

# Effects of Statins on Insulin-Like Growth Factor-1 Gene Expression in Fructose Induced Metabolic Syndrome in Rats

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**Abstract:** *Background and Purpose:* Insulin-like growth factor-1 (IGF-1) was found to have a role in both glucose homeostasis and cardiovascular disease. The present study was designed to compare the effects of fluvastatin and metformin on IGF-1 mRNA expression within the liver and on other individual components of the metabolic syndrome induced in rats by high fructose feeding.

*Experimental Approach:* Rats fed 60% fructose in diet for 6 weeks were treated daily with fluvastatin (3.75mg/kg/day) or metformin (200mg/kg/day) during the last 2 weeks and compared with untreated fructose fed group. Fasting levels of plasma cholesterol, triglyceride, glucose, insulin, nitric oxide products, IGF-1 and IGF-1 mRNA within the liver as well as systolic blood pressure and body weight were determined.

*Results:* Compared to control rats, the fructose fed group developed hypertension, hyperlipidemia, hyperinsulinemia, hyperglycemia and endothelial dysfunction as well as decreased levels of plasma IGF-1 and its mRNA within the liver. Fructose fed rats treated with fluvastatin or metformin for 2 weeks showed significant decrease in plasma cholesterol, triglyceride, insulin and glucose levels compared to untreated fructose fed group. Also, both drugs increased significantly plasma levels of nitric oxide products and IGF-1 together with significant increase in IGF-1 mRNA within the liver. However, only metformin treated rats showed significant decrease in systolic blood pressure compared to fructose fed group.

*Conclusions:* This study showed that in a rat model of insulin resistance, fluvastatin improves the metabolic profile and increases plasma level of IGF-1 and its gene expression as effective as metformin.

**Keywords:** IGF-1, metabolic syndrome, statins, metformin, fructose fed rats.

## INTRODUCTION

The metabolic syndrome or the insulin resistance syndrome is associated with increased morbidity and mortality for cardiovascular disease [1]. It represents a clustering of metabolic disorders and cardiovascular risk factors including: insulin resistance, hyperinsulinemia, central obesity, glucose intolerance or diabetes mellitus, atherogenic dyslipidemia, hypertension, prothrombotic and proinflammatory states and endothelial dysfunction [2].

Increasing evidence indicates that IGF-1 protects against endothelial dysfunction, atherosclerotic plaque development, the metabolic syndrome, clinical instability and ischemic myocardial damage [3]. Low plasma IGF-1 concentrations are independently related to impaired insulin sensitivity and other components of the metabolic syndrome as glucose intolerance, diabetes, abdominal obesity and atherogenic dyslipidemia [4]. In addition, subjects with angiographically documented coronary artery disease have low circulating IGF-1 concentrations [5]. It is possible that low IGF-1 levels may increase cardiovascular risk by affecting insulin

sensitivity and thus could be part of the polymetabolic syndrome [6].

Pharmacological therapy is a critical step in the management of patients with metabolic syndrome when lifestyle modifications fail to achieve the therapeutic goals. Other than weight loss, there is no single best therapy and treatment should consist of treatment of individual components of the syndrome [7]. It is also important to know whether drugs used in treatment, have a secondary effect, either favorable or unfavorable, on insulin resistance. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-COA) reductase inhibitors (statins) have beneficial effects in terms of cardiovascular morbidity and mortality. Statins have multiple pleiotropic actions independent of their classical effects on serum lipids [8]. Yet, there are controversies reports regarding the effects of statins on insulin resistance. Some reports indicate that statins worsen the insulin action or have no effect on the plasma insulin level [9]. Other reports indicate that statins improve insulin sensitivity [10, 11].

The present study was designed to evaluate the effects of fluvastatin on IGF-1 mRNA expression in liver tissues and on the other metabolic profiles in fructose induced insulin resistance in rats. Also, the effects of fluvastatin were

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compared with metformin, a well known insulin sensitizing agent. The fructose fed rat is used as an animal model of insulin resistance and is considered to parallel multiple metabolic syndrome (syndrome x) observed in humans [12].

## MATERIALS AND METHODS

### Experimental Animals

A total of 40 healthy male albino rats (aged 6 weeks) weighing 200-250 gram was used in this study. The animals were housed in regular cages situated in an animal room at constant temperature (22°C) and humidity with a 14 hour/10 hour light dark cycle. Ten rats were fed a standard rat chow diet for 6 weeks (Standard rat chow containing 60% vegetable starch, 5% fat and 18% protein). A total of 30 rats were fed fructose enriched diet (FED) consisting of 60% fructose, 5% fat and 18% protein of diet weight for 6 weeks. During the last 2 weeks 20 animals fed with FED were given either fluvastatin or metformin. All rats were given tap water to drink ad libitum. All animal studies were conducted according to the regulations for the use and care of experimental animals.

