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Proprotein convertase subtilisin kexin-9 level remains unchanged following glucocorticoid-induced dyslipidemia in Wistar rats

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ABSTRACT: Proprotein convertase subtilisin kexin type 9 (PCSK9) is a secreted liver enzyme that regulates low-density lipoproteins and is dysregulated in dyslipidemia of various etiology. Glucocorticoids are powerful anti-inflammatory agents that also induce dyslipidemia. The role of PCSK9 in glucocorticoid-induced dyslipidemia remains underexplored. In this study, we investigate sex-dependent changes in serum PCSK9 levels following glucocorticoid-induced dyslipidemia. Fourteen male and fourteen female Wistar rats, eight weeks old were administered normal saline or dexamethasone (dex) 2 mg/kg via the intraperitoneal route for seven consecutive days. Six hours after the last dose, cardiac blood was collected from anesthetized animals. Serum was analyzed for lipid profile, sex hormones and enzyme concentration using enzyme-linked immunosorbent assays. Data were analyzed using one-way analysis of variance followed by Sidak's test. Significance was accepted at $p \leq 0.05$. Dexamethasone administration induced significant hypercholesterolemia (94.55 ± 1.676 vs 150.6 ± 11 mg/dL; $p < 0.0001$) and dyslipidemia (24.13 ± 4.11 vs 72.46 ± 8.883 mg/dL; $p < 0.0001$) in both male and female rats. There was a trend towards increased circulating PCSK9 however increases were not statistically significant. Dexamethasone induced dyslipidemia without altering plasma concentration of PCSK9.

Keywords: dyslipidemia; glucocorticoids; pcsk9

Introduction

Endogenous glucocorticoids (GCs) such as cortisol (corticosterone in rats) are steroidal compounds involved in physiological response to stress and the regulation of glucose and lipid metabolism (1,2). Glucocorticoids via the glucocorticoid nuclear receptor are involved in the transcriptional regulation of key enzymes involved in hepatic gluconeogenesis (3,4), brain insulin action (5) and lipid metabolism (6). GCs have anti-inflammatory (7), anti-emetic (8), immunosuppressive (9) and antineoplastic effects (10) making them important drugs in the clinic. However, pharmacological glucocorticoid administration is usually associated with glucose and lipid dysmetabolism (11). For instance, GCs induce insulin resistance, hyperlipidemia and hypertriglyceridemia (12).

Proprotein convertase subtilisin kexin type 9 (PCSK9) is a key regulator of hepatic low-density lipoprotein receptor (LDLR) and thus involved in low-density lipoprotein (LDL) cholesterol homeostasis (13). PCSK9 is a 692 amino-acid enzyme expressed in the liver, small intestine and kidneys though primarily secreted into circulation by the liver (14). In the liver, the LDLR binds to circulating LDL resulting in the endocytosis of LDL and its eventual clearance from the plasma. The LDLR is subsequently recycled to the cell membrane to participate in further plasma LDL clearance. PCSK9 prevents LDLR recycling by two major mechanisms: association with extracellular and also intracellular LDLR thus targeting the receptor for lysosomal and endosomal degradation (14). Thus, PCSK9 serves as a negative regulator of LDL.

Consequently, functional changes in PCSK9 have been associated with dyslipidemia. For instance, null mutations in PCSK9 results in low LDL levels which protect against atherosclerosis (15). In the same vein, PCSK9 knockout mouse models have low LDL concentrations (16). Conversely, mutations that lead to overexpression of PCSK9 are associated with increases in circulating lipids (17). Furthermore, PCSK9 levels are elevated in metabolic syndromes such as in patients with familial hypercholesterolemia (18), obesity (19) and type 2 diabetes (20). Though PCSK9 may be associated with dyslipidemia of different etiologies this is not firmly established as lack of changes in PCSK9 concentration in metabolic disease has also been reported (21).

In this study, we investigated PCSK9 in glucocorticoid-induced dyslipidemia. We administered a glucocorticoid, dexamethasone to Wistar rats and analyzed lipid profile and circulating serum PCSK9 concentrations. Even though GC administration induced alterations in serum low- and high-density lipoproteins as well triglyceride concentration as expected, PCSK9 concentration was not significantly altered. We further investigated whether effect of the glucocorticoid on PCSK9 would be sex-dependent. However, PCSK9 was not modified sex-dependently. Further studies are required to investigate PCSK9 changes in the liver at the protein and transcriptional levels.

Materials and Methods

Drugs and Chemicals

Dexamethasone solution 4 mg/ml (Decadron, Merck, UK) used in this study was of pharmaceutical grade. The assay kits for estrogen, testosterone, lipids and PCSK9 was supplied by Fortress Diagnostics (Antrim, UK) chemicals.

