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Drospirenone-containing contraceptive exerts positive effects on cardiac uric acid and PAI-1 but not GSK-3: Improved safety profiles in contraception?

Oluwaseun A. Adeyanju[a,b], Olaniyi A. Soetan[a], Lawrence A. Olatunji[a,*]

[a] HOPE Cardiometabolic Research Team, Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria
[b] Cardiometabolic Research Unit, Department of Physiology, Afe Babalola University, Ado-Ekiti, Nigeria

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ABSTRACT

The use of combined oral contraceptives (COC) have been associated with increased risk of adverse cardiovascular events and elevated cardiac and circulating plasminogen activator inhibitor-1 (PAI-1) and glycogen synthase kinase-3 (GSK-3) have been implicated in these events. Contraceptives containing drospirenone, a progestin with anti-androgenic actions may have a positive or neutral effect on cardiac PAI-1 and GSK-3 levels. Studies on the favorable effects of oral contraceptives containing drospirenone when compared with other androgenic contraceptives have not been fully elucidated. We therefore sought to compare the effect of a contraceptive containing ethinyl estradiol and drospirenone (DSP) with a contraceptive containing ethinyl estradiol and levonorgestrel (LVG) on cardiac uric acid (UA), PAI-1, GSK-3 and some hematological parameters. Ten weeks old female Wistar rats were divided into three groups; control (CON), LVG or DSP treated rats. The treatment lasted for 8 weeks. Results showed that LVG and not DSP treatment led to increase in plasma and cardiac tissue UA, plasma and cardiac PAI-1 as well as granulocyte-lymphocyte ratio (GLR) and platelet-lymphocyte ratio (PLR). However, the DSP treatment affected the circulating GSK-3. Taken together, the findings showed that LVG and not DSP affected cardiac UA and PAI-1. These results suggest that COC containing drospirenone appears to have positive effects on cardiac UA and PAI-1 levels but do not affect GSK-3, hence, COC containing drospirenone may be a better and safer means of contraception compared to androgenic contraceptives.

1. Introduction

Since their introduction over fifty decades ago, the use of combined hormonal oral contraceptives (COCs) have been related to increased risk of cardiometabolic disorder of which the exact mechanism by which they do this is still unclear, although it is most likely multifactorial and complex [1,2]. Understanding of this mechanism is very important due to the fact that a large number of women in their reproductive age are using the COC [2] as it remains one of the most effective reversible contraceptive methods [3], howbeit, with unresolved cardiometabolic disorder despite newer generations [4–6]. When evaluating the different types of COCs, attention has been drawn to a formulation containing drospirenone (DSP), a progestin derived from spironolactone with anti-mineralocorticoid and anti-androgenic actions [7]. DSP is an analog of spironolactone, with biochemical and pharmacological profiles that are similar to those of endogenous progesterone, with an antimineralocorticoid property and a mild anti-androgenic activity [8]. The use of EE in low doses associated with DSP have been used as contraceptives for more than a decade [9,10] but their effects and/or comparison with other COCs have not been fully elucidated.

Glycogen synthase kinase-3 (GSK-3) is a serine/threonine kinase [11]. It serves to phosphorylate and inactivate glycogen synthase, aside its numerous other functions. Numerous evidences support the role of elevated GSK-3 as a contributing factor in various pathophysiological states [12,13]. Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor that controls the fibrinolytic system by inhibiting tissue-type and urokinase plasminogen activators [14,15]. Increased plasma PAI-1 is suggested to represent a risk factor for cardiovascular disease [16,17]. More so, high plasma level of uric acid accompanies cardiovascular disease (CVD) risk factors [18] and although it is an established true mediator/causal risk factor for CVD [19], its underlying pathophysiological mechanism is yet to be fully elucidated.

* Corresponding author at: Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin, 240003, Nigeria.
E-mail address: tunjilaw@unilorin.edu.ng (L.A. Olatunji).

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DSP-containing COC, because of their unique pharmacological profile that combines a potent progestogenic activity with antiandrogenic and antimineralocorticoid activities [20], have been beneficial [21,22], however, there is little information on the effects of non-androgenic COC on cardiac PAI-1, GSK-3 or hematological indices. We therefore sought to compare the effect of a contraceptive containing ethinyl estradiol and drospirenone (DSP) with a contraceptive containing ethinyl estradiol and levonorgestrel (LVG) on cardiac uric acid (UA), PAI-1, GSK-3 and some hematological parameters.

