EFFECT OF APELIN ON INSULIN RESISTANCE, BETA CELL FUNCTION AND LIPID PROFILE IN HEALTHY AND DIABETIC RAT MODELS

Mohammed I. Shehata, Soad A. Ahmed, Randa S. Gomaa and Mahmoud M. Abulmeaty
Physiology Department, faculty of medicine, Zagazig University

ABSTRACT

Background: Apelin acts as a regulating peptide of cardiovascular, hypothalamus-hypophysis, metabolic, gastrointestinal and immune systems. **Objective:** The goal of this study was to clarify the effect of apelin on insulin resistance, beta cell function and lipid profile in healthy and diabetic rats. **Design:** 60 healthy adult male albino rats were divided into 3 equal groups: group I (normally fed group), group II (HFD –obese and diabetic or type II diabetes group) and Group III: A Streptozotocin (STZ) induced diabetic (type I diabetes) group, each group was subdivided into 2 subgroups (distilled water injected controls and apelin injected group). In all groups the sera were examined for glucose, insulin, triglycerides, total, HDL and LDL cholesterol levels. These data were used to measure the homeostasis model assessment of insulin resistance [HOMA-IR] and β-cell function [HOMA- β]. **Results:** Intraperitoneal injection of apelin decreased significantly serum insulin, glucose and HOMA-IR with insignificant change in HOMA-β and significantly increased total cholesterol, LDL and TG but it decreased significantly serum HDL in healthy lean and type II diabetic rat model**.** In type I diabetic rat model, apelin decreased significantly serum glucose and increased significantly HOMA- β with insignificant change in serum insulin and HOMA-IR and it produced significant decrease in serum TG with insignificant change in serum total cholesterol, LDL and HDL. **Conclusion:** Apelin has a detectable role in glucolipidemic metabolism in healthy and diabetic rat models. Further studies are needed to convert these effects into pharmacological trials in management of obesity and diabetes.

Key words: Apelin, glucose, lipid profile, insulin resistance, type I & II diabetes.

INTRODUCTION

Apelin is a circulating peptide, present in different tissues but also produced and secreted by human and mouse adipocytes [(1)]. Apelin was identified as the endogenous ligand of the ubiquitously expressed G protein–coupled receptor named APJ [(2)]. The apelin/APJ system exerts a large number of physiological roles, including regulation of fluid homeostasis, cardiovascular, immune and gastrointestinal functions [(3)]. A role for apelin/APJ in energy metabolism also has emerged recently. Acute and chronic apelin treatment has been shown to regulate glucose homeostasis [(4-5)]. Beneficial effects of acute intravenous injection of apelin were observed in normal-chow diet (ND)-fed mice on glucose uptake, especially in skeletal muscle, through an AMP-activated protein kinase (AMPK)-dependent pathway [(4)]. It is interesting that obese and insulin-resistant mice, exhibiting higher plasma apelin concentration than ND-fed mice [(6)], benefit from an acute apelin treatment since glucose tolerance was improved and muscle glucose uptake increased during an euglycemic-hyperinsulinemic clamp [(4)]. Chronic apelin treatment also ameliorates insulin sensitivity in young *db/db* mice [(4)]. Conversely, APKO mice (mice deficient in the apelin gene) develop insulin resistance especially when fed a high-fat diet (HFD) [(6)]. Altogether, these studies support a physiological role for apelin in the regulation of glucose homeostasis.

Chronic apelin treatment also decreases lipid storage in adipose tissue since a reduction of triglycerides (TGs) in various fat depots has been observed in ND- and HFD-fed mice [(7)]. Paradoxically, acute apelin treatment has been shown very recently to inhibit lipolysis in isolated adipocytes of non obese mice [(8)] but not in human adipose tissue [(9)]. The fate of lipids mobilized by chronic apelin treatment in obese and insulin-resistant mice is thus still unclear. More specific, the effects of apelin on lipid and carbohydrate metabolism in different types of diabetes have not yet been addressed.

The goal of this study was to investigate the effects of apelin-13, the most effective form of apelin, on insulin resistance, beta cell function and lipid profile in healthy and diabetic rats.

MATERIAL AND METHODS

Animals:

A total number of 60 healthy, adult, male albino rats weighing 180-200 gm were used. The used rats were obtained from the animal house from faculty of veterinary medicine of Zagazig University. The animals were kept in steel wire cages (6-8/cage) in the physiology research laboratory and in animal house in faculty of medicine of Zagazig University under hygienic conditions. Animals had free access to water from graduated tanks, kept at room temperature and were maintained on a 12 hr light/dark cycle.

Groups: Animals were divided into 3 equal groups (each = 20):

Group I: ***Lean group (n=20 rats)***: in which rats were fed on normal diet that is the mixed commercial rat laboratory chow and further subdivided into 2 sub groups:-

Apelin non-treated group (control) ***(n = 10 rats):*** rats are injected intraperitoneally with distilled water (500 µl) once daily at1.30 PM (from 1 to 2 O'clock) for 14 days.

Apelin treated group ***(n = 10 rats):*** rats were injected intraperitoneally with apelin-13 once daily (100 nmol/kg) at 1.30 PM (from 1 to 2 O'clock) for 14 days

Group II: "*High fat diet induced obese and diabetic (diabesity or type II diabetes) group (n = 20 rats):* in which rats were fed on high fat diet (HFD) that generally contain protein 20%, carbohydrates 35% and fat 45% for 15 weeks [10-11]**.** Rats that show obesity if BMI > 0.68 gm/cm^2 [12] and blood glucose level > 160 mg/dl [13], this group was further divided into 2 subgroups***:***

Apelin non-treated group (Control) ***(n = 10 rats):*** rats are injected intraperitoneally with distilled water (500 µl) once daily at1.30 PM (from 1 to 2 O'clock) for 14 days.

Apelin treated group ***(n = 10 rats):*** rats were injected intraperitoneally with apelin-13 once daily (100 nmol/kg) at 1.30 PM (from 1 to 2 O'clock) for 14 days

Group III: *A Streptozotocin (STZ) induced diabetic (type I diabetes) group (n = 20 rats):* in which rats were fed on normal diet that is the mixed commercial rat laboratory chow and experimental diabetes was induced by intra peritoneal injection of a single dose of streptozotocin (STZ, 65mg/kg body weight) dissolved in 1% Na citrate solution adjusted at pH 4.5 [14]**.** Three days later, diabetes induction was confirmed through measurement of blood glucose level in each animal (from blood sampled from the tail vein) with the Bionime GM300 Glucometer [15]. This group was further divided into 2 subgroups***:***

Apelin non-treated group (control) ***(n = 10 rats):*** rats are injected intraperitoneally with distilled water (500 µl) once daily at1.30 PM (from 1 to 2 O'clock) for 14 days.

