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Drospirenone-containing oral contraceptives do not affect glucose regulation and circulating corticosterone

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Abstract:

Background: Combined oral contraceptive (COC) use has been associated with an increased risk of insulin resistance (IR) and other adverse cardiovascular events, despite efforts to reduce the dosage and/or progestin type. COC containing drospirenone (DRSP) is an analog of spironolactone, hence its antimineralocorticoid and antiandrogenic characteristics have been deemed beneficial, although the benefits and/or negative outcome of its usage have not been fully elucidated. We therefore hypothesized that COC with DRSP component will not affect glucose regulation and circulating corticosterone.

Method: Ten-week-old female Wistar rats were divided into three groups: control (CON), ethinylestradiol/-drospirenone COC (EE/DRSP)-treated, and ethinylestradiol/levonorgestrel COC (EE/LN)-treated rats. The treatment lasted for 8 weeks.

Results: Results showed that with the exception of lipid profiles, EE/LN but not EE/DRSP COC treatment affected body weight, glucose tolerance, plasma insulin, corticosterone, (IR), and pancreatic β -cell dysfunction.

Conclusion: Taken together, the findings showed that the beneficial effect of EE/DRSP could possibly be through the DRSP component. The result also implies that COCs containing DRSP may be a better and safer means of contraception than those with LN with less cardiovascular risks.

Keywords: CVD, drospirenone, estrogen-progestin oral contraceptive, insulin resistance, glucocorticoids

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Introduction

Cardiometabolic disorder (CMD), a cluster of metabolic disorders including insulin resistance (IR), hyperglycemia, dyslipidemia, etc. [1] that occur together and place the affected individuals to higher risk of cardiovascular events [2], remains the leading cause of morbidity and mortality globally [3]. Among these cluster of metabolic disorders, IR ranks almost highest with rapid surge over the last decade, as it now affects close to 30 million people [4]. It is the primary metabolic link for the development of cardiovascular disease (CVD) [5], as it results in lower levels of uptake of glucose from the blood into target tissues, thereby causing high blood glucose level with characteristic compensatory hyperinsulinemia, which is associated with atherosclerotic CVD and mortality independent of other risk factors [6]. Despite increased efforts to prevent, treat, and control IR and its sequelae, the prevalence has not declined.

Glucocorticoids (GCs), such as cortisol in humans and corticosterone in rodents, are stress and catabolic hormones that have strong anti-inflammatory properties [7]. They are primarily responsible for mobilizing glucose to the circulation from the liver and also inhibiting the uptake and utilization of glucose. However, individuals with elevated circulating GCs present a variety of systemic manifestations that are associated with increased atherothrombotic CVD risk, such as IR, hypertension, diabetes mellitus, and dyslipidemia [8], [9].

Combined oral contraceptive (COC) is one of the most commonly prescribed birth control methods and used by millions of women around the world [10]. The adverse contribution of COC to the pathogenesis of CMD remains unresolved [11], [12] and the mechanisms still need further studies although one of it is by causing metabolic disarrangements with IR [13]. The types and doses of the estrogen component of the COC have not helped alleviate these dysfunctions [14], [15], as recent studies have shown that even COCs with low

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hormone levels (ethinylestradiol: EE), combined with/without newer generations of progestogens, may still lead to CV risk in women using the COC [16]. Attention has been drawn to COCs with formulation containing drospirenone (DRSP) [17].

DRSP is the synthetic progestin most similar to the natural progesterone, and is structurally similar to spironolactone, acting as an antagonist of aldosterone receptors with clinically recognized antimineralocorticoid activity [18]. The combination of DRSP with EE has been shown to be effective in women using COCs [19], [20], yet the negative side effects with its use have not been fully explored and studies on these have been largely inconclusive [16], [21]. The aim of this study was therefore to look at the effect of COC with DRSP component on glucose regulation and circulating corticosterone.

Materials and methods

Animals

All experimental procedures were approved by the University of Ilorin Ethical Review Committee (UIERC) with protocol number UERC/ASN/2016/364 and the procedure was done in accordance with guidelines of the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals; every effort was made to minimize both the number of animals used and their suffering. Ten-week-old female Wistar rats were used for the study. Rats had unrestricted access to standard rat chow and tap water. After 1 week of acclimatization of the rats to stabilize in the new environment and promote their welfare and reproducible results, the rats were randomly assigned to three groups of $n = 5$ each. Rats were maintained under standard environmental conditions of temperature (26–28 °C), relative humidity (57–65%), and 12-h dark/light cycle.

