

Inhibition of dipeptidyl peptidase-4 averts free fatty acids deposition in the hearts of oral estrogen–progestin contraceptive-induced hyperinsulinemic female rats

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Abstract: Free fatty acid (FFA) deposition in non-adipose tissues such as the heart is a characteristic of insulin resistant states which feature hyperinsulinemia and dipeptidyl peptidase-4 (DPP-4) activation. Estrogen–progestin oral contraceptives (OC) treatment reportedly increased DPP-4 activity in rat tissue, and DPP-4 inhibitors have anti-diabetic and anti-inflammatory properties. This study aims to investigate the effects of DPP-4 inhibition on cardiac FFA deposition in estrogen–progestin-treated female rats. From our data, estrogen–progestin OC exposure in female rats led to elevated plasma insulin, cardiac DPP-4 activity, FFA and triglyceride (TG) accumulation, TG/high-density lipoprotein cholesterol (TG/HDL-C) ratio, adenosine deaminase/xanthine oxidase/uric acid pathway (ADA/XO/UA), lipid peroxidation, glycogen synthase activity, and alanine phosphatase; whereas cardiac glucose-6-phosphate dehydrogenase, Na^+/K^+ -ATPase and nitric oxide (NO) were decreased. However, DPP-4 inhibition resulted in decreased plasma insulin, cardiac DPP-4 activity, FFA, TG, TG/HDL-C ratio, and alkaline phosphatase. These were accompanied by reduced ADA/XO/UA pathway, lipid peroxidation, and augmented NO and Na^+/K^+ -ATPase in estrogen–progestin OC-treated rats. DPP-4 inhibition attenuated cardiac lipid deposition accompanied by reduced activity in the ADA/XO/UA pathway in estrogen–progestin OC-treated female rats. DPP-4 is therefore a plausible therapeutic target in cardiometabolic disorders.

Key words: cardiac lipid accumulation, cardiometabolic disorders, dipeptidyl peptidase-4, hormonal contraceptive, hyperinsulinemia.

Résumé : Le dépôt d'acides gras dans les tissus non adipeux comme le cœur est une caractéristique d'états de résistance à l'insuline liés à l'hyperinsulinémie et à l'activation de la dipeptidyl peptidase-4 (DPP-4). On a rapporté que les contraceptifs oraux (CO) d'œstrogènes et de progestine entraînent une augmentation de l'activité de la DPP-4 dans le tissu de rat et que les inhibiteurs de la DPP-4 sont dotés de propriétés antidiabétiques et anti-inflammatoires. Cette étude porte sur les effets de l'inhibition de la DPP-4 sur le dépôt d'acides gras libres (AGL) dans le cœur de rates auxquelles était administré un composé d'œstrogène et de progestine. Nos données ont montré que l'administration de CO d'œstrogènes-progestine à des rates menait à une augmentation des taux d'insuline plasmatique et de l'activité de la DPP-4 cardiaque, de l'accumulation des AGL et des triglycérides (TG), du ratio TG sur les lipoprotéines de haute densité (TG/HDL), de la voie de signalisation adénosine désaminase/xanthine oxydase/acide urique, de la peroxydation lipidique, de l'activité de la glycogène synthase, de l'alanine phosphatase, avec en contrepartie une diminution des taux de glucose-6-phosphate déshydrogénase, de Na^+/K^+ -ATPase et d'oxyde nitrique (NO). Cependant, l'inhibition de la DPP-4 a entraîné un abaissement des taux d'insuline plasmatique, de l'activité de la DPP-4 cardiaque, des AGL, des TG et du ratio TG/HDL-C ainsi que de la phosphatase alcaline. Ces phénomènes s'accompagnaient d'une atténuation de la voie de signalisation adénosine désaminase/xanthine oxydase/acide urique (ADA/XO/AU) et de la peroxydation lipidique, avec une hausse du NO et de la Na^+/K^+ -ATPase chez les rates auxquelles était administré le CO d'œstrogènes-progestine. L'inhibition de la DPP-4 permettait d'atténuer le dépôt de lipides dans le cœur avec une diminution de l'activité de la voie de signalisation ADA/XO/AU chez les rates auxquelles était administré le CO d'œstrogènes-progestine. La DPP-4 constitue donc une cible thérapeutique plausible dans les troubles cardiométaboliques. [Traduit par la Rédaction]

Mots-clés : accumulation de lipides dans le cœur, troubles cardiométaboliques, dipeptidyl peptidase-4, contraceptif hormonal, hyperinsulinémie.

