M.M.A. Abulmeaty[1],
S. Abdullah Ahmed[2],
A.M. Almajwal[3]

Apelin is promising in management of diabetes complicating high fat diet induced obesity in rats

Progress in Nutrition
Vol. 15, N. 4, 245-254, 2013

Titolo

Apelina è promettente nel trattamento del diabete correlato all'obesità indotta da una dieta ricca di grassi nei ratti

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

Parole chiave
Apelina, diabete tipo II, dieta ad alto contenuto di grassi (HFD), diabete indotto da streptozotocina, HOMA-IR, HOMA-β, obesità

[1]Clinical nutrition program, community health sciences department
King Saud University (KSU), Riyadh, KSA
[2]Physiology department
Zagazig University, Zagazig, Egypt
[3]Clinical nutrition program, community health sciences department
King Saud University (KSU), Riyadh, KSA

Adress for correspondence:
Dr. Mahmoud Mustafa Ali Abulmeaty
King Saud University (KSU), Riyadh, KSA
PO Box: 10219, Riyadh 11433
Tel: 00966548155983
E- mail: Dr.abulmeaty@gmail.com
E-mail: mabulmeaty@ksu.edu.sa

Summary

Background and aim of the work: Apelin, a novel adipokine is identified as an endogenous ligand of receptor APJ. The present study was carried out to assess the value of apelin in management of type II diabetes complicating high fat diet induced obesity vs. type I diabetes mellitus in rats. *Methods:* 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, insulin treated, apelin treated and apelin & insulin treated subgroups), while group II was further subdivided into 2 equal subgroups (saline treated control and apelin treated groups). In all groups 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). *Results:* Animals of group II showed a significant decrease in serum levels of glucose and insulin with resulting a significant decrease in HOMA-IR. *Conclusions:* It was concluded that 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.

Riassunto

Scopo del lavoro: Apelina, una nuova adipochina è stata identificata come un ligando endogeno del recettore APJ. Il presente studio è stato condotto per valutare il valore dell'apelina nella gestione del diabete di tipo II correlato all'obesità indotta da una dieta ricca di grassi contro il diabete mellito di tipo I nei ratti. *Metodi:* Sono stati utilizzati un numero totale di 60 ratti albini maschi adulti sani. I ratti sono stati divisi in 2 gruppi: gruppo I (n=40) affetti da diabete indotto da streptozotocina, modello di diabete di tipo I e gruppo II (n=20) affetti da diabete correlato all'obesità indotta da una dieta ricca di grassi, modello di diabete di tipo II. Il gruppo I è stato suddiviso in 4 sottogruppi uguali (sottogruppi: controllo trattato con soluzione salina, trattati con insulina, trattati con apelina e trattati con apelina e insulina), mentre il gruppo II è stato ulteriormente suddiviso in 2 sottogruppi uguali (controllo trattato con soluzione salina e gruppi trattati con apelina). In tutti i gruppi sono stati misurati glicemia, insulina, trigliceridi, colesterolo totale, colesterolo LDL e HDL e sono stati utilizzati per calcolare l'indice HOMA-IR, il modello omeostatico per la valutazione dell'insulino-resistenza. *Risultati:* Gli animali del gruppo II hanno mostrato una significativa diminuzione dei livelli sierici di glucosio e insulina con conseguente diminuzione significativa dell'HOMA-IR. *Conclusioni:* Si è concluso che nei modelli di diabete di tipo II, l'apelina ha indotto un miglioramento significativo dei livelli sierici di glucosio e ha aumentato la sensibilità all'insulina, che denota una significativa riduzione dell'HOMA-IR; questo non era il caso dei ratti affetti da diabete di tipo I ed è questo il motivo per cui è un promettente target terapeutico nella gestione del diabete di tipo II correlato all'obesità, piuttosto che del tipo I.

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 *APJ* endogenous *ligand* (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 king Saud university ethics committee. 40 animals were bred in a light-temperature-controlled animal house (12-h light, 12-h dark cycle, temp around 25 C°, 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%. These 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), 2nd group was treated with IP injection of apelin (dose = 100 nmol/kg) at 1.30 PM for 14 days (APL) (8), 3rd 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 4th 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: 1st subgroup was injected by normal saline (0.4 ml) via IP route and 2nd 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-Methylnitroso-carbamoyl -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 (5 ml and 1 ml).

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 were 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 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.)*

Effects of apelin on serum triglycerides, and total, LDL & HDL cholesterol levels in STZ-induced 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)*.

Figure 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 (E) values in STZ- induced diabetic groups. Vertical bars represents the mean ± SEM (*, P < 0.05).

