

## ADIPONECTIN REGULATION IN TYPE 2 DIABETIC RATS: EFFECTS OF INSULIN, METFORMIN AND DEXAMETHASONE

<sup>1,2</sup>Tamer Ahmed Ismail, <sup>1,3</sup>Mohamed Mohamed Soliman and <sup>4</sup>Shimaa Ahmed Ismail

<sup>1</sup>Department of Medical Laboratory, Faculty of Applied Medical Sciences, Turabah, Taif University, Saudi Arabia

<sup>2</sup>Department of Physiology, Faculty of Veterinary Medicine, Zagazig University, Egypt

<sup>3</sup>Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Egypt

<sup>4</sup>Department of Clinical Pathology, Faculty of Veterinary Medicine, Zagazig University, Egypt

Received 2013-11-12, Revised 2013-11-19; Accepted 2013-11-23

### ABSTRACT

Adiponectin is a protein synthesized from adipose tissue, increases peripheral glucose utilization in liver and skeletal muscle. Adiponectin expression and secretion are decreased during obesity and insulin resistance. In this study, the effect of insulin, metformin and dexamethasone on serum lipid profiles was examined in Type 2 Diabetic (T2D) rats. T2D was induced by feeding rats a high fat diet for 4 weeks plus medium dose of Streptozotocin (STZ, 35 mg kg<sup>-1</sup> BW). Adiponectin, adiponectin receptors (AdipoR-1 and AdipoR-2), leptin, Peroxisome Proliferator Activated Receptor gamma (PPAR-γ), Hormone Sensitive Lipase (HSL), Pyruvate Kinase (PK), enolase and Glucose Transporter-2 (GLUT-2) expression in epididymal adipose and liver tissue were examined using RT-PCR. Results showed that metformin improved insulin resistance by normalizing serum lipid profiles in diabetic rats, while dexamethasone did not alter it. Metformin up-regulated adiponectin, AdipoR-1 and AdipoR-2 expression, while insulin and dexamethasone down-regulated them. Leptin expression was decreased while PPARγ, HSL, PK, enolase and GLUT-2 expression was increased by metformin administration. Dexamethasone failed to improve insulin resistance in T2D rats. In conclusion, metformin ameliorates T2D through controlling adiponectin expression and its consequent genes of lipids and glucose metabolism.

**Keywords:** Adiponectin Regulation, Diabetes, Metformin, Insulin, Glucose and Lipid Metabolism

### 1. INTRODUCTION

Improvements in technology lead to a sedentary lifestyle and consequently, an increase in body weight and obesity (Goldstein and Scalia, 2004). T2D is a quickly growing global metabolic disease characterized by impaired insulin secretion from pancreatic β cells and insulin resistance in liver, muscle and adipose tissue (Ashcroft and Rorsman, 2012). Insulin resistance is closely linked with other metabolic symptoms such as hypertension and hyperlipidemia (Cornier *et al.*, 2008). Insulin resistance is frequently associated with obesity, however, the

molecular link between increased adiposity and reduced sensitivity of target tissues to insulin is not completely clear (Kahn and Flier, 2000; Winter *et al.*, 2006).

Kwon and Pessin (2013) reviewed that adipose tissue was considered as an inert energy storage organ that accumulates and stores triacylglycerols during energy excess and releases fatty acids in times of systemic energy need. However, over the last two decades adipose tissue depots have been established as highly active endocrine and metabolically important organs that modulate energy expenditure and glucose homeostasis. White adipose tissue is responsible for the secretion of an array of signaling

**Correspondence Author:** Mohamed Mohamed Soliman, Department of Biochemistry, Faculty of Veterinary Medicine, Benha University, Moshtohor, P.O. Box 13736, Egypt Tel: 00966501531640; 002012-3722404

molecules, termed adipokines such as free fatty acid (Shulman, 2000), leptin (Friedman, 2000), plasminogen activator inhibitor-1 (Shimomura *et al.*, 1996), resistin (Steppan *et al.*, 2001), TNF- $\alpha$  (Hotamisligil, 1999) and adiponectin (Beltowski, 2003). The dysregulation of adipokines has been implicated in obesity, type 2 diabetes and cardiovascular diseases (Kwon and Pessin, 2013).

Adiponectin is a protein which was originally identified by Scherer *et al.* (1995). It is encoded by the Adipo Q gene in humans and has recently attracted much more attention (Capeau, 2007; Kwon and Pessin, 2013). Adiponectin is a member of the soluble defense collagen superfamily and plasma adiponectin concentrations and mRNA expression have been shown to be decreased in obesity and insulin resistance (Brun *et al.*, 2003; Weyer *et al.*, 2001). Its primary structure contains an N-terminal signal sequence, a variable domain, a collagen-like domain and a C-terminal globular domain (Yamauchi *et al.*, 2003). Adiponectin stimulates fatty acid oxidation in liver and muscle, decreases plasma triglycerides and enhances glucose metabolism by increasing insulin sensitivity (Combs *et al.*, 2001; Kloting *et al.*, 2010; Yamauchi *et al.*, 2001). Adiponectin is involved in the regulation of energy balance and body weight (Fruebis *et al.*, 2001; Yamauchi *et al.*, 2001). Its synthesis and secretion are regulated by several factors including IGF-1 and insulin (Halleux *et al.*, 2001) and are affected by thiazolidinediones (Bodles *et al.*, 2006). Diez and Iglesias (2003) suggested that plasma level of adiponectin has been reduced in clinical conditions associated with insulin resistance, including obesity, type 2 diabetes, dyslipidemia and hypertension. Moreover, it has been reported that high molecular weight form of adiponectin may serve as a significant surrogate marker for the prediction of type 2 diabetes in Japanese population (Satoh *et al.*, 2010). Adiponectin has two adiponectin receptors, adipoR1 is the major receptor expressed in skeletal muscle, whereas adipoR2 is mainly expressed in liver (de Oliveira *et al.*, 2011a). Both receptors are expressed in various tissues and pancreatic cells (Beylot *et al.*, 2006).

Treatment of type 2 diabetes depends on oral hypoglycemic drugs that contain Peroxisome Proliferator Activated Receptor Gamma (PPAR $\gamma$ ) and thiazolidinediones, among which metformin is the most common. These drugs act on the improvement of hyperinsulinemia, an integral part in the development of type 2 diabetes (Gerich, 1998). Metformin is widely used

as a first line of treatment for patients with type 2 diabetes mellitus. Metformin improves glucose metabolism mainly by suppressing hepatic glucose production (Hundal *et al.*, 2000), but its exact mechanism of action remains unclear. Several reports have concluded that metformin does not significantly affect plasma adiponectin levels (Tiikkainen *et al.*, 2004).

