Research article

Treatment with acetate during late pregnancy protects dams against testosterone-induced renal dysfunction

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1. Introduction

Cardiometabolic syndrome (CMS) underlies the prevalence of cardiovascular diseases contributing immensely to its mortality and morbidity. Cardiometabolic disease is closely related to chronic kidney disease (CKD). Studies show that the odds ratio of CKD increases with increase in components of CMS [1]. In addition, it is well documented that CKD culminates in cardiovascular disease and/or hypertension owing to the position of the kidney in regulating hemodynamic cardiovascular functions. Also, cardiovascular disease with its risk factors and complications are responsible for increased mortality, approximately 18 million worldwide [2]. Nevertheless, gestational diabetes mellitus is the manifestation of CMS during pregnancy and has the potential to cause maternal hypertension and offspring future cardiovascular disease [3,4].

Gestational excess testosterone has been shown to induce gestational glucose dysregulation, hyperinsulinemia and hepatic tissue derangement associated with increased hepatic urea production and suppression of glucose-6-phosphate dehydrogenase-dependent antioxidant barrier [5] which are components of CMS. Apart from the maternal impact, gestational hyperandrogenism has also been shown to result in severe

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sub-optimal intra-uterine fetal growth, an indicator of intra-uterine growth restriction [6]. Nevertheless, a review by Sarafidis in 2006 revealed the major links between CMS and kidney disease. He illustrated the involvement of oxidant stress and endothelial dysfunction as a result of IR and hyperinsulinemia in CMS-related kidney disease [7,8,9,10]. In addition, plasma urea, creatinine and recently, urea-creatinine ratio are accepted as reliable scores for judging renal or extra-renal excretory functions [11]. Taken together, poor fetal development and maternal deranged renal tissue are possible events caused by glucose deregulation-mediated oxidative stress following exposure to gestational excess androgen.

Apart from PCOS, placental aromatase deficiency, luteoma, and fetal congenital adrenal hyperplasia are conditions that may cause elevated gestational androgen [12]. Conditions characterized by gestational androgen excess are associated with poor fetal outcome, offspring future CMS [12] and persistent post-gestational hyperandrogenism. Several studies have elucidated androgen-induced intra-uterine programming leading to offspring future predisposition to IR [12]. Nevertheless, the persistent hyperandrogenemia observed in post-gestational period of women with gestational hyperandrogenism is not clear and might consistently expose maternal tissues to injuries after delivery. These injuries might endanger the health and fertility of affected women. Worthy of note is that supra-physiological levels of testosterone have been associated with oxidative stress involving xanthine oxidase (XO) dysregulation among other mechanisms.

Oxidative stress causes tissue injury. It is usually triggered by dysregulation of ROS-producing pathways, such as the (XO) pathway in the production of UA. XO catalyses the last two steps in purine metabolism leading to UA production and liberates ROS in the process [13]. As a risk factor for macrovascular complications, UA status of tissues indicates tissue injury and via various mechanisms can engender tissue injury [14] in organs like kidney. It is acceptable that in conditions of high intramural UA production, antioxidant barrier may be suppressed leading to inflammatory response, fibrosis and collagen deposition/remodeling. The mechanism(s) of UA-mediated organ damage is only moderately understood. However, some research findings, demonstrated that elevated uric acid elicit hypertension and disturbs NO production in macula densa via stimulation of arginase a competitor for the NO precursor, L-arginine with nitric oxide synthase [15,16].

The products derived from fermentation of complex carbohydrate in the colon by microbiota, short chain fatty acids (SCFA) are mainly acetate, propionate and butyrate constituting over 90% of the SCFAs present in the colon [17,18]. However, approximately 65% of colonic SCFA is acetate and it reaches significant concentrations in the circulation [19] Reports have revealed that acetate suppressed production of intracellular reactive oxygen species induced by lipopolysaccharide with concomitant increase in glutathione [20]. Reports of findings also showed that elevated testosterone disturbed colonic microbiota functions resulting in dysbiosis [21] which may contribute to the deleterious effects of the supra-physiological testosterone levels in conditions like PCOS. Usman et al. recently found that treatment with acetate during pregnancy ameliorated late gestational excess testosterone-induced IR and hepatic dysmetabolic events: it averted lipid accumulation and augmented antioxidant barrier while restoring glucoregulation [5]. Based on the outcomes of previous studies, acetate clearly reduces IR and tissue injury (liver) in a hyperandrogenic milieu during gestation. Therefore, the present study tested the hypothesis that gestational acetate treatment in an excessive androgen milieu protects fetal health and maternal renal tissue during and after pregnancy. Hence, animals exposed to gestational testosterone were treated with acetate during pregnancy and re-exposed to testosterone after pregnancy without acetate treatment.

2. Materials and methods

2.1. Animals and grouping

The experiment was carried out in adherence to recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The procedure was read and approved by the ethical review committee, University of Ilorin, Ilorin, Nigeria. Number and suffering of animals were minimized. Female Wistar rats (130–150 g) were obtained from the Animal House, College of Health Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were housed under standard conditions of light, humidity, ventilation and temperature. Rats had free access to tap water and standard rat chow and dwelt in this condition for two weeks of acclimatization. The animals were mated on Day 5 of gestation. The mating process was started with forty-two female rats of which thirty-two of them became pregnant giving us a success rate of about seventy-six percent. Out of the successful pregnancies, thirty (30) pregnant rats were randomly grouped into three (3) of ten (10) animals per group. Each group received vehicle (Ctr), testosterone-treated (Tes) and acetate + testosterone (Tes + Ace) during gestation after which half of the animals in each group were sacrificed and the remaining half left to deliver and wean their pups and stay for 8 weeks during which the Tes and Tes + Ace groups received re-exposure to testosterone in the last six days of the 8-week post-gestation period (Figure 1).