Experimental Animal and Care

Male and female Wistar rats, eight weeks old weighing 150-200 g were used in this study. The animals were obtained from animal breeding facility of the Department of Pharmacology, University of Ilorin, Ilorin and acclimatized to housing for seven days. All animal procedures comply with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving animals. Institutional approval for this study was granted by the University of Ilorin Animal Ethics Review Board

(UIL/UERC/01/47KB021). The twenty-eight animals (14 males and 14 females) used in the study were grouped according to sex and housed in polystyrene cages with steel mesh layer in groups of seven per cage. Animals were fed with rat chow pellets from Top Feed limited and tap water *ad libitum*. Food and water intake were measured every other day.

Experimental Design

Animals were randomized into two group of seven rats each, male and female control group and the dexamethasone-treated groups (male and female). Dexamethasone (2 mg/kg of body weight) or normal saline (0.9% wt/vol, NaCl) was administered to animals via the intraperitoneal route one hour after daylight onset (8:00 – 9:00 am) for seven consecutive days.

Biochemical Assays

Six hours after the last dose, animals were euthanized by ketamine/xylazine. Blood was collected in plain tubes and allowed to clot for 30 min before centrifugation at 3500xg for 10 min in centrifuge (Eppendorf 18/14; SN WO11272). The serum was collected using clean Pasteur pipettes into microcentrifuge tubes placed on ice. Samples were immediately transported on ice to analysis facility and all analytes were evaluated within two hours by a third-party Bridge Biotech Science Company, blinded to the study.

Total cholesterol (TC), HDL-c and triglycerides were determined in the serum of the rats by following the manufacturer's protocol. LDL-c was calculated using the Friedewald formula: LDL-C=TC- [HDL-C + TG/5). Serum estradiol, testosterone and PCSK9 were determined from serum using an ELISA kit as prescribed by the manufacturer.

Data Analyses.

Statistical analysis was performed using GraphPad Prism 8 statistical package (GraphPad Software, USA). The data were analyzed by one-way analysis of variance (ANOVA) followed by Sidak's test. All the results were expressed as mean ± SE for 6/7 rats in each group. Statistical significance was considered at $p \leq 0.05$.

Results

Effects of Dexamethasone on Lipid Parameters and PCSK9

Although seven days administration of dexamethasone resulted in significant increase in total cholesterol (Figure 1A) and LDL (Figure 1B) in male and female Wistar rats, HDL (Figure 1B) remain unchanged in male but significantly lower in female rats when compared with the control groups. Even though there was a trend towards increases in PCSK9, this was not significantly different when compared to control groups (Figure 1E). Dexamethasone-induced changes did not result from changes in sex hormone concentration (Figure 2A, B).