2. Materials and methods

2.1. Animals

All experimental procedures were approved by the University of Ilorin Ethical Review Committee (UIERC) in accordance with guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and every effort was made to minimize both the number of animals used and their suffering. Ten weeks old female Wistar rats were used for the study. Rats had unrestricted access to standard rat chow and tap water. After one week of acclimatization, the animals were randomly assigned to 3 groups of n = 5 each. Rats were maintained under standard environmental conditions of temperature, relative humidity, and 12 -h dark/light cycle.

2.2. Treatments

Control group received distilled water (vehicle; *po*), COC-treated groups received (*po*) a combination of 1.0 μg ethinylestradiol and 5.0 μg levonorgestrel (Wyeth-Ayerst, Inc., Montreal, Canada) and a combination of 1.0 μg ethinylestradiol and 0.3 mg drospirenone (Schering AG, Berlin, Germany) respectively. The treatments were daily and lasted for 8 weeks.

2.3. Sample and tissue preparations

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). For the tissue, the heart was excised, cleared of adhering connective tissues, blotted and weighed immediately. After weighing, 100 mg of cardiac tissue was carefully removed and homogenized with a glass homogenizer. The homogenate was used for the measurement of cardiac PAI-1, GSK-3 and UA levels. Blood was collected by cardiac puncture into EDTA-coated and heparinized bottles, accordingly. Blood collected in heparinized bottle was centrifuged at 3000 rpm for 5 min. Plasma was stored frozen until needed for biochemical assay. Hematological parameters were determined using a multi-parameter, automated hematological analyzer, URIT-3010 (Xianyang, China) after collecting the blood in EDTA-coated bottles.

2.4. Visceral adiposity

Visceral fat pads were collected by mid-line section of the rats after anesthesia (pentobarbital sodium 50 mg/kg *i.p*). The fat pads collected were peri-renal, abdominal and peri-uterine fats. The fat pads were weighed and final visceral fat mass was adjusted for body weight. The quantification of the adiposity was determined in a blinded manner.

2.5. Biochemical assays

The plasma levels of PAI-1 and GSK-3 were estimated using ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China). Plasma uric acid (UA) was estimated by standardized enzymatic colorimetric methods using assay kit obtained from Fortress Diagnostics Ltd. Antrim, UK.

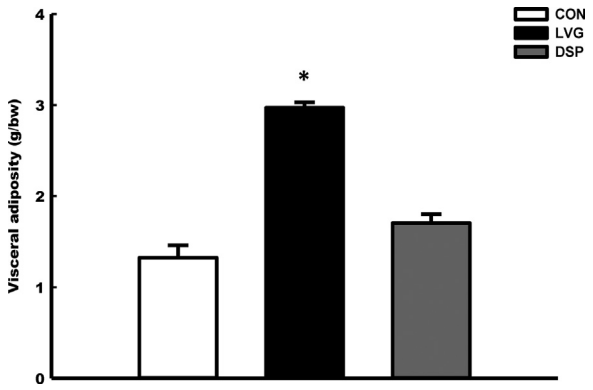

Fig. 1. Effect of ethinylestradiol with levonorgestrel (LVG) or drospirenone (DSP) treatment on visceral adiposity. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 5 rats per group (*$p < 0.05$ *vs* CON).

Table 1
Effect of Ethinylestradiol with levonorgestrel and drospirenone on hemogram in rats.

	CON	LVG	DSP
WBC ($10\hat{9}$ cells/L)	5.6 ± 0.3	5.3 ± 1.5	10.0 ± 0.1*
LYM ($10\hat{9}$ cells/L)	5.4 ± 0.3	3.6 ± 1.1*	7.9 ± 0.3*
GRAN ($10\hat{9}$ cells/L)	1.0 ± 0.3	1.3 ± 0.3	1.6 ± 0.3
RBC ($10\hat{1}2$ cells/L)	7.1 ± 0.3	7.2 ± 0.4	6.9 ± 0.2
HCT (%)	37.3 ± 0.3	54.9 ± 1.9*	38.7 ± 1.2
GRAN ($10\hat{9}$ cells/L)	376.7 ± 96.8	565.0 ± 28.6*	476.7 ± 46.4*

Data are expressed as mean ± SEM of 5 rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test (*p < 0.05 *vs* CON).

2.6. Statistical analysis

All experimental data were expressed as means ± SEM. Statistical significance for measured variables was determined by one-way analysis of variance (ANOVA) for the comparison of the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. The significance level was set at 0.05. Statistical group analysis was performed with SPSS statistical software.