Adiponectin is regulated by several hormones and factors in humans, rodents and cattle (Birkenfeld *et al.*, 2012; de Oliveira *et al.*, 2011a; Fasshauer *et al.*, 2002; Soliman *et al.*, 2011). Catecholamines (Fu *et al.*, 2007), growth hormones (Xu *et al.*, 2004), insulin (Fasshauer *et al.*, 2004) and glucocorticoids (Fasshauer *et al.*, 2001) are mostly examined. Both *in vitro* and *in vivo* studies have shown that glucocorticoids reduce plasma levels of adiponectin and inhibit adiponectin expression (Jang *et al.*, 2008). Moreover, Shi *et al.* (2010) observed decreased serum levels of adiponectin and reduced adiponectin mRNA expression in the adipose tissue of both obese and non-obese rats treated with glucocorticoids. Relatively few studies have analyzed the effects of glucocorticoids on the expression of adiponectin and its receptors during insulin resistance. Among glucocorticoids, dexamethasone is a synthetic glucocorticoids frequently used to rapidly generate insulin resistance in rodents (Besse *et al.*, 2005). In this study, we examined the effects of metformin, insulin and dexamethasone on insulin resistance with regarding to adiponectin and gene expression of lipids and glucose.

## 2. MATERIALS AND METHODS

### 2.1. Materials

Streptozotocin (STZ), insulin, metformin, dexamethasone were purchased from sigma Aldrich, USA. The Wistar albino rats were purchased from Egyptian Co for Experimental Animals Import, Helwan, Cairo, Egypt. Solvents and related materials were from ADWIA pharmaceutical company, Egypt. High fat diet was purchased from Qaha Co. Qaha, Kalubiya, Egypt. Biochemical kits for lipids profiles were from *Clini Lab*, Cairo, Egypt.

### 2.2. Induction of Type 2 Diabetes (Insulin Resistance) and Experimental Design

Male Wistar rats (50 rats), 4 weeks old, weighting 80-100 g, were selected randomly. Rats were exposed to 12 h/12h day light with free access to food and water.

The fifty rats were divided into five groups (10 rats per group). Control group was fed normal diet and the remaining 4 groups Fed High Fat Diet (HFD) for one month. The HFD constitutes 15.5% protein, 38.8% fat and 45.7% carbohydrates, by calories. Induction of T2D in HFD rats was based on the protocol of Srinivasan *et al.* (2005) (intraperitoneal injection of medium dose of STZ, 35 mg kg<sup>-1</sup> BW together with HFD). Diabetes was confirmed after 3 days, as serum glucose and lipid profiles were measured and values of glucose over 200 mg dL<sup>-1</sup> considered diabetic. All diabetic rats (n = 40) were subdivided into 4 subgroups, diabetic group (n = 10), diabetic plus insulin (n = 10) received insulin subcutaneously in a dose of 0.2 units/kg/day for 14 days, diabetic plus metformin (400 mg kg day<sup>-1</sup>) for 14 days and diabetic plus dexamethasone (0.2 mg 100<sup>-1</sup> g BW twice per day S/C) for 14 days.

After the end of experimental procedures, all rats were decapitated after overnight fasting and blood was collected for serum extraction. Liver and adipose tissues were preserved in TriZol for RNA extraction and gene expression.

### 2.3. Serum Chemistry Analysis

Serum Triglycerides (TG), Total Cholesterol (TC), Low Density Lipoproteins (VLDL), LDL and High Density Lipoproteins (HDL) were measured using commercial kits that based on spectrophotometric analysis.

### 2.4. RT-PCR Analysis and Gene Expression

Liver and epididymal adipose tissues were collected from rats, flash frozen in 1 mL TriZol reagent (Invitrogen, Carlsbad, CA) and subsequently stored at

-70°C. Frozen samples (approximately 100 mg of tissue per sample) were immediately homogenized using a Polytron 300 D homogenizer (Brinkman Instruments, Westbury, NY). Total RNA was extracted via chloroform extraction followed by nucleic acid precipitation with isopropanol. The pellet was washed with 75% ethanol and resuspended in molecular biology grade water. Nucleic acid concentration was measured using o.d. 260 nm (Smart-Spec; Bio-Rad Laboratories, Hercules, CA) and RNA integrity was evaluated using an Agilent bioanalyzer (model 2100; Agilent Technologies, Foster City, CA).

RNA (1 µg) was treated at 70°C for 5 min and reverse transcribed using 100 units of Moloney murine leukemia virus reverse transcriptase (Gibco), 50 pmol of poly (dT) primer and 20 nmol of dNTPs in a total volume of 10 µL at 37°C for 1 h. After heating at 94°C for 5 min, PCR amplification was performed with 2.5 units Taq polymerase (Perkin-Elmer, Foster City, CA, USA), 3 mM MgCl<sub>2</sub> and 50 pmol of forward and reverse primers specific for respective genes in a total volume of 25 µL. The PCR conditions of different tested genes are shown in **Table 1**. PCR product was visualized under UV lamp by electrophoresis in 1.5% agarose gel stained with ethidium bromide. Intensities of PCR bands will be analyzed densitometrically using NIH Image program (<http://rsb.info.nih.gov/nih-image/>).

### 2.5. Statistical Analysis

Results are expressed as means±S.E. for 5 independent rats per each group. Statistical analysis was done using ANOVA and Fischer's post hoc test, with p<0.05 being considered as statistically significant.

**Table 1.** PCR conditions of examined genes

mRNA expression	Forward primer	Reverse primer	PCR cycles and Annealing Temp
PK (229 bp)	5'-ATTGCTGTGACTGGATCTGC-3'	5'-CCCGCATGATGTTGGTATAG-3'	30 cycles, 52°C for 1 min
PEPCK (236 bp)	5'- TTTACTGGGAAGGCATCGAT-3'	5'-TCGTAGACAAGGGGGCAC-3'	30 cycles, 52°C for 1 min
GLUT-2 (330 bp)	5'- AAGGATCAAAGCCATGTTGG-3'	5'-GGAGACCTTCTGCTCAGTGG-3'	30 cycles, 55°C 1 min
LPL (269 bp)	5'-CCTGATGACGCTGATTTTGT-3'	5'-AGGCAAGCTGGTGAGGATCTG-3'	24 cycles, 60°C for 45 sec
Leptin (244bp)	5-'CCTGTGGCTTTGGTCCTATCTG-3'	5'-TATGCTTTGCTGGGGTTTC-3'	35 cycles, 61°C for 1 min
PPAR-γ (550 bp)	5'-CATTCTGCTCCACACTATGAA-3'	5'-CGGGAAGGACTTTATGTATGAG-3'	35 cycles 51°C for 1 min
Adipo (500 bp)	5'-CTCCACCCAAGGAAACTTGT-3'	5'-CTGGTCCACATTTTTCCT-3'	35 cycles, 59°C for 1 min
AdipoR-1 (398bp)	5'-AACTGGACTATTCAGGGA-3'	5'-TGGTTCCAGTCTCATCAG-3'	37 cycles, 58°C for 1 min
AdipoR-2 (233bp)	5'-ACCCACAACCTTCCTTCATC-3'	5'-GCTAGCCATGAGCATTAGCC-3'	37cycles, 58°C for 1 min
HSL (313 bp)	5'-TGCCCAGGAGTGTGTCTGAG-3'	5'-AGGACACCTTGGCTTGAGCG-3'	40 cycles, 61°C for 1 min
GAPDH (309 bp)	5'-AGATCCACAACGGATACATT-3	5-TCCCTCAAGATTGTCAAGAA-3'	25 cycles, 52°C 1 min

