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Research Article

Atorvastatin and Fenofibrate Modulate Certain Steroidal Hormones, Vitamin D and Bile Acids in Diabetic Dyslipidemic Rats

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ABSTRACT

Objectives: To investigate the effect of long term intake of traditionally used hypolipidemic drugs, atorvastatin and fenofibrate, on cholesterol derived products including steroidal hormones, vitamin D, and bile salts. Materials and methods: Male Wistar albino rats were divided into four groups including a normal control group and the other groups were rendered diabetic dyslipidemic (DD) and received placebo, atorvastatin (10 mg/kg body weight/day, p.o) "LD50 > 5000 mg/kg", or fenofibrate (100 mg/kg body weight/day, p.o) " LD50 > 2g/kg" for 4 and 8 weeks, respectively. Lipid profile, glycemic index and steroidal hormones levels were measured to evaluate the biological activities of these drugs. Results: DD rats demonstrated significant increase in serum glucose, fructosamine, total cholesterol (TC), triacylglycerol (TAG), atherogenic index (AI), and bile acids along with HDL-C decrease. Serum insulin and insulin resistance increased after 4 weeks and decreased after 8 weeks. Insulin, free testosterone, aldosterone, cortisol and vitamin D levels showed significant decrease. Atorvastatin or fenofibrate treatment resulted in significant decrease in serum glucose, fructosamine, TC, TAG, bile acids and vitamin D levels along with increase in HDL-C, insulin, aldosterone and cortisol but failed to achieve normal level of free testosterone. Fenofibrate improved insulin sensitivity in DD only after 8 weeks. Conclusions: Administration of hypolipidemic drugs seems to modulate certain cholesterol derived products in DD rats.

Key words: Aldosterone; Cortisol; Free testosterone

INTRODUCTION

Dyslipidemia represents a major risk factor for cardiovascular disorders in diabetics. It is characterized by increased levels of low density lipoprotein cholesterol (LDL-C), specifically its small dense particles and triacylglycerol (TAG) (1). This is mostly attributed to insulin deficiency and insulin resistance leading to higher flux of free fatty acids (2).

Previous studies demonstrated that plasma lipids and lipoproteins levels are usually affected by steroidal hormones whereas some sex hormones and their precursors can exert certain protective effect against CHD (3-5). It has been assumed that sex hormones such as adrenal androgens might affect certain enzymes involved in the metabolism of TAG and high density lipoprotein cholesterol (HDL-C) as well as lipolysis (4,6). However, fewer studies have examined only the influence of plasma lipid spectrum on endogenous steroid metabolism under physiological and pathological states (7,8).

Statins are the most widely used hypolipidemic drugs as compared to others like niacin, fibrates and ezetimibe (9). Statins acts through inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis (10). Fibrates are modulators for circulating lipids through activation of peroxisome proliferator-activated receptor-alpha (PPAR)-α (11). They enhance genes which regulates the catabolism and β-oxidation of fatty acids, reducing the risk of atherogenesis in diabetic patients (12,13). The administration of these hypolipidimic drugs can extend for long time, therefore the present study was designed to answer two questions:

1) What is the probability of cholesterol derived products modulation represented by steroidal hormones, vitamin D and bile acids in subsequent to any change in serum cholesterol level due to hypolipidimic medications?

2) Is there any beneficial effect for supplementation of cholesterol derived products for individuals scheduled on hypolipidemic medications for long term?

MATERIALS AND METHODS

Animals and interventions

Forty adult male Wistar Albino rats (6 week-old) weighing190 ± 10 g, purchased from the Egyptian Organization for Biological Products and Vaccines (Cairo, Egypt) were used in the present study. Rats were maintained under environmentally controlled conditions including ambient temperature of 25 ± 2°C and a 12 h light/dark cycle and allowed free access to standard rat chow diet and water. After acclimatization for 1 week, the rats were divided into four groups (ten rats per group): (I) normal control (NC) group; (II) diabetic dyslipidemic