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Introduction

Cardiovascular disease (CVD) has become a major disease burden with increasing morbidity and mortality rates worldwide. The reported global prevalence by the International Diabetic Federation shows that over 463 million adults are diabetic and are at risk of developing CVD (Saeedi et al. 2019; IDF 2019). Metabolic syndrome is a cluster of metabolic disease risk factors which contribute to the onset of diabetes and its progression to CVD. Current reports reveal that more than 1 billion of the world's adult population have metabolic syndrome (Saklayen 2018). The burden of metabolic syndrome-related CVD is shown to be higher in women, particularly at menopause as a result of decreased level of estradiol (Ford et al. 2002; Sarafidis et al. 2006); however, women of reproductive age are susceptible to metabolic syndrome, of which hormonal birth control pill usage is a common risk factor. Over a hundred million women are on estrogen–progestin oral contraceptives (OC) that act through suppression of gonadotropin releasing hormone, inhibiting follicular development and preventing ovulation (Caserta et al. 2014). But the associated cardiovascular impairments observed with OC intake remains a serious public health issue among women.

Hyperinsulinemia is an important metabolic syndrome feature that results from impaired insulin action on metabolic organs (i.e., insulin resistance (IR) phenomenon). Normally, increased insulin secretion is a response to elevated blood glucose in IR, an attempt to preserve insulin sensitivity and thus maintain glycemic level; however, hyperinsulinemia has been associated with pro-atherogenic, atherothrombotic and oxidative injury effects in several models of CVD including OC-induced cardiometabolic disorder (Sowers 2004; Perkins et al. 2015). Elevated free fatty acids (FFAs) from the systemic IR promote defective tissue-level insulin signaling by triggering downstream pro-inflammatory mediators. This mechanism involves activation of toll-like receptors by FFAs resulting in production of inflammatory cytokines which ultimately disrupts insulin signaling. (De Souza et al. 2005; Senn 2006). Triglyceride (TG) accumulation due to excessive FFA influx into metabolic organs, in part, worsens tissue inflammation via overproduction of uric acid (UA). Lipotoxicity and disrupted β -oxidation of FFAs cause decrease in tissues adenosine and ATP/ADP ratio. Adenosine deaminase (ADA), an enzyme that is activated in altered energy metabolism, initiates the irreversible hydrolytic deamination of adenosine to inosine. Inosine is further deaminated to hypoxanthine which is later converted to UA through the action of xanthine oxidase (XO) (Puddu et al. 2012). Hyperuricemia is an established contributing phenotype in the pathogenesis of endothelial dysfunction and cardiac injury (Perticone et al. 2013). Moreso, TG/high-density lipoprotein cholesterol (TG/HDL-C) ratio is a key predictor of adverse cardiovascular outcomes in experimental rats and heart disease patients. HDL-C, an essential lipid transport particle, has antioxidant, anti-inflammatory, and insulin sensitivity enhancing properties. Hence, an increase in the ratio of TG compared with HDL-C denotes imbalance between pro-atherogenic and anti-atherogenic lipoproteins (Zhou et al. 2016).

Dipeptidyl peptidases (DPP4) inhibitors (DPP-4i) (also known as gliptins) are a new class of oral hypoglycemic drugs that inhibit the enzymatic activity of DPP4 (Zhong et al. 2015). DPP4 is an integral membrane protein of widespread expression in several tissues including renal, vascular endothelial, and intestinal and immune cells. It acts on substrate, glucagon-like peptide-1, an insulinotropic hormone, catalyzing its degradation, thereby blotting its anti-hyperglycemic effects (Zheng et al. 2014). Pre-clinical studies identified enhanced DPP4 activity in rat models of type 2 diabetes mellitus and further suggested that inhibition of DPP4 activity would improve cardiometabolic issues (Williams et al. 2015; Rufinatscha et al. 2017). Also, data from existing studies in our laboratory revealed the role of DPP4 activity in pregnant (Badmus et al. 2018) and non-pregnant (Omolekulo et al. 2018) rats with metabolic perturbations. In this study we aim to determine the effect of DPP4

inhibition in the hearts of estrogen–progestin OC-induced hyperinsulinemic female rats.

Materials and methods

Animals

The investigation was conducted in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and was approved by the Institutional Ethical Review Board of University of Ilorin, Nigeria, and every effort was made to minimize both the number of animals used and their suffering. Ten-week-old female Wistar rats were obtained from the animal house of the College of Health Sciences, University of Ilorin. Rats had unrestricted access to standard rat chow and tap water. After 1 week of acclimatization, the animals were randomly assigned to four groups ($n = 6/\text{group}$). Rats were maintained under standard environmental conditions of temperature, relative humidity, and 12-hour dark/light cycle.

Treatment

Control group received distilled water (vehicle; p.o.) daily. Combined OC-treated group (COC) received (p.o.) a combination of 1.0 μg ethinyl estradiol and 5.0 μg levonorgestrel (Wyeth–Ayerst, Inc., QC, Canada) daily. The DPP-4i group received (p.o.) 100 mg sitagliptin (Januvia, Turkey) daily. The COC+DPP-4i group received (p.o.) a combination of COC steroids plus sitagliptin daily. The treatment lasted for 8 weeks.

Sample preparation

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood was collected by cardiac puncture into heparinized bottle and was centrifuged at 3000 rpm (100.62g) for 5 min at room temperature. Plasma was stored frozen until needed for biochemical assay. The heart was excised, cleared of adhering connective tissues, blotted, and weighed immediately. After weighing, 100 mg of heart tissue was carefully removed and homogenized with a glass homogenizer in cold phosphate-buffered saline.