Treatment

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

Sample preparation

At the end of treatment, the rats were anesthetized with pentobarbital sodium (50 mg/kg, ip). Blood was collected by cardiac puncture into EDTA-coated and heparinized bottles, accordingly. Blood collected in heparinized bottles was centrifuged at 3000 rpm for 5 min. Plasma was stored frozen until needed for biochemical assay. Plasma and blood viscosity were estimated according to the Vand formula [22] as adapted in our laboratory [23].

Oral glucose tolerance test (OGTT), IR, and pancreatic β -cell function

Glucose challenge test was performed 48 h before the end of the experiment. The rats were kept in fasting condition for 12 h overnight. Blood sample was collected by the tail-cut method before glucose load, and blood glucose level was assessed by a glucometer (ONETOUCH®-LifeScan, Inc., Milpitas, CA, USA); it was denoted as the 0th time point. Glucose (2 g/kg bw) was administered orally (po), and blood glucose was assessed subsequently after 30, 60, 90, and 120 min. Glucose tolerance was expressed as a function of the area under the OGTT curve (AUC) as previously described [24] and post-load glucose responses. IR was determined using the homeostasis model assessment for insulin resistance ($\text{HOMA-IR} = \text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{U/L)} / 22.5$) whereas $\text{HOMA-}\beta$ ($20 \times \text{fasting insulin (}\mu\text{U/L)} / \text{fasting glucose} - 3.5$) and 1-h post-load glucose levels were used to determine pancreatic β -cell function. Elevated 1-h post-load glucose level is a predictor of IR, pancreatic β -cell function, atherosclerotic CVD, and renal dysfunction [25], [26].

Biochemical assays

The plasma levels of corticosterone were estimated using ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China). Insulin was also determined using an ELISA kit (Ray Biotechnology, Cruz, Canada). The biological markers of dyslipidemia including total cholesterol (TC), triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C) were measured by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Antrim, UK). The atherogenic indices (TC/HDL-C and TG/HDL-C ratios) were also estimated.

Statistical analysis

All experimental data were expressed as means $\pm$ SEM. Statistical significance for the 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 pairwise comparison of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$. Statistical group analysis was performed with the SPSS statistical software.

Results

Effects of EE/LN vs. EE/DRSP treatments on body weight, food, and water intake

The initial body weight was comparable among all the experimental groups. However, for the EE/LN group, the final body weight was lower. Food and water intake was not significantly affected (Table 1).

Table 1: Effect of combined oral contraceptives containing levonorgestrel and drospirenone on body weight, food and water intake, fasting glycemia, and insulinemia in rats.

	CON	EE/LN	EE/DRSP
Body weight, g			
Initial	117.8 $\pm$ 10.0	117.7 $\pm$ 7.0	117.1 $\pm$ 8.0
Final	162.9 $\pm$ 10.9	131.3 $\pm$ 5.4 ^a	162.3 $\pm$ 6.4 ^b
Food intake, g/kg/day	72.8 $\pm$ 2.9	70.9 $\pm$ 6.7	78.0 $\pm$ 7.5
Water intake, mL/kg/day	192.4 $\pm$ 10.0	196.4 $\pm$ 7.0	207.3 $\pm$ 6.6
Fasting glycemia, mmol/L	3.5 $\pm$ 0.1	3.4 $\pm$ 0.2	3.5 $\pm$ 0.1
Fasting insulinemia, μ IU/mL	20.0 $\pm$ 1.1	31.1 $\pm$ 2.2 ^a	20.2 $\pm$ 1.5

Data are expressed as mean $\pm$ SEM of five rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test (^a $p < 0.05$ vs. CON; ^b $p < 0.05$ vs. EE/LN).

Effects of EE/LN vs. EE/DRSP treatments on glucose regulation and IR

Fasting glycemia was comparable in all the experimental groups (Table 1). EE/LN treatment led to significant increase in 1 and 1.5-h post-load glycemia changes, respectively, compared with CON, whereas EE/DRSP treatment at 1 and 1.5 h, respectively, did not change significantly compared with CON and EE/LN, respectively (Figure 1B,C). However, 2-h post-load glycemia was not affected in all the experimental groups (Figure 1D).

Figure 1: COCs containing drospirenone do not cause impaired glucose tolerance.

Effect of COCs containing levonorgestrel (EE/LN) or drospirenone (EE/DRSP) treatments on percentage post-load glycemic changes at 30, 60, 90, and 120 min (A–D, respectively) in female Wistar rats. EE/LN but not EE/DRSP caused impaired glucose tolerance and elevated 1- and 1.5-h post-load glucose response, respectively. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of five rats per group (^a $p < 0.05$ vs. CON; ^b $p < 0.05$ vs. EE/LN).