3. Results

3.1. Effects of LVG and DSP on visceral adiposity and UA

LVG treatment led to significant increase in visceral adiposity compared with the control whereas DSP treatment did not affect the visceral adiposity (Fig. 1). Furthermore, LVG treatment led to increased plasma and heart uric acid whereas, DSP had no effect on plasma and heart UA, compared with the control (Fig. 2a & b).

3.2. Effects of LVG and DSP on hematological parameters

DSP treatment led to significant increase in white blood cell and lymphocyte counts respectively compared with the control. LVG led to increase in platelet count and percentage hematocrit levels as well (Table 1). Platelet to lymphocyte ratio (PLR) was elevated in LVG compared with the control (Fig. 3).

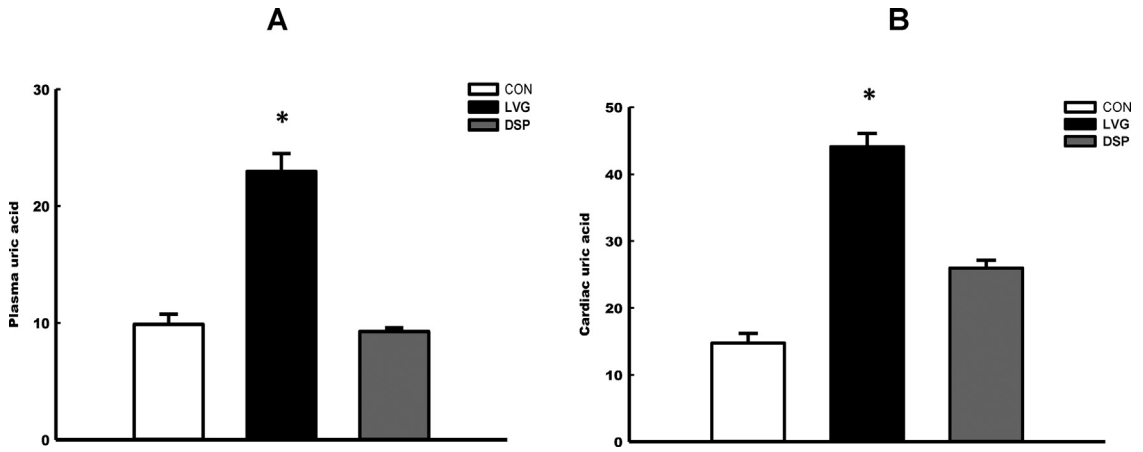

Fig. 2. Effect of ethinylestradiol with levonorgestrel (LVG) or drospirenone (DSP) treatment on plasma (A) and cardiac (B) uric acid. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 5 rats per group (*$p < 0.05$ *vs* CON).

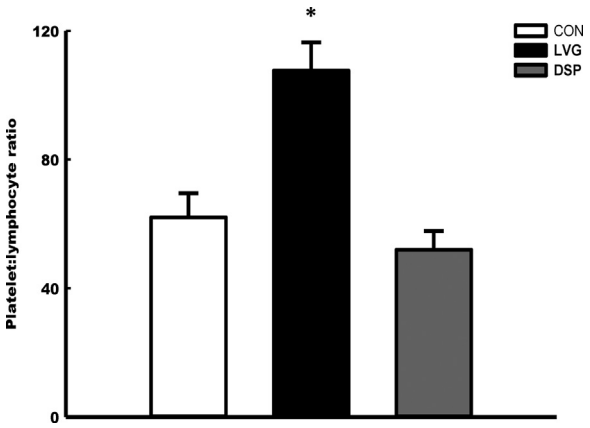

Fig. 3. Effect of ethinylestradiol with levonorgestrel (LVG) or drospirenone (DSP) treatment on platelet:lymphocyte ratio (PLR). Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 5 rats per group (*$p < 0.05$ *vs* CON).

3.3. Effects of LVG and DSP on plasma and cardiac PAI-1 and GSK-3

LVG treatment led to significant increase in plasma and cardiac plasminogen activator inhibitor-1 (PAI-1) compared with the control. DSP had no effect on the cardiac and plasma PAI-1 (Fig. 4a & b). Glycogen synthase kinase-3 (GSK3) was significantly raised in the cardiac and plasma of the group treated with LVG and DSP compared with CON (Fig. 5a & b).

4. Discussion

The results of the present study demonstrate that combined oral contraceptives (COCs) containing ethinylestradiol and levonorgestrel (LVG) caused an increase in visceral adiposity level which was accompanied by alteration in some hematological indices, elevated PLR, together with plasma and cardiac uric acid (UA), PAI-1 and GSK-3 levels. However, use of COC containing ethinylestradiol and drospirenone (DSP) did not affect these parameters except plasma and cardiac GSK-3.

