IS APELIN A PROMISING TARGET IN DIABETES MELLITUS OF RATS: TYPE I VS. TYPE II?

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ABSTRACT

Background: Apelin is a novel adipokine identified as an endogenous ligand of receptor APJ. Apelin and its receptor APJ are expressed in a wide variety of tissues including heart, brain, kidneys and lungs. Apelin in human physiology acts as a regulating peptide of cardiovascular, hypothalamus-hypophysis, metabolic, gastrointestinal and immune systems.

Objective: The present study was carried out to assess the value of apelin in management of type II vs. type I diabetes mellitus in rats.

Design: A total number of 60 healthy adult male albino rats were used. Rats were divided into 2 groups: group I (n=40) Streptozotocin induced diabetic group; model of type I diabetes and group II (n=20) high fat diet induced obesity and diabetic group; model of type II diabetes. Group I was subdivided into 4 equal subgroups (saline treated control, an insulin treated, an apelin treated and an apelin and insulin treated subgroups), while group II was further subdivided into 2 equal subgroups (a saline treated control and an apelin treated groups). In all groups the body weight, nose to anus length were measured, and these data were used to calculate the body mass index (BMI). In addition, serum glucose, insulin, triglycerides, total cholesterol, LDL and HDL cholesterol were estimated and used to calculate the homeostasis model assessment as a measure of insulin resistance (HOMA-IR) and the homeostasis model assessment as a measure of β cell function (HOMA-β).

Results: This study revealed that an intraperitoneal injection of apelin induced insignificant changes in most of parameters measured in animals of group I when compared with insulin treated subgroup. On the other hand, animals of group II showed a significant decrease in serum levels of glucose and insulin when compared with saline treated controls with resulting a significant decrease in HOMA-IR and a significant increase in HOMA- β. Furthermore, apelin injection induced a significant increase in serum triglyceride, total cholesterol and LDL cholesterol levels and insignificant changes in HDL cholesterol levels in group II rats.

Conclusion: In models of type II diabetes, apelin significantly induced amelioration of serum glucose level and enhance insulin sensitivity that was denoted by a significant reduction in HOMA-IR, this was not the case in rats of type I diabetes, so it is promising therapeutic target in management of obesity induced type II diabetes rather than type I.

Key words: Apelin, type II diabetes, high fat diet (HFD), Streptozotocin induced diabetes, HOMA-IR, HOMA-β, obesity.

INTRODUCTION

Apelin is a novel bioactive peptide originally isolated as the endogenous ligand of the orphan G protein- coupled receptor, APJ. It is named apelin after ***AP***J ***e***ndogenous ***li***ga***n***d **[1].** Apelin is secreted mainly from white adipose tissue (WAT) and expressed by each of the main adipose tissue depots in mice (*i.e.* intraabdominal and subcutaneous adipose tissue) **[2].** In addition, it is demonstrated that white adipocytes express apelin mRNA in excess amounts when compared with the other cell types present in this tissue or with organs known to express apelin such as kidney, heart, lung, endothelium, gastrointestinal tract, oxyntic cells of the stomach, Kupffer cells and many areas in the brain **[3].**

This novel peptide hormone exists in multiple molecular forms according to the amino acid sequence and number. Human preproapelin gene is located on chromosome Xq25-26.1. **[4].** Various forms of apelin could be generated from the 77-amino-acid precursor preproapelin. In vivo, the predominant forms of apelin are thought to be apelin-36, and the most effective forms are the pyroglutamylated form of apelin-13, apelin-17 and to a lesser extent apelin-12. In addition, there are other lower fragments of apelin which may have no APJ binding activity **[5].**

The apelin–APJ system has been postulated to play physiological roles in CVS, metabolism, CNS, GIT, pancreas, osteoblast, T lymphocyte,…etc **[6].** It was recently shown that acute apelin-13 treatment (200 pmol/kg intravenously) of high-fat diet (HFD) fed obese and insulin-resistant mice showed improved glucose tolerance, during euglycemic–hyperinsulinemic clamp **[7].** Also both short- and long-term apelin treatment improves insulin sensitivity in insulin-resistant obese mice **[8].** Thus, apelin is efficient in improving altered glucose metabolism, an effect that was found to be mediated mainly by an increase in glucose uptake in skeletal muscle **[7].** The condition is markedly questionable in cases of type I diabetes, despite of alleviating endoplasmic reticulum stress in the pancreas of Akita mice, a well-established type I diabetic model **[9],** apelin's role in glucose and lipid homeostasis herein type I diabetes was not studied good.

