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Full Length Research Paper

Adiponectin biochemical and histopathological effects on obesity/type-II diabetes mellitus and pancreatic ß-cell dysfunction in experimental rats

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A comparison was done for serum lipid profile, glucose, adiponectin and insulin levels with/without hyperlipidemia; and with/without diabetes mellitus-II Wistar rat; with histopathological and immunohistochemical studies on pancreatic Langerhans islets and ß-cells (endocrine secretary portion of insulin). Sixty four adult Wistar rats were divided into 8 groups with 14 weeks study duration. The study is an elevation of serum glucose levels and decrease in insulin and adiponectin levels in diabetic groups. Serum lipid profile: triacylglycerol and low density lipoprotein were elevated and high density lipoprotein was decreased in obesity groups. However, these items were in demanded balance after adiponectin treatments. Histopathological alterations were found in diabetic groups severely and moderately in obesity/without diabetes groups. Also, insulin investigation content showed strong immune-reaction in pancreatic β-cells in the control groups and light to null insulin immune-reaction in obesity and diabetic groups when stained with anti-insulin antibody. After adiponectin administration, there was a β-cells dysfunction recovery and moderate insulin immune- reaction.

Key words: Glucose, insulin, adiponectin, diabetes mellitus type-II, pancreatic ß-cells.

INTRODUCTION

Adiponectin is an adipocyte hormone which involves in glucose and lipid metabolism. It makes up about 0.01% of total human plasma proteins with varied concentrations. This concentration is valued 1000 times greater than other hormones, like leptin and insulin (Haluzik and Parizkova, 2004). Adiponectin is expressed and secreted only from white and brown adipose tissues (Ukkola and Santaniemi, 2002). Adiponectin has been proposed as a factor that may be considered as a link between the insulin resistance, excess obesity and beta cell dysfunction in the case of diabetes mellitus type-II (Kahn and Flier, 2000). It plays an important role in hyperglycemia, dyslipidemia and other inflammatory mechanisms (Shulman, 2000). It has been identified as a potential therapeutic target for the treatment of diabetes (Wild et al., 2004) for its anti-diabetic, anti-atherogenic and anti-inflammatory roles. Its production is inhibited by obesity beside diabetes mellitus type-II (DM-II) (Zhang et al., 2010).

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Abbreviations: DM-II, Diabetes mellitus type-II; **ND,** normocaloric groups; **HD,** hypercaloric diet.

Obesity is a complex multifactor disease characterized by an excessive accumulation of adipose tissue that may impair health. It is a major risk factor for the development of DM-II and is thought to confer increased risk for type-II diabetes through obesity-associated insulin resistance (Nathan et al., 2009). Expression and plasma levels of adiponectin decrease when insulin resistance and obesity are increased and increase when insulin sensitivity is improved and weight is lost (Wilson and Gyi, 2010). The fact that obesity is the state of adiponectin deficiency makes this hormone a target for possible therapeutic interventions, which focusing on the possibility of adiponectin treatment may improve obesity-related insulin resistance. Low adiponectin levels, in addition to insulin resistance, have been shown in DM-II, since adiponectin has been demonstrated to play an important role in response to insulin as its expression leads to decrease glucose levels (Butler et al., 2003). Consistent with the low circulating levels of adiponectin observed in DM-II, the concentration is inversely related to insulin resistance as its levels are decreased in DM-II (Retnakaran et al., 2005). Insulin resistance is the major characteristic of DM-II. Hypoadiponectinemia has a closer relationship with the degree of insulin resistance and hyperinsulinemia (Combs et al., 2001). In the Kingdom of Saudi Arabia, the rise in the prevalence of DM-II has been stated to gain attention years after rapid industrialization, which took place in the country (Alzaid, 1997).

In the pathogenesis of DM-II, progressive deterioration of ß-cell function leads to an inability to secrete sufficient insulin to compensate for insulin resistance (Hotta et al., 2000). In rodent models of obesity/without diabetes, there is an adaptive increase in ß-cells mass to meet metabolic demands (Milan et al., 2002). Obese humans with diabetes had a respective 40 and 63% deficit in relative ß-cells volume. The decreased ß-cells volume in patients with DM-II was due to a reduced number of ß–cells (Souza et al., 2005). Type-II diabetes is accompanied by chronic insulin resistance and a progressive decline in ß-cell function (Fruebis et al., 2005). Dysfunction of ß-cells or increased rates of ß-cells death (apoptosis) would result in reduced capacity to produce insulin (Reinehr et al., 2005). ß-cells mass is regulated by a balance of their replication and apoptosis, as development of new islets from exocrine pancreatic ducts (neogenesis). Disruption of any of the pathways of ß-cells formation or increased rates of ß-cells death would result in decreased ß-cell mass and thus reduce the capacity to produce insulin (Stefan et al., 2002). The amount of ß-cells in mammalian adults is tightly regulated and maintained at about 1% of the weight of the pancreas (Weyer et al., 2001). Thus, the purpose of this study was to evaluate the association of adiponectin level with the incident DM-II; with/without obesity. The hypothesis that the high glucose concentrations adversely affect the survival and cause apoptosis of pancreatic islets ß-cells was tested. To further insight, the differences of adiponectin levels accompanied by the baseline of lipid profile, glucose and insulin levels were investigated. Besides that, histopathological and immunohistochemistry studies were done on the pancreatic ß-cells.

MATERIALS AND METHODS

Animals

The experiments were carried out on male and female Wistar rats (aged: 75 to 90 days old) weighting about 250 to 300 g obtained from the Faculty of Pharmacy, King Saud University, Kingdom of Saudi Arabia. During the experiment, the animals were housed in clean properly ventilated cages under constant controlled climatic conditions: temperature (23°C) and lighting conditions (12 h light /12 h dark). Rat food and filtered tap water were provided *ad libitum* to all animals. They were acclimatized to their environment at least two weeks before starting the experiment. The practical part of the study was done in Northern Borders University, Faculty of Medicine, Arar and King Abd-Elaziz University, Faculty of Medicine, Jeddah.

Model design

Streptozotocin diabetic rat model

Adult Wistar rats weighting about 250 to 300 g (75 to 90 days old) were used for inducing diabetes. The animals were injected by streptozotocin at dose of 60 mg/kg of body weight intravenously. Streptozotocin induced diabetes within 3 days by destroying ß-cell (Yamauchi et al., 2003a). Animals received a standard rodent chow diet containing (by weight): 19.80% protein, 39.25% carbohydrate, 4.41% fat, 13.25% fibre, and 2.76 kcal/g of metabolizable energy, given to groups (3, 4, 7, and 8).

Obese rat model

Adult Wistar rats weighting about 250 to 300 g (75 to 90 days old) received a high-fat diet (20% fat content). Hypercaloric chow was used to make a mixture with 131.01 g of sucrose, 84.77 g of soy oil, 12.33 g of cholesterol and 1.23 g of cholic acid with 1000 g of a previously triturated standard chow. The dietary ingredients were homogenized in 60°C warm distilled water and homogenate was used to prepare the pellets. This continued for 10 to 14 weeks (Hug et al., 2004).