PCR cycle of respective genes are shown, while temperature and time of denaturation and elongation steps of each PCR cycle were 94°C, 30 sec and 72°C, 60 sec, respectively

### 3. RESULTS

#### 3.1. Effects of Insulin, Metformin and Dexamethasone on Lipid Profiles in Diabetic Rats

As shown in **Table 2**, induction of diabetes in rats increased serum cholesterol, TG, VLDL and LDL levels and decreased HDL levels. Administration of insulin and metformin significantly improved such amelioration in lipid profiles compared to control and diabetic rats. Dexamethasone administration failed to improve such changes in lipid profiles compared to diabetic rats.

#### 3.2. Effects of Insulin, Metformin and Dexamethasone on Insulin and Glucose Levels in Diabetic Rats

To test the effect of adiponectin regulators on insulin and glucose levels in type 2 diabetic rats, **Fig. 1** showed that feeding of high fat diet for 1 month together with small dose of STZ induced insulin resistance. Insulin resistance was characterized by increase in insulin and glucose levels. Insulin administration increased insulin levels in blood of diabetic rats but partially lower glucose concentrations. While metformin administration clearly improved insulin resistance, decreased insulin and consequently glucose

concentrations (**Fig. 1**). Dexamethasone is known as glucogenic glucocorticoid that increases glucose levels. Dexamethasone failed to normalize or improve insulin resistance seen in diabetic rats (**Table 2** and **Fig. 1**).

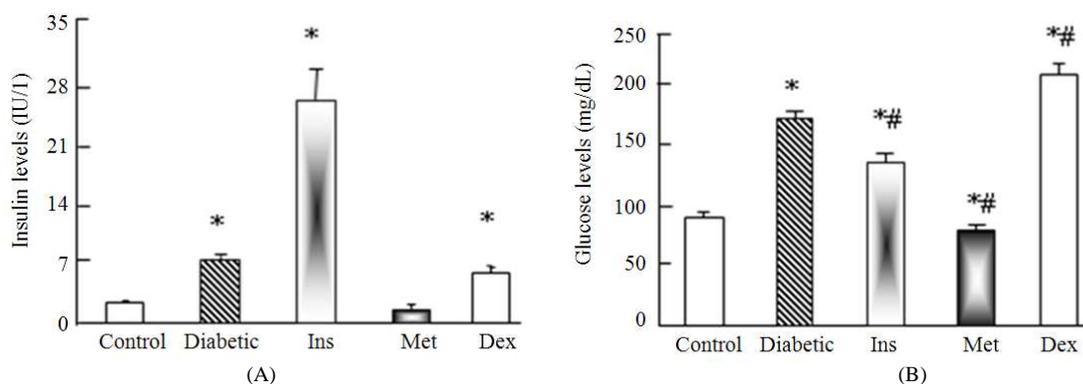
#### 3.3. Effects of Insulin, Metformin and Dexamethasone on Adiponectin and Adiponectin Receptor Expression in Epididymal Adipose Tissue

Diabetic rats showed a decrease in adiponectin expression relative to control (**Fig. 2A**) and insulin administration induced additive inhibitory effect on adiponectin expression, while metformin induced a significant and clear up-regulation in adiponectin expression. Dexamethasone administration induced inhibition in adiponectin expression. Regarding adiponectin receptor-1 expression (AdipoR-1), **Fig. 2B** showed that AdipoR-1 expression was down regulated in diabetic rats and insulin administration failed to recover this inhibition, while metformin up-regulate it. Dexamethasone partially normalized AdipoR-1 expression compared to control and diabetic rats. Next, we tested AdipoR-2 expression, as seen in **Fig. 2C**, AdipoR-2 expression was decreased in diabetic and insulin administered rats. AdipoR-2 expression increased by metformin and decreased by dexamethasone.

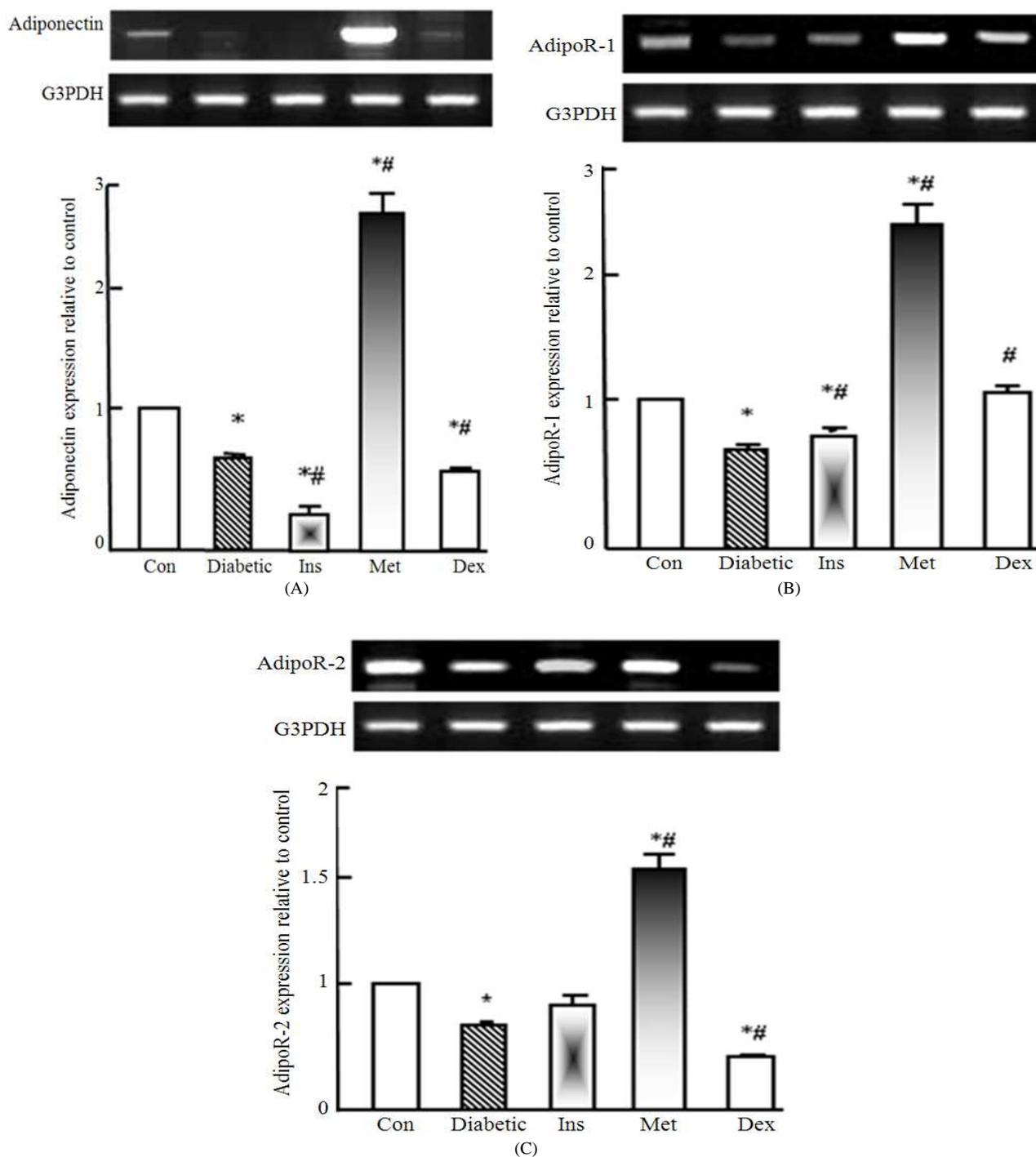
**Table 2.** Changes in lipid profiles after administration of insulin, metformin and dexamethasone into type 2 diabetic Wistar rats