Apelin treated group ***(n = 10 rats):*** rats were injected intraperitoneally with apelin-13 once daily (100 nmol/kg) at 1.30 PM (from 1 to 2 O'clock) for 14 days

Experimental protocol: Blood samples were obtained at the time of scarification and were allowed to clot for 2 hours at room temperature before centrifuging for 20 minutes at approximately 500 rpm [16]. The separated serum was stored at -20° C. Repeated freezing and thawing was avoided. Then, the serum was examined for level of glucose, insulin, triglycerides, total, HDL & LDL cholesterol [17]**.** These data were used to measure the homeostasis model assessment (HOMA), as a measure of insulin resistance [HOMA-IR=insulin (µU/mL)xglucose (mmol/L)/22.5] and β-cell function [HOMA- β = 20 x insulin (µU/mL)/(glucose-3.5)] [18]**.** LDL was calculated according to ***Friedewald et al., (1972)*** [19] as follows: LDL=TC-HDL-TG/5

Chemicals:

Apelin-13 trifluoroacetate salt: (Sigma- Aldrich co. USA), Glucose (anhydrous): ADWIC Laboratory Chemicals, Egypt, Streptozotocin, [*N* (Methyl nitro socarbamoyl)-α-D-glucosamine]: (SIGMA -ALDRICH Co.-USA), Kits for estimation of insulin: INS-EASIA, KAP1251 (BioSource Europe S.A.-Rue de l'Industrie, 4-A-1300 Nivelles-Belgium), Kits for estimation of serum glucose: INS-EASIA, KAP1251 (BioSource Europe S.A.-Rue de l'Industrie, 8-B-1400 Nivelles- Belgium), Kits for HDL level estimation (BioSource Europe S.A.-Rue de l'Industrie, 8-A- 1340 Nivelles-Belgium), Kits for TG level estimation (BioSource Europe S.A.-Rue de l'Industrie, 8-C- 1150 Nivelles-Belgium) and Kits for total cholesterol level estimation(BioSource Europe S.A.-Rue de l'Industrie, 8-B- 1400 Nivelles-Belgium)

Statistical Analysis:

The data obtained in the present study were expressed as mean ± SD for quantitative variables and statistically analyzed according to the methods described by ***Kirkwood (1989)*** [20]. Statistical significance was determined by unpaired Student's "t" test. P values less than 0.05 were considered to be significant. In statistical analysis; SPSS version 17 program for Windows (SPSS Inc. Chicago, IL, USA) was used**.**

RESULTS

Effect of apelin on insulin resistance, beta cell function and lipid profile in healthy rat model:

As shown in table 1 and figure 1, administration of apelin decreased significantly serum insulin level ($P<0.05$), glucose level and HOMA-IR ($P<0.01$) with insignificant change in HOMA-β ($P>0.05$). In addition apelin produced significant increase in serum level of total cholesterol, LDL ($P<0.05$) and TG ($P<0.01$) but it decreased significantly serum HDL ($P<0.05$) in healthy lean rat model.

Effect of apelin on insulin resistance, beta cell function and lipid profile in type II diabetic rat model:

As shown in table 1 and figure 2, administration of apelin decreased significantly serum glucose level, insulin level ($P<0.05$) and HOMA-IR ($P<0.01$) with insignificant change in HOMA-β ($P>0.05$). In addition apelin produced significant increase in serum level of total cholesterol, TG

(P<0.05) and LDL (P<0.01) but it decreased significantly serum HDL (P<0.05) in type II diabetic rat model.

Effect of apelin on insulin resistance, beta cell function and lipid profile in type I diabetic rat model:

As shown in table 1 and figure 3, administration of apelin decreased significantly serum glucose level (P<0.05) and increased significantly HOMA- β (P<0.05) with insignificant change in serum insulin level and HOMA-IR (P>0.05). In addition apelin produced significant decrease in serum level of TG (P<0.05) with insignificant change in serum total cholesterol, LDL and HDL (P>0.05) in type I diabetic rat mode

Table (1): Effect of apelin on insulin resistance, beta cell function and lipid profile in all studied groups

****** Significant when compared with the control of the same group (P<0.01).

***** Significant when compared with the control of the same group (P<0.05).

	Healthy rat model	Type II diabetic rat model	Type I diabetic rat model
Glucose (mmo/L)			
Control	**7.11± 0.28**	**8.83± 0.27**	**22.88± 3.06**
Apelin treated	**5.98± 0.46****	**7.44± 1.14***	**16.12± 5.31***
Insulin (µIU/ L)			
Control	**25.39 ± 4.06**	**32.37 ± 2.31**	**10.35 ± 1.4**
Apelin treated	**18.81 ± 4.3***	**28.05 ± 3.73***	**11.23 ± 1.89**
HOMA-IR			
Control	**7.99 ± 1.04**	**12.72± 1.25**	**10.35 ± 2.07**
Apelin treated	**5.03 ± 1.41****	**9.39± 2.47****	**8.04± 2.99**
HOMA-β			
Control	**142.6±34.45**	**121.38±5.27**	**10.88±2.24**
Apelin treated	**154.5±38.22**	**148.77±29.79***	**20.47±8.84 ***
Total Cholesterol (mg/dl)			
Control	**172.5 ± 5.3**	**180.16 ± 21.12**	**257.83 ± 22.63**
Apelin treated	**194.66 ± 18.26***	**215.54 ± 24.64***	**273± 36.32**
TG (mg/dl)			
Control	**164 ± 9.77**	**178.83 ± 18.75**	**246.33 ± 20.12**
Apelin treated	**207.77 ± 21.68****	**199.54 ± 19.02***	**184 ± 20.12 ***
LDL (mg/dl)			
Control	**81.16 ± 6.17**	**85.83 ± 15.94**	**107 ± 9.84**
Apelin treated	**116.88± 19.03***	**140.36± 22.41****	**120.75± 35.6**
HDL (mg/dl)			
Control	**60.5 ± 4.76**	**60.5 ± 6.53**	**56.16 ± 6.17**
Apelin treated	**46.77 ± 8.18***	**56.63 ± 4.69**	**53.25± 9.91**