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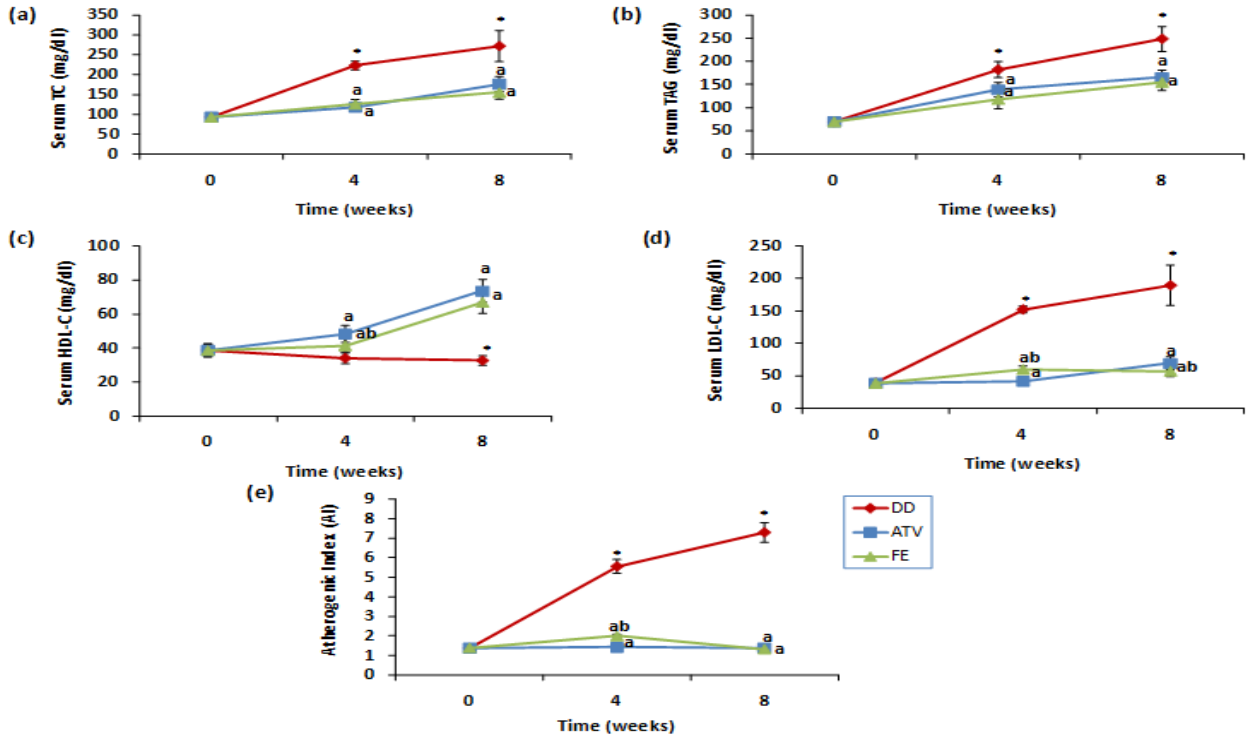

Figure 1: Effect of daily oral administration of atorvastatin and fenofibrate on serum lipid profile in diabetic dyslipidemic rats. NC group: Normal control rats were fed a normal diet; DD group: diabetic dyslipidemic rats received alloxan and a high-fat-diet for 4 and 8 weeks without any treatment; ATV group: diabetic dyslipidemic rats received atorvastatin (10 mg/kg/d) and FE group: diabetic dyslipidemic rats received fenofibrate (100 mg/kg/d). Data are expressed as mean ± SD, n = 6. TC: total cholesterol; TAG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. *p<0.05vs normal control group, ap<0.01vs diabetic dyslipidemic group, bp<0.05vs atorvastatin.

(DD) group (14-16). Induction of hypercholesterolemia was done by addition of 1% cholesterol (El Gomhorya Co., Egypt), 0.5% saturated fat and 0.5% cholic acid to rat chow diet and 0.01% thiouracil in drinking water and supplementation to rats for eight weeks (17-19). Rats which achieved a serum cholesterol level greater than 200 mg/dl were selected for this study (20). Induction of diabetes was done in hypercholesterolemic rats by intraperitoneal injection of a single dose of 90 mg/kg body weight of freshly prepared alloxan monohydrate (Sigma-Aldrich, USA) dissolved in ice-cold saline, followed by four hours post fasting period. Development of diabetes was verified after seven days through monitoring of serum glucose level (21); (III) DD rats, received atorvastatin (Lipinorm®, "MUP", Abou Sultan, Ismailia, Egypt) suspended in distilled water using gum acacia at 10 mg/kg body weight once daily by gastric gavage (p.o) along with high cholesterol diet for 8 weeks (ATV) group (22); (IV) DD rats received fenofibrate (Finorate®, Eva Pharmaceutical Company, Egypt) suspended in distilled water using gum acacia orally at 100 mg/kg body weight/day concomitantly with high cholesterol diet for 8 weeks (FE) group (23). All procedures were carried out in accordance with the Guidelines of Ethical Care of Experimental Animals and the protocol was approved by the Ethical Committee for animal handling at Faculty of Pharmacy, Zagazig University, Egypt (ECAHZU).