In the light of previous data, this study was carried out to demonstrate the effect of apelin on glucolipidemic homeostasis and insulin sensitivity in type I vs. type II diabetes mellitus in rats.

MATERIAL AND METHODS

Animals:

A total number of 60 healthy adult male albino rats, 12 to 15 weeks old and weighing 180-200 gm were used after approval of the experimental protocol by a Zagazig university ethics committee. 40 animals were bred in a light-temperature-controlled animal house (12-h light, 12-h dark cycle, and temp around 25 C^{o}, respectively) and fed the mixed commercial rat laboratory chow with free access to water till the time of the experiment. The remaining rats were fed on high fat diet (HFD) that generally contained protein 20%, carbohydrates 35% and fat 45%. This fat was mainly in form of lard and soy bean **[10].** Then the following protocols were performed:

Group I "normally fed group" (n = 40) that had been fed on normal chow for 12 weeks, an experimental model of type I diabetes was induced by intra peritoneal injection of streptozotocin (STZ, 55mg/kg body weight) dissolved in Na citrate solution adjusted at pH 4.5 **[11].** To avoid incidental puncture of the intestines, which would have decreased the success rate for induction of diabetes, all animals were fasted overnight **[12].** Because of streptozotocin's ability to induce fatal hypoglycemia as a result of massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 hours of STZ administration for the next 48 hours to prevent hypoglycemia. Rats with diabetes (blood glucose > 160 mg/dl) were selected for experiment **[13].** Furthermore, the diabetic rats were divided into 4 equal subgroups: 1st subgroup was used as a control and injected by saline (0.4 ml) via the intraperitoneal (IP) rout **(C)**, 2^{nd} group was treated with IP injection of apelin (dose = 100 nmol/kg) at 1.30 PM for 14 days **(APL) [8]**, 3^{rd} subgroup was treated with insulin (Humulin 70/30) by subcutaneous injection twice daily, in a dose designed according to blood glucose level that is measured before injection **(INS)**, while the 4^{th} subgroup in addition to insulin therapy, it was exposed to IP injection of apelin **(APL-INS)**. Unfortunately 3 rats in the control group had been died during the experiment.

Group II "HFD fed group" (n= 20), when get obese (BMI > 0.68 gm/cm^2**) [14],** and diabetic (blood glucose > 160 mg/dl) **[13],** it had been divided into 2 equal sub groups: 1^{st} subgroup was injected by normal saline (0.4 ml) via IP route and 2^{nd} subgroup was exposed to IP injection of apelin (dose = 100 nmol/kg) at 1.30 PM for 14 days **[8],** and daily changes in body weight were measured at 1 O'clock

Chemicals:

Apelin-13 trifluoroacetate salt: (Sigma- Aldrich co. USA).

Streptozotocin[N-Methylnitrosocarbamoyl -α-D-glucosamine]: (SIGMA-ALDRICH Co.-USA).

Insulin [(70/30) 100 iu/ml, 70% isophane insulin and 30% soluble insulin of rDNA origin = Humulin 70/30 (LILLY Egypt under license of ELI LILLY CO – USA)]

Kits for estimation of apelin: (Phoenix Pharmaceuticals, Belmont, CA).

Kits for estimation of insulin: (BioSource Europe S.A.).

Kits for estimation of serum glucose: INS-EASIA, KAP1251 (BioSource Europe S.A.)

Kits for triglycerides, total cholesterol and HDL level estimation (BioSource Europe S.A.).

Equipments:

Digital scale, centrifuge & syringes (5ml and 1ml)**.**

Blood sampling:

Blood samples (8 ml/rat) were obtained after sacrification of rats after overnight fast, and allowed to clot for 2 hours at room temperature before centrifugation for 20 minutes at approximately 500 rpm **[15].** The separated serum was stored at -20 °C. till the time of measurement. Repeated freezing and thawing were avoided **[16].**

Methods

1- Measuring serum glucose levels: Glucose was determined after enzymatic oxidation in the presence of glucose oxidase according to **[17].**

2- Measuring serum insulin levels: insulin ELISA Kits were used, which include an enzyme immunoassay for the quantitative determination of insulin in sera of rats according to **[18].**

3- Measuring serum apelin levels: using a blood sample from the saphenous vein without anesthesia **[19],** then sandwich enzyme immunoassay was used, according to the protocol designed by (Phoenix Pharmaceuticals).