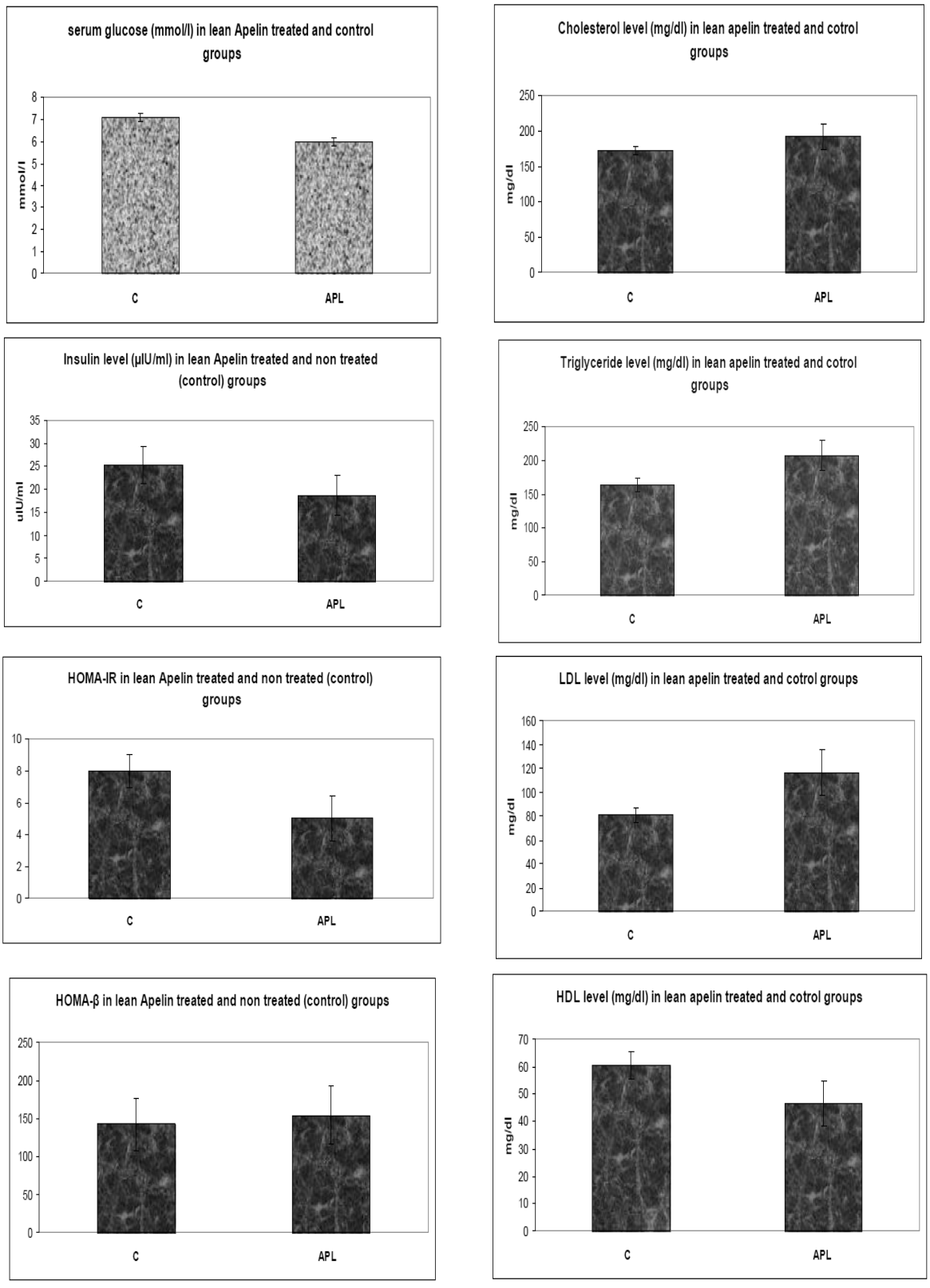

Figure (1): Effect of apelin on insulin resistance, beta cell function and lipid profile in healthy rat model.

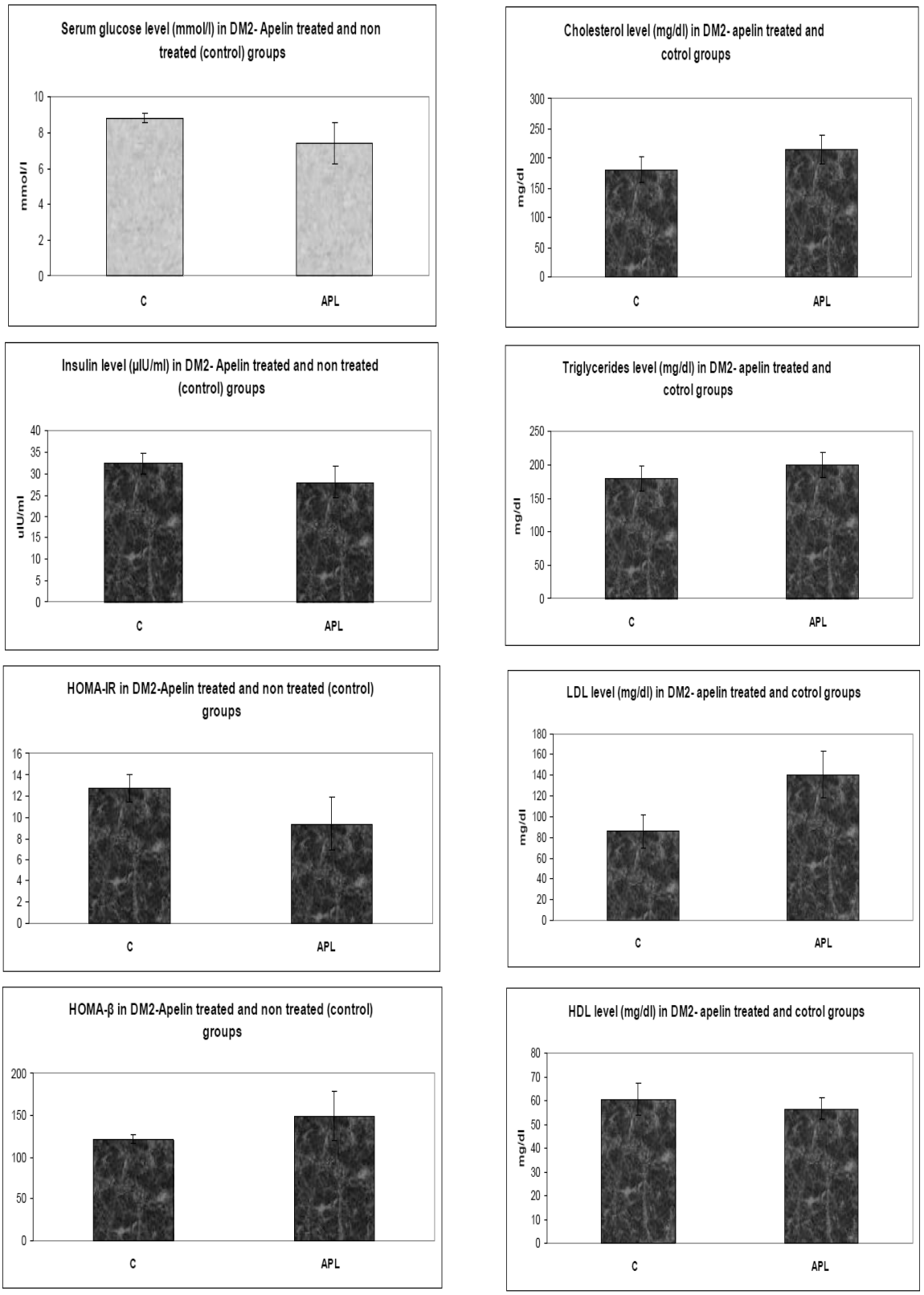

Figure (2): Effect of apelin on insulin resistance, beta cell function and lipid profile in type II diabetic rat model.