Hypercaloric chow containing: 15.25% protein, 43.34% carbohydrate, 11.86% fat, 10.20% fibre (by weight) and 3.41 kcal/g of metabolizable energy were given to groups (2, 4, 5, and 8).

Adiponectin treatment rat model

Matched on body weight, Wistar rats were infused with 1.5 mg/kg by intraperitoneally injected (i.p.) (Wang et al., 2007). The treatment continued for 7 days and was given to groups (5, 6, 7, and 8).

Group design

Group 1 (n=8): control Wistar rats not diabetic, not obese, nor

adiponectin administration; group 2 (n=8): obese Wistar rats, without streptozotocin induction for DM-II, nor adiponectin administration; group 3 (n=8): Wistar rats with streptozotocin induction for DM-II, without obesity, nor adiponectin administration; group 4 (n=8): diabetic/obese Wistar rats without adiponectin administration; group 5 (n=8): effect of adiponectin on control Wistar rats, without diabetic rats, nor obese rats; group 6 (n=8): effect of adiponectin on obese Wistar rats without diabetic rats; group 7 (n=8): effect of adiponectin administration on diabetic/non obese Wistar rats; group 8 (n=8): effect of adiponectin on diabetic/obese Wistar rats.

Body mass index determinations

The animals were anaesthetized (0.1 ml i.p. of 1% sodium barbiturate) for measurement of body length (nose-to-anus, or nose-anal length) (Pannacciulli et al., 2003). The body weight and body length were used to determine body mass index (g./cm^2) once a week.

Biochemical studies

The animals were anaesthetized (0.1 ml i.p. of 1% sodium barbiturate) and blood was collected intravenously (i.v.) of rats tails using capillary tubes containing heparin. The blood samples were collected at same time of measurements day and that was repeated weekly. The food was withdrawn 10 h before blood collection. The blood samples were stored in ethylenediaminetetraacetic acid (EDTA)-coated tubes.

1. Measurement of lipid profile; low-density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol and triacylglycerol (TG) (Trayhurn et al., 2001).
2. Measurement of serum glucose level (Trinder and Stern, 1959).
3. Insulin (Amherdt et al., 1974) and adiponectin (Imagawa et al., 2002) levels were determined using ELISA kits.

Histopathological and Immunohistochemical studies

By the end of the experiment, the rats were anaesthetized by ether and sacrificed by decapitation. The pancreas was extracted. The specimens were immersed in 10% formalin neutral buffered for 24 h, washed, dehydrated, cleared and embedded in paraffin. Then, specimens were processed into 5 µm sections for light microscopic examination routine hematoxylin and eosin stain (H&E) (luiz and Jose, 2010). Immunohistochemical staining occurred by applying anti-insulin antibodies stain method; which stain ß-cells (endocrine secretary portion in pancreatic islets of Langerhans) as follow: Ab-7842 added at 1/1000 dilution. The sections were fixed with formaldehyde and blocked with 5% serum prior for incubation with primary antibody for 12 h. A Cy2 conjugated goat polyclonal antibody was used as secondary antibody. ß-cells, unit of insulin secretion, were stained in green or reddish brown (Fujita et al., 2008).

Statistical analysis

The results were presented as means ± standard deviations (SD). Analysis of variance (ANOVA) for two variables (Two Way-ANOVA) was used together with student t-test. Significant analysis of variance results were subjected to post hoc. Statistical significance was set at $P<0.05$ and high significance was set at $P\leq 0.01$ (Fisher, 1970).

RESULTS

Body mass index determinations

Table 1 summarizes body mass index (g/cm^2) during the study period (14-weeks) for normocaloric groups (ND) which include (groups 1, 3, 5, and 7) and hypercaloric diet (HD) groups which include (groups 2, 4, 6, and 8). ND and HD groups started with a similar body weight at week 0. There was significant separation of body weight between ND and HD groups at week 5 till week 9; but a high significant separation was observed till study end (week 14).

Biochemical studies

Determination of diabetes

Animals were injected with streptozotocin and became markedly diabetic with type-II as manifested by fasting serum glucose levels (Table 2). Baseline values of glucose were relatively similar in day 0 in all studied groups. Fasting serum glucose levels were significantly high in diabetic groups when compared with non-diabetic groups/with or without obesity and without adiponectin injection and also high from those with adiponectin injection. There was no significant variation in serum glucose levels between adiponectin injection groups (Tables 2). After adiponectin treatment, a high significant decrease in serum glucose levels was noticed when compared with those without adiponectin treatment in diabetic/with or without obesity groups.

Lipid profile

Serum levels of triacylglycerol increased in HD groups than ND groups (Table 3). HDL was lower in HD rats than ND rats (Table 4). LDL was increased in HD rats than ND rats (Table 5). Triacylglycerol, HDL and LDL levels do not vary among diabetic/without obesity groups. There were significances for triacylglycerol, HDL and LDL levels in 5th week till 9th week, but there was a high significant difference till 14th week. After adiponectin induction, a high significant decrease was observed in serum LDL and triacylglycerol levels and a high significant increase in serum HDL levels when compared with those without adiponectin treatment in obesity groups.

Hormonal profile

Baseline values of adiponectin and insulin levels were

similar among the all groups in day 0. Serum adiponectin levels were highly decreased significantly and insulin levels were highly increased in diabetic groups/without adiponectin treatment significantly when compared with other non-diabetic groups/without adiponectin induction (Tables 6 and 7). Adiponectin levels decreased significantly in obese group without adiponectin treatment in the 5th week till week 9, with high significance till week 14. After adiponectin induction, a high significant decrease was observed in serum insulin levels and a high significant increase in adiponectin levels in groups with adiponectin treatment when compared with those without adiponectin treatment to be in the demanded balance (relatively similar to control group) (Tables 6 and 7). There was no significant variation in the serum adiponectin and insulin levels between adiponectin injection groups.

Histopathological and immunohistopathological studies

Histopathological studies

For histopathological studies of pancreatic tissue sections by routine hematoxylin and eosin stain (H&E) (Figure 1), the comparison between these sections showed pancreatic Langerhans islets of diabetic rats' degeneration with necrotic changes. There was a severe vaculation of pancreatic islets with an extensive fibrosis and loss of architecture. Also, there were hyaline and amyloidal droplets which were due to extracellular proteinaceous materials deposition. These of all alterations were less than those of the obese cases. On the other hand, after adiponectin treatment, there were regeneration for pancreatic Langerhans islet cells and a reduction in vaculation and fibrosis, also hyaline and amyloidal droplets were lightly observed.

Immunohistochemical studies

For a comparison of insulin content, anti-insulin antibody stain was applied. Strongly positive immune-reaction was observed in the control and adiponectin groups. After adiponectin administration, diabetic/with or without obesity groups showed moderate positive immune-reaction of insulin in pancreatic β-cells; as adiponectin helped in regeneration of pancreatic β-cells, then insulin secretion became in the demanded balance (Figure 2). On the other hand, at diabetic/with or without obesity groups, but without adiponectin induction, there was a light to null immune-reaction of insulin in pancreatic β-cells.