Fasting blood glucose (FBG) level and TG-glucose index (TyG)

Terminal FBG was determined after the rats had a 12-h overnight fast on the day of sacrifice. Blood glucose levels were determined with a glucometer (ONETOUCHR-LifeScan, Inc., Milpitas, CA, USA). TyG was expressed as $\text{Ln}[(\text{TG} \times \text{FBG})/2]$.

Biochemical assays

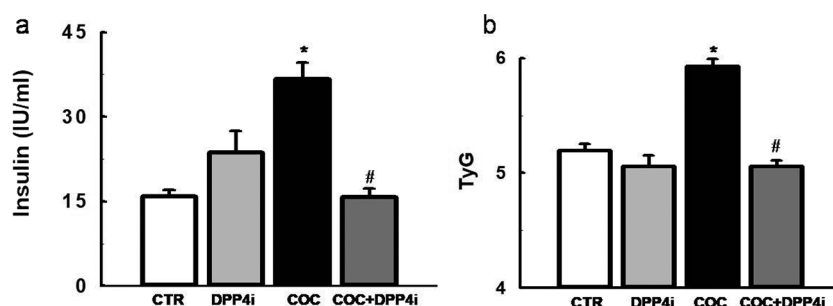
DPP-4 activity was estimated by fluorescence method using assay kit obtained from BioVision Inc. (Milpitas). Plasma insulin was determined using ELISA kit from Ray Biotech, Inc. (GA, USA). Cardiac FFA, TG, HDL-C, lactate, lactate dehydrogenase (LDH), glycogen, glycogen synthase, aspartate transaminase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase, glucose-6-phosphate dehydrogenase (G6PD), plasma urea, and creatinine were measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Antrim, UK). Cardiac UA level, ADA, and XO activities were measured by standardized enzymatic colorimetric method using assay kit obtained from Fortress diagnostics (Antrim, UK). Cardiac nitric oxide (NO) was measured by quantification of nitrite using Griess Reagent, and cardiac malondialdehyde, as marker of oxidative stress, was measured by standardized enzymatic colorimetric methods using assay kits from Oxford Biomedical Research, Inc. (Oxford, MI, USA). Nicotinamide dinucleotide phosphate (NADPH) and both reduced and oxidized glutathione were measured by spectrophotometric method. Na^+/K^+ -ATPase activity was determined by monitoring the hydrolysis of ATP to ADP using the coupled assay of Barnett. TG/HDL-C ratio was estimated as index of atherogenic dyslipidemia.

Table 1. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on body weight, cardiac weight, and fasting blood glucose level (FBG).

	CTR	DPP4i	COC	COC + DPP4i
Body weight (g)				
Week 0	123.3±4.3	120.0±6.2	125.9±2.8	117.1±2.5
Week 8	153.9±6.3	113.4±5.2*	138.9±4.7*	145.2±5.2#
Cardiac weight (g/kg)	4.7±0.1	4.8±0.5	4.5±0.5	3.5±0.1*#
Cardiac weight/tibia length (g/100 g)	21.4±0.7	15.0±0.1*	16.1±1.6	14.0±0.2*#
FBG (mmol/L)	4.2±0.4	4.0±0.2	4.1±0.2	3.9±0.4

Note: Data were presented as mean ± standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs. COC. Data were analyzed by two-way ANOVA and Bonferroni post hoc test. CTR, control; COC, combined oral contraceptive.

Fig. 1. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on insulin and triglyceride glucose index (TyG) in combined oral contraceptive (COC) – treated female Wistar rats. DPP-4i reduced (a) plasma insulin and (b) TyG. COC with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4i alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i. Data were presented as mean ± standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs. COC. Data were analyzed by one-way ANOVA and Bonferroni post hoc test.



Data analysis and statistics

All data were expressed as means ± standard error of the mean. Statistical group analysis was performed with SPSS statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparisons of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

Results

DPP-4i increases and decreases body and cardiac weight, respectively, in COC-treated rats

COC and DPP-4i treatment led to a significant reduction in body weight when compared with control; however, COC+DPP-4i treatment significantly increased body weight when compared with COC-treated rats. Cardiac weight was not significantly affected by COC treatment compared with control, whereas COC+DPP-4i treatment significantly reduced cardiac weight and cardiac weight/tibia length when compared with COC-treated rats. FBG level was not affected in all experimental groups (Table 1).

DPP-4i improves glucoregulation in COC-treated rats

There was a significant increase in plasma insulin level in COC-treated rats when compared with control. Nevertheless, DPP-4i treatment in COC-treated rats reduced plasma insulin level when compared with COC-treated rats (Fig. 1a). The TyG index, a marker of glucose regulation, was significantly increased in COC-treated rats when compared with control (Fig. 1b); however, COC+DPP-4i treatment significantly reduced TyG index compared with COC-treated rats.