### Drugs and Chemicals

Fluvastatin (Novartis, Egypt) and Metformin (Minapharm, Egypt) were supplied as white powder, soluble in water freshly prepared as aqueous solution to be given as a single daily oral dose of 3.75 mg/kg/day [13] and 200mg/kg/day [14] respectively. Fructose was purchased from El Gomherya Company, Giza, Egypt.

### Experimental Design

The animals were divided into four groups (10 rats each):

Group I: Control group (C) received standard rat chow diet. Group II: Fructose fed group (FFG) received FED for 6 weeks. Group III: Fluvastatin treated fructose fed group (FFG+F) fed with FED for 6 weeks with fluvastatin treatment (3.75mg/kg/day) given orally during the last 2 weeks. Group IV: Metformin treated fructose fed group (FFG+M) fed with FED for 6 weeks with metformin treatment (200mg/kg/day) given orally during the last 2 weeks.

Blood samples were taken from the retroorbital sinus under anesthesia after an overnight fast (10 h) in collecting heparinized capillary tubes. The samples were centrifuged at 1000 Xg and plasma was split and frozen in polypropylene tubes at -20°C until assayed.

### Plasma Analysis

Plasma glucose, triglycerides and cholesterol were measured calorimetrically. Plasma insulin level was

measured by radioimmunoassay (Behringer Mannheim, Marburg, Germany). Nitric oxide products (nitrates and nitrites) levels were determined [15]. Plasma IGF-1 levels were determined by radioimmunoassay (RIA) (BioSource, Nivelles, Belgium, Europe) after separation of IGFs from IGF-binding proteins by acid-ethanol extraction.

### Gene Expression of IGF-1 by RT-PCR

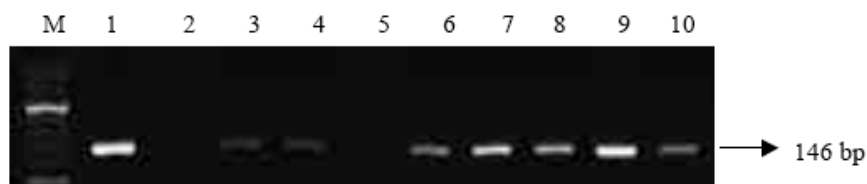
Total RNA from the liver tissues was extracted by the acid guanidinium thiocyanate-phenol chloroform method. The extracted RNA was quantitated by spectrophotometer at 260 nm. The sequence of the primers used to amplify IGF-1 gene is as follows: IGF-1-F: GTGTGGACCGAGGGGCTT TTAATTC and IGF-1-R: GCTTCAGTGGGGCACAGTACATCTC. RT-PCR was done using the extracted RNA for detection of the expression of IGF-1 genes. Reverse transcription was done using the RT-PCR kit (Promega, Madison, WI). Total RNA (1µg), 6 µl dNTPs (10 mM each), 6 µl 5X buffer and 30 pmol specific downstream primer were brought up to a total volume of 28 µl. The mixture was heat shocked for 5 min. at 65°C and chilled on ice for 5 min. Then, 0.5µ RNasin (40U/µL) and 1µL AMV (10U/µL) were added and the mixture was incubated at 42°C for 2 hours. Then, 5 µL of cDNA was added to the following PCR mixture: 200 µmol deoxynucleoside triphosphates, 2.5 U Taq polymerase (Quiagen Taq Polymerase 250U), and 3.3 mM MgCl<sub>2</sub> in a final volume of 30 µl. The reaction mixture was placed in a Hybaid Express thermal cycler for 3 minutes at 95°C, and then subjected for 32 cycles of 95°C for 1.5 minutes, 55°C for 1 minute, and 72°C for 1.5 minutes. This was followed by a final step at 72°C for 10 minutes. Fifteen µl of the PCR products were applied to the specified wells into an ethidium bromide-stained 2% agarose gel in known order. 100 base pair DNA ladder was used. The PCR products were semiquantitated using the gel documentation system (Bio Doc Analyze) supplied by Biometra (Fig. 1).

