway (24). JAK2 is able to phosphorylate and activate the downstream antiapoptotic target signals (25). Also, the lowering of glucose level could be due to that EPO induced increase in glucose uptake by increasing

glucose transporter 4(GLUT4) trafficking toward the plasma membrane in the adipocyte cells (26).EPO also may lower the fasting glucose by increasing sensitivity to insulin.

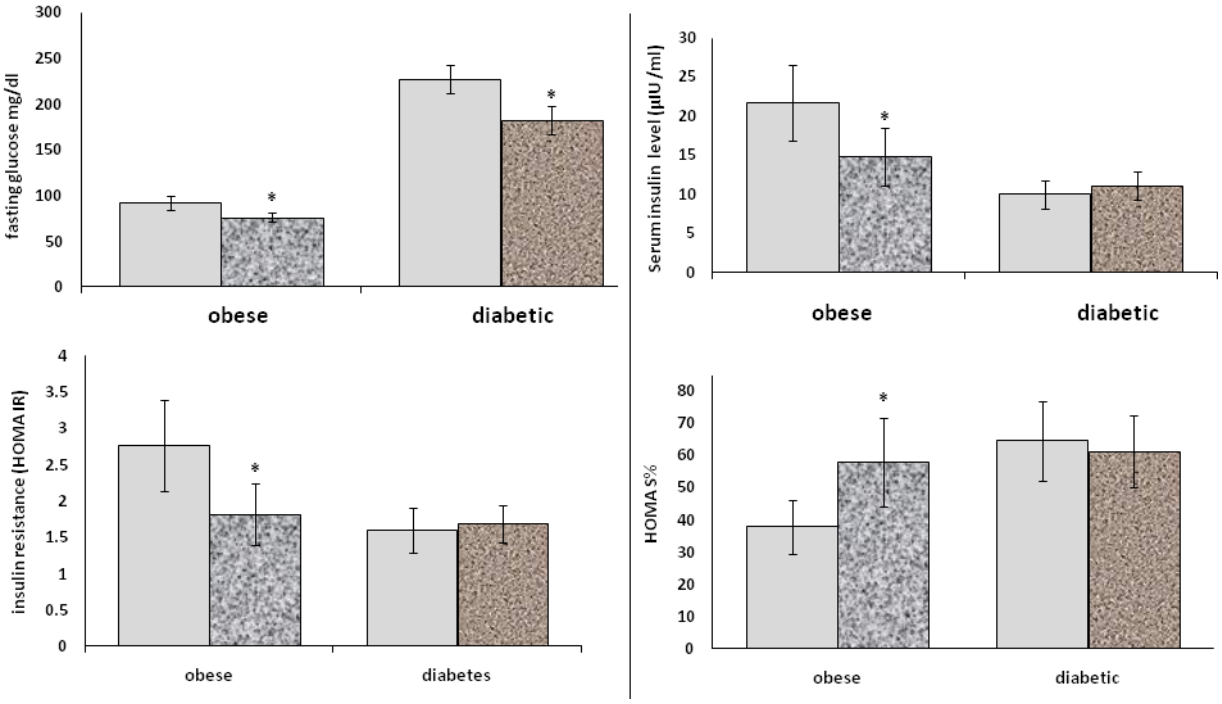

Fig. (3): Fasting glucose, fasting insulin, HOMA IR, HOMA S% in obese, erythropoietin treated obese, diabetic and erythropoietin treated diabetic groups. *Denote statistical significance vs control group.