Serum and tissue sampling

At the end of the experiment, rats were fasted overnight, and blood samples were collected *via* retro-orbital bleeding after 0,4 and 8 weeks, centrifuged at 4500 rpm for 20 min and fresh sera were processed immediately for the determination of glucose level. The remaining serum samples were stored as aliquots at -20°C for further estimation of fructosamine, TC, TAG, HDL-C, LDL-C as well as bile acids, insulin, free testosterone, aldosterone, cortisol and vitamin D levels.

Biochemical analysis

Serum levels of glucose, TC, TAG and HDL-C were determined enzymatically using commercially available kits (Spinreact Co., Spain) according to the manufacturer's instructions. LDL-C was calculated from Friedwald formula: LDL-C = TC-(TAG/5+HDL-C). Atherogenic indexes were calculated from the formula: AI = TC-HDL-C/HDL-C.

Serum fructosamine level was estimated according to the method of Schleicher (24) using QCA kit, Spain. Insulin, free testosterone, aldosterone and cortisol levels were measured in serum by Rat enzyme linked immunosorbent assay (ELISA) kits. Rat insulin Enzyme Immunoassay (EIA) kit was supplied from SPI; Société de Pharmacologieet d'Immunologie – BIO, Parcd'Activités du Pas du Lac – Bertin Group (Montigny Le Bretonneux, France). The Bio-Line Free testosterone ELISA kit was from Bio-Line S.A., Bruxelles, Belgium. Rat Aldosterone