Oral contraception remains one of the most effective reversible contraceptive methods commonly used worldwide by over 100 million women [3]. The adverse contribution of COC to the pathogenesis of cardiometabolic disorders remains unresolved despite reduction in the ethinylestradiol dosage and recently, change in progestin type [5]. However, the pathophysiological role of hyperuricemia, high cardiac PAI-1 and GSK-3 levels in cardiometabolic disorder and the fact that these can be induced by the use of COC calls for a COC with lesser negative effect to be used as this holds great clinical and public health relevance.

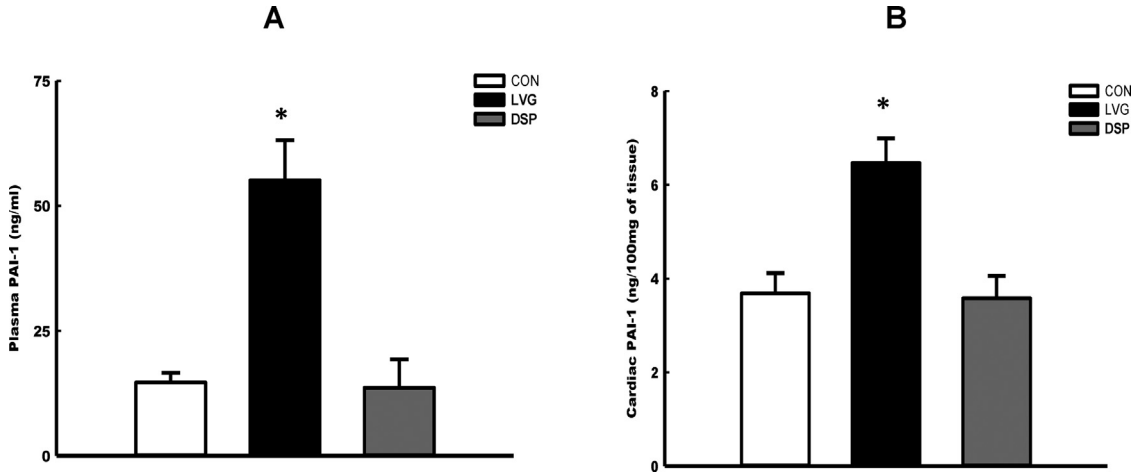

Fig. 4. Effect of ethinylestradiol with levonorgestrel (LVG) or drospirenone (DSP) treatment on plasma (A) and cardiac (B) PAI-1. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 5 rats per group (*$p < 0.05$ *vs* CON).

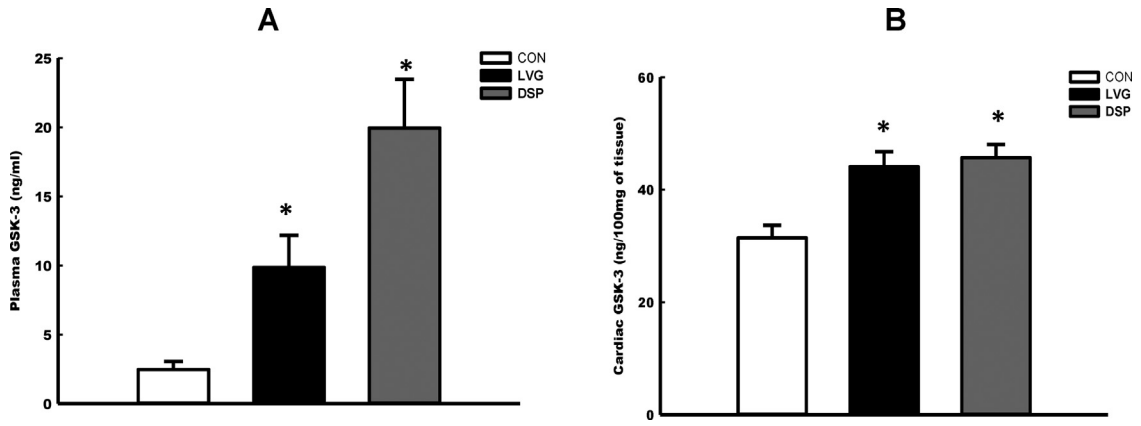

Fig. 5. Effect of ethinylestradiol with levonorgestrel (LVG) or drospirenone (DSP) treatment on plasma (A) and cardiac (B) GSK-3. Data were analyzed by one-way ANOVA followed by Bonferroni *post hoc* test. Values are expressed as mean ± SEM of 5 rats per group (*$p < 0.05$ *vs* CON).