4- Calculating the homeostasis model assessment as a measure of insulin resistance (HOMA-IR): by using the following equation; [HOMA-IR = insulin (μU/mL) x glucose (mmol/L) / 22.5] **[20-21].**

5- Calculating the homeostasis model assessment as a measure of β cell function (HOMA-β): according to the following equation; [HOMA- β = 20 x insulin (μ U/mL) / (glucose - 3.5)] **[22].**

6- Estimation of serum Triglycerides: firstly triglycerides are enzymatically hydrolyzed to glycerol then measured according to **[23].**

7- Estimation of serum cholesterol levels: total cholesterol was estimated according to the method described by **Tietz et al, [17].**

8- Estimation of serum HDL-cholesterol: it was determined enzymatically according to **[24].**

9- Determination of LDL cholesterol: According to **(25),** LDL was calculated as follows: LDL=TC-HDL-TG/5.

Statistical Analysis:

Data were presented as mean ± SEM. Statistical significance was determined by unpaired Student's "t" test and ANOVA with a post hoc test was used to analyze differences in multiple comparisons. P values < 0.05 were considered significant.

In statistical analysis, SPSS version 19 program for Windows (SPSS Inc. Chicago, IL, USA) was used**.**

RESULTS

Serum apelin level after IP injection

Blood samples taken from the saphenous vein of conscious apelin treated rats after IP injection by 2 hours showed rise of mean ± SEM of the serum apelin level to about 3 times the serum level in controls *(60.50 ± 2.66 ng/ml vs. 18.81±1.43 ng/ml) (P < 0.05, Fig. 1A.)*

Effects of apelin on serum glucose, insulin levels, HOMA-IR and HOMA-β in STZ-induced diabetic rats.

Figure 1B shows the serum glucose level (mmol/l) in STZ- induced diabetic studied subgroups in which there were a significant decrease (P < 0.05) in serum glucose level when compared with that of the control (C) group. Also, there was a significant decrease in serum glucose level between apelin treated (APL) and insulin treated (INS) groups *(APL vs. INS: 16.12 ± 1.87 vs. 9.47 ± 0.55, P < 0.05).* On the

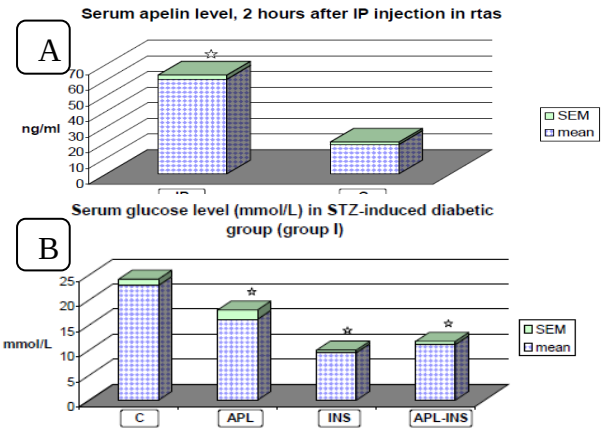
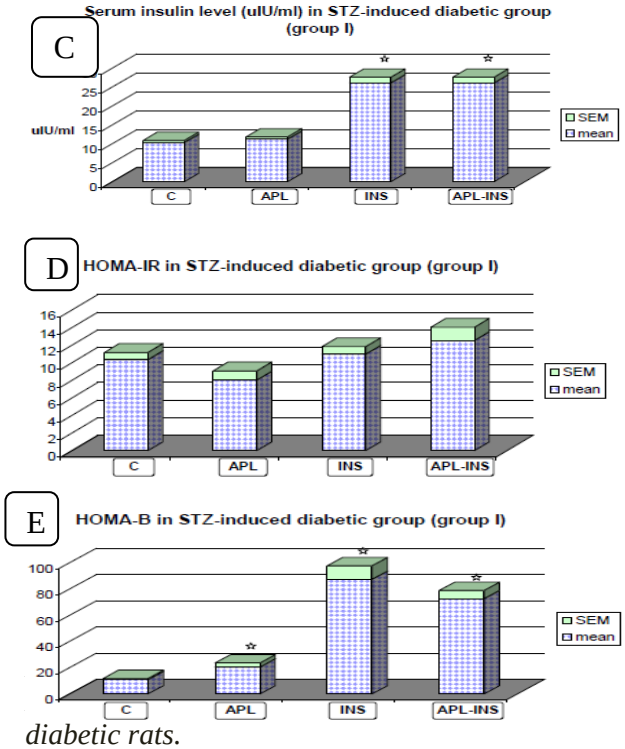