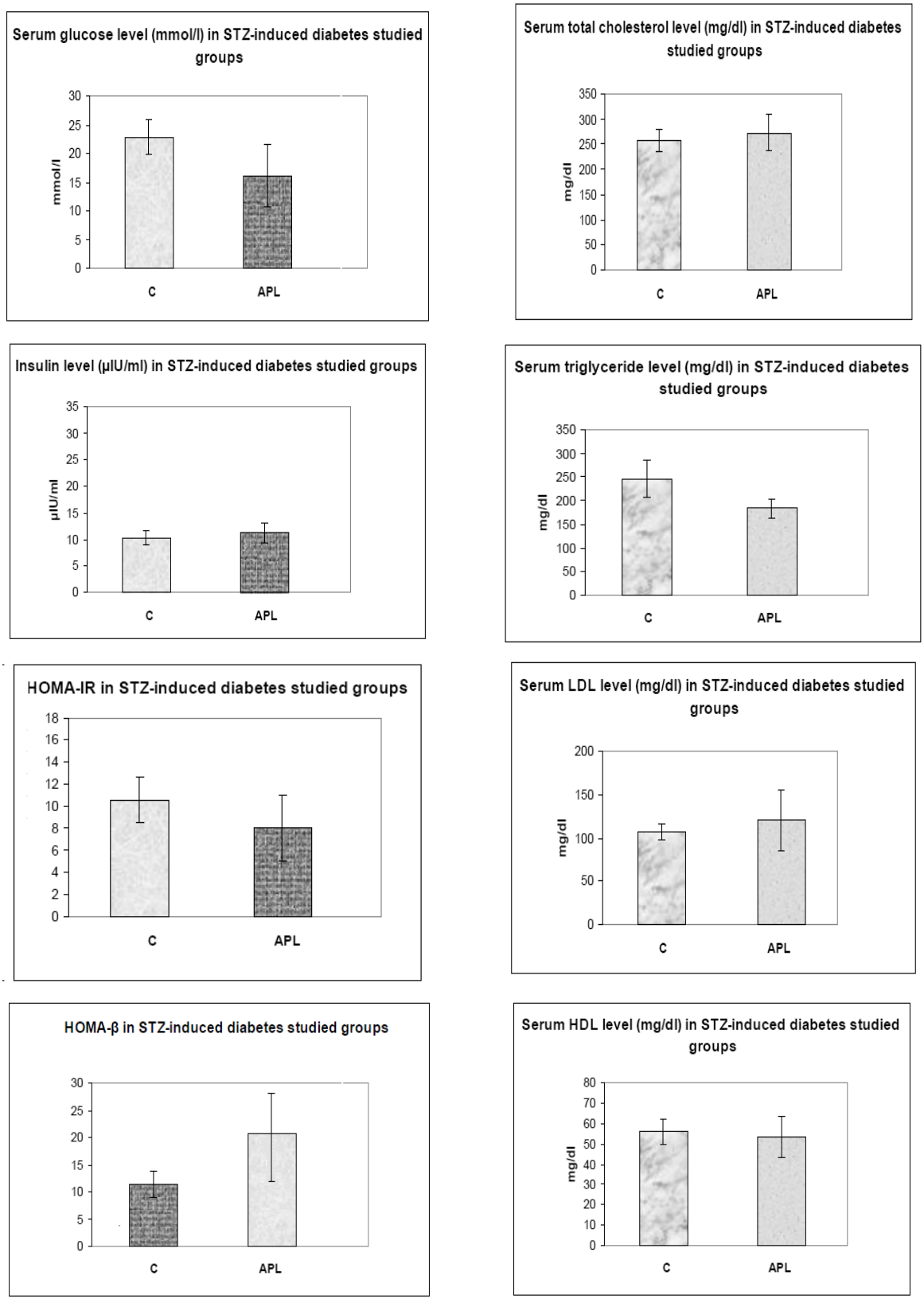

Figure (3): Effect of apelin on insulin resistance, beta cell function and lipid profile in type I diabetic rat model.]t

DISCUSSION

The present study shows that apelin administration improved in vivo glucose metabolism in normal and insulin-resistant high fat fed obese rats. These data were consistent with that of ***Dray et al, (2008)*** [4] who had found that a bolus of increasing concentrations of apelin injected intravenously into mice every 45 min produced a significant reduction of glycemia (25% decreases in blood glucose at the end). Also high fat fed mice (which display hyperinsulinemia, hyperglycemia, and obesity) is glucose intolerant or frankly diabetic, apelin injection was significantly improved glucose tolerance. In line and to confirm the above data, ***Yue et al, (2010)*** [10] created a line of mice deficient in the apelin gene (APLN -/- or APKO). In general, APKO mice were viable, fertile and no gross or histological abnormalities were observed in any major organs, but these mice are insulin resistant and serum glucose was also increased (but not overtly diabetic). In addition to the above insulin-stimulated Akt phosphorylation (an intracellular pathway named after, AKT8 virus oncogene cellular homolog) was decreased significantly. High-fat, high-sucrose diet exacerbated insulin resistance and hyperglycemia in APKO mice. This gives a further support to apelin's role in maintaining insulin sensitivity and glycemia. Moreover, restoration of apelin (dose; 2 mg/kg/day) to APKO mice leads to reversal of the features of insulin resistance including hyperglycemia by increasing glucose uptake, and increases Akt phosphorylation in skeletal muscles. Apelin-induced glucose uptake and Akt phosphorylation are sensitive to compound C; an AMPK inhibitor, suggesting the involvement of AMPK and Gi stimulation could result in AMPK activation. Furthermore ***Higuchi et al. (2007)*** [7] found that apelin-treated normal and diet-induced obese mice showed reduced levels of blood glucose after treatment, compared with controls during the intraperitoneal glucose tolerance test and decreased serum insulin levels in both groups. From these observations, it is suggested that apelin treatment increased insulin sensitivity.