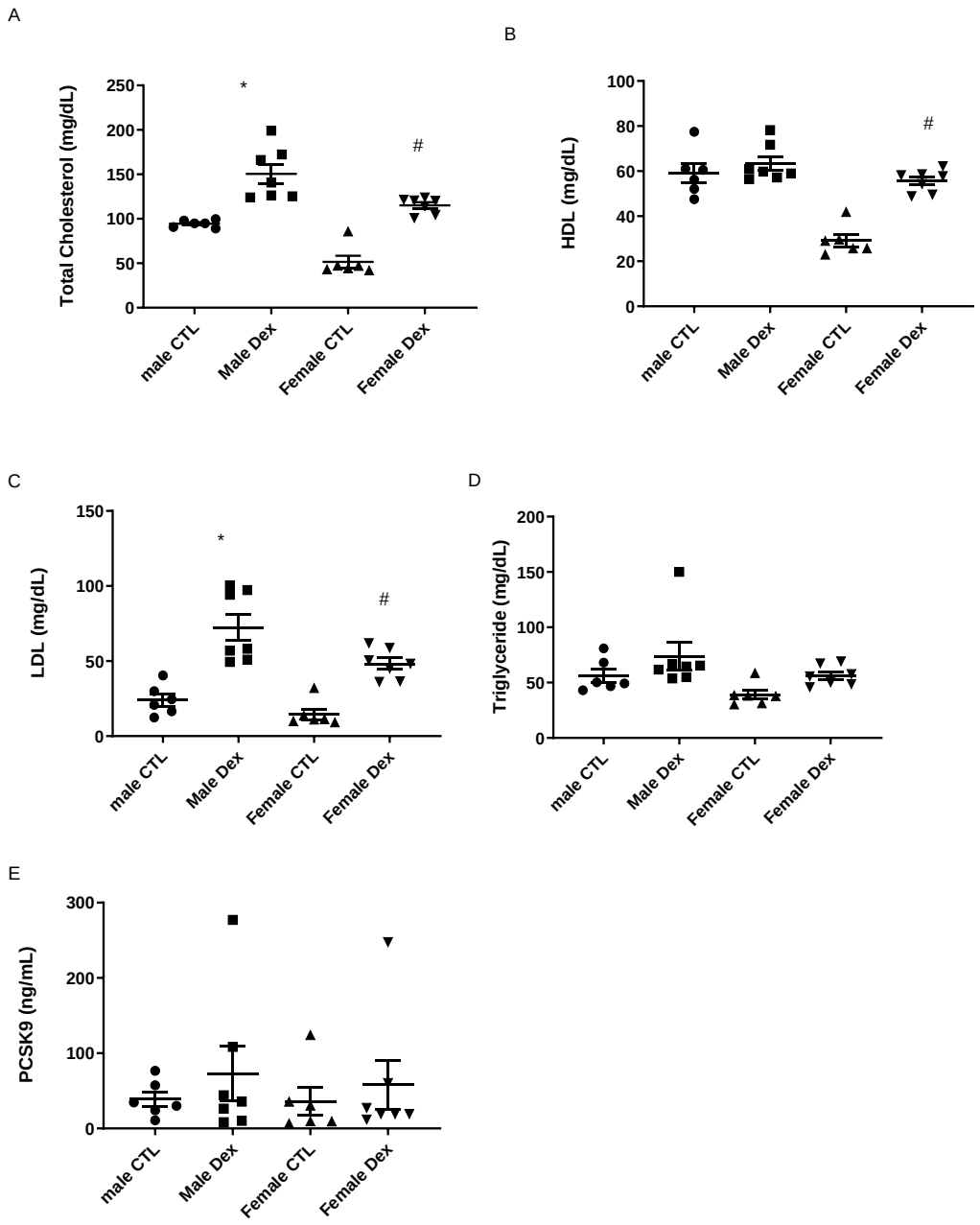

Fig 1: Comparison of lipid profile. Effect of dexamethasone 2 mg/kg in Wistar rats on (A) TC (B) HDL (C) LDL (D) TG. (E**)** PCSK9**.**

Data are presented as mean ± sem (n=6/7). Significance is indicated on graphs as follows: *p ≤ 0.05 is significant when compared to male control animals, # p ≤ 0.05 is significant when compared to female control animals. CTL; Control animals, Dex; Dexamethasone, LDL; low-density lipoprotein, TG; triglyceride, TC; Total Cholesterol, HDL; High-density lipoprotein; PCSK9, Proprotein convertase subtilisin kexin-9.

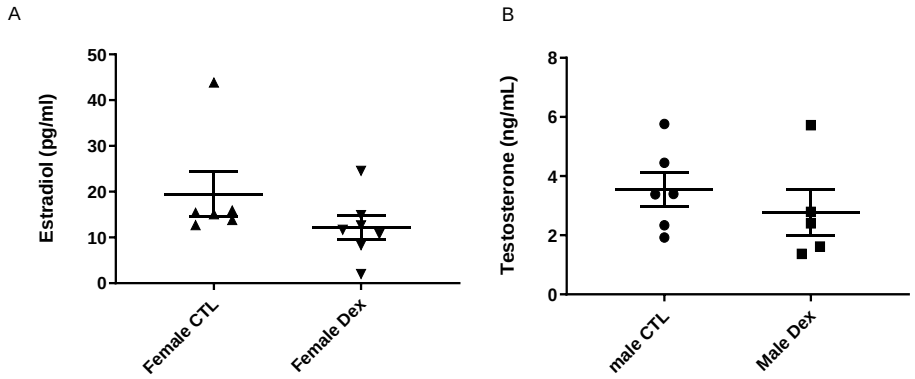

Fig 2: Effect of dexamethasone (2 mg/kg) treatment on (A) Estradiol (B) Testosterone. Estradiol (in female rats only) and testosterone (in male rats only) were assayed in plasma.
Data are presented as mean ± sem (n=6/7). Significance is indicated on graphs as *$p \leq 0.05$ when compared to control. CTL; Control animals, Dex; Dexamethasone.