Fig. 1. Serum apelin level after IP injection of apelin (A), effects of apelin on the serum glucose level (B), serum insulin level (C), HOMA-IR values (D) and HOMA β (E) values in STZ-induced diabetic groups. Vertical bars represents the mean ± SEM (*, P < 0.05).

Other hand, there was an insignificant difference in serum glucose level in (INS) group when compared with that of the (APL-INS) group range *(INS vs. APL-INS; P > 0.5).*

Figure 1C demonstrates an insignificant difference in the serum insulin level between apelin treated group (APL) and control (C) groups *(APL vs. C; P > 0.5).* Furthermore, there was an insignificant difference in means ± SEM between INS and APL-INS groups *(INS vs. APL-INS; P > 0.5)* suggesting that apelin added nothing to the serum insulin level. In contrast, the apparent significant rise in serum insulin level among INS & APL-INS vs. C groups was attributed to exogenous insulin injections.

As regarding HOMA-IR and HOMA-β, there were an insignificant difference in means ± SEM of HOMA-IR among APL, INS & APL-INS vs. C groups, while INS & APL-INS vs. APL demonstrated a significant rise in HOMA-IR *means ± SEM (10.97 ± 0.86, 12.53 ± 1.51 vs. 8.04 ± 1.05) (P < 0.05, Fig. 1D).* Moreover, there was insignificant changes in HOMA-β in mean ± SEM between INS vs. APL-INS group *(P > 0.05, Fig. 1E),* while APL vs. C showed a significant rise in HOMA-*β (20.47±3.12 vs. 10.88 ±0.91) (P < 0.05, Fig. 1E.)*



diabetic rats.

Figure 2A shows the effect of apelin on serum triglyceride level in rats of group I, in which there was a significant reduction in serum triglyceride level in PAL, INS & APL-INS vs. C *(P < 0.05).* As regarding total, LDL and HDL serum levels there were an insignificant difference in means ± SEM between APL vs. C or between INS vs. APL-INS subgroups *(Fig 2; B, C & D respectively).*