Visceral fat accumulation is a located upstream of metabolic syndrome/disorders [23]. The result of this study shows that DSP has a positive effect on the visceral adiposity as against LVG. More so, hyperuricemia has been considered a factor that frequently accompanies metabolic disorder [18] and in fact a causal risk factor for atherothrombotic cardiovascular disease (CVD) such as myocardial infarction, hypertension, preeclampsia, and stroke [19,24,25]. DSP did not alter the uric acid level as against LVG which resulted in hyperuricemia both in the cardiac tissue and plasma. This further shows the effectiveness of DSP over LVG. This is in consonance with a recent study [22] in which antiandrogenic COC has no effect on CVD risk factors. A possible mechanism could be that DSP component of the COC mimics endogenous progesterone which can attenuate the vascular effects of ethinylestradiol component and can therefore block the effect [7].

Hemogram an easily available and inexpensive test that provides important data regarding the quantitative and qualitative properties of various blood cells has been shown to be important in CVD [26]. Platelets (PLTs) are reported to be important in CVD risk factors [27,28]. However, despite all these conditions, CV occurrences in relation to PLT count has not been detailed and conclusive [29,30]. Thus, PLT counts has emerge as another potentially useful marker of CVD risk. Lymphocytes (LYMs) have been shown to play a broader role in the modulation of the inflammatory response at each phase of the atherosclerotic process [31]. Lymphopenia has been considered a worse prognosis of systemic stress and other CVD risk factors [32] in experimental [33] and clinical [34] studies. LVG led to a decrease and increase respectively in LYM and PLT count respectively whereas opposite effects were observed in the DSP treated group, further emphasizing the positive effect of DSP compared with LVG.

In our study, LVG led to an increase in PLT/LYM ratio. The interactions between PLTs and LYMs have been found to be one of the main pathophysiologic mechanisms involved in the development of atherothrombotic events [35,36]. Because the PLT/LYM is a ratio, it is relatively more stable than individual blood parameters that can be altered by several variables like dehydration, overhydration and blood specimen handling. More so, the advantage of the ratio could be that it reflects the condition of both aggregation and inflammatory pathways, and it may be more valuable than either PLT or LYM count alone in the prediction of various inflammatory diseases including CVD [37,38]. Hence, PLT/LYM ratio is a pathophysiologic mechanism for the development of thrombogenesis [35]. PLT/LYM ratio in DSP treated group was comparable with the control.

Reports exist that elevated GSK-3 can cause CVD risk factors [39] and its inhibition can reduce the risks [40]. Since its discovery over three decades ago, GSK-3 has been implicated in the regulation of many additional biological processes apart from glycogen metabolism. Most of the diseases and/or disorders linked to CVD have a common denominator; GSK-3 [41,42]. It regulates several physiologic processes and their dysregulation are proposed to be involved in the pathogenesis of numerous diseases [42]. The potential role of GSK-3 in diseases has led to the proposal that therapeutic target of GSK-3 is beneficial and relevant [43], hence, cardiac or plasma GSK-3 is a strong mediator of CVD [44]. Additionally, increased plasma PAI-1 is suggested to represent a risk factor for CVD [16,17]. The myocardium is a key insulin-responsive tissue and is thus particularly vulnerable to diabetic glucose/insulin homeostatic shifts. Multiple studies have demonstrated the central importance of PAI-1 as a profibrotic mediator in cardiac and vascular tissues [45][41] as it also promotes clot formation, thereby contributing to myocardial infarction, stroke, and other atherothrombotic CVD events [46][42]. More so, numerous studies have implicated dysregulation of GSK-3 in the pathogenesis of IR, type 2 diabetes and myocardial diseases [12,13,43]. Increase in circulating and cardiac PAI-1 and GSK-3 in LVG rats in this study are consistent with previous studies that connected cardiac diseases to elevated GSK-3 and PAI-1 [44,47–49]. LVG resulted in increased level of cardiac and plasma PAI-1 and GSK-3 respectively. Whereas DSP did not cause an increase in PAI-1, it did aggravate the cardiac and plasma GSK-3.

In conclusion, the present study demonstrates that ethinylestradiol with levonorgestrel and not with drospirenone leads to increase in cardiac and circulating PAI-1. The beneficial effects of ethinylestradiol with drospirenone are however not associated with plasma and cardiac GSK-3. Since the goal of this research was limited to the parameters estimated, there is need to carry out further studies on COC containing drospirenone with other types of COC formulations in order to reach a conclusion on its real impact on cardiac and vascular pathophysiology.

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