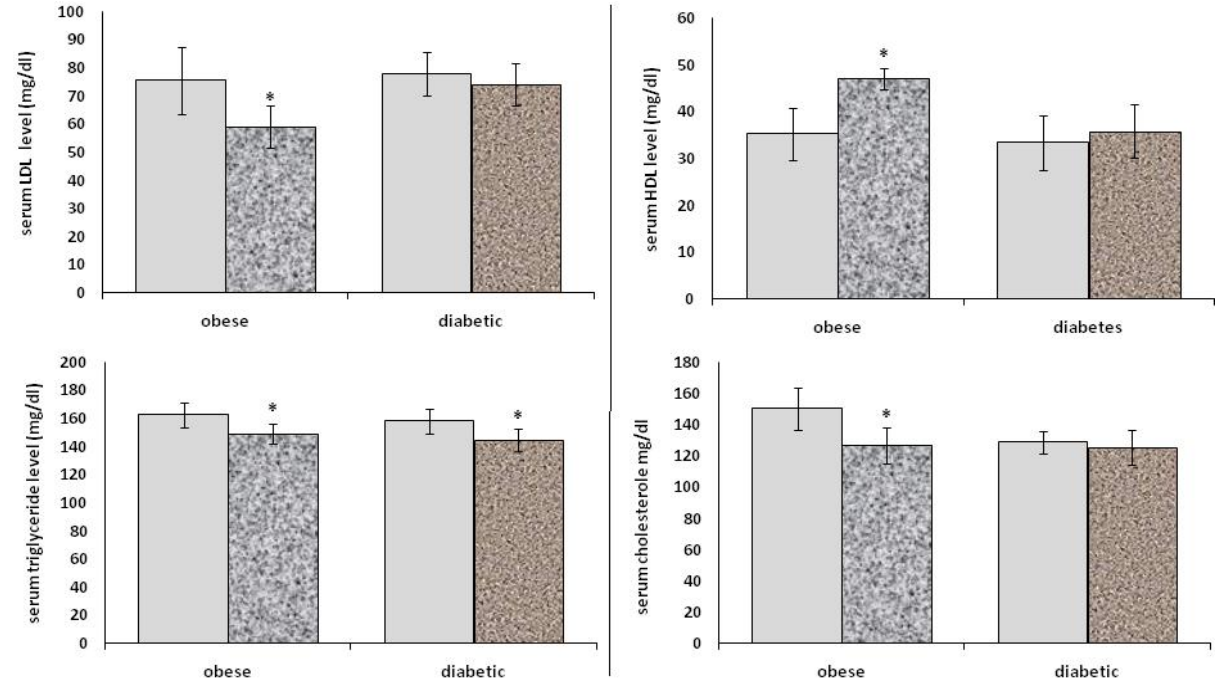

Fig. (4): Serum LDL, HDL, triglycerides and cholesterol levels in obese, erythropoietin treated obese, diabetic and erythropoietin treated diabetic groups. *Denote statistical significance vs control group.

Table (2): Comparison between all parameters studied in erythropoietin treated obese and erythropoietin treated diabetic groups

Parameters	Obese group (n=10)	Erythropoietin treated obese group (n=10)	Diabetic group (n=10)	Erythropoietin treated diabetic group (n=10)	P_1 value	P_2 value
Fasting glucose (mg/dl)	91.94±7.70	75.83±4.84*	227.19±15.40	182.21±14.96#	0.0001	0.0001
Serum insulin (µIU/ml)	21.76±4.85	14.91±3.70*	9.98±1.81	11.14± 1.76	0.0023	0.1645
HOMA IR	2.76±0.63	1.82±0.43*	1.60±0.31	1.69±0.26	0.0010	0.4872
HOMA S%	37.83±8.42	57.91±13.64*	64.37±12.31	61.08±11.16	0.0009	0.5391
Serum LDL (mg/dl)	75.54±12.13	59.30±7.47*	77.87±7.87	74.19± 7.59	0.0020	0.3004
Serum HDL (mg/dl)	35.29±5.57	47.12±2.22*	33.38±5.92	35.81±5.67	0.0001	0.3609
Serum TG(mg/dl)	162.64±8.97	149.27±7.16*	158.16±9.14	144.68±7.96#	0.0017	0.0025
Serum cholesterol (mg/dl)	150.46±13.41	126.96±7.05*	128.90±11.40	125.71± 11.25	0.0001	0.5371
BMI (g/cm)	0.78±0.70	0.59±0.05*	0.44±0.05	0.42±0.05	0.0001	0.2751
Glycosylated Hb (%)	----	----	7.4%±0.52	6.4%±0.52#	-----	0.0004