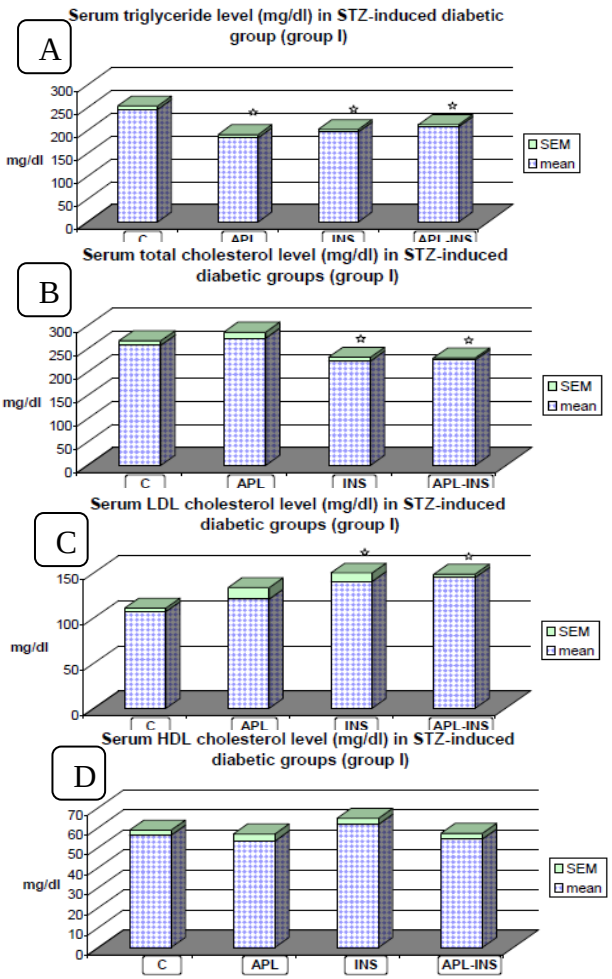


Fig. 2. Effects of apelin on the serum triglyceride level (A), serum total cholesterol (B), serum LDL-cholesterol (C) and HDL-cholesterol (D) levels in STZ- induced diabetic groups. Vertical bars represents the mean ± SEM (*, P < 0.05).

Effects of apelin on BMI, serum glucose, insulin levels, HOMA-IR and HOMA-β in type II diabetic rats.

Table (1) shows the effect of apelin injection on BMI, serum glucose, insulin levels, HOMA-IR and HOMA-β in obesity-induced diabetic group. It was found that apelin injection, produced a significant decrease in the mean ± SEM values of serum glucose level in apelin treated subgroup (APL) when compared to that of the control (C) subgroup *(APL vs. C: 7.44 ± 0.34 vs. 8.83 ± 0.11mmol/l, P < 0.05; Fig 3A).*

In figure 3B there was a significant decrease in the mean values ± SEM of insulin level in APL vs. C *(APL vs. C: 28.05 ± 1.12 vs. 32.37 ± 0.94 µIU/ml, P < 0.05).*

Moreover, chronic IP administration of apelin produced a significant reduction in HOMA-IR values in APL vs. C *(APL vs. C: 9.39 ± 0.74 vs. 12.72 ± 0.51, P < 0.05; Fig 3C)* this might be explained by the combined reducing effect of apelin on serum glucose and insulin. In addition, there was a significant increase in the mean values of HOMA-β in APL vs. C (*APL vs. C:* 148.77 ± 8.98 vs. 121.38 ± 2.15, P < 0.05*; Fig 3D).*

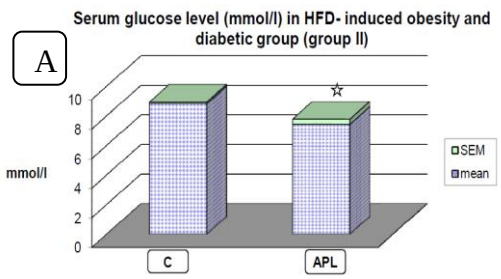
Effects of apelin on serum triglycerides, and total, LDL & HDL cholesterol levels in type II diabetic rats.

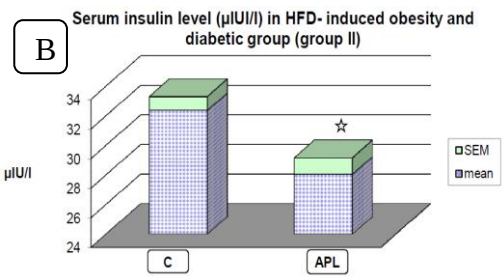
Figure 4A shows the effect of apelin on serum triglyceride levels in rats of type II diabetic group. There was a significant increase in the mean values ± SEM of serum triglyceride levels in rats of APL subgroup when compared with that of C subgroup *(APL vs. C: 199.54 ± 5.73 vs. 178.83 ± 7.65 mg/dl, P < 0.05).*

Also, there was a significant increase in the mean values ± SEM of serum total and LDL cholesterol levels in APL when compared to that of C *(both P < 0.05; Fig. 4, B and C).* In the other hand, figure 4D demonstrated an insignificant change in the mean values ± SEM of serum HDL-cholesterol levels in APL vs. C *(P > 0.05).*

Table (1): Effect of apelin IP. injection on BMI, serum glucose, insulin. HOMA-IR, HOMA-β and lipid profile in type II diabetic rats (group II).