	Control	Diabetic group	Diabetic + Insulin	Diabetic + Metformin	Diabetic + Dex
Cholesterol	118.6±2.9	190±4.4	107.3±4.9#	103.6±3.5#	201.6±8.1
TG	98.3±6	195.7±10.5*	100.5±2.9#	80.6±5.2#	152±10.2
LDL	57.6±4.4	208.6±9.6*	55.6±5.2#	58.6±1.8#	149.3±1.8
VLDL	19.6±2.1	38±2.1*	20.6±0.9#	17.3±1.8#	43.6±1.3
HDL	34±1	23.6±2.1	33.3±0.8#	32.6±1.2#	24±3.2

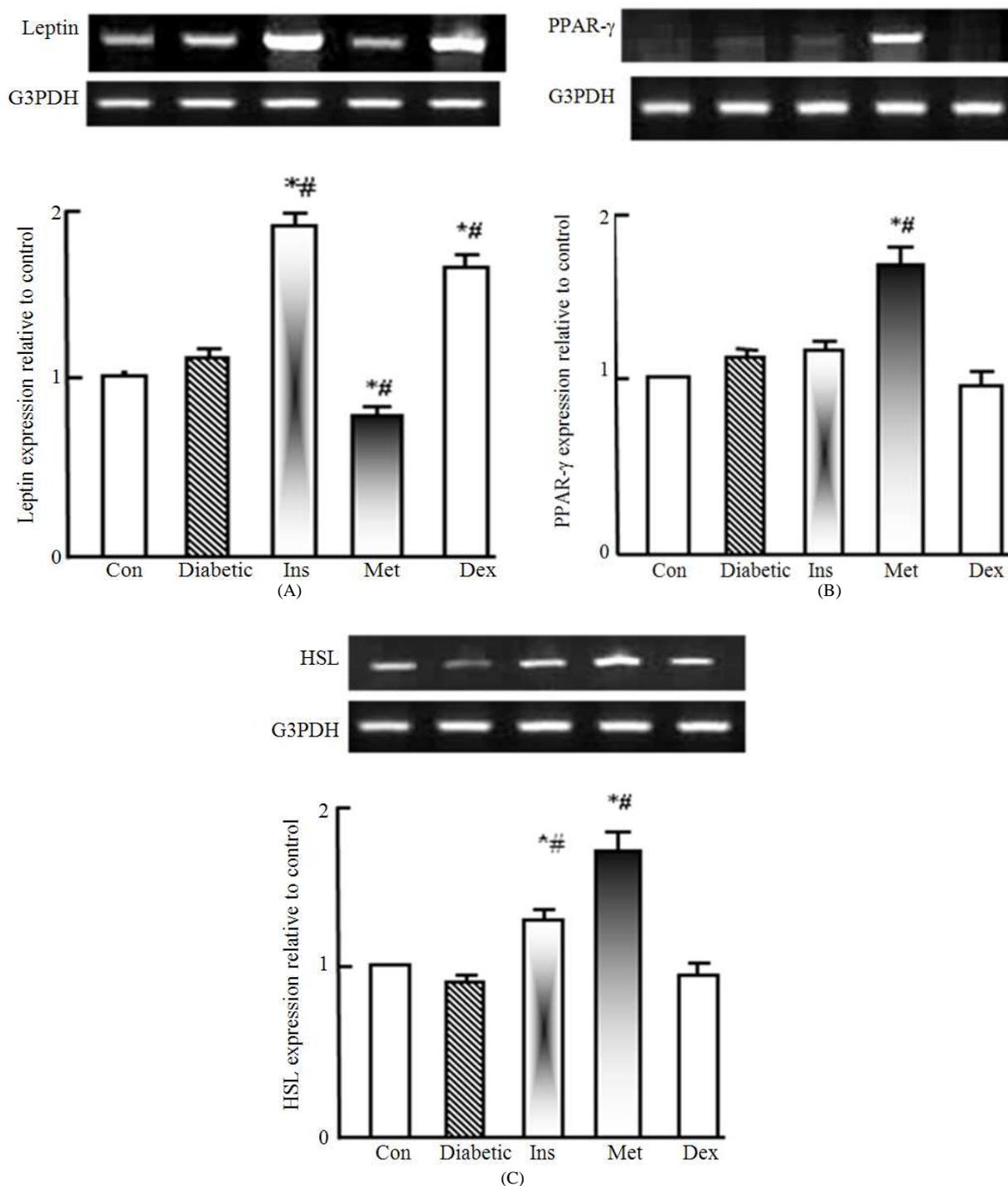
Data are presented as (means ± S.E.). S.E = Standard error for 5 different rats per each group. \*p<0.05 Vs control and #p<0.05 Vs diabetic rats