In addition, the AUC of OGTT was higher in the EE/LN-treated group than in CON but reduced in the EE/DRSP group compared with EE/LN (Figure 2A). EE/LN treatment led to an increase in fasting plasma insulin (Table 1) HOMA-IR (Figure 2B), and HOMA- β (Figure 2C) compared with the CON group, which were all not affected by EE/DRSP treatment compared with EE/LN treatment.

Figure 2: COCs containing levonorgestrel and drospirenone both affected atherogenic dyslipidemia.

Effect of COCs containing levonorgestrel (EE/LN) or drospirenone (EE/DRSP) treatments on (A) insulin resistance, (B) pancreatic β -cell function, and (C) area under the curve of oral glucose tolerance test in female Wistar rats. EE/LN but not EE/DRSP caused insulin resistance, impaired pancreatic β -cell function and elevated area under the curve of oral glucose tolerance test. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of five rats per group (^a $p < 0.05$ vs. CON; ^b $p < 0.05$ vs. EE/LN).

Effects of EE/LN vs. EE/DRSP treatments on atherogenic lipids and blood viscosity

The plasma HDL cholesterol levels were significantly lowered in all the treated groups compared with the control. EE/LN treatment led to significant increases in biological markers of dyslipidemia such as plasma TG, TC and LDL cholesterol levels compared with the control and EE/DRSP-treated groups (Table 2). EE/LN and EE/DRSP treatments led to significant increase in the atherogenic indices (TG/HDL-C and TC/HDL-C) compared with the CON group (Figure 3A,B). Plasma viscosity was comparable in all the groups (Figure 4A). However, blood viscosity (Figure 4B) and percentage hematocrit (Figure 4C) levels were significantly raised in the EE/LN-treated group compared with the CON and EE/DRSP group, respectively.

Table 2: Effect of combined oral contraceptives containing levonorgestrel and drospirenone on triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein (LDL-C) in rats.

	CON	EE/LN	EE/DRSP
TG, mg/dL	61.7 ± 6.6	138.2 ± 1.4 ^a	120.1 ± 1.9 ^{a,b}
TC, mg/dL	46.5 ± 2.6	91.9 ± 3.4 ^a	71.9 ± 3.5 ^{a,b}
HDL-C, mg/dL	70.6 ± 14.2	39.5 ± 5.6 ^a	35.3 ± 3.8 ^a
LDL-C, mg/dL	2.7 ± 1.9	30.9 ± 3.4 ^a	14.9 ± 3.7 ^{a,b}

Data are expressed as mean ± SEM of five rats per group. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test (^ap < 0.05 vs. CON; ^bp < 0.05 vs. EE/LN).

Figure 3: COCs containing drospirenone do not cause insulin resistance.

Effect of COCs containing levonorgestrel (EE/LN) or drospirenone (EE/DRSP) treatments on atherogenic indices (A, B) in female Wistar rats. EE/LN and EE/DRSP caused elevated TG/HDL-C and TC/HDL-C ratios. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean ± SEM of five rats per group (^ap < 0.05 vs. CON; ^bp < 0.05 vs. EE/LN).

Figure 4: COCs containing levonorgestrel altered the whole blood viscosity.

Effect of COCs containing levonorgestrel (EE/LN) or drospirenone (EE/DRSP) treatments on (A) plasma viscosity, (B) whole blood viscosity, and (C) hematocrit level in female Wistar rats. EE/LN but not EE/DRSP increased whole blood viscosity and hematocrit levels. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of five rats per group (^a $p < 0.05$ vs. CON; ^b $p < 0.05$ vs. EE/LN).

Effects of EE/LN vs. EE/DRSP treatments on circulating GCs

EE/LN treatment led to significant increase in circulating corticosterone level compared with the CON and EE/DRSP-treated groups. However, there was no change in the level of corticosterone level in the EE/DRSP group compared with the CON group (Figure 5).

Figure 5: Effect of COCs containing levonorgestrel (EE/LN) or drospirenone (EE/DRSP) treatments on circulating corticosterone in female Wistar rats.

EE/LN but not EE/DRSP increased circulating corticosterone. Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. Values are expressed as mean $\pm$ SEM of five rats per group (^a $p < 0.05$ vs. CON; ^b $p < 0.05$ vs. EE/LN).

Discussion

The results of the present study demonstrate that EE/LN caused IR, which was accompanied by pancreatic β -cell dysfunction, glucose deregulation, dyslipidemia, as well as increased blood viscosity and circulating corticosterone. The alterations in these parameters were however not noticed in the EE/DRSP-treated group.