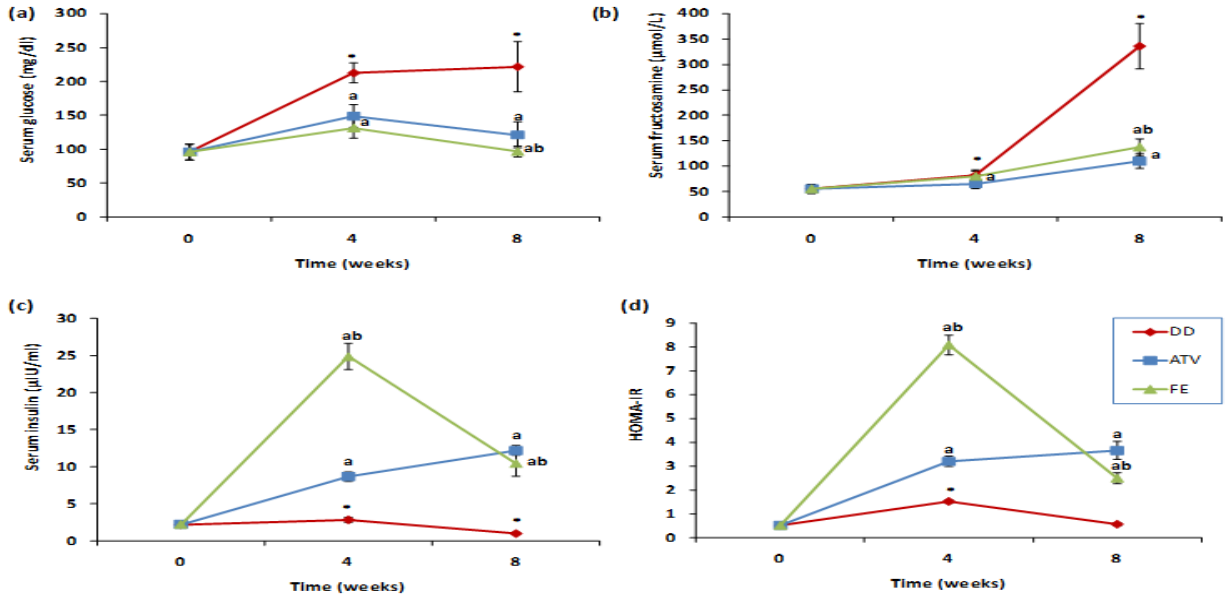

Figure 2: Effect of oral administration of atorvastatin and fenofibrate daily on serum glucose, fructosamine, insulin and hemostatic model of insulin resistance in diabetic dyslipidemic rats. NC group: Normal control rats were fed a normal diet; DD group: diabetic dyslipidemic rats received alloxan and a high-fat-diet for 4 and 8 weeks without any treatment; ATV group: diabetic dyslipidemic rats received atorvastatin (10 mg/kg/d) and FE group: diabetic dyslipidemic rats received fenofibrate (100 mg/kg/d). Data are expressed as mean ± SD, n = 6. TC: total cholesterol; TAG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. *p<0.01vs normal control group, ap<0.01vsdiabetic dyslipidemic group, bp<0.05 vs atorvastatin.

(ALD) ELISA kit was obtained from K-ASSAY (KAMIYA BIOMEDICAL COMPANY, Seattle, WA). Rat Cortisol ELISA Kit was provided by Wuhan Eiaab Science Co., LTD "EIAaB®" (Wuhan, China).The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using serum glucose and insulin levels as previously described by Matthews et al. (25): HOMA-IR = (glucose X insulin)/405.

Vitamin D level in serum was determined in its hormonally active form 1,25-dihydroxycholecalciferol (calcitriol) using radioimmunoassay (RIA) kit (Diasorin Inc., Stillwater, Minnesota, USA). Serum levels of bile acids (cholic acid, chenodeoxycholic acid, and deoxycholic acid) were estimated by using combined gas-liquid chromatography and mass spectrometry according to Ahlberg et al. (26).

Statistical analysis

All results were expressed as means ± SD. The significance of differences among groups was evaluated using one-way analysis of variance (ANOVA) for multiple comparisons, and Student's *t*-test was performed for differences between two groups using SPSS (version 16; SPSS Inc., Chicago, IL, USA). The statistical associations between functional parameters were assayed using Pearson's correlation analysis.

RESULTS

Effect of atorvastatin/fenofibrate on serum lipids, lipoproteins, glucose, fructosamine, and insulin levels

Diabetes and high cholesterol diet induced significant increase in total cholesterol (TC), TAG and LDL-C at weeks 4 and 8 compared to NC (p<0.0001) in a timed dependent manner. On the other hand, HDL-C demonstrated only significant decrease after 8 weeks of alloxan and high fat diet treatment (p<0.05). Either atorvastatin or fenofibrate markedly raised HDL-C above baseline values compared with DD group (p<0.01). The atherogenic indexes were subsequently increased in DD group in comparison with NC group and these indexes were significantly reduced by both drugs. All lipid profile results are shown in Figure 1.

Treatment with either atorvastatin or fenofibrate significantly lowered serum glucose and fructosamine levels compared to DD group (p<0.0001). Serum insulin level showed a significant increase in DD group at the end of 4 weeks and turned to be decreased at the end of 8 weeks compared with NC at p<0.01 (Fig.2). The increase in insulin was greater in fenofibrate than in atrovastatin after 4 weeks and this condition was reversed after 8 weeks (p<0.05). Regarding insulin resistance, we found that alloxan and DD induced a significant increase in HOMA-IR (p<0.0001) only at the end of 4 weeks. Either atorvasatin or fenofibrate treatment resulted in significant increases in HOMA-IR in comparison with DD group (p<0.0001 at 4 weeks). However, HOMA-IR was increased in atorvastatin group and decreased in fenofibrate group after 8 weeks.

Effect of atorvastatin/fenofibrate on steroidogenesis

Steroidal hormones include free testosterone, cortisol and aldosterone as well as Vitamin D and bile acids. All these steroids showed a significant decrease in DD rats except for bile acids which demonstrated a significant increase as compared with NC ones (p<0.0001, Fig.3). Serum cortisol and aldosterone levels increased significantly