DISCUSSION

Adiponectin may provide a link between obesity and diabetes. It exerts profound anti-diabetic, anti-atherogenic and anti-inflammatory roles (Fasshauer et al., 2002). This study demonstrates that serum adiponectin levels were altered after the induction of DM-II and/or hyperlipidemia. Serum adiponectin levels were decreased in diabetic rats when compared with their non-diabetic counterparts. This finding agreed with previous studies showing that adiponectin levels decrease in DM-II (Mao et al., 2006). Streptozotocin-induced diabetes resembles human DM-II and reduces adiponectin, due to decreased insulin-mediated glucose metabolism in adipose tissue (Fasshauer and Paschke, 2003). The present results showed that serum adiponectin levels were negatively associated with the body mass index, so obesity is characterized by hypoadiponectinemia (Trayhurn and Beattie, 2001) and were decreased in DM-II (Yamauchi et al., 2003b). This study results agreed with the result of Abbasi et al. (2004).

Insulin resistance is a major feature in the etiology of obesity and type-II diabetes, and the relationship between insulin and adiponectin was studied. There are some conflicting reports concerning the influence of insulin on the adiponectin gene expression (Matsushita et al., 2007). This study found decreased adiponectin levels in hyperlipidemic rats, in DM-II animals, as well as in those ones having both DM-II and hyperlipidemia. The reason may be the lack of an immune mechanism that leads to β-cell damage and insulin deficiency in our model of streptozotocin-induced diabetes (Dubiel et al., 2012). In this study, although animals were fed with high-fat diet presented with hypercholesterolemia, little difference was observed in adiponectin levels among non diabetic groups/with obesity induction as there is difference in serum lipid profile. This finding is in agreement with weak correlation between adiponectin hormone and levels of total cholesterol (Kern et al., 2003).

In this study, LDL levels increased, HDL levels decrease and triglyceride levels increased, because saturated fat resulted due to blood cholesterol increase. These results resemble the results of Ouchi et al. (2003) which stated that eating too much fat may cause hyperlipidemia with increase in the total triglyceride and LDL and decrease in HDL. The increase in total cholesterol is associated with the decrease in HDL as cholesterol is present in all tissues and lipoproteins plasma and free cholesterol is expelled from tissues by HDL (Andreelli et al., 2006). HDL cholesterol is often called "good" because it is a lipoprotein that transports lipids from the periphery (extra-hepatic) to the liver. However, LDL is a lipoprotein that transports lipids from liver to peripheral (extra-hepatic) and is often called "bad"cholesterol. The state of hypercholesterolemia is characterized

Table 1. Body mass index (gm/cm). Values were expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	1.872±0.234	1.871±0.232	1.868±0.228	1.871±0.232	1.869±0.23	1.872±0.234	1.873±0.236	1.871±0.232
2	1.875±0.234	1.878±0.238	1.872±0.234	1.875±0.236	1.871±0.232	1.876±0.237	1.875±0.235	1.874±0.233
3	1.879±0.234	1.882±0.313	1.874±0.465	1.88±0.396	1.873±0.236	1.879±0.239	1.877±0.238	1.878±0.238
4	1.882±0.232	1.885±0.269	1.875±0.468	1.887±0.368	1.876±0.37	1.881±0.233	1.879±0.375	1.882±0.235
5	1.887±0.235	1.895*±0.475	1.878±0.371	1.891*±0.236	1.878±0.371	1.885*±0.269	1.88±0.396	1.884*±0.376
6	1.894±0.313	1.929*±0.321	1.88±0.396	1.925*±0.321	1.88±0.396	1.889*±0.231	1.882±0.313	1.889*±0.271
7	1.895±0.315	1.935*±0.241	1.882±0.313	1.932*±0.386	1.882±0.313	1.892*±0.473	1.884±0.312	1.893*±0.236
8	1.899±0.323	1.947*±0.384	1.883±0.315	1.944*±0.215	1.885±0.269	1.897*±0.32	1.885±0.269	1.897*±0.247
9	1.922±0.348	1.966*±0.245	1.888±0.23	1.963*±0.245	1.887±0.235	1.925*±0.321	1.887±0.269	1.927*±0.249
10	1.926±0.355	1.973**±0.219	1.892±0.473	1.972**±0.325	1.89±0.27	1.936**±0.381	1.888±0.377	1.934**±0.253
11	1.929±0.358	1.981**±0.396	1.894±0.313	1.982**±0.33	1.892±0.473	1.944**±0.215	1.891±0.27	1.945**±0.387
12	1.932±0.376	1.992**±0.332	1.896±0.318	1.989**±0.281	1.894±0.317	1.956**±0.384	1.893±0.474	1.952**±0.277
13	1.935±0.379	1.995**±0.384	1.897±0.32	1.992**±0.249	1.895±0.318	1.967**±0.245	1.894±0.316	1.965**±0.28
14	1.937±0.384	1.998**±0.249	1.899±0.323	1.997**±0.332	1.897±0.321	1.977**±0.219	1.895±0.321	1.975**±0.326

*Significant; 0.01≤P≤0.05 and **High significant; P<0.01.

Table 2. Mean of serum glucose levels (mg/dl), values expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	56.28±7.07	56±7.02	54.89±6.86	56.14±7.01	55.76±6.97	55.84±6.98	56.2±7.02	54.97±6.87
2	56.36±7.09	56.34±7.04	55.23±6.9	56.43±7.05	55.89±6.98	56.23±7.02	56.57±7.07	55.12±6.89
3	56.59±7.1	56.48±7.06	55.5±6.93	56.9±7.11	56.24±7.03	56.49±7.06	56.87±7.1	55.35±6.91
4	56.83±7.15	56.68±7.08	56.64**±7.08	57.55**±7.19	56.45±7.05	56.61±7.07	56.32**±7.11	55.58**±6.94
5	57.18±7.18	57.12±7.14	57.48**±7.18	58.72**±7.34	56.78±7.09	56.72±7.09	57.23**±7.15	55.74**±6.96
6	57.39±7.22	57.53±7.19	58.12**±7.26	59.12**±7.39	56.91±7.11	56.89±7.11	57.35**±7.16	55.82**±6.97
7	57.54±7.24	57.82±7.22	59.33**±7.41	60.42**±7.55	57.32±7.16	57.35±7.16	57.51**±7.18	55.92**±6.99
8	57.75±7.24	58.21±7.27	60.27**±7.53	61.42**±7.67	57.62±7.2	57.57±7.19	57.68**±7.21	56.23**±7.02
9	57.96±7.34	58.52±7.31	61.33**±7.66	62.36**±7.79	57.81±7.22	57.68±7.21	57.79 **±7.22	56.34**±7.04
10	58.17±7.35	58.81±7.35	62.42**±7.8	63.78**±7.97	57.9±±7.23	57.83±7.22	57.92**±7.24	56.53**±7.06
11	58.32±7.39	59.26±7.4	63.6**±7.95	64.47**±8.05	58.24±7.28	58.15±7.26	58.15**±7.26	56.68**±7.08
12	58.54±7.41	59.43±7.45	64.34**±8.04	65.32**±8.16	58.41±7.3	58.35±7.29	58.36**±7.29	56.76**±7.09
13	58.74±7.45	59.64±7.49	65.42**±8.17	66.18**±8.27	58.68±7.33	58.69±7.33	58.43**±7.3	56.8**±7.1
14	58.89±7.47	59.85±7.51	66.33**±8.29	67.34**±8.41	58.84±7.35	58.86±7.35	58.68**±7.33	56.93**±7.12

*Significant; 0.01≤P≤0.05 and **High significant; P < 0.01.