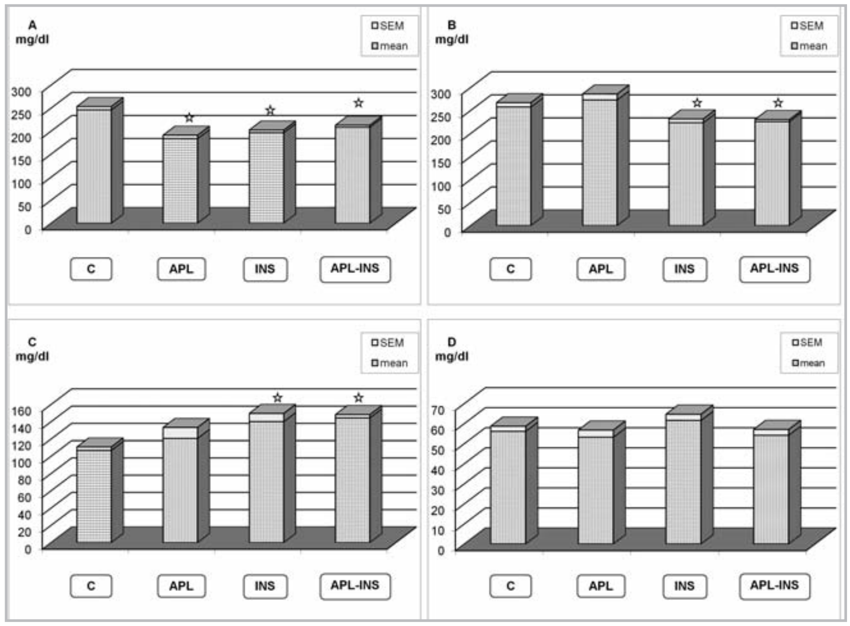
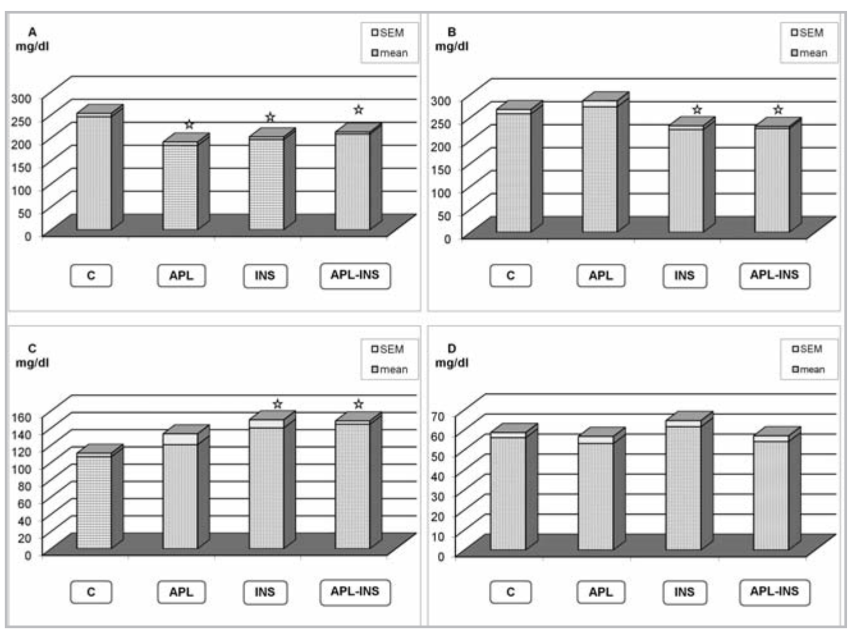


Figure 2 - Effects of apelin on the serum triglyceride level (A), serum total cholesterol (B), serum LDL-cholesterol (C) and HDL-cholesterol (D) levels in STZ-induced diabetic groups. Vertical bars represents the mean ± SEM (*, P < 0.05).



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).*

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

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).*

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

Figure 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).