DPP-4i attenuated cardiac lipid accumulation in COC-treated rats

Treatment with COC increased cardiac FFA, TG, and TG/HDL-C ratio compared with control (Fig. 2). Treatment with DPP-4i

increased cardiac FFA but not TG and TG/HDL-C ratio; however, COC+DPP-4i treatment did not significantly affect cardiac FFA but significantly reduced cardiac TG and TG/HDL-C ratio compared to COC-treated rats (Figs. 2a and 2b).

DPP-4i reduced UA production, lipid peroxidation, and increased NO levels

Cardiac UA level, ADA, and XO activities were increased in COC-treated rats when compared with control (Figs. 3a and 3c). Lipid peroxidation and NO also increased in the heart of COC-treated rats (Figs. 3d and 3e). Treatment of animals with only DPP-4i increased ADA and UA level but not XO activity, whereas lipid peroxidation and NO levels were not affected by the treatment. COC+DPP-4i treatment significantly reduced cardiac UA level, ADA, and XO activities when compared with COC-treated rats.

DPP-4i reduces cardiac lipid peroxidation, increases cardiac NO bioavailability, and improves liver functions in COC-treated rats

Cardiac AST and ALP activities were significantly increased in COC-treated rats when compared with control, whereas COC+DPP-4i treatment significantly reduced cardiac AST and ALP activities when compared with COC-treated rats. Cardiac gamma-glutamyl transferase was comparable among the experimental group (Fig. 4).

DPP-4i has no effect on cardiac lactate and glycogen production, and LDH activity but increases glycogen synthase activity in COC-treated rats

Cardiac LDH activity and lactate and glycogen levels were comparable among the experimental groups (Figs. 5a and 5b); however, cardiac glycogen synthase activity was significantly increased in COC-treated rats when compared with control whereas COC+DPP-4i treatment significantly reduced glycogen synthase activity when compared with COC-treated rats (Figs. 5c and 5d).

Fig. 2. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on cardiac free fatty acid (FFA), triglyceride (TG), and TG/high-density lipoprotein cholesterol (HDL-C) ratio. DPP-4i reduced (a) cardiac FFA, (b) TG, and (c) TG/HDL. Combined oral contraceptive (COC) with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4i alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i. Data were presented as mean $\pm$ standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs. COC. Data were analyzed by one-way ANOVA and Bonferroni post hoc test.

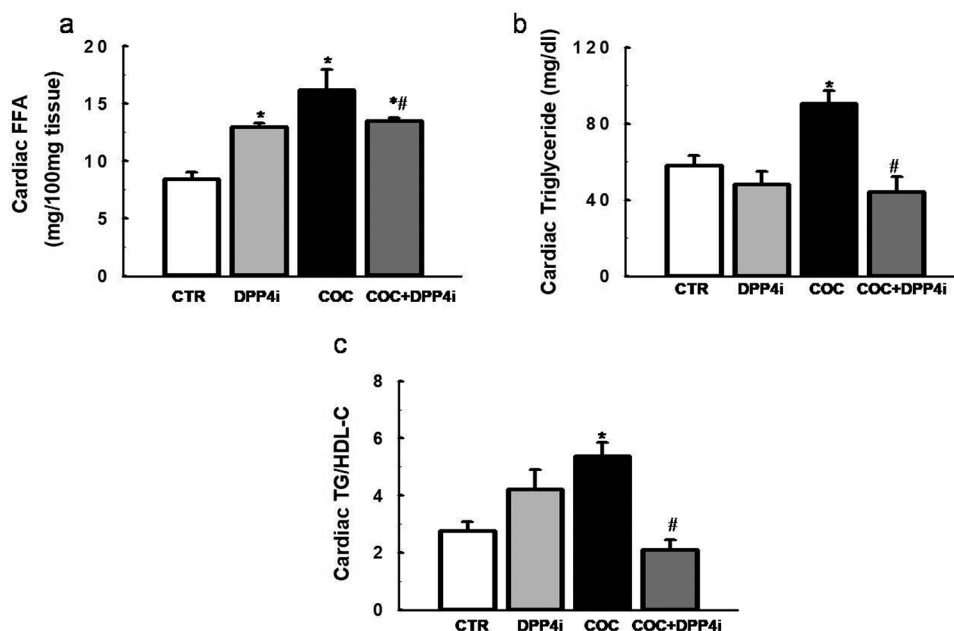


Fig. 3. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on cardiac uric acid (UA) production, malondialdehyde (MDA) and nitric oxide (NO). DPP-4i reduced ((a), (b) and (c)) UA, xanthine oxidase (XO), and adenosine deaminase (ADA), reduced (d) lipid peroxidation MDA, and increased (e) NO. Combined oral contraceptive (COC) with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4i alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i inhibitor. Data were presented as mean $\pm$ standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs. COC. Data were analyzed by one-way ANOVA and Bonferroni post hoc test.