Table 3. Mean serum triacylglycerol levels (mg/dl). Values expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	92.15±11.51	92.22±11.53	92.13±11.52	92.23±11.53	92.18±11.52	92.19±11.53	92.18±11.52	92.14±11.51
2	92.32±11.54	92.73±11.59	92.27±11.53	92.5±11.56	92.28±11.53	92.23±11.53	92.28±11.53	92.22±11.53
3	92.47±11.55	93.15±11.64	92.68±11.58	92.78±11.6	92.35±11.54	92.45±11.56	92.57±11.56	92.56±11.57
4	92.56±11.57	93.81±11.72	92.88±11.61	93.44±11.68	92.67±11.58	92.74±11.59	92.78±11.59	92.87±11.6
5	92.71±11.58	94.14**±11.76	93.15±11.64	93.85**±11.73	92.85±11.61	92.89**±11.62	92.92±11.61	92.93**±11.62
6	93.12±11.64	94.93**±11.86	93.32±11.664	94.38**±11.79	93.17±11.65	93.21**±11.65	93.15±11.64	93.14**±11.65
7	93.35±11.66	95**±11.87	93.72±11.72	94.82**±11.85	93.25±11.66	93.38**±11.67	93.56±11.69	93.42**±11.67
8	93.42±11.67	95.23**±11.9	93.89±11.73	95.41**±11.93	93.55±11.69	93.68**±11.71	93.71±11.71	93.75**±11.72
9	93.67±11.71	95.89**±11.98	94.21±11.77	95.82**±11.97	93.74±11.71	93.82**±11.73	93.85±11.74	93.86**±11.73
10	93.89±11.74	96.28**±12.03	94.54±11.82	96.35**±12.04	93.84±11.73	93.99**±11.75	93.94±11.74	93.98**±11.75
11	93.94±11.74	96.58**±12.07	94.65±11.83	96.54**±12.06	93.92±11.74	94.12**±11.76	94.16±11.77	94.11**±11.76
12	94.15±11.76	96.78**±12.09	94.76±11.85	96.82**±12.1	93.94±11.74	94.18**±11.77	94.21±11.77	94.25**±11.78
13	94.56±11.82	97.11**±12.138	95.29±11.9	97.24**±12.15	94.28±11.78	94.35**±11.79	94.32±11.79	94.65**±11.83
14	94.97±11.87	98**±12.25	95.75±11.97	98.44**±12.3	94.72±11.86	94.87**±11.86	94.78±11.85	94.88**±11.88

*Significant; 0.01≤P≤0.05 and **High significant; P < 0.01.

Table 3. Mean serum triacylglycerol levels (mg/dl). Values expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	92.15±11.51	92.22±11.53	92.13±11.52	92.23±11.53	92.18±11.52	92.19±11.53	92.18±11.52	92.14±11.51
2	92.32±11.54	92.73±11.59	92.27±11.53	92.5±11.56	92.28±11.53	92.23±11.53	92.28±11.53	92.22±11.53
3	92.47±11.55	93.15±11.64	92.68±11.58	92.78±11.6	92.35±11.54	92.45±11.56	92.57±11.56	92.56±11.57
4	92.56±11.57	93.81±11.72	92.88±11.61	93.44±11.68	92.67±11.58	92.74±11.59	92.78±11.59	92.87±11.6
5	92.71±11.58	94.14**±11.76	93.15±11.64	93.85**±11.73	92.85±11.61	92.89**±11.62	92.92±11.61	92.93**±11.62
6	93.12±11.64	94.93**±11.86	93.32±11.664	94.38**±11.79	93.17±11.65	93.21**±11.65	93.15±11.64	93.14**±11.65
7	93.35±11.66	95**±11.87	93.72±11.72	94.82**±11.85	93.25±11.66	93.38**±11.67	93.56±11.69	93.42**±11.67
8	93.42±11.67	95.23**±11.9	93.89±11.73	95.41**±11.93	93.55±11.69	93.68**±11.71	93.71±11.71	93.75**±11.72
9	93.67±11.71	95.89**±11.98	94.21±11.77	95.82**±11.97	93.74±11.71	93.82**±11.73	93.85±11.74	93.86**±11.73
10	93.89±11.74	96.28**±12.03	94.54±11.82	96.35**±12.04	93.84±11.73	93.99**±11.75	93.94±11.74	93.98**±11.75
11	93.94±11.74	96.58**±12.07	94.65±11.83	96.54**±12.06	93.92±11.74	94.12**±11.76	94.16±11.77	94.11**±11.76
12	94.15±11.76	96.78**±12.09	94.76±11.85	96.82**±12.1	93.94±11.74	94.18**±11.77	94.21±11.77	94.25**±11.78
13	94.56±11.82	97.11**±12.138	95.29±11.9	97.24**±12.15	94.28±11.78	94.35**±11.79	94.32±11.79	94.65**±11.83
14	94.97±11.87	98**±12.25	95.75±11.97	98.44**±12.3	94.72±11.86	94.87**±11.86	94.78±11.85	94.88**±11.88

*Significant; 0.01≤P≤0.05 and **High significant; P < 0.01.

Table 5. Mean serum LDL levels (mg/dl). Values expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	32.62±4.07	33±4.125	33.4±4.17	33.6±4.2	32.8±4.1	32.6±4.07	33.4±4.17	33.3±4.16
2	32.87±4.11	33.74±4.2	33.64±4.2	34.51±4.3	33.15±4.14	32.89±4.11	33.88±4.23	33.43±4.17
3	33±4.12	34.12±4.26	33.85±4.23	35.45±4.43	33.28±4.16	33.12±4.14	33.96±4.24	33.55±4.19
4	33.25±4.15	34.89±4.36	33.93±4.24	36.58±4.57	33.38±4.17	33.25±4.15	34.25±4.28	33.78±4.22
5	33.32±4.16	35.55*±4.44	34.25±4.28	37.95*±4.74	33.45±4.18	33.64**±4.2	34.35±4.29	34.17**±4.27
6	33.41±4.17	36.12*±4.5	34.45±4.3	38.47*±4.8	33.67±4.21	33.89**±4.24	34.54±4.32	34.35**±4.29
7	33.65±4.21	37.34*±4.66	34.67±4.33	39.67*±4.95	33.83±4.22	34.35**±4.29	34.68±4.33	34.75**±4.34
8	33.72±4.22	38.75*±4..84	34.79±4.34	40.73*±5.09	33.97±4.24	34.78**±4.34	34.77±4.34	35.29**±4.4
9	33.89±4.23	39.28*±4.91	34.97±4.37	41.19*±5.14	34.27±4.28	34.92**±4.36	34.82±4.35	35.48**±4.43
10	34.17±4.27	40.87**±5.1	35.47±4.43	42.38**±5.29	34.36±4.29	35.35**±4.41	34.92±4.36	35.76**±4.447
11	34.32±4.29	41.69**±5.2	35.69±4.46	45.88**±5.73	34.47±4.31	35.75**±4.46	35.14±4.39	36.19**±4.52
12	34.44±4.3	42.71**±5.33	35.75±4.46	46.97**±5.87	34.58±4.32	35.89**±4.48	35.22±4.4	36.45**±4.55
13	34.58±4.32	43.84**±5.48	35.87±4.48	47.57**±5.94	34.86±4.35	36.52**±4.56	35.39±4.42	36.76**±4.59
14	34.76±4.34	44.62**±5.57	36.12±4.5	48.64**±6.08	34.98±4.37	36.88**±4.61	35.48±4.43	36.83**±4.6

*Significant; 0.01≤P≤0.05; **High significant; P<0.01.