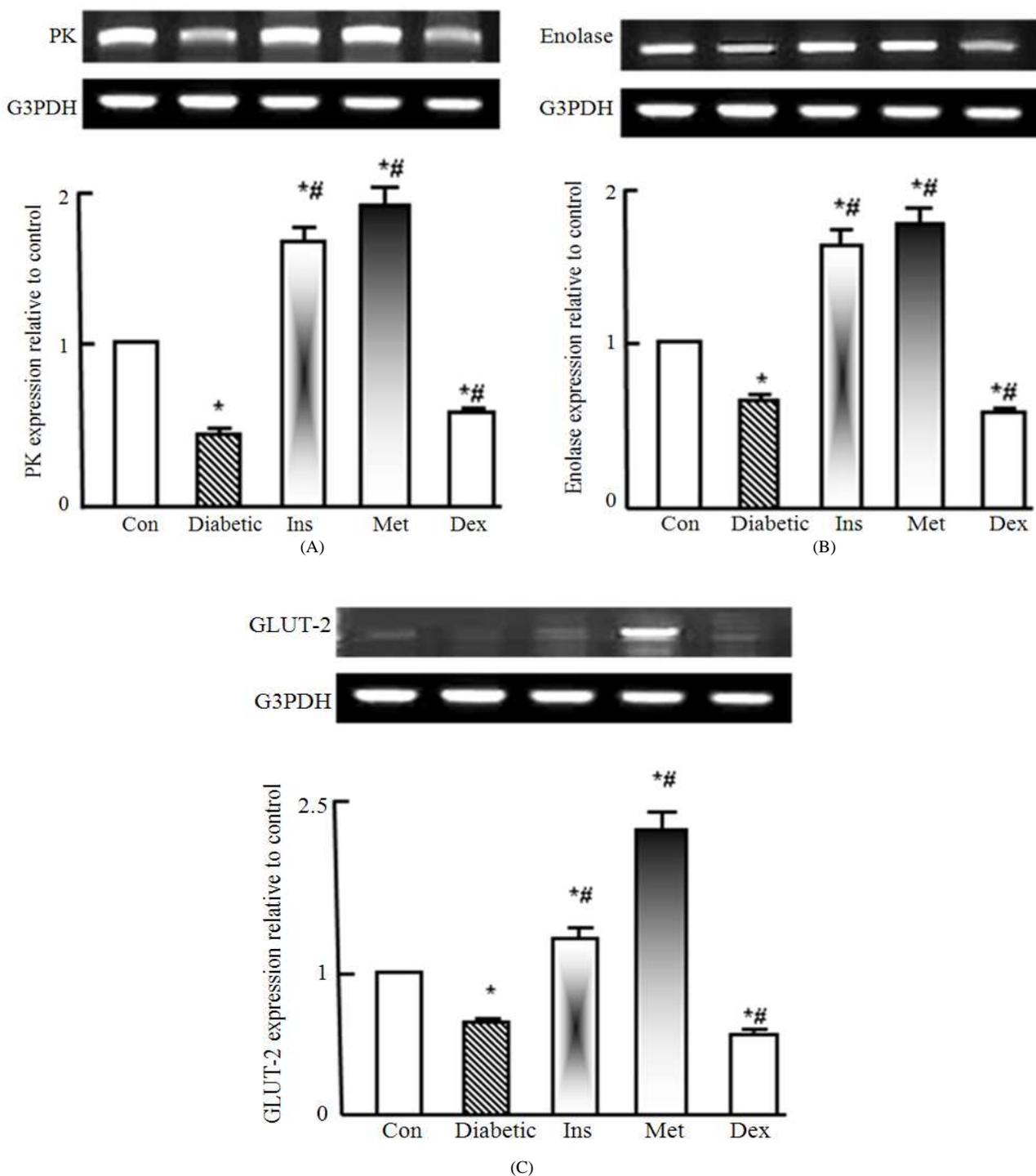
**Fig. 1.** Serum changes in insulin and glucose after administration of insulin, metformin and dexamethasone into type 2 diabetic Wistar rats. Results are expressed as means ± SE for 5 different rats. Insulin and glucose was measured using commercial kits.



**Fig. 2.** RT-PCR analysis of adiponectin, AdipoR-1 and AdipoR-2 expression in epididymal adipose tissue after administration of insulin, metformin and dexamethasone into type 2 diabetic Wistar rats. RNA was extracted and reverse transcribed (1 µg) and RT-PCR analysis was carried out for adiponectin, AdipoR-1 and AdipoR-2 expression as described in materials and methods



**Fig. 3.** RT-PCR analysis of leptin, PPAR- $\gamma$  and HSL expression in epididymal adipose tissue after administration of insulin, metformin and dexamethasone into type 2 diabetic Wistar rats. RNA was extracted and reverse transcribed (1  $\mu$ g) and RT-PCR analysis was carried out for leptin, PPAR- $\gamma$  and HSL expression as described in materials and methods



**Fig. 4.** RT-PCR analysis of PK, enolase and GLUT-2 expression in liver tissue after administration of insulin, metformin and dexamethasone into type 2 diabetic Wistar rats. RNA was extracted and reverse transcribed (1  $\mu$ g) and RT-PCR analysis was carried out for PK, enolase and GLUT-2 expression as described in materials and methods

### 3.4. Effects of Insulin, Metformin and Dexamethasone on Lipolytic Gene Expression in Epididymal Adipose Tissue

In type 2 diabetic rats, the expression of leptin, PPAR- $\gamma$  and HSL were not changed (**Fig. 3**). Insulin administration induced significant increase in leptin and HSL expression without changes in PPAR- $\gamma$ . Metformin induced significant increase in expression of PPAR- $\gamma$  and HSL and a decrease in the expression of leptin (**Fig. 3**). While, dexamethasone induced upregulation in leptin expression without changes in PPAR- $\gamma$  and HSL expression.

### 3.5. Effects of Insulin, Metformin and Dexamethasone on PK, Enolase and GLUT-2 Gene Expression in Liver Tissue

In diabetic rats PK, enolase and Glucose Transporter-2 (GLUT-2) expression was decreased (**Fig. 4**). Insulin administration increased PK and enolase expression in a way to improve insulin resistance. Moreover, metformin administration improved the decrease in glycolysis recorded in diabetic rats and increased the expression of PK and enolase. Dexamethasone inhibited expression of PK and enolase and failed to improve insulin resistance in diabetic rats. As seen in **Fig. 4C**, diabetes inhibited GLUT-2 expression and administration of insulin and metformin up-regulate (1/2 fold for insulin and 1.5 fold for metformin respectively), while, dexamethasone down regulate GLUT-2 expression in liver of diabetic rats.

## 4. DISCUSSION

Results of this study showed the importance of adiponectin in regulation of T2D. Administration of metformin and to less extent insulin improved insulin sensitivity (**Table 2**) and decreased glucose levels in blood (**Fig. 1**). Metformin and/or troglitazone are commonly used in treatment of T2D and mediate their insulin sensitizing actions via distinct metabolic pathways (Ciaraldi *et al.*, 2002). In adipocytes, troglitazone, a member of the thiazolidinediones (TZD), seems to enhance anabolic pathways, leading to an increase in adipocyte size, while metformin appears to stimulate catabolic pathways, which results in a reduced adipocyte size (Ciaraldi *et al.*, 2002). It is possible that both metformin and troglitazone cause differences in production of adiponectin. Adiponectin is reduced in type 2 diabetes (Weyer *et al.*, 2001). Moreover, metformin increased adiponectin gene expression and circulating hormone levels (Phillips *et al.*, 2003). Our

results showed that metformin (PPAR- $\gamma$  agonist) increased adiponectin and its receptors expression in T2D rats. Thus the insulin-sensitizing effect of troglitazone appears to be mediated through an elevation in adiponectin expression (Yu *et al.*, 2002). It was suggested that modulation of adipocytes function including upregulation of adiponectin synthesis and secretion, is an important mechanism by which thiazolidinediones improve insulin action (Phillips *et al.*, 2003; Tiikkainen *et al.*, 2004; Yu *et al.*, 2002). Our findings confirmed that metformin is a more efficient in improving insulin sensitivity and T2D. This is supported by the findings of Bailey and Day (2004) and Fonseca *et al.* (2000).