Data are given as mean ± SD. (P_1) erythropoietin treated obese group vs control obese. (P_2) Erythropoietin treated diabetic group vs control diabetic. *Denote statistical significance vs control obese. #Denote statistical significance vs control diabetic. It is not possible to analyze Glycosylated Hb (%) in erythropoietin treated obese group vs control obese because SD is zero.

Results of the present work revealed significant decrease in serum insulin and HOMA- IR when EPO is administrated to obese rats which could be due to reducing the transcription factor enhancing binding protein alpha (CEBP/α). This transcription factor play role in insulin sensitivity as it is required during adipogenesis for development of insulin-stimulated glucose uptake (27). EPO therapy significantly decreased plasma cell differentiation antigen 1 (PC-1) activity to the normal value. Elevated levels of PC-1 play a role in the development of insulin resistance in obesity as PC-1 inhibits insulin signaling either at the level of the insulin receptor or downstream at a post receptor site (28) Also, the mechanism by which EPO affects sensitivity to insulin is that EPO may operate via an increase in NO which is a powerful vasodilator as well as insulin sensitizer (29). Improvement in insulin sensitivity caused by EPO receptor agonist was largely due to enhancement of insulin-stimulated glucose uptake in skeletal muscle and heart by activation of non-oxidative mechanisms of glucose utilization in skeletal muscle and heart (30).

Concerning lipid metabolism it is evident from the present work that EPO when administrated to obese rats causes significant decrease in serum triglyceride, cholesterol, LDL-cholesterol, on the other hand EPO result in significant increase in

HDL-cholesterol. The significant decrease of triglyceride level may be related to an improved response to insulin, science it is known that patients with increased insulin resistance had diminished lipoprotein lipase activity, while the triglyceride production remains normal and thus results in hypertriglyceridemia (6).

Siamopoulos et al., (31) reported that the increase in serum HDL levels could be attributed to the improvement in tissue oxygenation which increases activity of ATP-binding cassette transporter (ABCA1) or other enzymes involved in HDL maturation and lead to the increase in HDL levels.It is known that ABCA1 mediates the efflux of cholesterol and phospholipids to lipid-poor apolipoproteins (apo-A1andapo-E), which then form HDL (32). Epo treatment improves glucose utilization and reduces insulin resistance, and it is known that high insulin plasma levels stimulate cholesterol synthesis (33). As regard the BMI it is evident from the present work that EPO significantly reduce the BMI in obese group, increased energy consumption or expenditure is a possible mechanism (34).

It was reported that rhEPO not only increased the serum level of leptin, but up- regulated the expression levels of hepatic leptin receptors (Ob-Ra and Ob-Rb). Therefore, it is accepted that EPO might be involved in leptin-mediated weight loss (35). EPO treated diabetic group produced insignificant change in all studied parameters with exception of significant decrease in fasting glucose, serum triglyceride and glycosylated Hb% when these results compared to control diabetic group . As regard to the hypoglycemic effect of EPO could be due to promotion of β -cell growth and survival it inhibits apoptosis in β cells during diabetes progression (36). Glycosylated Hb % is an indicator for improvement of blood glucose level in diabetic (37), thus the reduction of glycosylated Hb% could be due to the hypoglycemic effect of the EPO in the present work. The significant decrease of triglyceride level may be related to an improved response to insulin (6)

Conclusion

It can be concluded that EPO can be considered as adjunct anti-diabetic/obesity drug to reduce blood glucose, hypolipidemic effect and attenuate weight gain.

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