Table 6. Mean of serum adiponectin (ng/ml) levels. Values expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	127.54±15.94	127.42±15.92	127.42±15.92	127.35±15.919	127.5±15.939	127.32±15.917	127.44±15.937	127.33±15.918
2	127.68±15.96	127.33±15.9	127.23±15.9	127.11±15.88	127.79±15.973	127.28±15.91	127.16±15.895	127.12±15.892
3	127.78±15.97	127.28±15.89	127.18±15.897	126.75±15.843	127.61±15.951	127.17±15.896	127.04±15.88	127.07±15.883
4	127.91±15.98	127.19±15.86	126.49**±15.811	126.12**±15.765	128.01±16	126.97±15.87	126.85**±15.856	126.87**±15.858
5	128.12±16.01	126.72*±15.84	126.12**±15.765	125.87**±15.733	128.15±16.018	126.83**±15.853	126.67**±15.833	126.65**±15.832
6	128.24±16.03	126.64*±15.8	125.84**±15.73	125.29**±15.66	128.27±16.03	126.77**±15.84	126.23**±15.77	126.46**±15.81
7	128.35±16.04	126.25*±15.78	125.25**±15.656	124.74**±15.592	128.39±16.04	126.61**±15.826	125.78**±15.722	126.21**±15.776
8	128.41±16.05	126.18*±15.77	124.68**±15.585	124.1**±15.51	128.47±16.05	126.58**±15.822	125.56**±15.695	125.72**±15.715
9	128.52±16.06	125.89*±15.73	123.79**±15.473	123.94**±15.49	128.58±16.07	126.45**±15.81	125.28**±15.66	125.44**±15.68
10	128.61±16.07	125.52**±15.71	122.92**±15.365	123.23**±15.4	128.65±16.08	126.39**±15.7	124.96**±15.62	125.17**±15.64
11	128.74±16.09	125.12**±15.67	122.22**±15.277	122.68**±15.335	128.77±16.09	126.22**±15.77	124.84**±15.6	124.89**±15.6
12	128.81±16.1	124.75**±15.64	121.65**±15.2	122.32**±15.29	128.82±16.03	126.18**±15.77	124.56**±15.57	124.74**±15.59
13	128.92±16.11	124.56**±15.59	120.56**±15.07	119.76**±14.97	128.95±16.11	125.84**±15.73	124.33**±15.54	124.52**±15.56
14	129.11±16.13	124.38**±15.57	119.68**±14.96	119.04**±14.88	129.06±16.132	125.76**±15.72	124.21**±15.526	124.2**±15.525

*Significant; 0.01≤P≤0.05; **High significant; P<0.01.

Table 7. Mean of serum insulin levels (mg/dl). Values expressed as means ± standard deviation.

Duration (weeks)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
1	0.621±0.077	0.623±0.077	0.622±0.077	0.624±0.078	0.624±0.078	0.622±0.077	0.622±0.077	0.621±0.076
2	0.627±0.078	0.631±0.077	0.632±0.079	0.637±0.079	0.628±0.078	0.632±0.079	0.624±0.078	0.625±0.077
3	0.632±0.79	0.638±0.078	0.648±0.081	0.65±0.082	0.632±0.079	0.646±0.08	0.628±0.078	0.627±0.078
4	0.638±0.79	0.642±0.079	0.668**±0.083	0.681**±0.085	0.638±0.079	0.652±0.0815	0.632**±0.079	0.638**±0.078
5	0.642±0.08	0.647±0.08	0.694**±0.086	0.718**±0.089	0.642±0.08	0.661±0.083	0.639**±0.079	0.643**±0.08
6	0.648±0.081	0.652±0.081	0.713**±0.089	0.732**±0.092	0.648±0.081	0.669±0.087	0.644**±0.08	0.658**±0.082
7	0.655±0.082	0.659±0.082	0.737**±0.092	0.755**±0.094	0.655±0.082	0.672±0.084	0.648**±0.081	0.662**±0.083
8	0.662±0.083	0.664±0.083	0.756**±0.094	0.779**±0.097	0.662±0.083	0.678±0.084	0.658**±0.082	0.676**±0.084
9	0.667±0.083	0.678±0.084	0.768**±0.096	0.792**±0.099	0.667±0.083	0.682±0.085	0.67**±0.082	0.682**±0.085
10	0.671±0.084	0.681±0.085	0.787**±0.098	0.822**±0.12	0.671±0.084	0.687±0.085	0.679**±0.084	0.689**±0.086
11	0.678±0.085	0.692±0.086	0.819**±0.12	0.842**±0.13	0.678±0.085	0.692±0.086	0.683**±0.085	0.698**±0.087
12	0.682±0.086	0.717±0.089	0,836**±0.14	0.867**±0.15	0.682±0.085	0.697±0.087	0.689**±0.086	0.719**±0.089
13	0.689±0.087	0.727±0.09	0.854**±0.16	0.882**±0.18	0.689±0.086	0.712±0.089	0.717**±0.089	0.721**±0.09
14	0.7±0.088	0.736±0.092	0.882**±0.18	0.91**±0.19	0.699±0.087	0.716±0.089	0.725**±0.09	0.734**±0.092

*Significant; 0.01≤P≤0.05; (**): High significant; P<0.01.

by increasing blood cholesterol levels above normal; then obesity is caused (Stefan et al., 2002).

On the other hand, after adiponectin treatment for obese and diabetic groups, the levels of adiponectin increased and insulin levels were in balance. Adiponectin is a possible insulin-sensitizing agent (Kim et al., 2006). Recently, it has been reported that adiponectin levels in diabetic rats were suppressed when compared with non-diabetic rats (Kahn, 2003). Plasma adiponectin levels were shown to be a marker of glucose metabolism and obesity. After recombinant adiponectin treatment, reduced serum glucose was observed in diabetic rodents stimulating insulin secretion, which was in agreement with the study of Bergman et al. (2002). Recently, adiponectin treatment has been shown to improve insulin resistance with attenuation of body weight gain (Okamoto et al., 2003). Another potential mechanism for adiponectin protective effect is improved insulin secretion and action, which has been shown recently (Matsuzawa et al., 2002).