parameters	Sub-Groups	Mean ± SEM	P value
BMI	Control	0.83 ± 0.00	NS
	Apelin treated	0.80 ± 0.01	
serum glucose mmol /l	Control	8.83 ± 0.11	P < 0.05
	Apelin treated	7.44 ± 0.34	
Insulin µIU / ml	Control	32.37 ± 0.94	P < 0.05
	Apelin treated	28.05 ± 1.12	
HOMA-IR	Control	12.72 ± 0.51	P < 0.05
	Apelin treated	9.39 ± 0.74	
HOMA-β	Control	121.38 ± 2.15	P < 0.05
	Apelin treated	148.77 ± 8.98	
Serum triglycerides mg /dl	Control	178.83 ± 7.65	P < 0.05
	Apelin treated	199.54 ± 5.73	
Serum total Cholest mg /dl	Control	180.16 ± 8.62	P < 0.05
	Apelin treated	215.54 ± 7.43	
Serum LDL Cholest mg /dl	Control	85.83 ± 6.50	P < 0.05
	Apelin treated	140.36 ± 6.75	
Serum HDL cholestmg /dl	Control	60.50 ± 2.66	NS
	Apelin treated	56.63 ± 1.41	





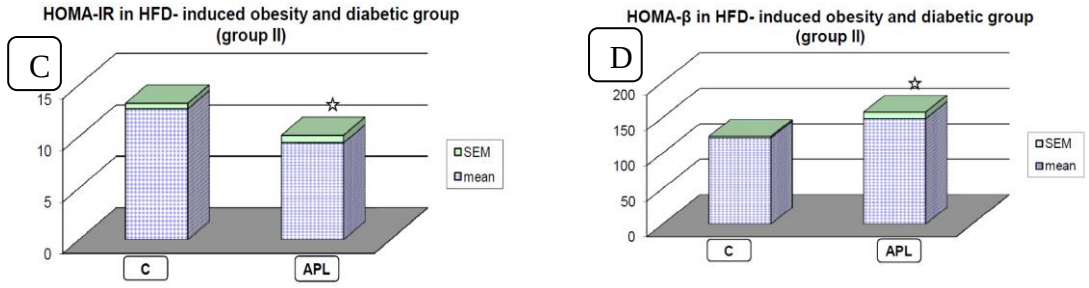


Fig. 3. Effects of apelin on the serum glucose level (A), serum insulin level (B), HOMA-IR values (C) and HOMA β (D) values in type II diabetic groups. Vertical bars represents the mean ± SEM (* , P < 0.05).