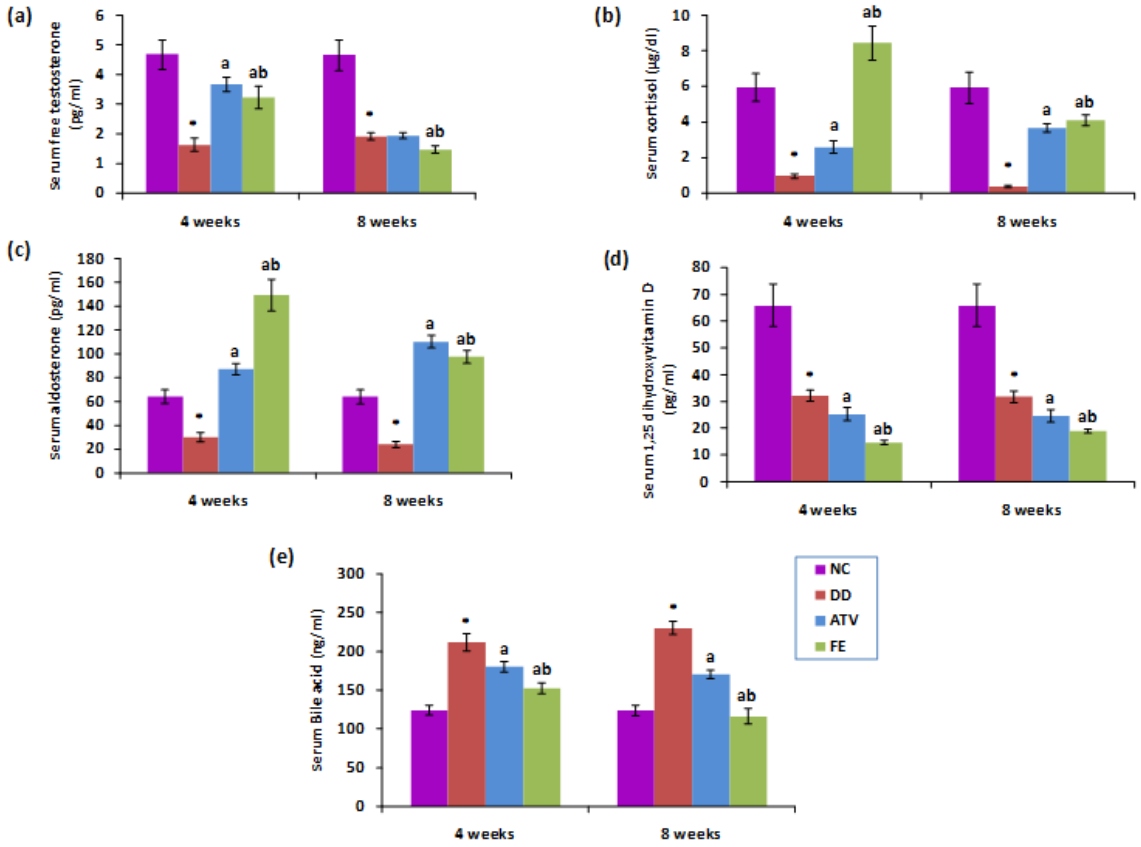

Figure 3: Effect of daily oral administration of atorvastatin and fenofibrate on serum steroids in diabetic dyslipidemic rats. NC group: Normal control rats were fed a normal diet; DD group: diabetic dyslipidemic rats received alloxan and a high-fat-diet for 4 and 8 weeks and did not receive any treatment; ATV group: diabetic dyslipidemic rats received atorvastatin (10 mg/kg/d) and FE group: diabetic dyslipidemic rats received fenofibrate (100 mg/kg/d). Data are expressed as mean ± SD, n = 6. TC: total cholesterol; TAG: triacylglycerol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol. *p<0.0001 vs normal control group, ap<0.001vsdiabetic dyslipidemic group, bp<0.05 vs atorvastatin.

after 4 and 8 weeks in either atorvastatin or fenofibrate–treated rats as compared to DD group (p<0.0001) (Fig.3). Cortisol and aldosterone did not return to normal values by either treatment after 8 weeks. Free testosterone displayed significant increases following either atorvastatin or fenofibrate treatments only after 4 weeks with respect to DD group (p<0.0001). On the other hand, at the end of 8 weeks treatment free testosterone level was markedly reduced in fenofibrate group compared to DD group (p<0.0001). Atorvastatin/fenofibrate significantly reduced serum levels of Vitamin D and bile acids compared to DD group (p<0.001 at 4 and 8 weeks). The reduction achieved by fenofibrate was greater than atorvastatin (p<0.001).