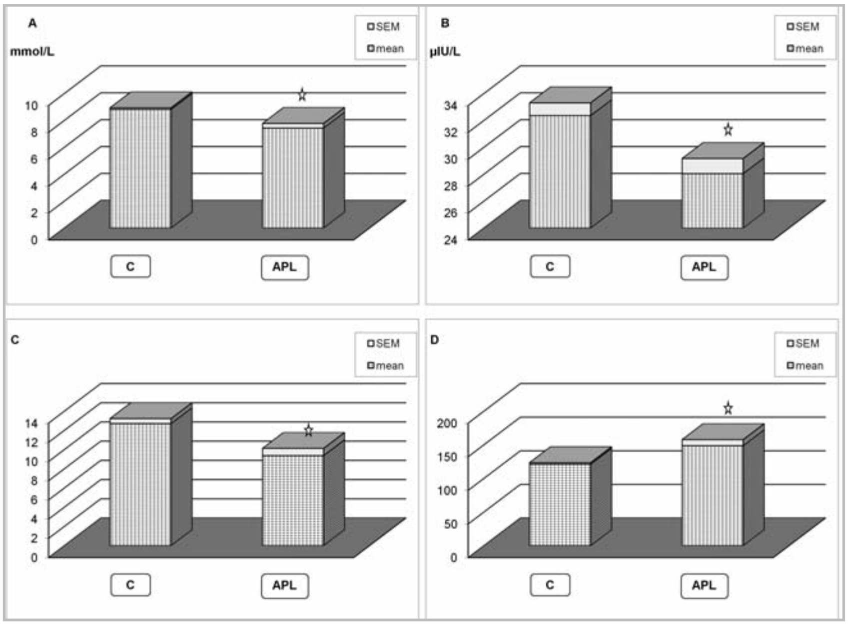
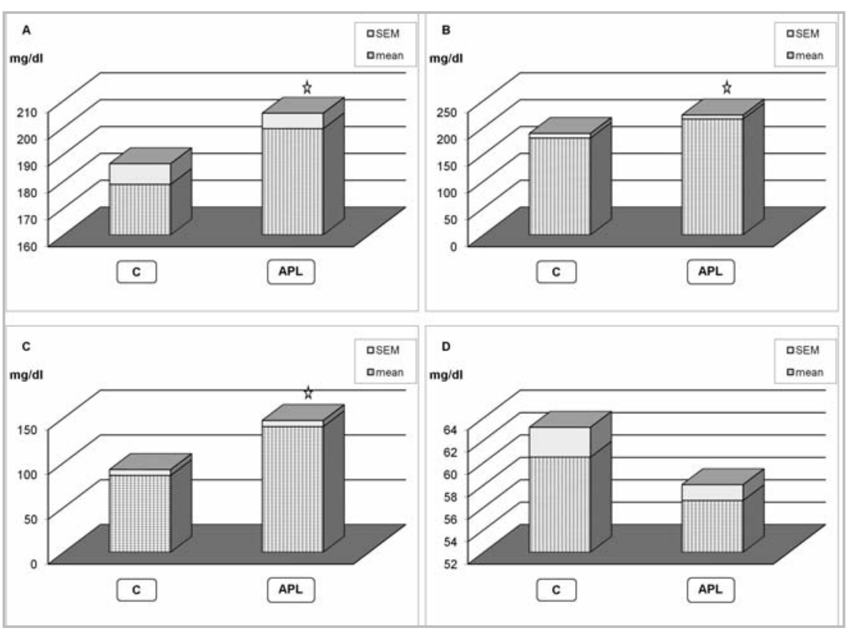


Figure 4 - Effects of apelin on the serum triglyceride level (A), serum total cholesterol (B), serum LDL-cholesterol (C) and HDL-cholesterol (D) levels in type II diabetic groups. Vertical bars represents the mean ± SEM (*, P < 0.05).



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 increasing 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 (29).

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.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at King Saudi University, KSA for funding the work through the research group project no RGP-VPP-193).

References

1. Tatemoto K, Takayama K, Zou M, et al. The novel peptide apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regulatory Peptides 2001; 99: 87–92.
2. Sunter D, Hewson AK, Dickson SL. Intracerebroventricular injection of apelin-13 reduces food intake in the rat. Neuroscience Letters 2003; 353: 1-42.
3. Dray C, Debard C, Jager J, et al. Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. Am J Physiol Endocrinol Metab 2010, 298(6): E1161-9.
4. Cheng X, Cheng XS, Pang CC: Venous dilator effect of apelin, an endogenous peptide ligand for the orphan APJ receptor, in conscious rats. European Journal of Pharmacology 470: 171– 175, 2003.
5. Yue P, Jin H, Aillaud M, et al. Apelin is necessary for the maintenance of insulin sensitivity. Am J Physiol Endocrinol Metab 2010; 298: E59–E67.
6. Casten-Laurel I, Boucher J, Dray C, Daviaud D, Guigné C, Valet P. Apelin, a novel adipokine over-produced in obesity: friend or foe? Mol Cell Endocrinol 2005; 245: 7–9.
7. Dray C, Knauf C, le Daviaud D, et al. Apelin stimulates glucose utilization in normal and obese insulin-resistant mice. Cell Metabolism 2008; 8: 437–445.
8. Higuchi K, Masaki T, Gotoh K, et al. Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of uncoupling proteins in mice. Endocrinology 2007; 148: 2690–2697.
9. Chen H, Zheng C, Zhang X, et al. Apelin alleviates diabetes-associated endoplasmic reticulum stress in the pancreas of Akita mice. Peptides 2011; 32: 1634–1639.
10. Nascimento A, Sugizaki M, Leopoldo S, et al. Misclassification probability as obese or lean in hypercaloric and normocaloric diet. Biol Res 2008; 4: 253-259.
11. Karen EI, Owen, Jessica TY. The effect of long-term insulin treatment with and without antecedent hypoglycemia on neuropeptide and corticosteroid receptor expression in the