### Measurement of Systolic Blood Pressure (SBP)

The SBP was measured in conscious rats at baseline, after 4 and 6 weeks by the indirect tail cuff method (Harvard Rat Tail Blood Pressure Monitor System). The animals were kept at 37°C for 30 minutes before measurements. The average of five consecutive readings was used for SBP evaluation.

### Statistical Analysis

Data were entered using the statistical package SPSS version 11. Data were summarized using mean and standard deviation for the quantitative variable. Comparisons between groups were done using ANOVA (analysis of variance) followed by post hoc test for multiple comparisons between



**Fig. (1).** Agarose gel electrophoresis 2% stained with ethidium bromide showing PCR products of IGF-1 in rat livers of different groups at 146 bp. M: molecular DNA marker (50 bp each). Lane 1: control liver. Lanes 2, 5 negative control. Lanes 3, 4: FFG (6 w). Lanes 6, 7: FFG +F. Lanes 8, 9: FFG+M. Lane 10: FFG (4w).

**Table 1. Body Weight and Biochemical Parameters of the Different Experimental Groups**

Parameters	Group I C	GroupII FFG(6 w)	Group III FFG+F	GroupIV FFG +M
Total cholesterol (mg/dl)	60.2±6.4	81.8±8.8*	67.6±4.7**	71.7±7.9**
Triglyceride(mg/dl)	77.3±8.3	142.5±27.9*	84.3±14.3**	118.8±26.2**
Nitric oxide products(umol/ ml)	21.8±1.8	6.6±1.6*	23.0±3.8**	23.7±2.66**
Glucose (mg/dl)	133.8±7.0	169.3±7.8*	121.4±8.2**	119.6±13.3**
Insulin (uU/ml)	22.1±4.2	42.7±13.6*	31.1±11.2**	32.2±10.7**
IGF-1 (ng/ml)	741.0±69.8	603.0±93.5*	733.7±59.3**	727.6±50.8**
IGF-1 gene(ug/mg tissue)	900.4±85.7	406.5±124.1*	772.9±83.7**	864.9±79.7**
Body weight (g)	236.8±5.9	238.2±12.9	236.8±7.2	238.8±10.3

Values are means ± SD (n = 10 each group).

\*p < 0.05 compared with control group.

\*\* p < 0.05 compared with FFG. C=control group; FFG=fructose fed group; FFG+F= fluvastatin treated fructose fed group; FFG+M= metformin treated fructose fed group.

each 2 groups. The results were considered significant when  $p < 0.05$ .

## RESULTS

The values of the plasma cholesterol, triglyceride, nitric oxide products, glucose, insulin, IGF-1 and IGF-1 mRNA within the liver as well as body weight at baseline and after 6 weeks in all the studied groups, were illustrated in Table 1.

### Effects of FED

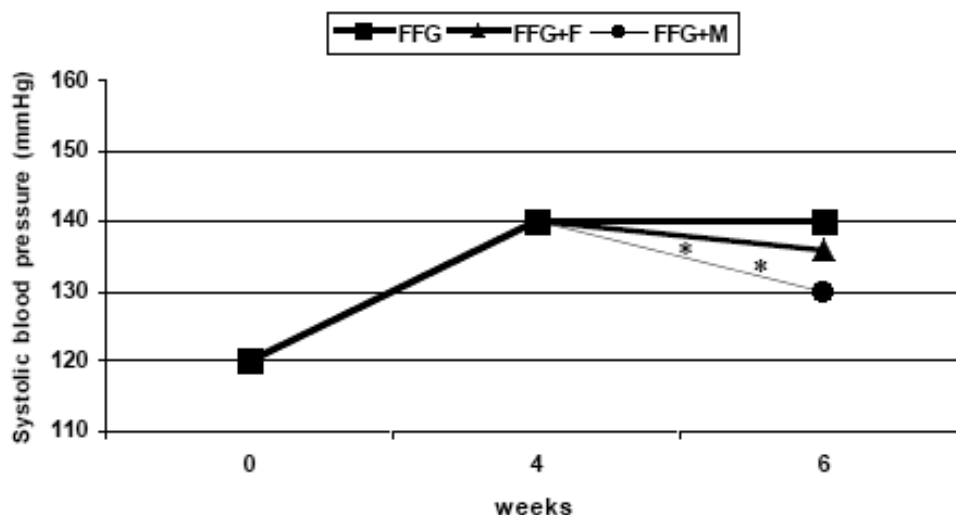
In the present study, there was insignificant change in body weight in fructose fed group throughout the experimental period compared with control group (Table 1). However, the group on FED showed significant increase in SBP after 4 weeks and 6 weeks compared to control (122±5.19 mmHg). This increase was 16% and 17% respectively above the control value ( $p < 0.05$ , Fig. 2). There was significant increase in plasma levels of cholesterol, triglyceride, glucose and insulin after 6 weeks in rats fed FED compared to control rats. This increase was 35%, 84%, 26% and 173% respectively ( $p < 0.001$ ) above the control