Biochemical correlations

Correlation analysis among individual groups after 8 weeks revealed that the 1,25-dihydroxy vitamin D correlated positively with bile acids, free testosterone and aldosterone, cortisol (Figs.4-6). Insulin was strongly correlated with cortisol (Fig.7).

DISCUSSION

Present study indicated that induction of diabetic dyslipidemic state significantly decreased free testosterone, aldosterone, cortisol, and vitamin D levels after 4 and 8 weeks. This is mainly attributed to decreased production of steroid acute regulatory (STAR) protein level as mediated through down-regulation of STAR gene expression (27). Generally, cholesterol exists in serum either bound to lipoprotein (LDL or HDL) or as cholesterol sulphate. Reported study referred that hyperlipidemia has certain effects on benzodiazepine receptors (PBR), translocation of cholesterol to the inner mitochondrial membrane of steroidogenic cells leading to decrease of STAR protein, pregnenolone and in turn other steroidal hormones namely aldosterone, cortisol and testosterone (28).

A previous study referred also to the positive correlation between high cholesterol level and low fertility index (serum testosterone level) in experimental rats and simvastatin administration to these rats improved to certain extent the reproductive efficiency which is mostly attributed to its hypocholesterolemic effect (29).

Decreased serum and testicular testosterone was attributed also to decreased binding of testicular LH/hCG where LH receptors showed down-regulation in the testes of DD rats (30) in addition to a reduction in the spermatid

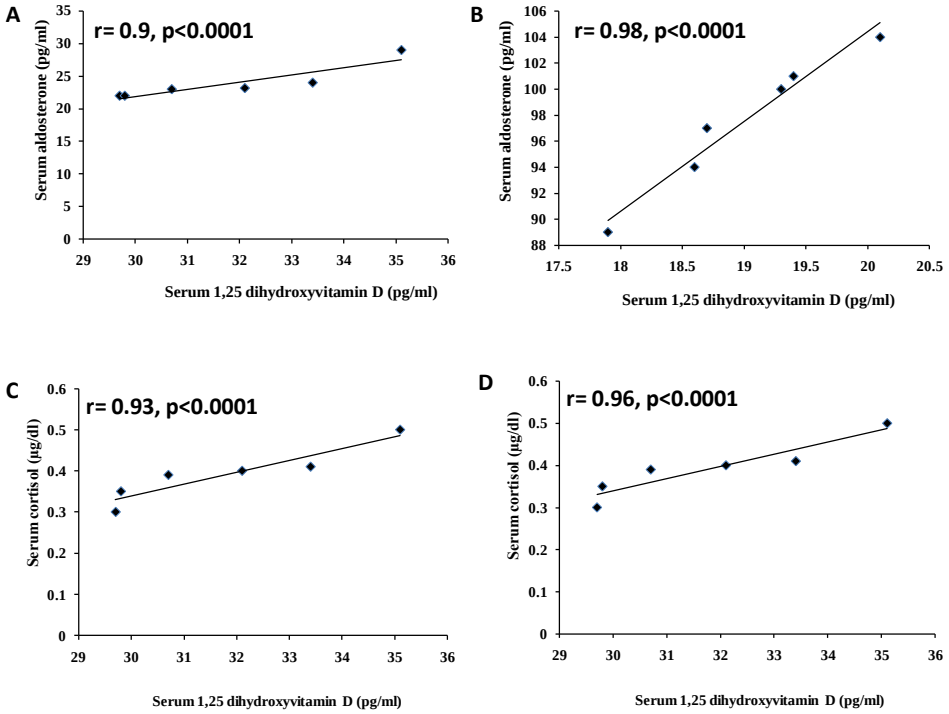

Figure 4: Correlation among serum 1,25 dihydroxyvitamin D, aldosterone and cortisol: A,C refer to DHC group and B,D refer to Fenofibrate group. The modulation of adrenal steroids was 1,25 dihydroxyvitamin D-dependent. 1,25 dihydroxyvitamin D was strongly correlated with aldosterone and cortisol.