In obese and hyperinsulinemic type II diabetic groups, it was reported that higher levels of blood apelin than that in controls were found [1]. It could be hypothesized that the high levels of circulating apelin found in obesity help to delay the onset of insulin resistance. Over time, the endogenous apelin might be either insufficient or inefficient. Apelin peptides are subjected to enzymatic degradation leading to inactive forms of apelin [3]**.** These inactive forms cannot be discriminated from the active ones in the assay used. Moreover, ***Vallae et al. (2008)*** [21] demonstrated that in vitro, apelin-13 was progressively converted to [Pyr1] apelin and no other breakdown products were found. Another hypothesis is that the high levels of apelin lead to apelin resistance. However, ***Zhong et al. (2007)*** [22] showed that even if there is a depressed expression of apelin receptors in aortic rings of diabetic mice (that have apelin resistance), apelin enhanced phosphorylation of eNOS and Akt. These apelin-mediated biological effects observed here in insulin-resistant mice might be due to the added exogenous active form of apelin-13 in the bloodstream.

In a trial to understand mechanisms of apelin-induced glycemic control ***Dray et al, (2008)*** [4] reported that apelin effect on glycemia might be either a direct action on glucose utilizing tissues or the result of an increased insulin sensitivity. Hemodynamic effects of apelin have been suggested to be associated with glucose utilization; vasodilatation is associated with enhanced insulin sensitivity, whereas vasoconstriction results in decreased glucose utilization [23]. Apelin was shown to cause endothelium dependent vasodilatation by triggering the release of NO [24]**.** The absence of apelin effect in vivo in eNOS -/- mice could result from a crosstalk between hemodynamic and direct metabolic effect of apelin on glucose uptake. Alternatively, NO may act on apelin-stimulated glucose uptake, independently of its vascular action since eNOS is expressed in skeletal muscle [25]. Indeed, apelin stimulates both eNOS phosphorylation and glucose uptake in muscles of mice, and this effect is completely suppressed in eNOS -/- mice. Taken together these data indicate that eNOS activation is essential for central apelin to exert its effect on glucose uptake [26]. Furthermore ***Attané et al., (2011)***[9] proved that apelin stimulated AMPK phosphorylation in a dose-dependent manner in human adipose tissue, which was associated with increased glucose uptake since C compound (20 μM), an AMPK inhibitor, and completely prevented apelin-induced glucose uptake. Finally ***Zhu et al., (2011)*** [27] suggest that apelin stimulates glucose uptake through the PI3K/Akt pathway, promotes GLUT4 translocation from the cytoplasm to the plasma membrane, and modulates inflammatory responses in insulin-resistant adipocytes. Another opinion was reported by ***Sorhede Winzell et al, (2005)*** [28] concerning apelin-glucose relationship. They reported 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 ***Sorhede Winzell et al. (2005)*** [28] experiments, mice were anesthetized or that apelin-36 was used

instead of apelin-13. By investigating serum insulin level, insulin homeostasis model assessment as a measure of insulin resistance (HOMA-IR) and insulin homeostasis model assessment as a measure of β cell function (HOMA-β) in the studied groups, it was found that apelin IP injection significantly reduced the serum insulin levels in lean, high fat diet induced diabesity groups when compared by distilled water injected controls, while showed an insignificant change in STZ-induced diabetic rats. HOMA-IR was also significantly reduced in lean, HFD-induced diabesity and in STZ-induced diabetic groups when compared with distilled water-injected controls i.e. peripheral apelin administration improving insulin sensitivity. Moreover, apelin injection fails to raise β cell function (HOMA-β) in comparison to control healthy group. Reducing effect of apelin on serum insulin level is agreed by ***Sorhede Winzell et al., (2005)***[28] who concluded that the APJ receptor is expressed in pancreatic islets and that iv injection of apelin-36 inhibits glucose stimulated insulin secretion both in vivo and in vitro. This may suggest that the islet beta-cells are targets for apelin-36. ***Higuchi et al, (2007)*** [7] also found that apelin IP injection decreased serum insulin levels both in normal and diet-induced obese mice. In addition, apelin-treated mice showed reduced levels of blood glucose after treatment, compared with controls during the IP glucose tolerance test. From these observations, it is suggested that apelin treatment increased insulin sensitivity in vivo. These data are consistent also with ***Guo et al., (2009)*** [29] who examined effect of apelin on insulinoma cell extracts and found that apelin over the concentration range of 1-10 nmol/L inhibited the insulin response to glucose and GLP-1 and the concentration effect was biphasic. This effect of apelin was abolished when insulin secretion was induced with cAMP analogues and selective inhibitors of cAMP and PI3-kinase completely prevent the apelin effect on insulin secretion and cAMP accumulation. These findings suggest that apelin exerts direct inhibitory actions on the pancreatic beta-cells by activating PI3-kinase and subsequently suppressing of cAMP levels. In addition to above ***Ringstrom et al., (2010)*** [30] concluded that apelin is a novel insulin-regulating islet peptide. Islet apelin expression is negatively regulated by glucocorticoids, and upregulated by lipotoxicity and type II DM. The presence of apelin receptors in islets suggests a role for apelin as a paracrine or autocrine messenger within the islets. On the other hand, ***Dray et al, (2008)*** [4] stated that no significant modification of insulin blood levels was found between apelin- and saline injected mice. This discrepancy could be explained by ***Dray et al. (2008)*** [4] performed their experiment ex vivo, whereas this work was conducted in vivo.

As regarding apelin effect on insulin resistance, results of this thesis are in line with ***Yue et al., (2010)*** [5] who found that apelin ameliorates insulin resistance in (db/db) mice and also decreases insulin resistance in condition of established insulin resistance. Additionally, apelin-treated mice had significantly decreased insulin levels as well as increased adiponectin levels. Taken together, it is unknown whether apelin affects insulin sensitivity by secondarily influencing the systemic environment of insulin resistance (e.g., altering hormone secretion, lipolysis, inflammation, etc.) or by a primary effect on individual cells.

Also in this study, apelin injection failed to raise β cell function (HOMA-β) in comparison to control except in diabesity-apelin treated group. This result can be explained by apelin's action on body adiposity and glucose turnover i.e. reduction of adiposity and increase in glucose uptake lead to some sort of beta cell rest, encouraging it to improve its function. This principle of beta cell rest in human models of obesity induced type II diabetes is well known, ***Hu et al., (2011)*** [31] for example stated that intensive glycemic control therapy in newly diagnosed type II diabetes not only partially restored β-cell function but also greatly restored insulin sensitivity. In another similar study on type 1 diabetic rat ***Chen et al., (2011)*** [32] clarified one of molecular mechanisms of the effects of apelin on endoplasmic reticulum (ER) stress in the pancreas of type 1 diabetic mice model. Apelin-13 (400 pmol/kg) was injected in the tail vein for 10 weeks resulting in amelioration of diabetes-induced reduction in pancreatic islet mass and insulin content as well as alleviation of ER stress. Taken together, these results suggest a novel physiological role of apelin in alleviating ER stress in the pancreas as a mechanism of amelioration of type 1 diabetes. Also ***Meral et al., (2010)*** [33] found that children with type 1 DM have significantly increased circulating apelin levels when compared with healthy controls.