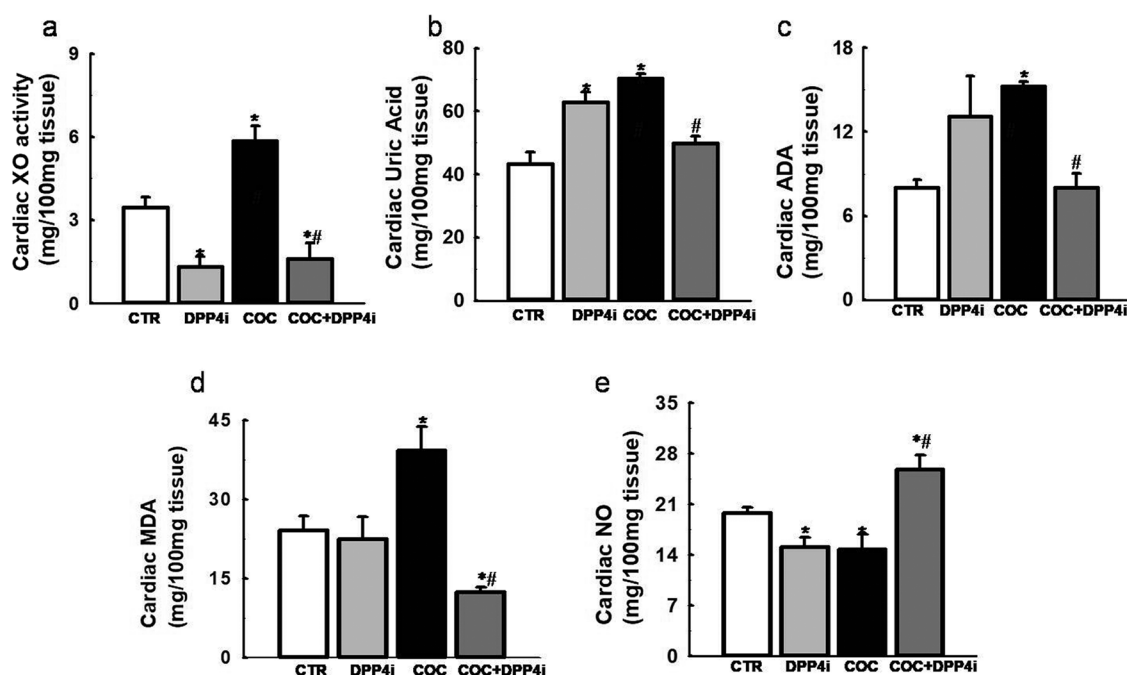


Fig. 4. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on cardiac alkaline phosphatase (ALP), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT). DPP-4i reduced (a) cardiac ALP and (b) AST, but did not affect (c) GGT. Combined oral contraceptive (COC) with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4i alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i. Data were presented as mean $\pm$ standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs. COC. Data were analyzed by one-way ANOVA and Bonferroni post hoc test.

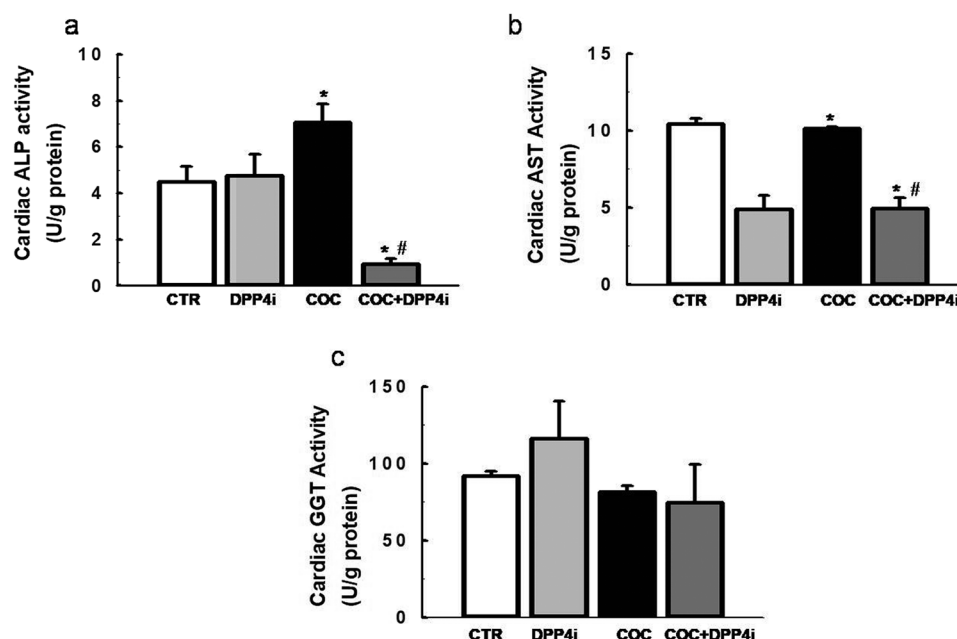


Fig. 5. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on cardiac lactate production and glycogen synthesis. DPP-4i did not affect cardiac (a) lactate production and (b) lactate dehydrogenase (LDH) activity. Glycogen synthesis did not affect (c) cardiac glycogen but increased (d) glycogen synthase activity. Combined oral contraceptive (COC) with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4i alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i. Data were presented as mean $\pm$ standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs. COC. Data were analyzed by one-way ANOVA and Bonferroni post hoc test.