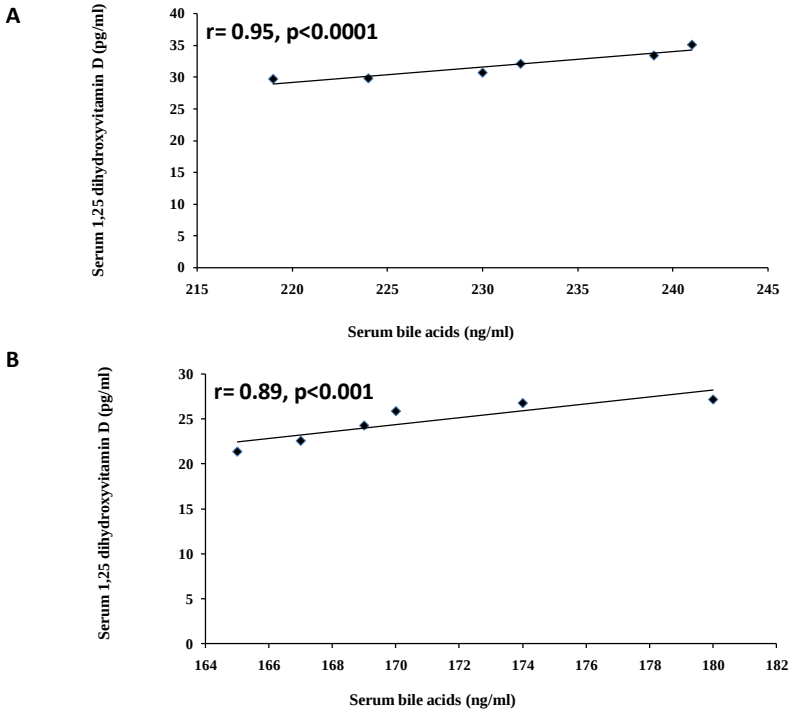

Figure 5: Correlation between serum 1,25 dihydroxyvitamin D and bile acids: A refers to DHC group and B refers to Atorvastatin group. The modulation of bile acids was 1,25 dihydroxyvitamin D-dependent. 1,25 dihydroxyvitamin D was strongly correlated with bile acids.

cell population, Leydig cells count and their nuclear dimensions (31,32). Another study indicated that testicular testosterone concentration and luteinizing hormone (LH) receptors were reduced in alloxan-induced diabetic rats (33) and diabetic individuals (34). This led other investigators to conclude that severely uncontrolled type 1 diabetics had lower serum testosterone than mild cases (35).

Hypercholesterolemia can also impair testicular steroidogenesis in mice through the renin–angiotensin system (RAS). The bioactive peptides of RAS localized in the gonads are greatly involved in the relation between

cholesterol and testosterone through alteration of steroidogenesis and inhibition of testosterone production (36).

Present study demonstrated a tendency of free testosterone to increase after 4 weeks of atorvastatin or fenofibrate administration which turned to a marked decrease after 8 weeks treatment by fenofibrate only. This is in agreement with a previous experimental study which indicated that statin administration at high doses reduced testosterone level (37). The most likely reason that free testosterone levels were unaltered by atorvastatin here in spite of lower total cholesterol level is a homeostatic mechanism *via* reduced total testosterone and sex hormone binding globulin (SHBG) production (38). An alternative hypothesis is that atorvastatin causes a primary reduction in SHBG with consequent reductions in total testosterone. SHBG is produced in the liver, the primary site of statins action, but the mechanism by which statins could alter SHBG is unknown. SHBG levels are known to be modulated by a number of factors including down-regulation by insulin resistance and upregulation by estrogens (39). It was reported that fenofibrate therapy in men suffering from mixed hyperlipidemia showed a significant decrease of dehydroepiandrosterone, a precursor for testosterone biosynthesis, level. This may account for reduced testosterone level observed herein following fenofibrate treatment for 8 weeks (40). Certain controversy was reported while some studies have demonstrated reduced testosterone levels due to simvastatin treatment **(**41,42). Others however reported no effect (43-45). The mechanisms by which prostatic tissue maintains tissue androgens may include metabolism of adrenal androgens or de novo synthesis from cholesterol (46).