This discrepancy of apelin effect on serum insulin level, HOMA-IR and HOMA-β is due to many reasons; rout of apelin injection (ip, iv or icv), structural form of apelin (apelin-13 or apelin-36), used dose, other adipokines involvement (specially visfatin and adiponectin), nutritional status, metabolic disorder as diabetes, species variation… etc.

The next metabolic effect of apelin studied in this study was the effect of apelin on lipid profile.

In this work apelin IP injection resulted in a significant increase in serum triglyceride (TG) level in normal rats. In obesity induced-type II DM, apelin injection could significantly increase TG level in the serum but fail to significantly affect TG in STZ induced diabetic group. Interestingly apelin injection significantly increased total and LDL-cholesterol and decreased HDL- cholesterol in the serum of normal and type II diabetic rats when compared to saline injected controls, while insignificant changes are reported in STZ-induced diabetic rats. Data of this study in normal and diabesity groups were in agreement with ***Yue et al.,(2011)*** [8] who investigated serum FFA, glycerol, and leptin concentrations, as well as abdominal adiposity, in apelin-null and wild-type mice and found that serum FFA and glycerol are significantly increased in apelin-null vs. wild-type mice; these changes were ameliorated in response to exogenous apelin. Apelin also reduced isoproterenol-induced FFA release in adipocytes isolated from wild-type but not APJ-null mice. Apelin's inhibition was reversed by the G(q) inhibitor and the AMP-activated protein kinase inhibitors. In addition to this ***Xu et al., (2011)*** [34] reported that apelin negatively regulates catecholamine-mediated lipolysis but information reported by ***Tasci et al., (2007)*** [35] are against the present study, as they stated that plasma apelin is decreased in non-obese, non-diabetic and normotensive patients with elevated LDL-cholesterol. Low apelin levels in hypercholesterolemia seem to be associated with insulin resistance. Interestingly reduction in LDL-cholesterol levels in otherwise healthy people with isolated dyslipidemia results in an increase in plasma apelin concentration [36]**.** Third opinion stated by ***Attané et al., (2011***) [9] who studied lipolysis and glucose uptake ex vivo, in response to apelin on isolated adipocytes and explants from adipose tissue of the subcutaneous region of healthy subjects. Apelin had no significant effect on basal and isoprenaline-stimulated lipolysis. Also ***Kourtis et al., (2011)*** [37] recently reported that apelin was negatively correlated with LDL and HDL-cholesterol in the pregnant ladies.

Moreover results of this study, as regard TG in diabesity group are in line with ***Soriguer et al., (2009)*** [38] who study apelin and TG levels in morbidly obese patients with diabetes and found that apelin levels correlated significantly in the morbidly obese patients with serum triglycerides. This discrepancy in apelin effect on lipid homeostasis need further detailed work to clarify the possible reasons but as a conclusion apelin effect on lipid markedly dependant on the metabolic conditions of the body including obesity and diabetic types for example, apelin in normal rats decreases lipolysis and increases TG level taken together with hemodynamic action of apelin and as a stimulant of angiogenesis, may contribute to development of obesity. Thus Inhibition of apelin signaling through the use of anti-apelin antibodies may represent an additional strategy for development of novel anti-obesity treatments ***(Rayalam et al., 2008)*** [39] furthermore reduction of total and LDL cholesterol add more beneficial role to apelin as fighter against cardiovascular co-morbidities.

It could be concluded that apelin has a detectable role in glucolipidemic metabolism in healthy and diabetic rat models. Further studies are needed to convert these effects into pharmacological trials in management of obesity and diabetes.

REFERENCES:

1) Boucher, J.; Castan-Laurell,I.; Daviaud,D.; Guigne,C.; Buleon, M.; Carpene, C.; Saulnier-Blache, J.S. and Valet P.(2005): Adipokine expression profile in adipocytes of different mouse models of obesity. Horm Metab Res.; 37(12):761-7.

2) Tatemoto K, Hosoya M, Habata Y, Fujii R, Kakegawa T, Zou MX, Kawamata Y, Fukusumi S, Hinuma S, Kitada C, Kurokawa T, Onda H and Fujino M. (1998): Isolation and characterization of a novel endogenous peptide ligand for the human APJ receptor. Biochem Biophys Res Commun; 251:471–476pmid:9792798.

3) Carpéné C, Dray C, Attané C, Valet P, Portillo MP, Churruca I, Milagro FI and Castan-Laurell I. (2007): Expanding role for the apelin/APJ system in physiopathology. J Physiol Biochem; 63:359–373.

4) Dray,C.; Knauf, C.; le Daviaud,D.; Waget, A.; Boucher,J.; Bule M.; Cani, P.; Attane, C.; Guigne, C.; Carpéné C.; Burcelin, R.; Castan-Laurell, I. and Valet, P. (2008): Apelin Stimulates Glucose Utilization in Normaland Obese Insulin-Resistant Mice. Cell Metabolism 8, 437–445.

5) Yue, P.; Jin, H.; Aillaud, M.; Deng, A.; Azuma, J.; Asagami,T.; Kundu, R.; Reaven,G.; Quertermous, T. and Tsao,P.(2010): Apelin is necessary for the maintenance of insulin sensitivity. Am J Physiol Endocrinol Metab 298: E59–E67.

6) Dray, C.; Debard, C.; Jager, J.; Disse, E.; Daviaud, D.; Martin, P.; Attan, C.; Wanecq, E.; Guign, C.; Bost, F.; Tanti, JF.; Laville, M.; Vidal, H.; Valet, P and Castan-Laurell I. (2010): Apelin and APJ regulation in adipose tissue and skeletal muscle of type 2 diabetic mice and humans. Am J Physiol Endocrinol Metab. 298(6):E1161-9.

7) Higuchi, K.; Masaki, T.; Gotoh, K,; Chiba, S.; Katsuragi, I.; Tanaka, K.; Kakuma, T. and Yoshimatsu, H (2007): Apelin, an APJ receptor ligand, regulates body adiposity and favors the messenger ribonucleic acid expression of

uncoupling proteins in mice, Endocrinology 148, 2690–2697.

8) Yue, P.; Jin, H.; Xu, S.; Aillaud, M.; Deng, A.C.; Azuma, J.; Kundu, R.K.; Reaven, G.M.; Quertermous, T. and Tsao, P.S. (2011): Apelin decreases lipolysis via G(q), G(i), and AMPK-Dependent Mechanisms. Endocrinology; 152(1):59-68.

9) Attané, C.; Daviaud, D.; Dray, C.; Dusaulcy, R.; Masseboeuf, M.; Prévot, D.; Carpéné, C.; Castan-Laurell, I. and Valet, P. (2011): Apelin stimulates glucose uptake but not lipolysis in human adipose tissue ex vivo. J Mol Endocrinol. 2011 Jan 19; 46(1):21-8.