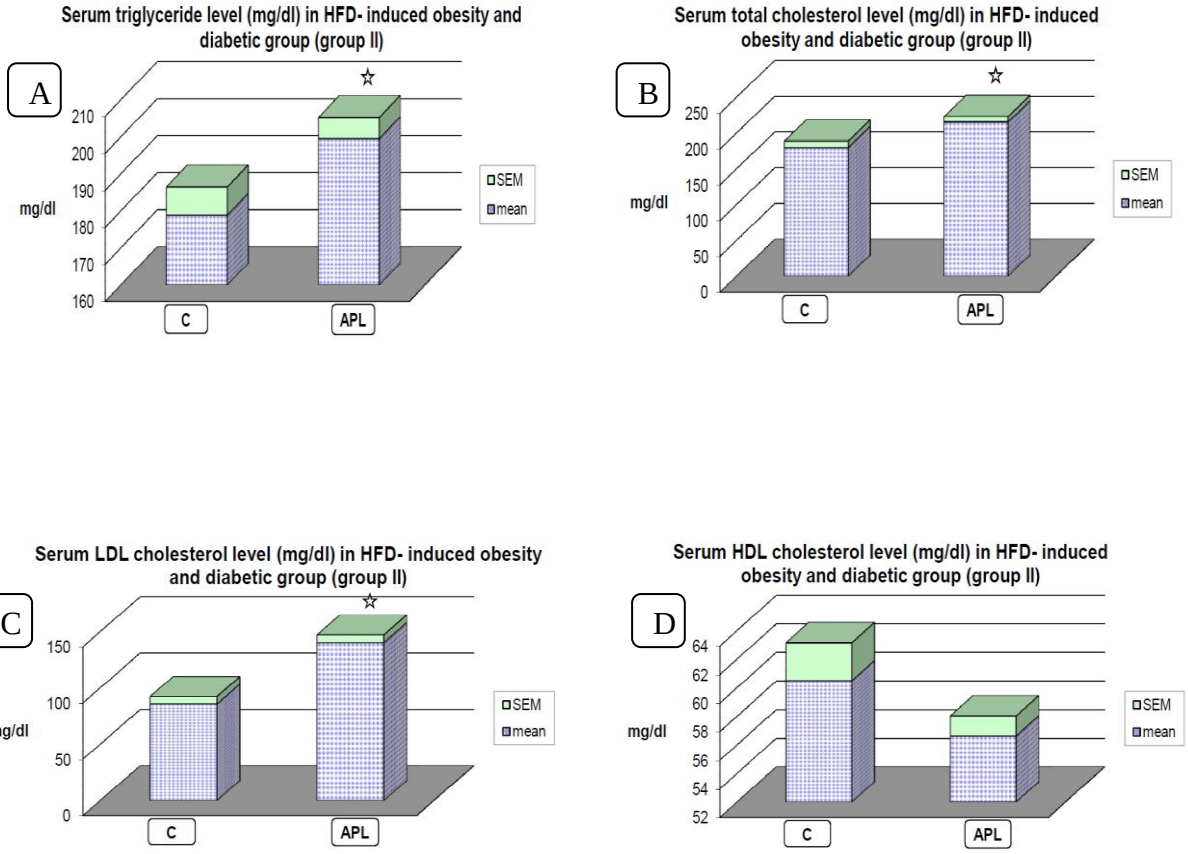


Fig. 4. Effects of apelin on the serum triglyceride level (A), serum total cholesterol (B), serum LDL-cholesterol (C) and HDL-cholesterol (D) levels in type II diabetic groups. Vertical bars represents the mean ± SEM (* , P < 0.05).

DISCUSSION

Apelin is a novel adipokine, involved in regulation of the metabolic, cardiovascular, gastrointestinal, and immune functions, as well as in bone physiology, fluid homeostasis and cardiovascular system embryonic development **[1].** The apelinergic system has been demonstrated to be involved in the pathogenesis of a number of high prevalence conditions – such as hypertension, heart failure (HF), obesity, glucose intolerance and diabetes mellitus type II (TIIDM), as well as HIV infections, diabetes insipidus, gastric ulcer and osteoporosis **[26]**.

In this study, apelin ameliorated the blood glucose resulting in a significant reduction in serum glucose level in apelin treated obesity-induced diabetic group (type II DM) when compared with control subgroup, while it failed to induce the same effect in APL-INS vs. INS subgroups of STZ-induced diabetes (type I DM) in the equal period of treatment. Furthermore, it also significantly decreases the serum insulin level in obesity induced diabetic group while, inducing insignificant changes on serum insulin in APL-INS vs. INS subgroups. In addition to above finding, apelin induced a significant reduction in HOMA-IR together with a significant rise in HOMA-β in rats of type II diabetes without any similar significant effect in rats of type I diabetes (APL-INS vs. INS). These data collectively support the hypothesis stats that apelin in conditions of obesity induced diabetes, has a peripheral unique insulin independent effect on glucose homeostasis, this effect may be increasing glucose uptake and improving insulin sensitivity status in insulin responsive tissues as adipose tissue and skeletal muscles resulting in improvement of beta cell function. This is not the case in type I diabetes models due to different pathophysiology of type I DM, despite of some beneficial effects of apelin on beta cells as reducing endoplasmic reticulum stress **[9]**, but there is no state of insulin resistant or adipokines dysregulation.

These data were consistent with that of **Dray et al. [7]** who had found that a bolus of increasing concentrations of apelin injected intravenously into mice significantly improved glucose tolerance in high fat fed mice which were glucose intolerant or frankly diabetic. Also this opinion was supported by **Xu et al. [27]** who demonstrated that apelin increased myocardial glucose uptake through a pathway involving AMPK. Apelin also facilitates IRS-1 Ser-789 phosphorylation, suggesting a novel mechanism for its effects on glucose uptake. In line with this theory, our results in type I diabetic rats apelin induced a significant reduction in serum glucose level combined with significant rise of HOMA-β in APL vs. C *(both $P < 0.05$)*.