Hyperglycemia promoted by the high-fat diet with medium dose of STZ was accompanied by a reduction in adiponectin, AdipoR1 and AdipoR2 gene expression (**Fig. 2**) in the epididymal adipose tissue, suggesting the involvement of adiponectin in T2D incidence. Other studies have suggested that increased insulin levels due to a high-fat diet may affect expression of the adiponectin receptors (Bullen *et al.*, 2007; Liu *et al.*, 2007). Our findings supported this idea as insulin decreased adiponectin and AdipoR1 expression in adipose tissue. It is well established that increased insulin levels due to a high-fat diet and medium dose STZ affected the expression of the adiponectin receptors (Bullen *et al.*, 2007; Liu *et al.*, 2007). Insulin represses the expression of both adiponectin receptors in muscle and liver (Liu *et al.*, 2007), that AdipoR1, but not AdipoR2, is repressed by insulin (Inukai *et al.*, 2005). Therefore and possibly because insulin levels were high in T2D than control group, we can assume that the changes in the expression of adiponectin and its receptors is due to the increase in insulin levels.

Insulin resistance induced by glucocorticoids is associated with a decrease and an alteration in adiponectin receptors and consequently is the main cause of hyperglycemia (de Oliveira *et al.*, 2011b) and that are consistent with our findings (**Table 2 and Fig. 2**). Serum adiponectin levels are negatively associated with the BMI in healthy individuals and are decreased in T2D (Margoni *et al.*, 2011). Therefore, adiponectin may be a local regulator for glucose utilization in the adipocytes and adipose tissue via its regulation of PPAR- $\gamma$ , glucose and lipids transcriptional factors expression (Ajuwon and Spurlock, 2005; Yamauchi *et al.*, 2001).

Unlike adiponectin, metformin inhibited leptin expression while insulin up-regulate it. Therefore, metformin suppress leptin secretion by a selective molecular mechanism that may contribute to the

anorexigenic effect of metformin in diabetic patients (Klein *et al.*, 2004). So, probably the increase in adiponectin expression is coincided with an increase in PPAR- $\gamma$  and HSL expression to stimulate lipolysis.

Adiponectin is well documented to reduce plasma concentration of fatty acids and triglycerides in mice models of obesity and hyperlipidemia (Yamauchi *et al.*, 2001). The effect is mediated by acceleration of fatty acid oxidation in muscle cells, which leads to decrease in cellular triglyceride content (Fruebis *et al.*, 2001). In skeletal muscle, adiponectin increases expression of the proteins involved in fatty acid metabolism, such as acyl-CoA oxidase and Uncoupling Protein-2 (UCP-2) and increases body temperature, suggesting a stimulatory effect on energy expenditure (Fruebis *et al.*, 2001). Adiponectin has no direct effect on adipose tissue hormone-sensitive lipase (Combs *et al.*, 2001; Fruebis *et al.*, 2001), indicating that reduction of plasma fatty acids results from accelerated tissue uptake rather than inhibition of lipolysis, while metformin has the stimulatory action on lipolysis and its related gene expression (Klein *et al.*, 2004).

Glucocorticoids secretion from adrenal gland is primarily controlled by the hypothalamic-pituitary-adrenal axis. Glucocorticoids act in a variety of physiological processes, including the immune response, the regulation of cardiovascular activity, brain function, glucose and lipid metabolism, insulin secretion and contribute to the development of obesity and insulin resistance (Ruzzin *et al.*, 2005). Glucocorticoids have been demonstrated to cause insulin resistance *in vivo* (Andrews and Walker, 1999) and are negatively regulate adiponectin expression (Halleux *et al.*, 2001). Treatment with dexamethasone caused a decrease in adiponectin levels in serum of adrenalectomized rats, which may be related to the effects of glucocorticoids in promoting insulin resistance (de Oliveira *et al.*, 2011b). Here, dexamethasone potentially inhibited gene expression of adiponectin and AdipoR2 in adipose tissue. Therefore, adiponectin down-regulation is the mechanism by which glucocorticoids impair insulin sensitivity (Fasshauer *et al.*, 2002) and more studies are needed to outline this alteration. However, Jang *et al.* (2008) found that dexamethasone inhibited AdipoR2 mRNA expression in non-diabetic subjects and that support our hypothesis.

Glucose influx is inhibited in the absence of insulin and recovered on insulin treatment (Vats *et al.*, 2004). Our findings showed that administration of insulin and metformin but not dexamethasone to type 2 diabetic rats induced a significant increase in PK and enolase mRNA expression, a key enzymes in regulation of glycolysis. The PK activity decreases due to diabetes and increases

by the administration of insulin to diabetic rats in the liver tissues (Yamada and Noguchi, 1999). The increase in activity of PK in the liver tissue of rats is the cause of increased glycolysis and decreased gluconeogenesis as indicated by PEPCK mRNA expression (Taylor and Agius, 1988). Moreover, GLUT-2 expression is increased after insulin and metformin administration. GLUT-2 is trans membrane carrier protein, that enables passive transport of glucose across cell membrane. GLUT-2 regulates passage of glucose between liver and blood and it is responsible for renal glucose reabsorption (Freitas *et al.*, 2005). Therefore, metformin up-regulate GLUT-2 expression to increase glucose metabolism in liver through up-regulation of adiponectin expression in adipose tissue.

## 5. CONCLUSION

In conclusion, our findings clarify that some regulators such as metformin regulated adiponectin expression to treat type 2 diabetic rats possibly at the transcriptional levels of some lipid and glucose metabolism.

## 6. ACKNOWLEDGMENT

This study was supported by a grant in aid of The Deans of Scientific Affairs, Taif University, Kingdom of Saudi Arabia (Research project number 2220-1-1434).