Present results showed higher insulin resistance in either atorvastatin or fenofibrate group after 4 weeks. However, insulin resistance was reduced by fenofibrate after 8 weeks. Sena et al. (47) has reported that atorvastatin treatment improves glucose metabolism (fasting glucose and 2 h glucose tolerance). Additionally, it was able to decrease free fatty acids (FFAs) and HOMA index. Supporting this notion, previous studies showed that statin therapy can improve the parameters of glucose metabolism in diabetic and non-diabetic patients (48,49). This effect may be attributed to decreased gluconeogenesis since atorvastatin was reported to decrease glucose-6-phosphatase expression in an animal model of type II diabetes (50) or by increased uptake of glucose in muscle, or both. Previous studies indicated that the flux and turnover of portal free fatty acids (FFAs) is crucial for the development of insulin resistance (51). In confirm, we found that atorvastatin and fenofibrate significantly reduced serum glucose and TG levels, suggesting reduction of FFAs uptake. Fenofibrate enhanced insulin sensitivity, decreased serum glucose and TG, and decreased liver glycogen/lipid levels in the mice induced by high fat diet and small-dose strepotozotocin (52). This is concordant with our findings. Fenofibrate lowered serum TG by activating lipoprotein lipase (53) and accelerated the beta-oxidation of fatty acid by activating PPAR alpha (54). An increased supply of free fatty acids for oxidation leads to a reduced rate of glucose oxidation and interferes with the inhibitory action of insulin on hepatic glucose production (55). So, decreased serum TG or FFAs may improve the insulin sensitivity leading to a better glycemic control. Also, the decrease in serum TG may bring about a secondary decrease in liver glycogen storage and TG accumulation. This preventive effect may be useful for type 2 diabetes.

Current study illustrated a decrease in vitamin D level in diabetic hyperlipidemic state. This may be attributed to increased level of serum TAG that is inversely correlated with seasonal changes in vitamin D status (56). Present findings indicated also that atorvastatin administration significantly reduced vitamin D level in DD rats. However, certain controversy in this concern was reported before. Some clinical studies indicated that statins may increase 25-OH-D_3 and/or 1,25-$(OH)_2$-D_3 level in humans (57,58), while others demonstrated no effect (59). Recent study indicated that certain patients with stable coronary heart disease receiving atorvastatin displayed vitamin D deficiency or insufficiency (7.2%) and (41.4%) while other patients (3.0%) had elevated vitamin D (60). This was attributed to variable effect of atorvastatin on vitamin D level among patients. Another study indicated that atorvastatin reduced plasma 25-OH-D_3 but had no effect on 1,25-(OH)(2)-D_3. Since 25-OH-D_3 has beneficial metabolic effects and increased insulin sensitivity, atorvastatin-induced 25-OH-D(3) reduction may account for insulin resistance (61). Fenofibrate administration for 6 months to patients suffering from familial hyperlipoproteinaemia caused a significant decline in plasma 25-hydroxy vitamin D and a rise in 1,25-dihydroxy vitamin D_3. Thus, fibrates could influence plasma concentrations of vitamin D metabolites either directly or indirectly by reducing cholesterol plasma levels (62).

Regarding aldosterone, its observed increase following administration of hypolipidemic drugs may be due to the compensatory mechanism involving activation of certain cytochrome P_{450} family member to balance its decrease in DD rats but this needs further justification. The same observation may be applied also for cortisol which exhibited a trend to increase as compared to DD group in spite of being significantly lower than normal.

Bile acids demonstrated also a significant increase after 4 and 8 weeks of high cholesterol diet. This is mostly attributed to activation of cytochrome P450 7 α hydroxylase (CYP7A1) in the endoplasmic reticulum or cytochrome P450 27 hydroxylase (CYP27A1) in the mitochondria (63). Administration of atorvastatin for 4 and 8 weeks resulted in a significant decrease of serum bile acids which is mostly attributed to decreased activity of hepatic cholesterol 7 α-hydroxylase. Aoki et al. (64) reported that mRNA expression of such enzyme showed a significant decrease by atorvastatin. Similar decrease in serum bile acids was also observed after fenofibrate administration. The latter drug promotes fecal excretion of total lipids, cholic acid, and deoxycholic acid leading to reduction in serum bile acid level (65). However, a

previous study demonstrated a significant increase of blood corticosterone level in subsequent to fenofibrate treatment which is attributed to PPARα activation (23), in agreement with our study.

CONCLUSION

Hypolipidemic drugs used in the present study exerted certain influences on various cholesterol bi-products. They restored cortisol and aldosterone depletion as well as bile acids elevation induced by diabetic dyslipidemia. However, their prolonged administration remarkably reduced free testosterone and vitamin D. Therefore, these hormones must be monitored during long term intake of such drugs taking in consideration that appropriate replacement therapy of these products can be instituted upon urgent need but after approval of such situation clinically.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to disclose.

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