10) Nascimento, A.; Sugizaki, M.; Leopoldo, S.; Lima-Leopoldo, A.; Nogueir,C.; Novelli, E.; Padovani C. and Cicogna, A. (2008): Misclassification probability as obese or lean in hypercaloric and normocaloric diet. Biol Res 41: 253-259.

11) Francia-Farje LA, Silva DS, Volpato GT, Fernandes GS, Carnietto N, Cicogna AC, Kempinas and Wde G. (2010): Sibutramine effects on the reproductive performance of pregnant overweight and nonoverweight rats. J Toxicol Environ Health A.; 73(13-14):985-90.

12) Novelli, E.; Diniz, Y.; Galhardi, C.; Ebaid, G.; Rodrigues,H.; Mani, F.; Fernandes,A.; Cicogna, A. and Novelli Filho, J. (2007): Anthropometrical parameters and markers of obesity in rats Laboratory Animals Ltd. Laboratory Animals 41, 111–119.

13) Palsamy, P. and Subramanian, S. (2008): Resveratrol, a natural phytoalexin, normalizes hyperglycemia in streptozotocinnicotinamide induced experimental diabetic rats. Biomedicine & Pharmacotherapy 1-8.

14) Karen, E.I.; Owen, C. and Jessica, T.Y. (2008): 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, 43-51.

15) Yves, M.H. and Theo, F.M. (2007): The effect of low-dose insulin on mechanical sensitivity and allodynia in type I diabetes neuropathy. Neuroscience Letters 417:149–154.

16) Gui, Y.; Silha, V. and Murphy, L (2004): Sexual Dimorphism and Regulation of Resistin, Adiponectin, and Leptin Expression in the Mouse Obesity Research 12:1481-1491.

17) Matthews, D.R.; Hosker, J.P.; Rudenski, A.S.; Naylor, B.A.; Treacher, D.F. and Turner, R.C. (1985): Homeostasis model assessment: insulin resistance and beta cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia; 28:412–9.

18) Sun, G.; Bishop, J.; Khalili, S.; Vasdev, S.; Gill, V.; Pace, D.; Fitzpatrick, D.; Randell, E.; Ya- Xie, G. and Zhang, H. (2007): Serum visfatin concentrations are positively correlated with serum triacylglycerols and down-regulated by overfeeding in healthy young men. Am J Clin Nutr ; 85:399–404.

19) Friedewald, W. T.; Levy, R.I. and Fredrickson, D.S. (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem., 18: 499-502.

20) Kirkwood BR (1989): Essentials of medical statistics. *Blackwell Scientific Publication, Oxford, London, PP151,*.

21) Vallae, A.; Hoggard, N.; Adams, AC.; Roca, P. and Speakman, JR. (2008): Chronic central administration of apelin-13 over 10 days increases food intake, body weight, locomotor activity and body temperature in C57BL/6 mice. J Neuroendocrinol.; 20 (1) :79- 84.

22) Zhong, J.C.; Yu, X.Y.; Huang, Y.; Yung, L.M.; Lau, C.W. and Lin, S.G. (2007): Apelin modulates aortic vascular tone via endothelial nitric oxide synthase phosphorylation pathway in diabetic mice. Cardiovasc. Res. 74, 388–395.

23) Juan, C.C.; Chien, Y.; Wu, L.Y.; Yang, W.M.; Chang, C.L.; Lai, Y.H.; Ho, P.H.; Kwok, C.F. and Ho, L.T. (2005): Angiotensin II enhances insulin sensitivity in vitro and in vivo. Endocrinology 146, 2246–2254.

24) Tatemoto, K.; Takayama , K.; Zou , M.; Kumaki, I.; Zhang, W.; Kumano, K. and Fujimiya, M.(2001): The novel peptide Apelin lowers blood pressure via a nitric oxide-dependent mechanism. Regulatory Peptides; 99: 87–92.

25) Kapur, S.; Be´dard, S.; Marcotte, B.; Cote´, C.H. and Marette, A. (1997): Expression of nitric oxide synthase in skeletal muscle: a novel role for nitric oxide as a modulator of insulin action. Diabetes 46, 1691–1700.

26) Duparc, T.; Colom, A.; Cani, P.D.; Massaly, N.; Rastrelli, S.; Drougard, A.; Le-Gonidec, S.; Mouldous, L.; Frances, B.; Leclercq, I.; Llorens-Cortes, C.; Pospisilik, J.A.; Delzenne, N.M.; Valet, P.; Castan-Laurell, I.and Knauf, C. (2011): Central apelin controls glucose homeostasis via a nitric oxide-dependent pathway in mice. Antioxid Redox Signal. 15; 15(6):1477-96.

27) Zhu, S.; Sun, F.; Li, W.; Cao, Y.; Wang, C.; Wang, Y.; Liang, D.; Zhang, R.; Zhang, S.; Wang, H. and Cao, F. (2011): Apelin stimulates glucose uptake through the PI3K/Akt pathway and improves insulin resistance in 3T3-L1 adipocytes. Mol Cell Biochem; 353(1-2):305-13.

28) Sorhede-Winzell, M,; Magnusson, C. and Ahren, B, (2005): The apj receptor is expressed in pancreatic islets and its ligand, apelin, inhibits insulin secretion in mice. Regulatory Peptides 131, 12 –17.

29) Guo, L.; Li, Q.; Wang, W.; Yu, P.; Pan, H.; Li, P.; Sun, Y. and Zhang, J. (2009): Apelin inhibits insulin secretion in pancreatic beta-cells by activation of PI3-kinase phosphodiesterase 3B. Endocr Res.; 34(4):142-54.

30) Ringstrom, C.; Nitert, M.; Bennet, H.; Fex, M.; Valet, P.; Rehfeld, J.; Friis-Hansen, L. and

Wierup, N. (2010): Apelin is a novel islet peptide. Regulatory Peptides 162, 44–51.