To gives a further support to apelin's role in maintaining insulin sensitivity and glycemia, **Yue et al. [5]** created a line of mice deficient in the apelin gene (APLN -/- or APKO) then, they had injected apelin into APKO mice that resulted in reversal of the features of insulin resistance including hyperglycemia by increasing glucose uptake, and increases Akt phosphorylation in skeletal muscles. Moreover, it was reported that the high level of blood apelin in obese and hyperinsulinemic type II diabetic cases helps to delay the onset of insulin resistance **[28].** Over time, the endogenous apelin might be either insufficient or inefficient. Apelin peptides are subjected to enzymatic degradation leading to inactive forms of apelin **[27].**

Another opinion concerning apelin-glucose relationship was reported by **Sörhede-Winzell et al. [30]** who stated that apelin had no effect on basal levels of glucose**.** This discrepancy with the results of this study, could be due to the fact that, in **Sörhede-Winzell et al.** experiments, mice were anesthetized or that apelin-36 was used instead of apelin-13.

The condition in STZ-induced diabetic group is markedly different, apelin and insulin in (APL-INS) did not induce a significant additive effect with respect to glucose and insulin serum levels when compared to insulin-treated controls (INS). In line with these data the finding in the children with Type I DM whose have significantly increased circulating apelin levels when compared to healthy controls and there were no significant relation between the apelin and BMI, glucose, lipids, adiponectin levels, and insulin sensitivity **[31].** On contrary, **Dray et al. [7]** reported that apelin and insulin are synergistic in this regard. This apparent discrepancy could be explained by the fact that **Dray et al. [7],** performed their experiment ex vivo, whereas this work was conducted in vivo.

As regarding apelin effect on lipid profile that represent an important issue in management of diabetes, surprisingly apelin IP injection for 2 weeks, significantly increased serum levels of triglycerides, total and LDL cholesterol an failed to significantly change HDL level in obesity induced diabetic group when compared with saline treated controls, while, it produced a significant reduction in serum triglyceride in STZ-induced diabetic subgroups when compared with the control subgroup (APL vs. C), but there was an insignificant difference in serum levels of these parameters between INS and APL-INS.

These results in obesity induced diabetic group which fed on high fat diet even during the experiment period, were in line and explained by **Than et al. [32]** who proved that the exogenous application of apelin decreased lipid droplet formation, intracellular triglyceride, expression of adipogenic transcriptional factors in adipose tissue via an autocrine mechanism, that includes either increased phosphorylation of hormone sensitive lipase (HSL) and acetyl CoA carboxylase leading to inhibition of lipolysis **[33]** or apelin-induced inhibition of expression of fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). As FAS and ACC are also adipogenic markers **[32]**. Thus the exogenous lipids can elevate the serum levels of triglycerides and cholesterol; this can be assessed later in a future study using low fat diet during apelin treatment of obesity induced diabetic rats. Moreover results of this study, as regard triglycerides in diabesity group were in line with **Soriguer et al. [34]** who studied apelin and triglyceride levels in morbidly obese patients with diabetes and found that apelin levels correlated significantly with serum triglycerides.

In the other hand, these data were disagreed by **Higuchi et al. [8]** who found that the triglyceride content of the epididymal white adipose tissue was decreased and serum triglyceride levels were reduced in the apelin treated high fat diet fed group, compared with controls, but these rats were not diabetic. Also **Kourtis et al. [35]** reported that apelin was negatively correlated with ox-LDL and HDL-cholesterol in the pregnant ladies. Furthermore, **Hashimoto et al. [36]** who studied the role of apelin in the mechanism of atherosclerosis in APJ knockout mice, had reported that the APJ+/+ animals having normal diet (not a high cholesterol diet) had interestingly lower blood LDL-cholesterol level compared to those knockout (APJ−/−) mice. This may imply that in case of normal or low cholesterol diet increased APJ expression is associated with a low blood LDL-cholesterol.

Finally we can conclude that; (a) apelin has hypoglycemic effect in normal and in both types of diabetes independent on insulin, might be due to enhanced glucose uptake, (b) apelin maintains insulin sensitivity in type II diabetes (c) apelin improved β cell function in type I diabetes, (d) apelin decreased serum triglycerides in type I diabetes while raise it in type II fed on high fat diet, & (e) apelin is more promising target in the management of type II rather than type I diabetes in rats.

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