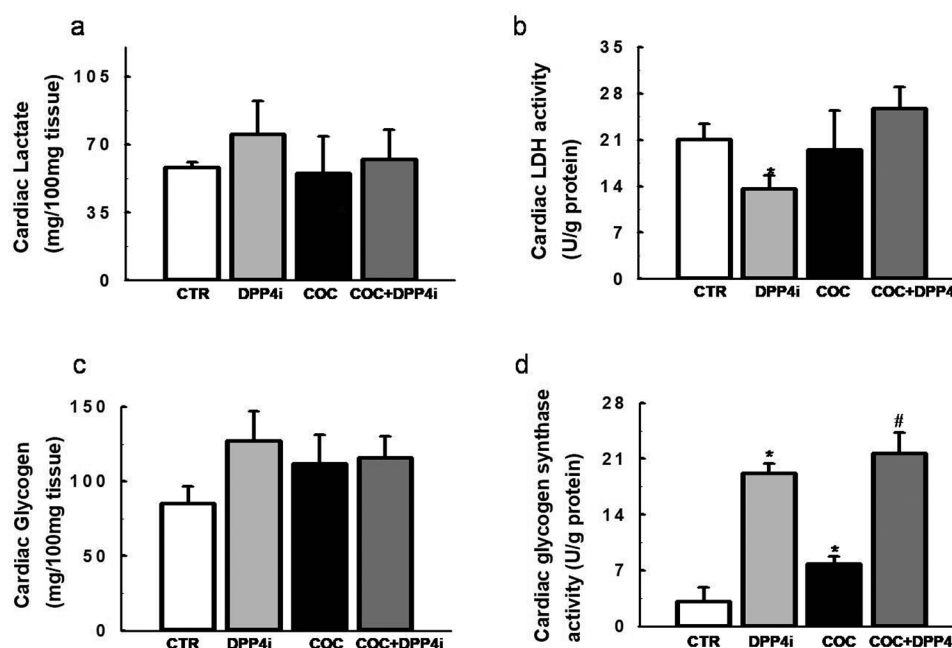
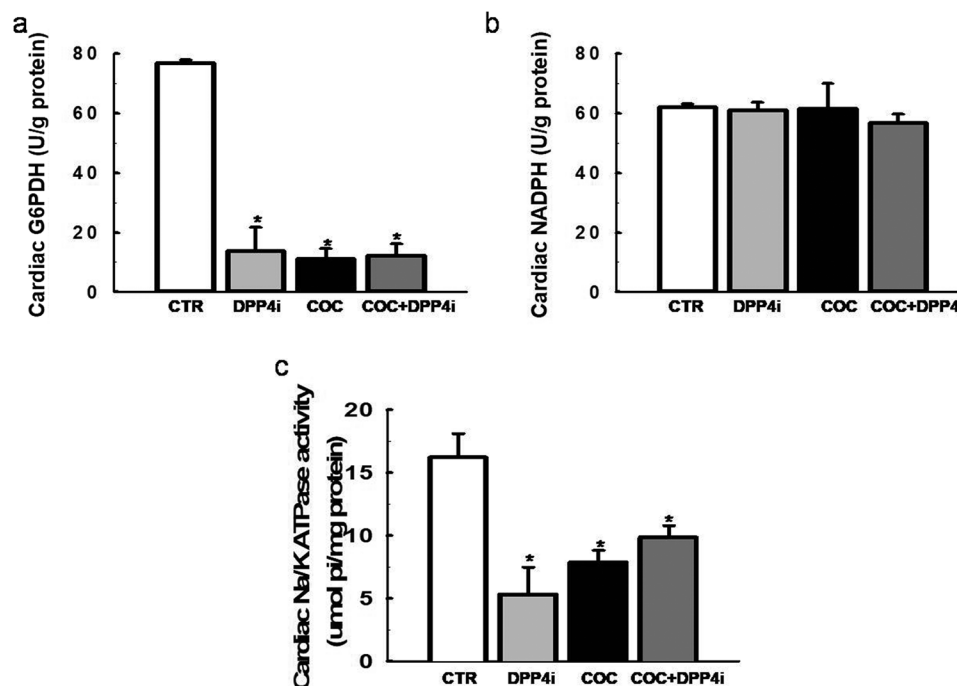


Fig. 6. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on cardiac glucose-6-phosphate dehydrogenase (G6PDH), reduced nicotinamide adenine dinucleotide phosphate (NADPH) and Na^+/K^+ -ATPase activity. DPP-4i did not affect (a) G6PDH, (b) NADPH, and (c) Na^+/K^+ -ATPase activity. Combined oral contraceptive (COC) with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4 inhibitor alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i. Data were presented as mean $\pm$ standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs COC. Data were analyzed by one-way ANOVA and Bonferroni post hoc test.