IR has been shown to be the hallmark of CVD risk factors [27]. It is associated with decreased glucose uptake in insulin-sensitive tissues such as the skeletal muscle and liver. IR is rapidly becoming one of the major public health challenges, signifying an enormous global economic burden [28], particularly when accompanied by elevated fasting and 1-h post-load glycemia [29], [30]. Elevated 1-h post-load glycemia has been shown to be a reliable, independent determinant of impaired pancreatic β -cell function and IR, which are accompanied by increased atherosclerotic CVD even in individuals otherwise regarded as having normal glucose tolerance [31]. Hence, factors or intervention that could promote or prevent the development of IR, impaired 1-h post-load glycemia, and its associated atherogenic dyslipidemia will be of great clinical and public health importance, particularly in COC users. Our study showed evidence that EE/DRSP treatment prevented IR and elevated 1-h post load. This may be due to the DRSP component, which is known to exhibit a potent anti-aldosterone activity as it blocks the mineralocorticoid receptor (MR) that can bring about IR and other CVD risk factors if activated [17]. More so, it has been demonstrated in our laboratory that EE/LN can activate MR [12], further proving the beneficial use of EE/DRSP, possibly due to the DRSP component. The findings that 2-h post-load glycemia and fasting glycemia (Figure 1D) were comparable in all the experimental groups do not undermine the effectiveness of EE/DRSP in glucose homeostasis, as elevated 1-h post-load glycemia has been shown to be a stronger independent predictor of pancreatic β -cell dysfunction and cardiometabolic complications than 2-h post-load glycemia [32]. In addition, the anti-aldosterone effect of DRSP can reduce estrogen-related sodium and water retention, which regulates sodium and water balance. This may translate into weight benefits as noticed in our result [33]. Interestingly, there was reduction in body weight in the EE/LN-treated rats.

Dyslipidemia, characterized by elevated TG, TC, TG/HDL-C, and TC/HDL-C, was observed in both EE/LN- and EE/DRSP-treated rats. The dyslipidemia observed in the EE/LN-treated rats may be due to the observed hyperinsulinemia and IR that was noticed in the group [33]. Studies have demonstrated that atherogenic lipid ratios (TG/HDL-C and TC/HDL-C) are useful markers in identifying individuals at risk for devel-

oping CVD [34]. Although not significant, the ratios were reduced in the EE/DRSP-treated group compared with EE/LN-treated rats. This is in consonance with previous reports [35], [36]. More so, previous reports have shown the beneficial effect of EE/DRSP on some cardiovascular risk factors such as hypertension yet little or no impact on other cardiometabolic profiles such as dyslipidemia [37], [38]. Hence, EE/DRSP treatment had lesser risk on lipid metabolism. However, it should be noted that the variability might be due to the combined actions of EE and DRSP, as the action of DRSP has been shown to be cardioprotective [36], [39]. Altered blood rheology has been linked to the development of atherothrombotic complications, and increased systemic inflammation seems to be an important clinical correlate [40], [41]. Studies have shown that altered levels of blood rheological markers, which affect blood flow in both macro-and micro-circulations, are associated with incident atherothrombotic CVD. These markers include blood viscosity and its major determinants, namely hematocrit and plasma viscosity [40]. Blood viscosity and hematocrit level were elevated in the EE/LN-treated rats, but were however not affected in the EE/DRSP-treated animals, compared with CON, further confirming the effectiveness and superiority of EE/DRSP COC over EE/LN COC. More so, blood viscosity, which is an index of blood rheology, is positively correlated with blood pressure and has been related to future cardiovascular events, as was shown in a previous report [42].

GCs have been reported to cause impaired insulin action/dysmetabolism [43], [44]. The elevated GC in the EE/LN group is consistent with previous studies in our laboratory [11], [12]. An interesting finding in this work is the anti-GC activity of EE/DRSP for the first time. This also is possibly due to the DRSP component. This is contrary to a previous report that DRSP lacks anti-GC or even GC activity [45]. Although aldosterone was not estimated, which is one of the limitations of this work, it has been shown that both corticosterone and aldosterone have equal affinity to MR [46].

Conclusion

The present study demonstrates that DRSP-containing COC seems not to affect glucose regulation, IR, and circulating corticosterone but did affect lipid metabolism, though, to a lesser extent compared with EE/LN. The beneficial effects of EE/DRSP could have largely been through the DRSP component of the COC. Taken together, the findings demonstrated that COCs containing DRSP may be a better and safer means of contraception than that those containing LN, with lesser cardiovascular risks. Since the goal of this research is limited to the parameters estimated, there is great need to further our knowledge of DRSP-containing COCs with studies that analyze various types of other COC formulations in order to reach any conclusion on the real benefit of this COC on vascular pathophysiology.

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