value (Table 1). Administration of FED for 6 weeks caused significant decrease in plasma nitric oxide products level by 69% compared to control group ( $p < 0.001$ ; Table 1). As shown in Fig. (3), IGF-1 mRNA expression determined by RT-PCR in the liver tissues from fructose fed rats was significantly decreased after 6 weeks compared to control rats. The decrease was by 54% below control value ( $p < 0.001$ ). This was accompanied by significant decrease in plasma IGF-1 level by 18% ( $p < 0.01$ ) in fructose fed rats compared to control group (Fig. 4).

### Effects of Pharmacologic Intervention

#### Effects of Fluvastatin

In the present study, fluvastatin treated fructose fed rats showed significant reduction in plasma cholesterol (by 17%), triglycerides (by 40%), glucose (by 28%) and insulin levels (by 45%) compared to untreated fructose fed group ( $p < 0.001$ , Table 1). Fluvastatin increased significantly nitric oxide products level (by 248%,  $p < 0.001$ ) compared to untreated fructose fed rats (Table 1). As shown in Fig. (3), IGF-1 mRNA expression within the liver was significantly



**Fig. (2).** Systolic blood pressure in different experimental groups. \* P < 0.05 compared to FFG.

increased by 90% ( $p < 0.001$ ) with significant increase in plasma IGF-1 level by 21% ( $p < 0.01$ , Fig. 4) in fluvastatin treated rats compared to untreated fructose fed group. However, fluvastatin produced insignificant reduction in SBP in fructose fed rats ( $p > 0.05$ , Fig. 2).

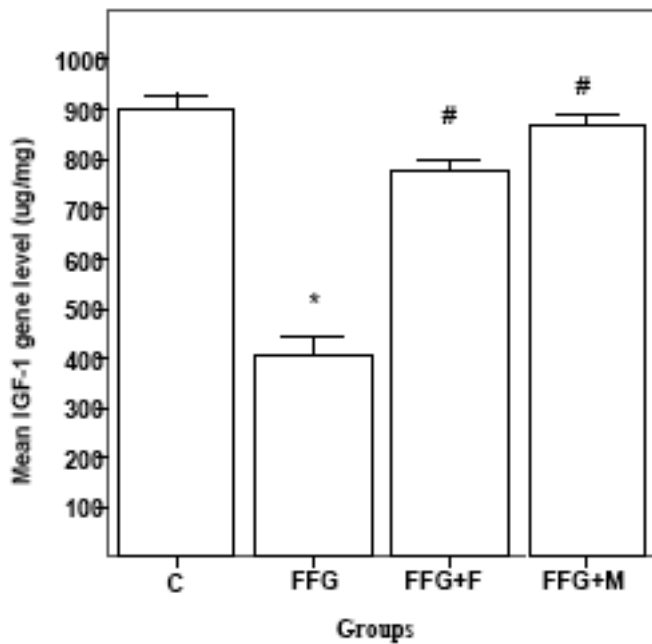


Fig. (3). Comparison of IGF-1 gene levels between different groups. \* $p < 0.001$  compared with control group. # $p < 0.001$  compared with fructose fed. Group. Values are means  $\pm$  SE.

#### Effects of Metformin

Metformin treatment improved all the disorders caused by high fructose diet. Compared with fructose group, the plasma cholesterol, triglyceride, glucose and insulin levels were significantly lower in metformin treated fructose fed rats. This decrease was by 12% ( $p < 0.05$ ), 16% ( $p < 0.05$ ), 29% ( $p < 0.001$ ) and 45% ( $p < 0.001$ ) respectively (Table 1). There was significant increase in plasma nitric oxide products level in metformin treated rats by 259% ( $p < 0.001$ ) compared with untreated fructose fed rats (Table 1). Metformin administration caused significant increase in plasma IGF-1 by 20% ( $p < 0.01$ , Fig. 4) and IGF-1 mRNA expression in liver tissues by 112% ( $p < 0.001$ , Fig. 3) compared to fructose fed group. There was also significant reduction in SBP by 7% ( $p < 0.05$ ) in metformin treated rats compared to untreated fructose fed rats (Fig. 2).