31) Hu, Y.; Li, L.; Xu, Y.; Yu, T.; Tong, G.; Huang, H.; Bi, Y.; Weng, J. and Zhu, D. (2011): Short-term intensive therapy in newly diagnosed type 2 diabetes partially restores both insulin sensitivity and B-cell function in subjects with long-term remission. Diabetes Care; 34(8):1848-53. Epub 2011 Jun 16.
32) Chen, H.; Zheng, C.; Zhang, X.; Li, J.; Zheng, L. and Huang, K. (2011): Apelin alleviates diabetes-associated endoplasmic reticulum stress in the pancreas of Akita mice. Peptides; 32(8):1634-9.
33) Meral, C.; Tascilar, E.; Karademir, F.; Tanju, I.A.; Cekmez, F.; Ipcioglu, O.M.; Ercin, C.N.; Gocmen, I.; Dogru, T. (2010): Elevated plasma levels of apelin in children with type 1 diabetes mellitus. J Pediatr Endocrinol Metab.; 23(5):497-502.
34) Xu S, Tsao PS and Yue P. (2011): Apelin and insulin resistance: another arrow for the quiver? J Diabetes; 3(3):225-31.
35) Tasci, I.; Dogru, T.; Naharci, I.; Erdem, G.; Yilmaz, M.I.; Sonmez, A.; Bingol, N.; Kilic, S.; Bingol, S. and Erikci, S. (2007): Plasma apelin is lower in patients with elevated LDL-cholesterol. Exp Clin Endocrinol Diabetes; 115(7):428-32.
36) Tasci, I.; Erdem, G.; Ozgur, G.; Tapan, S.; Dogru, T.; Genc, H.; Acikel, C.; Ozgurtas, T. and Sonmez, A. (2009): LDL cholesterol lowering increases plasma apelin in isolated hypercholesterolemia. Atherosclerosis; 204(1):222-8.
37) Kourtis, A.; Gkiomisi, A.; Mouzaki, M.; Makedou, K.; Anastasilakis, A.D.; Toulis, K.A.; Gerou, S.; Gavana, E. and Agorastos, T. (2011): Apelin levels in normal pregnancy. Clin Endocrinol (Oxf); 75(3):367-71.
38) Soriguer, F.; Garrido-Sanchez, L.; Garcia-Serrano, S.; Garcia Almeida, J.M.; Garcia-Arnes, J; Tinahones, F. and Garcia-Fuentes, E. (2009): Apelin Levels Are Increased in Morbidly Obese Subjects with Type 2 Diabetes Mellitus. OBES SURG, 19:1574–1580
39) Rayalam, S.; Della-fara, M. A.; Krieg, P. A.; Christopher, M. C. and Baile, C. (2008): A putative role for apelin in the etiology of obesity. Biochemical and Biophysical research communications 368: 815-819.

تأثيرالأبيلين على دلالات مقاومة الإنسولين و الحالة الوظيفية لخلايا بيتا و الشاكلة الشحمية في نموذج الجرذان الصحية والسكري

إن هرمون الأبلين من الهرمونات الببتيدية المكتشفة حديثا والتي وجد أن لها تأثيرات مختلفة على الجهاز الدوري والغدة النخامية وتحت مهاد المخ والتمثيل الغذائي وحدوث السمنة وكذلك نشوب بعضي المضاعفات المصاحبة للسمنة مثل البوال السكري من النوع الثاني. وعلاقة هذا الهرمون بعملية أيض الكربوهيدرات والشحوم ما زالت غير واضحة. لذلك فقد أجريت هذه الدراسة لاستبيان تأثير هذا الهرمون على بعض قياسات الإستقلاب الحيوي للجلوكوز والشحوم في نموذج للجرذان الطبيعية وأيضا نموذج الجرذان المصابة بداء السكري بنوعيه الأول والثاني و لتحقيق هذا الهدف فقد تم استخدام عدد 60 من ذكور الجرذان البيضاء البالغة و تم تقسيمها إلي ثلاث مجموعات متساوية كما يلي:

المجموعة الأولي: المجموعة التي أطعمت بطعام عادى وقد تم تقسيمها إلى مجموعتين صغيرتين :

- مجموعة ضابطة. تم حقنها بماء مقطر بجرعة 500ميكرولتر داخل البروتون يوميا لمدة 14 يوما (10 جرذان)
- مجموعة تم حقنها بهرمون الأبلين بجرعة 100 نانومول / كجم داخل البروتون يوميا لمدة 14 يوما (10 جرذان).

. **المجموعة الثانية**: تم تغذيتها بطعام عالي الدهون حتى أصبحت بدينةومصابة بداء السكري من النوع الثاني وأيضا قسمت إلى مجموعتين :

- مجموعة ضابطة. تم حقنها بماء مقطر بجرعة 500ميكرولتر داخل البروتون يوميا لمدة 14 يوما (10 جرذان)
- مجموعة تم حقنها بهرمون الأبلين بجرعة 100 نانومول / كجم داخل البروتون يوميا لمدة 14 يوما (10 جرذان).

. **المجموعة الثالثة**: تم تغذيتها بطعام عادى وحقنها بعقار استربتوزتوسين لإحداث داء السكري من النوع الأول وأيضا قسمت إلى مجموعتين :

- مجموعة ضابطة. تم حقنها بماء مقطر بجرعة 500ميكرولتر داخل البروتون يوميا لمدة 14 يوما (10 جرذان)
- مجموعة تم حقنها بهرمون الأبلين بجرعة 100 نانومول / كجم داخل البروتون يوميا لمدة 14 يوما (10 جرذان).

وتم قياس مستويات الجلكوز-الانسولين-الدهون الثلاثية- الكولسترول (الكلي-منخفض وعالي الكثافة)في مصل الجرذان بعد ذبحها في نهاية التجربة ثم استخدمت البيانات الناتجة في حساب بعض المعادلات الدالة علي حساسية الجسم للانسولين ووظيفةخلايا بيتا بجزر لانجرهانز بالبنكرياس في كل المجموعات

وقد أسفر هذا البحث عن النتائج الآتية:

1) يحدث الأبيلين إنخفاضا ذا دلالة إحصائية في مستوي الجلوكوز والإنسولين مصحوبا بتحسن في معاملات حساسية الجسم للإنسولين في نموذج الجرذان الطبيعية والمصابة بالسكري من النوع الثاني في حين ان الأبيلين أحدث إنخفاضا في مستوي الجلكوز فقط ولم يؤثر علي مستوي الإنسولين في نموذج الجرذان المصابة بالسكري من النوع الأول بينما أحدث نقصا ذا دلالة إحصائية في معامل مقاومة الجسم للإنسولين وزيادة ذات دلالة إحصائية في كفاءة خلايا البنكرياس المفرزة للإنسولين

2) يؤدي الأبيلين إلي زيادة ذات دلالة إحصائية في مستوي الدهون الثلاثية في جميع نماذج الجرذان قيد البحث والكوليستسرول الكلي ومنخفض الكثافة في نموذج الجرذان الطبيعية والمصابة بالسكري من النوع الثاني والكوليستسرول عالي الكثافة في نموذج الجرذان الطبيعية.

و من مجمل هذه النتائج يتضح أن هرمون الأبيلين قد تختلف تأثيراته على حسب وزن الجسم وطبيعة الحالة المرضية و بناء على هذه الدراسة فقد يفتح التعامل الدوائي مع هرمون الأبيلين الباب أمام علاج جديد لمرض السمنة وكذلك المضاعفات المصاحبة لها مثل البول السكري .