Adipocyte-derived mediator has been proposed as a factor that link insulin resistance, excess obesity and ß-cell dysfunction in type-II diabetes (Meyerhardt et al., 2008). Type-II diabetes is characterized by a combination of insulin resistance and alterations in ß-cell function (Shargorodsky, 2009). The latter may be ascribed to a certain extent as deleterious effect of chronic hyperglycemia is referred to glucotoxicity (Wolpin et al., 2008). This evidence for a link between hyperglycemia and dysfunction in pancreatic islets of Langerhans comes from *vivo* and *in vitro* studies in animal models (Oda, 2008).

The main features of apoptosis are architecture of the islet not maintained (Shargorodsky, 2009). A novel animal model of type 2 diabetes, showed that there was hyperglycemia development which was associated with pancreatic ß-cell failure (Yan, 2008) and this was shown in the histopathological and immunohistochemical studies on the diabetic rats in this study. However, adiponectin injection helped in neogenesis of the pancreatic ß-cells and regeneration of the pancreatic islets of Langerhans. Several studies have shown that chronic elevation of blood glucose concentrations in both humans and experimental animal models leads to ß-cell dysfunction in terms of insulin secretion and insulin synthesis (Waki et al., 2003), despite some evidence for glucose toxicity leading to cell death (Pollak, 2007).

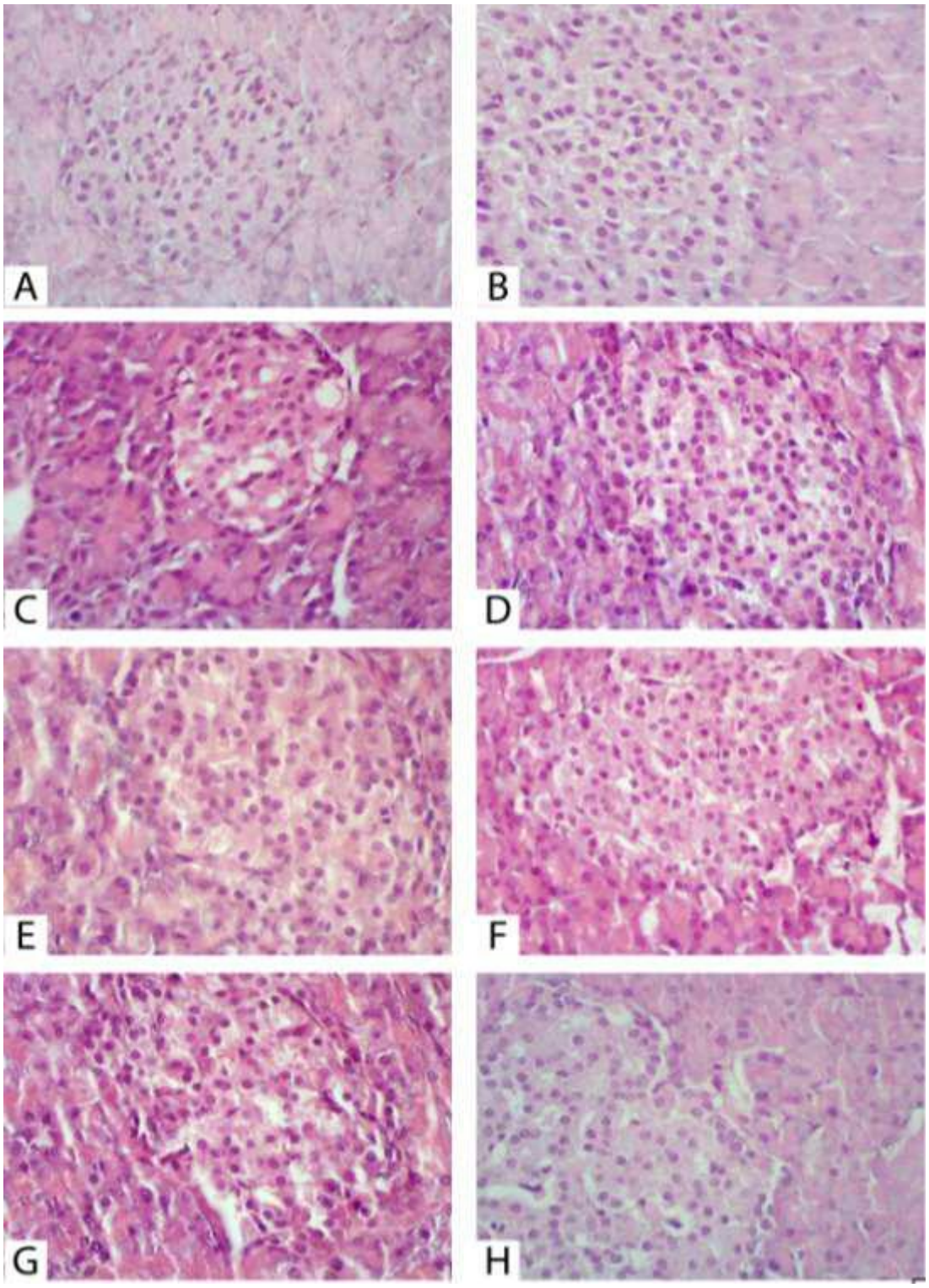

Figure 1. The histopathology of pancreatic islet of Langerhans. Normal appearance in control group (*a*). Normal appearance in adiponectin/without diabetes nor obesity group (b). Diabetic rats/without adiponectin treatment with a severity of vaculation of pancreatic Langerhans islets and extensive fibrosis. There was severe degenerative and necrotic changes with obvious amyloid and hyaline droplets (e), (g). The degeneration and necrotic changes in Langerhans islet were less than those in obese rats/without adiponectin treatment (*c*). Islets of Langerhans with light to null degenerative changes and light vacuoles in rats after adiponetin treatment (d*), (*f), (h). (H&E x400).