## 7. REFERENCES

- Ajuwon, M.K. and M.E. Spurlock, 2005. Adiponectin inhibits LPS-induced NF- $\kappa$ B activation and IL-6 production and increases PPAR- $\gamma$ 2 expression in adipocytes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.*, 288: R1220-R1225. PMID: 15604306
- Andrews, R.C. and B.R. Walker, 1999. Glucocorticoids and insulin resistance: Old hormones, new targets. *Clin. Sci. (Lond)*, 96: 513-23. PMID: 10209084
- Ashcroft, F.M. and P. Rorsman, 2012. Diabetes mellitus and the beta cell: The last ten years. *Cell*, 148: 1160-1171. PMID: 22424227
- Bailey, C.J. and C. Day, 2004. Avandamet: Combined metformin-rosiglitazone treatment for insulin resistance in type 2 diabetes. *Int. J. Clin. Pract.*, 58: 867-876. PMID: 15529521
- Beltowski, J., 2003. Adiponectin and resistin-new hormones of white adipose tissue. *Med. Sci. Monit.*, 9: RA55-RA61. PMID: 12601307

- Besse, C., N. Nicod and L. Tappy, 2005. Changes in insulin secretion and glucose metabolism induced by dexamethasone in lean and obese females. *Obes. Res.*, 13: 306-311. PMID: 15800288
- Beylot, M., C. Pinteur and O. Peroni, 2006. Expression of the adiponectin receptors AdipoR1 and AdipoR2 in lean rats and in obese Zucker rats. *Metabolism*, 55: 396-401. PMID: 16483885
- Birkenfeld, A.L., M. Boschmann, S. Engeli, C. Moro and A.M. Arafat *et al.*, 2012. Atrial natriuretic peptide and adiponectin interactions in man. *PLoS One*, 7: e43238-e43238. PMID: 22916229
- Bodles, A.M., A. Banga, N. Rasouli, F. Ono and P.A. Kern *et al.*, 2006. Pioglitazone increases secretion of high-molecular-weight adiponectin from adipocytes. *Am. J. Physiol. Endocrinol. Metab.*, 291: E1100-1105. PMID: 16803857
- Brun, J.M., A.S. Lihn, C. Verdich, S.B. Pedersen and S. Toubro *et al.*, 2003. Regulation of adiponectin by adipose tissue-derived cytokines: *In vivo* and *in vitro* investigations in humans. *Am. J. Physiol. Endocrinol. Metab.*, 285: E527-E533. PMID: 12736161
- Bullen, J.W. S. Blucher, T. Kelesidis and C.S. Mantzoros, 2007. Regulation of adiponectin and its receptors in response to development of diet-induced obesity in mice. *Am. J. Physiol. Endocrinol. Metab.*, 292: 1079-1086. PMID: 17164441
- Capeau, J., 2007. The story of adiponectin and its receptors AdipoR1 and R2: To follow. *J. Hepatol.*, 47: 736-738. PMID: 17599632
- Ciaraldi, T.P., A.P. Kong, N.V. Chu, D.D. Kim and S. Baxi *et al.*, 2002. Regulation of glucose transport and insulin signaling by troglitazone or metformin in adipose tissue of type 2 diabetic subjects. *Diabetes*, 51: 30-36. PMID: 11756319
- Combs, T.P., A.H. Berg, S. Obici, P.E. Scherer and L. Rossetti, 2001. Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. *J. Clin. Invest.*, 108: 1875-1881. PMID: 11748271
- Cornier, M.A., D. Dabelea, T.L., Hernandez, R.C. Lindstrom and A.J. Steig *et al.*, 2008. The metabolic syndrome. *Endocr. Rev.*, 29: 777-822. PMID: 18971485
- de Oliveira, C., A.B. de Mattos, C. Biz, L.M. Oyama and E.B. Ribeiro *et al.*, 2011a. High-fat diet and glucocorticoid treatment cause hyperglycemia associated with adiponectin receptor alterations. *Lipids Health Disease.*, 1-14. PMID: 21244702
- de Oliveira, C., C. Iwanaga-Carvalho, J.F. Mota, L.M. Oyama and E.B. Ribeiro *et al.*, 2011b. Effects of adrenal hormones on the expression of adiponectin and adiponectin receptors in adipose tissue, muscle and liver. *Steroids*, 76: 1260-1267. PMID: 21745490
- Diez, J.J. and P. Iglesias, 2003. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur. J. Endocrinol.*, 148: 293-300. PMID: 12611609
- Fasshauer, M., J. Klein, S. Neumann, M. Eszlinger and R. Paschke, 2002. Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. *Biochem. and Biophys. Res. Commun.*, 290: 1084-1089. PMID: 11798186
- Fasshauer, M., J. Klein, S. Kralisch, M. Klier and U. Lossner *et al.*, 2004. Growth hormone is a positive regulator of adiponectin receptor 2 in 3T3-L1 adipocytes. *FEBS Lett.*, 558: 27-32. PMID: 14759511
- Fasshauer, M., J. Klein, S. Neumann, M. Eszlinger and R. Paschke, 2001. Adiponectin gene expression is inhibited by beta-adrenergic stimulation via protein kinase A in 3T3-L1 adipocytes. *FEBS Lett.*, 507: 142-6. PMID: 11684087
- Fonseca, V., J. Rosenstock, R. Patwardhan and A. Salzman, 2000. Effect of metformin and rosiglitazone combination therapy in patients with type 2 diabetes mellitus: A randomized controlled trial. *JAMA*, 283: 1695-1702. PMID: 10755495
- Freitas, H.S., B.D. Schaan, P.M. Seraphim, M.T. Nunes and U.F. Machado, 2005. Acute and short-term insulin-induced molecular adaptations of GLUT2 gene expression in the renal cortex of diabetic rats. *Mol. Cell. Endocrinol.*, 237: 49-57. PMID: 15869838
- Friedman, J.M., 2000. Obesity in the new millennium. *Nature*, 404: 632-634. PMID: 10766249
- Fruebis, J., T.S. Tsao, S. Javorschi, D. Ebbets-Reed and M.R.S. Erickson *et al.*, 2001. 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. USA.*, 98: 2005-2010. PMID: 11172066
- Fu, L., K. Isobe, Q. Zeng, K. Suzukawa and K. Takekoshi *et al.*, 2007. Beta adrenoceptor agonists down regulate adiponectin, but upregulate adiponectin receptor 2 and tumor necrosis factor- $\alpha$  expression in adipocytes. *Eur. J. Pharmacol.*, 569: 155-162. PMID: 17574233