DPP-4i has no effect on G6PDH activity in COC-treated rats

NADPH activity was comparable among the experimental groups, whereas G6PDH and Na^+/K^+ ATPase activities were significantly reduced in all treated groups (Fig. 6).

DPP-4i suppress DPP-4 activity in COC-treated rats

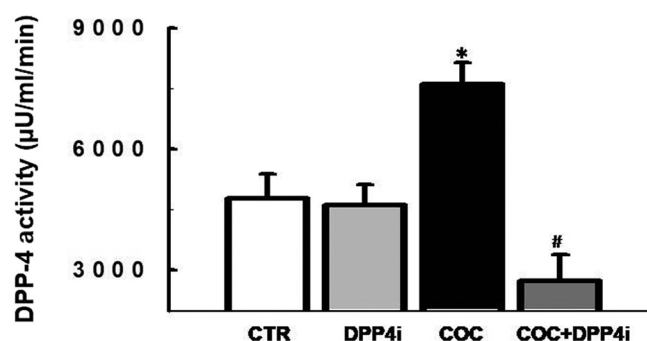
COC treatment led to a significant increase in DPP-4 activity when compared with control which was significantly reduced by COC+DPP-4i treatment compared with COC-treated group (Fig. 7).

Discussion

The current results show that treatment with estrogen-progestin OC caused hyperinsulinemia that is accompanied by lipid accumulation, activation of ADA/XO/UA pathway, increased tissue injury markers, and depressed activities of G6PDH and Na^+/K^+ -ATPase in the heart. These changes were associated with increased activity of DPP-4 enzyme in the heart of estrogen-progestin OC-treated rats; however, inhibition of DPP-4 activity by sitagliptin attenuated estrogen-progestin OC-induced hyperinsulinemia and accumulation of cardiac lipids and UA, without averting depressed cardiac G6PDH and Na^+/K^+ -ATPase activities.

Poor cardiometabolic profile in women and female experimental rodents on estrogen-progestin OC steroids remains a major health threat that has been consistently reported (Le-Ha et al. 2013; Olatunji et al. 2016a). The TyG index is an indicator of disrupted glucose and lipid regulation. The prediction of metabolic syndrome by TyG has been shown to be stronger than plasma insulin, glucose, or TG in experimental models and humans (Unger et al. 2014; Kang et al. 2017). Our data shows that estrogen-progestin OC led to hyperinsulinemia and increased TyG index that is not associated with increased body weight gain and cardiac weight. This suggests that the dysmetabolism induced by OC in this study is not due to body weight gain or related to cardiac hypertrophy as

Fig. 7. Effect of dipeptidyl peptidase-4 inhibitor (DPP-4i) on cardiac DPP-4 activity. DPP-4i reduced cardiac DPP-4 activity. Combined oral contraceptive (COC) with or without DPP-4i and DPP-4i alone were administered simultaneously for 6 weeks. CTR: animals treated with distilled water orally; DPP-4i: animals treated with DPP-4i alone; COC: animals treated with combined oral contraceptive; COC+DPP-4i: animals treated with COC plus DPP-4i. Data were presented as mean $\pm$ standard error of mean and $p < 0.05$ was taken as statistically significant. * $p < 0.05$ vs. CTR; # $p < 0.05$ vs COC Data were analyzed by one-way ANOVA and Bonferroni post hoc test.



observed in previous studies (Michael et al. 2020; Adegoke et al. 2020).

Elevated insulinemia has been shown to establish a pro-inflammatory state due to defective insulin receptor signaling leading to dysregulation of the mitogen activated protein kinase pathway. In this pathogenic mechanism, plasma insulin acts as a growth factor that mediates downstream production of inflammatory cytokines which may in turn impair insulin receptor substrate phosphorylation (Wang et al. 2004). The pathophysiology

of OC-induced IR-related cardiac dysfunction is not completely understood, but it involves triggering of inflammatory and oxidative stress pathways by excessive UA generation in the heart (Chen and Kotani 2012; Olaniyi et al. 2020; Michael et al. 2020). Adenosine, an important glucose and lipid signaling-enhancing molecule, is depleted by ADA in the heart, a process of catalyzing the initial step of purine degradation. Increased activity of XO completes the last two steps leading to UA generation. Besides, XO is a sole inducer of superoxide generation during the process of producing UA (Puddu et al. 2012). Hence, the increased activity of cardiac ADA and XO activities including elevated UA implies activation of ADA/XO/UA pathway in the heart. This may provide explanation for increased lipid peroxidation and depressed activity of G6PDH.