#### DISCUSSION

The results of the present work resemble those previously reported by Ackerman *et al.* [16] in which rats fed 60% fructose in diet are a suitable model for non obese rats with some aspects of the metabolic syndrome such as hypertension, hyperinsulinemia and hypertriglyceridemia.. The reduced IGF-1 levels observed in the present work were explained by Robins [17] in which he reported that reduced IGF-1 levels are independently associated with glucose intolerance, diabetes, abdominal obesity and atherogenic dyslipidemia.

In the present work, fructose fed rats treated with fluvastatin (3.75 mg/kg/day) for 2 weeks showed significant

reduction of both plasma cholesterol and triglyceride levels by 17% and 40% respectively with significant elevation in plasma nitric oxide level by 248%, compared to values obtained from untreated fructose fed group. In accordance with the results of the present work Jokubaitis *et al.* [18] compared the effects of fluvastatin 20 mg and 40 mg in patients with non insulin dependent diabetes mellitus and hyperlipidemia for 6 weeks and they found that both dosages resulted in a significant reduction of LDL and VLDL cholesterol and triglyceride levels compared with placebo with significant elevation of high density lipoprotein (HDL) cholesterol. Bevilacqua *et al.* [19] studied the effects of fluvastatin extended release 80 mg for 2 months in patients with type 2 diabetes with the lipid triad (increase small dense LDL, decrease HDL, hypertriglyceridemia) and they found that after fluvastatin therapy, LDL, apolipoprotein B, intermediate density LDL, small dense LDL and triglyceride levels were significantly decreased and HDL and apolipoprotein A1 were increased. On the contrary, Sejda *et al.* [20] found no significant difference on triglyceride, HDL cholesterol and apolipoprotein A1 in patients with dyslipidemia after a 3 month treatment with fluvastatin despite significant reduction in total cholesterol, LDL cholesterol and apolipoprotein B.

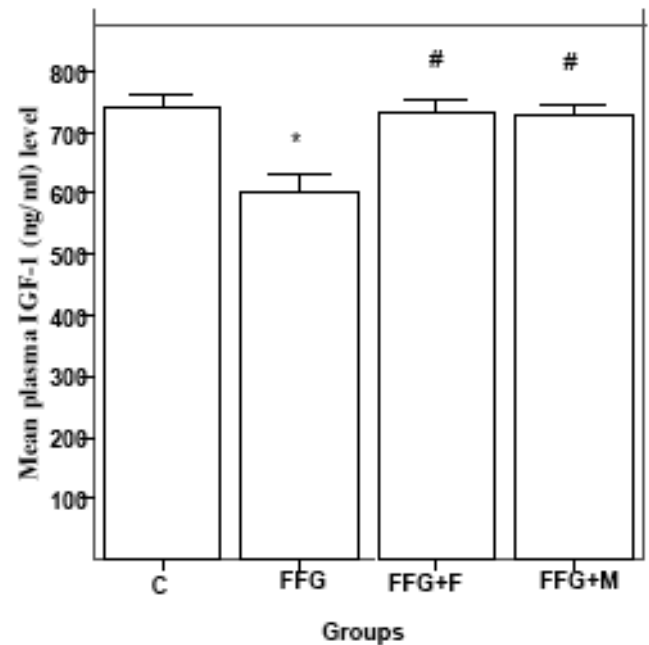


Fig. (4). Comparison of plasma IGF-1 levels between different groups. \* $p < 0.01$  compared with control group. # $p < 0.01$  compared with fructose fed. Group Values are means  $\pm$  SE.

The beneficial effects of fluvastatin on serum lipids and nitric oxide observed in the present work were explained by previous findings. Statins may exert cholesterol independent or pleiotropic vascular effects which appear to restore or improve endothelial function through increasing the bioavailability of nitric oxide, promoting reendothelialization, reducing oxidative stress and inhibiting inflammatory responses [21]. The results of this study showed that fluvastatin significantly reduced plasma glucose and insulin levels by 28% and 45% respectively in fructose treated rats compared with control group. Consistent with our results, Sonmez *et al.* [22] found that fluvastatin 40mg/d for 8 weeks

decreased significantly plasma insulin and lipid levels in non diabetic dyslipidemic patients. A study by Huptas *et al.* [23] evaluated the effect of 6 weeks of atorvastatin (10mg/day) in insulin resistant subjects and found that atorvastatin resulted in a significant reduction in fasting C peptide, glucose and a borderline reduction of insulin ( $p=0.08$ ). Yet, Jokubaitis *et al.* [18] found that fasting glucose and glycosylated hemoglobin levels were not markedly affected by treatment with fluvastatin 20 or 40 mg for 6 weeks in hyperlipidemic patients with non insulin dependent diabetes mellitus. Also, Altunbas *et al.* [24] found that simvastatin 20 mg/ day for 2 months in patients with primary hypercholesterolemia did not affect insulin sensitivity determined by euglycemic hyperinsulinemic clamp method.