- Gerich, J.E., 1998. The genetic basis of type 2 diabetes mellitus: Impaired insulin secretion versus impaired insulin sensitivity. *Endocr. Rev.*, 19: 491-503. PMID: 9715377
- Goldstein, B.J. and R. Scalia, 2004. Adiponectin: A novel adipokine linking adipocytes and vascular function. *J. Clin. Endocrinol. Metab.*, 89: 2563-2568. PMID: 15181024
- Halleux, C.M., M. Takahashi, M.L. Delporte, R. Detry and T. Funahashi *et al.*, 2001. Secretion of adiponectin and regulation of apM1 gene expression in human visceral adipose tissue. *Biochem. Biophys. Res. Commun.*, 288: 1102-1107. PMID: 11700024
- Hotamisligil, G.S., 1999. The role of TNF and TNF receptors in obesity and insulin resistance. *J. Intern. Med.*, 245: 621-625. PMID: 10395191
- Hundal, R.S., M. Krssak, S. Dufour, D. Laurent and V. Lebon *et al.*, 2000. Mechanism by which metformin reduces glucose production in type 2 diabetes. *Diabetes*, 49: 2063-2069. PMID: 11118008
- Inukai, K., Y. Nakashima, M. Watanabe, N. Takata and T. Sawa *et al.*, 2005. Regulation of adiponectin receptor gene expression in diabetic mice. *Am. J. Physiol. Endocrinol. Metab.*, 288: 876-882. PMID: 15613685
- Jang, C., W.J. Inder, V.R. Obeyesekere and F.P. Alford, 2008. Adiponectin, skeletal muscle adiponectin receptor expression and insulin resistance following dexamethasone. *Clin. Endocrinol. (Oxf.)*, 69: 745-50. PMID: 18363889
- Kahn, B.B. and J.S. Flier, 2000. Obesity and insulin resistance. *J. Clin. Invest.*, 106: 473-481. PMID: 10953022
- Klein, J., S. Westphal, D. Kraus, B. Meier and N. Perwitz *et al.*, 2004. Metformin inhibits leptin secretion via a mitogen-activated protein kinase signalling pathway in brown adipocytes. *J. Endocrinol.*, 183: 299-307. PMID: 15531718
- Kloting, N., M. Fasshauer, A. Dietrich, P. Kovacs and M.R. Schon *et al.*, 2010. Insulin-sensitive obesity. *Am. J. Physiol. Endocrinol. Metab.*, 299: 506-515. PMID: 20570822
- Kwon, H. and J.E. Pessin, 2013. Adipokines mediate inflammation and insulin resistance. *Frontiers Endocrinol.*, 4: 1-11. PMID: 23781214
- Liu, Y., M.D. Michael, S. Kash, W.R. Bensch and B.P. Monia *et al.*, 2007. Deficiency of adiponectin receptor 2 reduces diet-induced insulin resistance but promotes type 2 diabetes. *Endocrinology*, 148: 683-692. PMID: 17068142
- Margoni, A., D.N. Perrea., I. Vlachos, G Prokopaki and A. Pantopoulou *et al.*, 2011. Serum leptin, adiponectin and tumor necrosis factor- $\alpha$  in hyperlipidemic rats with/without concomitant diabetes mellitus. *Mol. Med.*, 17: 36-40. PMID: 20838752
- Phillips, S.A., T.P. Ciaraldi, A.P.S. Kong, R. Bandukwala and V. Aroda *et al.*, 2003. Modulation of circulating and adipose tissue adiponectin levels by antidiabetic therapy. *Diabetes*, 52: 667-674. PMID: 12606507
- Ruzzin, J., A.S. Wagman and J. Jensen, 2005. Glucocorticoid-induced insulin resistance in skeletal muscles: Defects in insulin signalling and the effects of a selective glycogen synthase kinase-3 inhibitor. *Diabetologia*, 48: 2119-2130. PMID: 16078016
- Satoh, H., S. Fujii and H. Tsutsui, 2010. The High Molecular Weight (HMW) form of adiponectin is a significant surrogate marker for the prediction of type 2 diabetes mellitus in the Japanese population. *Nutr. Metab. Cardiovasc. Dis.*, 20: e9-10. PMID: 19695856
- Scherer, P.E., S. Williams, M. Fogliano, G. Baldini and H.F. Lodish, 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J. Biol. Chem.*, 270: 26746-26749. PMID: 7592907
- Shi, J.H., W.H. Du, X.Y. Liu, Y.P. Fan and X.L. Hu *et al.*, 2010. Glucocorticoids decrease serum adiponectin level and WAT adiponectin mRNA expression in rats. *Steroids*, 75: 853-858. PMID: 20471407
- Shimomura, I., T. Funahashi, M. Takahashi, K. Maeda and K. Kotani *et al.*, 1996. Enhanced expression of PAI-1 in visceral fat: Possible contributor to vascular disease in obesity. *Nat. Med.*, 2: 800-803. PMID: 8673927
- Shulman, G.I., 2000. Cellular mechanisms of insulin resistance. *J. Clin. Invest.*, 106: 171-176. PMID: 10903330
- Soliman, M.M., T.A. Ismail, H.F. Attia and O.A. Ragab, 2011. Bovine adiponectin, Genetic expression and regulation in bovine adipocyte. *Seria Med. Vet.*, 54: 79-91.
- Srinivasan, K., B. Viswanad, L. Asrat, C.L. Kaul and P. Ramarao, 2005. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol. Res.*, 52: 313-320. PMID: 15979893
- Steppan, C.M., S.T. Bailey, S. Bhat, E.J. Brown and R.R. Banerjee *et al.*, 2001. The hormone resistin links obesity to diabetes. *Nature*, 409: 307-312. PMID: 11201732

- Taylor, R. and L. Agius, 1988. The biochemistry of diabetes. *Biochem. J.*, 250: 625-640. PMID: 3291853
- Tiikkainen, M., A.M. Hakkinen, E. Korshennikova, T. Nyman and S. Makimattila *et al.*, 2004. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance and gene expression in adipose tissue in patients with type 2 diabetes. *Diabetes*, 53: 2169-2176. PMID: 15277403
- Vats, V., S.P. Yadav and J.K. Grover, 2004. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. *J. Ethnopharmacol.*, 90: 155-160. PMID: 14698524
- Weyer, C., T. Funahashi, S. Tanaka, K. Hotta and Y. Matsuzawa *et al.*, 2001. Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. *J. Clin. Endocrinol. Metab.*, 86: 1930-1935. PMID: 11344187
- Winter, W., J. Dejongh, T. Post, B. Ploeger and R. Urquhart *et al.*, 2006. A mechanism-based disease progression model for comparison of long-term effects of pioglitazone, metformin and gliclazide on disease processes underlying Type 2 Diabetes Mellitus. *J. Pharmacokinet. Pharmacodyn.*, 33: 313-343. PMID: 16552630
- Xu, A., L.C. Wong, Y. Wang, J.Y. Xu and G.J. Cooper *et al.*, 2004. Chronic treatment with growth hormone stimulates adiponectin gene expression in 3T3-L1 adipocytes. *FEBS Lett.*, 572: 129-134. PMID: 15304336
- Yamada, K. and T. Noguchi, 1999. Nutrient and hormonal regulation of pyruvate kinase gene expression. *Biochem. J.*, 337: 1-11. PMID: 9854017
- Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi and N. Kubota *et al.*, 2001. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat. Med.*, 7: 941-946. PMID: 11479627
- Yamauchi, T., J. Kamon, Y. Ito, A. Tsuchida and T. Yokomizo *et al.*, 2003. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, 423: 762-769. PMID: 12802337
- Yu, J.G., S. Javorschi., A.L. Hevener, Y.T. Kruszynska and R.A. Norman *et al.*, 2002. The effect of thiazolidinediones on plasma adiponectin levels in normal, obese and type 2 diabetic subjects. *Diabetes*, 51: 2968-2974. PMID: 12351435