In addition, increased production of UA may worsen cardiac lipid status by altering cardiomyocytes mitochondrial functions. Elevated UA production may result in mitochondrial oxidative stress and cause lipid accumulation (Lin et al. 2009). Also, increased cardiac TG may be facilitated by elevated FFA influx from circulation and (or) impaired tissue oxidation of FFAs. In line with these, our findings reveal that elevated insulinemia induced by OC may underscore excess deposits of FFA and TG accumulation in the heart possibly through activation of ADA/XO/UA pathway. Nevertheless, cardiac tissue lipotoxicity has been reported in cardiomyopathy and failing heart in humans and in experimental metabolic syndrome (Sharma et al. 2004; McGavock et al. 2007; Puliniakunnil et al. 2013). An increase in the ratio of TG to HDL-C concentration was also observed in OC-treated rats in this study which accentuates a growing lipotoxicity as the glucoregulatory, antioxidant, and anti-atherogenic HDL-C was reduced relative to the oxidative and inflammatory TG. This condition predisposes the heart to lipid peroxidation and tissue damage.

Cardiovascular tissues are usually vulnerable to peroxidation of lipid products in perturbed metabolism due to increased oxidants' reaction with lipids, yielding species that are capable of causing inflammatory damage to cells particularly of the vascular endothelium and myocardium (Anderson et al. 1995). Also, elevated cardiac UA as realized with OC treatment in the present study can cause NO deficiency, a risk factor for endothelial dysfunction and tissue damage by activating arginase (Zharikov et al. 2008) and (or) decreasing the interaction between endothelial NO synthase and calmodulin (Park et al. 2013). In congruence, OC treatment in this study presents with increased level of malondialdehyde and depressed NO concentration in the heart. The mechanistic role of NO in the regulation of cardiomyocellular electrolyte homeostasis and contractile function is inconclusive. Some reports hypothesized the modulatory role of NO synthesis on Na^+/K^+ -ATPase functional activity (Zhou et al. 2000). But it is well known that alteration in Na^+/K^+ -ATPase activity is associated with cardiovascular pathologies such as hypertension and heart disease (Müller-Ehmsen et al. 2002). Similarly, we observed that OC-treatment elicits a decrease in the activity of Na^+/K^+ -ATPase in the heart of female rats which may be due to injurious modifications by oxidants. The present study showed that markers of tissue injury, ALP and AST, were elevated in the heart of OC-treated rats. Importantly, our OC model of cardiac dysmetabolism showed increased activity of DPP-4 enzyme indicating its likely involvement in OC-related cardiac dysfunction. DPP-4 activity is reported to play significant role in inflammatory and metabolic dysregulation associated with type 2 diabetes condition (Ben-Shlomo et al. 2013).

In cardiometabolic disorders, DPP-4 is an emerging therapeutic target due to its ability to degrade cardioprotective incretins glucagon-like peptide-1 and gastric inhibitory peptide. Reports from our laboratory and others have shown that the inhibition of DPP4 confers improved glucoregulation and protects liver from metabolic damage (Ikeda et al. 2016; Omolekulo et al. 2018); however, the role of DPP-4 in OC-induced perturbed cardiac lipid

metabolism is not completely understood. This study provides evidence that increased DPP-4 activity is involved in cardiac lipid accumulation caused by OC treatment. Here, inhibition of DPP-4 activity by sitagliptin in OC-treated female rats decreased plasma insulin and TyG index as expected. Nevertheless, sitagliptin also depressed cardiac DPP-4 activity and resolved cardiac lipid accumulation in OC-treated rats. This effect of DPP-4 inhibition is accompanied by normalized cardiac ADA/XO/UA pathway which led to reduced cardiac UA levels. In line with this finding, a previous study from our laboratory had shown that DPP-4 inhibition by sitagliptin attenuated hepatic ADA/XO/UA pathway activity in OC-treated animals (Omolekulo et al. 2019). In addition, DPP-4 inhibition also attenuated cardiac lipid peroxidation, injury markers, and augmented NO level but did not restore cardiac G6PDH and Na^+/K^+ -ATPase activities in OC-treated rats. This shows that DPP-4 inhibition in OC-treated animals could resolve cardiac hyperinsulinemia-related UA and lipid dysmetabolism irrespective of the G6PDH level and Na^+/K^+ -ATPase activities in the heart. Despite the fact that deficiency of G6PDH can predispose cells to oxidative damage, it is surprising that DPP-4 inhibition could avert the oxidant-releasing elevated ADA/XO/UA pathway, lipid peroxidation, and injury markers without affecting G6PDH. As DPP-4 inhibition also depressed cardiac G6PDH and Na^+/K^+ -ATPase activities in animals that were not treated with OC, and as these molecules play important roles in cardiac functions, there is need to further investigate appropriateness of the use of sitagliptin in the management of cardiomyopathy.

In conclusion, our data validates that estrogen-progestin OC treatment to female animals causes hyperinsulinemia with elevated cardiac lipid accumulation, depressed NO levels, and reduced G6PDH and Na^+/K^+ -ATPase activities through elevated ADA/XO/UA pathway and DPP-4 activities. DPP-4 inhibition by sitagliptin in OC-treated female rats has cardio-protective effects mediated via suppression of UA production and lipid accumulation independent of G6PDH and Na^+/K^+ -ATPase activities.

Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Conflict of interest

Authors state no conflict of interest.

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