brains of diabetic rats. Brain research Bulletin 2008, 43-51.
12. Yves MH, Theo FM. The effect of low-dose insulin on mechanical sensitivity and allodynia in type I diabetes neuropathy. Neuroscience Letters 2007, 417:149–154.
13. Palsamy P, Subramanian S. Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocin-nicotinamide induced experimental diabetic rats. Biomedicine & Pharmacotherapy 2008; 1-8.
14. Novelli E, Diniz Y, Galhardi C, et al. Anthropometrical parameters and markers of obesity in rats Laboratory Animals Ltd. Laboratory Animals 2007; 41: 111–119.
15. Gui Y, Silha V, Murphy L. Sexual Dimorphism and Regulation of Resistin, Adiponectin, and Leptin Expression in the Mouse. Obesity Research 2004; 12: 1481-1491.
16. Nishizawa H, Shimomura I, Kishida K. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. Diabetes 2002; 51: 2734–41.
17. Tietz NW, Cook T, McNiven MA. Clinical Guide to Laboratory Tests. WB Saunders Co 1995; 509-512.
18. Temple RC, Clark PM, Hales CN. Measurement of insulin secretion in type 2 diabetes: problems and pitfalls. Diabetic Medicine 1992; 9: 503-512.
19. Hoff J. Methods of blood collection in the mouse. Lab Animal 2000; 29: No. 10.
20. Soonthornpun S, Setasuban W, Thamprasit A, Chayanunnukul W, Rattarasarn C, Geater A. Novel Insulin Sensitivity Index Derived from Oral Glucose Tolerance Test. J Clin Endocrinol Metab 2003; 88: 1019-1023.
21. Cacho J, Sevillano J, de-Castro J, Herrera E, Ramos M. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. Am J Physiol Endocrinol Metab 2008; 295: E1269–E1276.
22. Sun G, Bishop J, Khalili S, et al. Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. Am J Clin Nutr 2007; 85: 399–404.
23. Fossati P, Principle Lab. Clin Chem 1982; 28: 2077-2079.
24. Nauck M, Marz W, Jarausch J. Multicenter evaluation of a homogenous assay for HDL-Cholesterol without sample pretreatment Clin Chem 1997; 43: 1622-1629.
25. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
26. Ladeiras-Lopes R, Ferreira-Martins J, Leite-Moreira AF. The Apelinergic System: The Role Played in Human Physiology and Pathology and Potential Therapeutic Applications. Arq Bras Cardiol 2008; 90(5): 343-349.
27. Xu S, Han P, Huang M, Wu J, Chang C, Tsao P, Yue P. In vivo, ex vivo, and in vitro studies on apelin's effect on myocardial glucose Uptake. Peptides 2012; 37: 320–326.
28. Boucher J, Castan-Laurell I, Daviaud D, et al. Adipokine expression profile in adipocytes of different mouse models of obesity. Horm Metab Res 2005; 37(12): 761-7.
29. Carpéné C, Dray C, Attané C, et al. Expanding role for the apelin/APJ system in physiopathology. J Physiol Biochem 2007; 63: 359–373.
30. Sörhede-Winzell M, Magnusson C, Ahre´n B. The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. Regulatory Peptides 2005; 131: 12–17.
31. Meral C, Tascilar E, Karademir F, et al. Elevated plasma levels of apelin in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab 2010; 23(5): 497-502.
32. Than A, Cheng Y, Foh L, et al. Apelin inhibits adipogenesis and lipolysis through distinct molecular pathways. Molecular and Cellular Endocrinology 2012; 362: 227–241.
33. Yue P, Jin H, Xu S, et al. Apelin decreases lipolysis via G(q), G(i), and AMPK-Dependent Mechanisms. Endocrinology 2011; 152(1): 59-68.
34. Soriguer F, Garrido-Sanchez L, Garcia-Serrano S, et al. Apelin Levels Are Increased in Morbidly Obese Subjects with Type 2 Diabetes Mellitus. OBES SURG 2009; 19: 1574–1580.
35. Kourtis A, Gkiomisi A, Mouzaki M, et al. Apelin levels in normal pregnancy. Clin Endocrinol (Oxf) 2011; 75(3): 367-71.
36. Hashimoto T, Kihara M, Imai N. Requirement of apelin–apelin receptor system for oxidative stress-linked atherosclerosis. Am J Pathol 2007; 171: 1705–12.