The results of the present work concerning the effects of fluvastatin on plasma glucose and insulin levels could be attributed to the corresponding significant statistical elevation of both plasma IGF-1 and its mRNA expression in the liver by 21% and 90% respectively in fructose fed rats treated with fluvastatin compared to control group. IGF-1, an important metabolic regulatory hormone synthesized and released mainly by the liver and kidneys, has beneficial effects on glucose homeostasis due to its glucose lowering and insulin sensitizing action [25]. IGF-1 has hypoglycemic effects and enhances insulin sensitivity in both experimental animals and human subjects. In skeletal muscle cells of type 2 diabetic patients, IGF-1 was found to be a more potent stimulant of glucose transport than insulin itself [26].

The molecular mechanisms by which low IGF-1 concentrations may induce insulin insensitivity are unclear. It has been proposed that an increased proportion of hybrid insulin/IGF-1 receptors would reduce insulin sensitivity in target tissues of insulin action, contributing to cellular insulin resistance. Thus low circulating IGF-1 levels can lead to upregulation of IGF-1 receptors resulting in increased formation of hybrid insulin/IGF-1 receptors which might contribute to impair insulin action in target tissues by sequestering insulin receptors in a less responsive form [27]. Yakar *et al.* [28] reported that low plasma IGF-1 concentrations may provide inadequate negative feedback at the level of the hypothalamus and/or pituitary thus resulting in growth hormone hypersecretion and a decrease in insulin sensitivity particularly in the skeletal muscle.

The hypoglycemic effects of IGF-1 may be also due to increasing endothelial constitutive nitric oxide synthase (NOS) activity. IGF-1, by interacting with a tyrosine kinase membrane receptor linked to the insulin receptor substrate 1 and 2, causes phosphorylation of phosphatidyl inositol 3-kinase (PI3-K) which activates the serine/threonine kinase Akt signaling pathway [3]. This pathway phosphorylates constitutive NOS leading to nitric oxide production with multiple metabolic and vascular protective effects including glucose transport in muscle and fat and inhibition of hepatic gluconeogenesis [29]. Wang *et al.* [30] suggested that the activation of endothelial NOS with a low dose of pitavastatin involves phosphoinositide 3-kinase and the Akt pathway and produces nitric oxide in endothelial cell and this effect may be mediated through IGF-1.

In the present study treatment of fructose fed rats with metformin improves all the metabolic profile compared to control group. There was a significant reduction in blood

pressure, plasma cholesterol, triglyceride, glucose and insulin levels by 7%, 12%, 16%, 29% and 45% respectively with significant elevation of plasma nitric oxide level by 259%. These results are coincided with Inzucchi *et al.* [31]. Metformin improves lipid profiles and has direct vascular effects beyond its hypoglycemic actions [32].

Consistent with our results, Katakam *et al.* [33] found that fasting insulin, lipid profiles and mean arterial pressure were improved after 2 weeks treatment with metformin (300mg/kg) in fructose fed insulin resistant rats. Moreover, acetylcholine induced relaxation in mesenteric arteries from these rats was restored to control levels after treatment with metformin. On the contrary, Chen *et al.* [34] reported that metformin treatment for 6 weeks was not effective in reducing the systolic blood pressure in fructose fed rats despite significant reduction in insulin and triglyceride concentrations which are in accordance with our results. The mechanism of blood pressure lowering effect of metformin observed in the present work may be due to its insulin sensitizing effects [35]. Compensatory hyperinsulinemia has been thought to be a cause of hypertension because insulin could cause sodium retention, sympathetic nerve activation and vascular smooth muscle cell proliferation [36].

In the present work metformin treatment caused significant elevation of plasma IGF-1 and its gene by 20% and 112% respectively compared to control group which could be attributed to the beneficial metabolic effects of the drug observed in this study as well as to its insulin sensitizing effect.

## CONCLUSION

Fluvastatin and metformin are equally effective in improving the metabolic profile in an animal model of insulin resistance and this improvement correlated positively with IGF-1 levels.

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