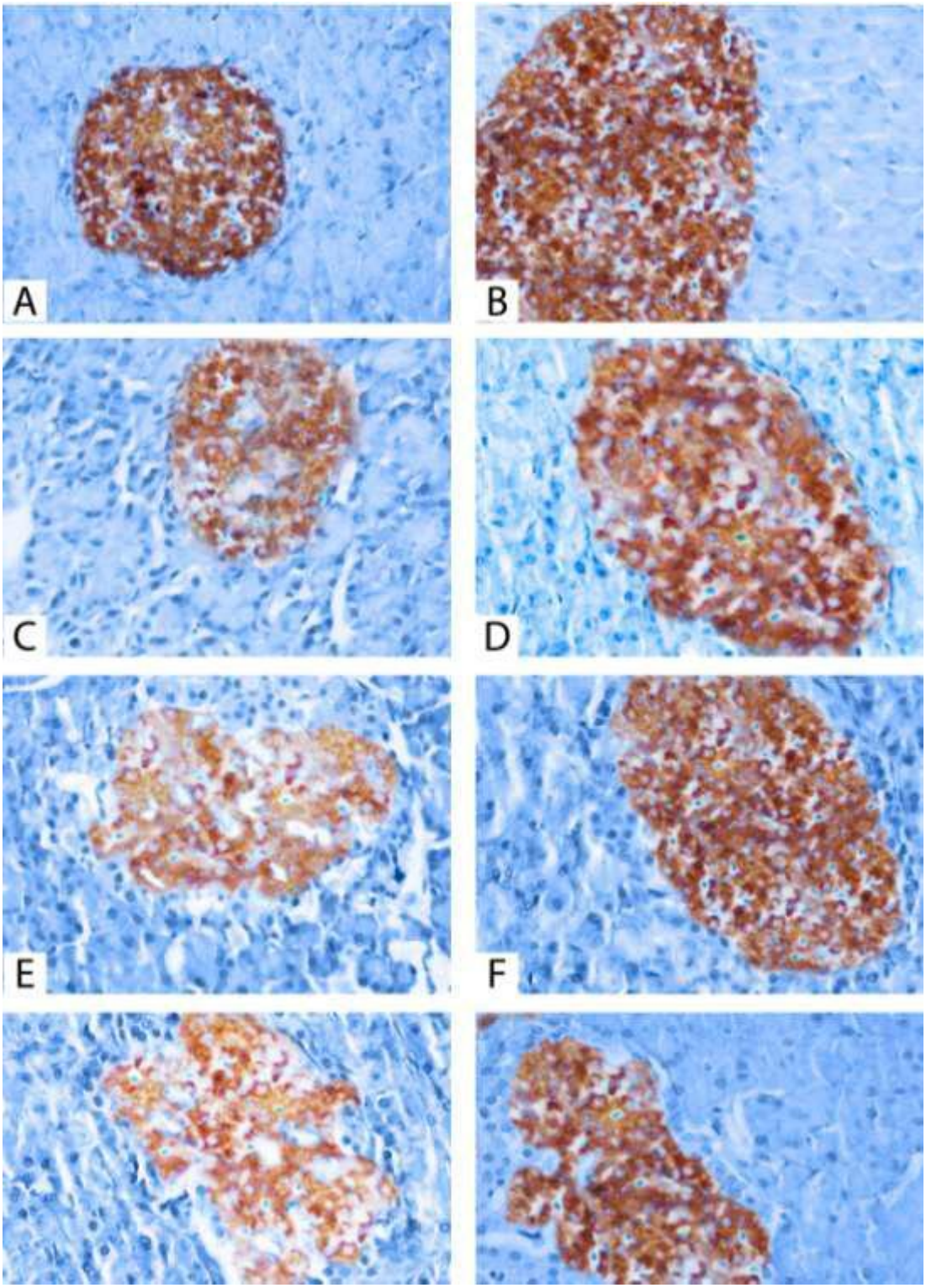

Figure 2. Immunohistochemicaal studies of pancreatic islet of Langerhans. Strongly stained β-cells with anti-insulin antibody immune reactive stain in control group (*a*) and in adiponectin/without diabetes nor obesity group (b). Moderate staining β-cells in Langerhans islets in adiponectin treatment groups (obesity/diabetic rats) (d), (f) and (h) as there was increased number of insulin immune-reactive granules in β-cells. There is light to null staining β-cells in Langerhans islets in the groups without adiponectin treatment (diabetic rats) (e), (*g*) (obesity rats) (*c*). (Anti-insuline antibody stain x400).

CONCLUSIONS AND FUTURE DIRECTIONS

The discovery of adiponectin undoubtedly represents a very important step in our attempts to further understand the mechanism of obesity-induced insulin resistance. Adiponectin concentrations were decreased in obese and type II diabetic individuals and it caused histopathological and immunohistochemical alterations, but recovered after adiponectin treatment. This fact together with the promising results of experimental studies suggests the possibility of adiponectin replacement as a new pharmacological approach to insulin resistance treatment. Despite this promising implication, there was still number of crucial steps to be performed to fully understand the biology of adiponectin, e.g. identification of the adiponectin receptor and the exact mechanism of adiponectin action. From the clinical point of view, human studies with recombinant adiponectin administration will be necessary in order to test the effectiveness of this compound in the treatment of insulin resistance in clinical medicine.

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REFERENCES

Abbasi FK, Chu JF, Lamendola CS (2004). Discrimination between obesity and insulin resistance in the relationship with adiponectin. J. Diabetes 53:585-590.

Amherdt MB, Harris VF, Renold AH, Orci LP, Unger RO (1974). Hepatic autography in uncontrolled experimental diabetes and its relationships to insulin and glucagon. J. Clin. Invest. 54:188-193.

Alzaid AJ (1997). Time to declare war on diabetes. Ann. Saudi Med. 17:154-155.

Andreelli F, Foretz M, Knauf C, Cani PD, Perrin C, Iglesias MA, Pillot B, Bado A, Tronche F, Mithieux G, Vaulont S, Burcelin R, Viollet B (2006). Liver adenosine monophosphate-activated kinase-alpha2 catalytic subunit is a key target for the control of hepatic glucose production by adiponectin and leptin but not insulin. Endocrinology 147:2432-2441.

Bergman RR, Finegood DH, Kahn SE (2002). The evolution of â-cell dysfunction and insulin resistance in type 2 diabetes. Eur. J. Clin. Invest. 32:35-45.

Butler AO, Janson JU, Bonner-Weir SK, Ritzel RG, Rizza RK, Butler PP (2003). Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. Diabetes 52:102-110.

Combs TW, Berg AK, Obici SY, Scherer PR, Rossetti LO (2001). Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. J. Clin. Invest. 108:1875-1881.

Dubiel EK, Lambert PW, Clark PT (2012). In vitro morphogenesis of PANC-1 cells into islet-like aggregates using RGD-covered dextran derivative surfaces. Colloids Surf. B. Biointerfaces 89:117-25.

Fasshauer MK, Klein JT, Neumann SS, Eszlinger MN, Paschke RY (2002). Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem. Biophys. Res. Commun. 290:1084-1090.

Fasshauer MF, Paschke RL (2003). Regulation of adipocytokines and insulin resistance. Diabetologia 46:1594-1603.

Fisher RI (1970). Statistical method for research workers. In: Edinburgh (ed.), 14 Oliver and Boyed. pp. 140-144.

Fruebis JV, Tsao TF, Javorschi SX, Erickson MS, Yen FD, Bihain BT, Lodish HO (2005). Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc. Natl. Acad. Sci. 98:2010-2001.

Fujita NQ, Itoh TK, Omori HR, Fukuda MV, Noda TR, Yoshimori TO (2008). The Atg16L complex specifies the site of LC3 lipidation for membrane biogenesis in autophagy. Mol. Biol. Cell 19:2092-2100.

Haluzik MA, Parizkova JY (2004). Adiponectin and Its Role in the Obesity-Induced Insulin Resistance and Related Complications. Physiol. Res. 53:123-129.

Hotta KG, Funahashi TR, Arita YT (2000). Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. J. Biol. 20:1595-1599.

Hug CY, Wang JT, Ahmad NM, Bogan JU, Tsao TO, Lodish HH (2004). T-cadherin is a receptor for hexameric and high-molecular-weight forms of Acrp30/adiponectin. Proc. Natl. Acad. Sci. 101:10308-10313.