Discussion

PCSK9 inhibitors are the most recently introduced drugs in the clinical management of dyslipidemias. The first PCSK9 inhibitor was approved primarily for patients with familial hypercholesterolemia. Due to salutary effects on atherogenic lipids and a marked reduction in risk of cardiovascular events, there has been interest in expanding the therapeutic coverage of PCSK9 inhibitors. (22). This class of drugs have since been evaluated in other diseases associated with dyslipidemia. Thus, PCSK9 inhibitors improve lipid profile in type 2 diabetes patients with elevated PCSK9 levels (23). To identify more indications for PCSK9 inhibition, several studies evaluated changes in PCSK9 concentrations in known dyslipidemic conditions. PCSK9 has been observed to be elevated in drug-naïve and protease inhibitor-treated HIV patients (24,25), sepsis (26,27), metabolic syndrome (28) and rheumatoid arthritis (29). However, studies evaluating PCSK9 in polycystic ovarian syndrome did not observe increase circulating PCSK9 despite the presence of dyslipidemia (30). Furthermore, despite low LDL concentrations in patients co-infected with HIV and hepatitis B, PCSK9 are elevated (31). No studies to our knowledge have evaluated the effect of glucocorticoids on circulating PCSK9.

This study reveals that dexamethasone administration after seven days did not modulate plasma concentrations of PCSK9 in Wistar rats. This is in line with previous observations that social stress in animals which is usually associated with elevated glucocorticoid concentrations did not elevate PCSK9 concentration (32). This lack of an effect may be due to absence of glucocorticoid response elements (GREs) in the regulatory sites of the PCSK9 gene. In global genome analyses investigating presence of glucocorticoid response elements, PCSK9 was not reported as a target for direct glucocorticoid receptor (GR) binding (14). Rather, important regulators of cholesterol metabolism such as sterol regulatory element binding protein (SREBP) and hepatocyte nuclear factor-1 alpha (HIF-α) have been reported to directly regulate the expression of PCSK9 (14). On the other hand, GR is known to modulate gene expression indirectly via transactivation and transrepression. An indirect effect of GR on PCSK9 may therefore not be ruled out since GR has been reported to transactivate both HIF-α and SREBP.

Alternatively, the lack of significant change in PCSK9 may be due to reported wide variations in PCSK9 concentration. An analysis of studies reporting PCSK9 concentration shows a wide biological variation within and amongst studies suggesting this variation may underlie a lack of significant change observed in several human and animal studies (21).

Despite the lack of effect of dexamethasone on PCSK9, plasma concentration of low-density lipoproteins (LDL) was elevated in both male and female Wistar rats following dexamethasone administration. This is in line with some studies showing LDL elevation following administration of glucocorticoids (33). However, several other studies did not observe changes in LDL concentration following glucocorticoid administration (34,35). A careful review of these results suggests differences in LDL outcome following glucocorticoid administration may be due to differences in the type of glucocorticoid, duration and age of animals receiving glucocorticoid. While dexamethasone increased LDL concentration following high dose (10mg/kg) for 10 days (35), a much lower dose (0.5 mg/kg) produced the same effect even though for a longer duration (33). The lack of change in LDL concentration reported by (34) despite a high dose of dexamethasone 10 mg/kg may be due to oral administration. As observed in this study, dexamethasone consistently increases cholesterol concentration in animals administered a glucocorticoid (35,36). However, despite changes in cholesterol and LDL following dexamethasone administration, the strongest association was found between PCSK9 and triglycerides (Data not shown). This is in line with a human study reporting strong association of PCSK9 with triglyceride concentrations in an African population (28).

We observed sexually dimorphic effects of dexamethasone on some lipid parameters. While HDL was elevated in female rats exposed to the glucocorticoid, HDL levels remained unchanged in male rats. This is in accordance with the study of (37) that reported increased HDL concentrations after glucocorticoid administration in humans. This has usually been attributed to protective effects of female sex hormones – estrogen. While estrogen may appear as a negative regulator of PCSK9, testosterone may not (38). PCSK9 levels rise in menopausal women compared to premenopausal women - the rise is not seen in adult men of similar age. PCSK9 levels is also correlated with estrogen level fluctuations during menstrual cycle (39). Testosterone however has been reported to increase PCSK9 expression in hepatic cells (40) while pharmacological testosterone levels also lower PCSK9 in males (39). In this study, neither of the sex hormones appear to significantly influence circulating PCSK9.

However, a lack of change in PCSK9 levels in dexamethasone-treated rats does not preclude the evaluation of PCSK9 inhibitors in glucocorticoid-induced dyslipidemia. It has been observed than even in healthy subjects without elevated LDL and PCSK9 concentrations, PCSK9 inhibitors further lower LDL levels (18,41,42). PCSK9 inhibitors may therefore still be used as adjunct in glucocorticoid-treated patients that do not attain lipid targets despite already receiving statin therapy since statin therapy itself is known to upregulate PCSK9 which may contribute to limiting its efficacy (43). In conclusion, glucocorticoid-induced dyslipidemia appears not to be associated with elevated circulating PCSK9. However, more studies are needed to clarify the potential role of GCs in PCSK9 regulation and the therapeutic potential of PCSK9 inhibitors in stress and glucocorticoid-induced dyslipidemia.

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