Imagawa AT, Funahashi TK, Nakamura TG, Nishizawa HJ (2002). Elevated serum concentration of adipose-derived factor, adiponectin, in patients with type 1 diabetes. Diabetes Care 25:1665-1666.

Kahn BZ, Flier JV (2000). Obesity and insulin resistance. J. Clin. Invest. 106:473-481.

Kern PU, Gregorio GL, Lu TO, Ranganathan GP (2003). Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance and tumor necrosis factor-alpha expression. J. Diabetes 52:1779-1785.

Kim CT, Park EG, Kang CL, Ahn BW, Cha SP, Kim HG (2006). Comparison of body fat composition and serum adiponectin levels in diabetic obesity and non-diabetic obesity. J. Obes. 14:1164-1171.

luiz DE, Carlos JI, Jose CV (2010). Pasic histology and histological techniques. McGraw-Hill Publishing Co. 12th edition. Chapter 16, pp. 142-150.

Mao XY, Kikani CC, Riojas RW, Langlais PE (2006). APPL1 binds to adiponectin receptors and mediates adiponectin signalling and function. Nat. Cell. Biol. 8:516–523.

Matsushita MS, Suzuki NW, Obara KR, Fujioka YR, Ohsumi YT, Inagaki FP (2007). Structure of At g5.Atg16, a complex essential for autophagy. J. Biol. Chem. 282:6763-6772.

Matsuzawa YT, Stumvoll ML, Weyer CV, Tataranni PA (2002). Low plasma adiponectin concentrations do not predict weight gain in humans. J. Diabetes 51:2964–2967.

Meyerhardt JG, Niedzwiecki DF, Hollis DE (2008). Impact of body mass index and weight change after treatment on cancer recurrence and survival in patients with stage III colon cancer. J. Clin. Oncol. 26:4109-4115.

Milan GH, Granzotto MG, Scarda AR, Calcagno AY, Federspil GE, Vettor RC (2002). Resistin and adiponectin expression in visceral fat of obese rats: Effect of weight loss. Obes. Res. 10:1095-1103.

Nathan DJ, Buse JL, Davidson MB, Ferrannini ER, Holman RH, Sherwin RT, Zinman BP (2009). Medical management of hyperglycemia in type 2 diabetes mellitus: A consensus algorithm for the initiation and adjustment of therapy: A consensus statement from American Diabetes Association and European Association for the Study of Diabetes. Diabetologia 52:17-30.

Oda NP (2008). The ratio of leptin to adiponectin can be used as an index of insulin resistance. J. Metab. 57:268-273.

Okamoto YT, Ohashi KD, Nagaretani HG, Kishida KJ (2003). Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. J. Circ. 107:671–674.

Ouchi NB, Kihara SR, Funahashi TE, Nakamura TP (2003). Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. J. Circ. 107:671–674.

Pannacciulli NM, Vettor RH, Milan GU, Catucci AF, Federspil GE, Giorgino RP, Pergola GU (2003). Anorexia nervosa is characterized by increased adiponectin plasma levels and reduced nonoxidative

glucose metabolism. J. Clin. Endocrinol. Metab. 88:1748-1752. Pollak MB (2007). Insulin-like growth factor related signaling and cancer development. Recent Results Cancer Res. 174:49-53.

Reinehr TA, Kratzsch JE, Kiess WR, Andler WF (2005). Circulating soluble leptin receptor, leptin, and insulin resistance before and after weight loss in obese children. Int. J. Obes. 29:1230-1235.

Retnakaran RU, Hanley JY, Raif NN, Hirning CR, Conolley PU, Kahn ET, Zinman BE (2005). Adiponectin and betdiabta cell dysfunction gestational diabetes pathophysiological implications. Diabetologia 48:993-1001.

Shargorodsky MW, (2009). Adiponectin and vascular properties in obese patients: Is it a novel biomarker of early atherosclerosis? Int. J. Obes. 33:553-580.

Shulman GS (2000). Cellular mechanisms of insulin resistance. J. Clin. Invest. 106: 171–176.

Souza RY, Allebrandt KE, Furtado LP, Chautard-Freire-Maia EL (2005). Possible influence of BCHE locus of butyrylcholinesterase on stature and body mass index. Am. J. Phys. Anthropol. 326:329-334.

Stefan NF, Bunt JF, Salbe AG, Funahashi TR, Matsuzawa YW, Tataranni PO (2002). Plasma adiponectin concentrations in children: Relationships with obesity and insulinemia. J. Clin. Endocrinol. Metab. 87:4652-4656.

Trayhurn PP, Beattie JG (2001). Physiological role of adipose tissue: White adipose tissue as an endocrine and secretory organ. Proc. Nutr. Soc. 60:329–339.

Trinder PI, Stern ML (1959). Determination of blood glucose using aminophenazone. J. Clin. Pathol. 22:246-250.

Ukkola OP, Santaniemi MW (2002). Adiponectin: A link between excess adiposity and associated co-morbidities. J. Mol. Med. 80:696-702.

Waki HH, Yamauchi JR, Kamon YO, Ito SI Uchida ST, Kita KQ, Hara YU, Nagai TP (2003). Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J. Biol. Chem. 278:40352-40363.

Wang CC, Mao XR, Wang LD, Liu MI (2007). Adiponectin sensitizes insulin signaling by reducing p70 S6 kinase-mediated serine phosphorylation of IRS-1. J. Biol. Chem. 282:7991-7996.

Weyer CP, Funahashi TE, Tanaka SA, Hotta KM, Matsuzawa YR, Tataranni PP (2001). Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. J. Clin. Endocrinol. Metab. 86:1930-1935.

Wild SA, Rogli GC, Green AN, Sicree RK, King HR (2004). Global prevalence of diabetes estimates for the year 2000 and projections for 2030. Diabetes Care 27:1047-1053.

Wilson GF, Gyi AL (2010). The status and perspective of diabetes health education in China: Inspiration from Australia. Int. J. Nurs. Pract. 16:92-98.

Wolpin BH, Meyerhardt AL, Chan AP (2008). Insulin, the insulin-like growth factor axis, and mortality in patients with nonmetastatic colorectal cancer. J. Clin. Oncol. 27:176-185.

Yamauchi TG, Hara KK, Kubota NM, Terauchi YI, Tobe KE (2003a). Dual roles of adiponectin/acrp30 in vivo as an anti-diabetic and anti-atherogenic adipokine. Curr Drug Targets Immune. Endocr. Metabol. Disord. 3:243-254.

Yamauchi TU, Kamon JR, Ito YW, Tsuchida AP, Yokomizo TO, Sugiyama TT, Hara KY (2003b). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 423:762-769.

Yan EI (2008). Insulin, hs-CRP, leptin, and adiponectin. An analysis of their relationship to the metabolic syndrome in an obese population with an elevated waist circumference. Metab. Syndr. Relat. Disord. 6:64-73.

Zhang PG, Zhang XK, Brown JU, Vistisen DH, Sicree RE, Shaw JW, Nichols GY (2010). Global healthcare expenditure on diabetes for 2010 and 2030. Diabetes Res. Clin. Pract. 87:293-301.