2.2. Administration and treatment

The Ctr animals received distilled water (po) and olive oil (sc), Tes received testosterone propionate (0.5 mg/kg; sc) only and Tes + Ace received combination of SCFA, acetate (200 mg/kg; po) and testosterone (0.5 mg/kg; sc) between gestational days 14 and 19. During the last six (6) days of 8-week post-gestation, Ctr received olive oil (sc), Tes received testosterone propionate (0.5 mg/kg; sc) and Tes + Ace also received testosterone (0.5 mg/kg; sc) only.

2.3. Sample preparation

On gestational day 20 and immediately after 8-week post-gestational period, animals were anesthetized with sodium pentobarbital (50 mg/kg, ip) and sacrificed to obtain blood sample and. Blood was collected by retro-orbital puncture into heparinized bottles. Centrifugation of blood sample was carried out at 3000 rpm for 5 min to obtain plasma. The
kidneys were also excised, cleared of adhering connective tissues, blotted and weighed. After weighing, 100 mg of tissue was carefully removed and homogenized in cold phosphate buffer solution (PBS) with a glass homogenizer, centrifuged at 10000 rpm for 10 min at 4 °C. The homogenate was used for the measurement of renal biochemical parameters.

2.4. Biochemical assay

Plasma testosterone was determined using ELISA kit from Elabscience (Wuhan, China) with a sensitivity of 0.01 nmol/L, detection range of 0.01–0.70 nmol/L and the specificity is recognition of testosterone in sample. No significant cross-reactivity or interference between testosterone and analogues was observed and coefficient of variation is <10%. Plasma urea and creatinine were determined by standard colorimetric methods using reagents purchased from Randox Laboratory Ltd. (Co. Antrim, UK). Renal adenosine deaminase (ADA), glutathione peroxidase (GPx), lactate dehydrogenase (LDH) and gamma glutamyl transferase (GGT) were determined by standard enzymatic colorimetric methods using assay reagents from Elabscience (Wuhan, China). Similarly, renal uric acid (UA), free fatty acid (FFA), triglyceride (TG), total cholesterol (TC) and malondialdehyde (MDA) were determined by standard colorimetric methods using reagents purchased from Randox Laboratory Ltd. (Co. Antrim, UK).

2.5. Statistical analysis

The distribution of the data was confirmed using Shapiro-Wilk test and the data were normally distributed. All data were expressed as means ± SEM. Statistical group analysis was performed with SPSS statistical software (version 20.0; IBM Corp, Armonk, N.Y., USA). One-way ANOVA was used to compare the mean values of variables among the groups. Bonferroni’s test was used for post hoc analysis. Statistically significant differences were accepted at p < 0.05.

3. Results

3.1. Acetate treatment during pregnancy improves placental and fetal outcome in late gestational testosterone-exposed Wistar rats

Late gestational testosterone exposure led to reduced placental weight (p = 0.0001, F = 205.7), fetal weight (p = 0.0001, F = 749.0) and increased resorption (p = 0.0001, F = 85.6) (Figure 2a, b and d). The fetal number was not significantly affected by testosterone exposure (p =...
0.0628, F = 3.3695) (Figure 2c). However, gestational acetate treatment significantly increased fetal and placental weight and decreased fetal resorption in Tes + Ace when compared with testosterone-exposed group (Figure 2).

3.2. Acetate treatment during pregnancy improves renal function in late gestational and post-gestational testosterone-exposed Wistar rats

Gestational and post-gestational testosterone exposure significantly increased plasma creatinine (p = 0.0001, F = 385.4 and p = 0.0002, F = 15.2 for gestational and post-gestational creatinine respectively) and urea (p = 0.0001, F = 300.9 and p = 0.01, F = 13.8 for gestational and post-gestational urea respectively) when compared to the control groups, which were significantly decreased by gestational acetate (Figure 3a, b, c, d).

3.3. Acetate treatment during pregnancy augments renal glutathione peroxidase activity and ameliorates lipid peroxidation in late gestational and post-gestational testosterone-exposed Wistar rats

Both gestational and post-gestational testosterone exposure reduced GPx activity (p = 0.0001, F = 28.92 and F = 27.54 for gestational and post gestational GPx respectively) and increased gestational MDA level (p = 0.0001, F = 45.96) but not post-gestational MDA. However, gestational acetate treatment in gestational and post-gestational testosterone-exposed rats increased gestational GPx, reduced gestational and post-gestational MDA (p = 0.01, F = 5.70 for post-
gestational MDA), but did not affect post-gestational GPx compared with testosterone-exposed groups. Nevertheless, MDA significantly increased in gestational testosterone-exposure, whereas GPx decreased in post-gestational testosterone-exposure when compared to the control (Figure 4).

3.4. Acetate treatment during pregnancy ameliorates renal lipid in late gestational and post-gestational testosterone-exposed Wistar rats

There was a significant increase in renal FFA (p = 0.0001, F = 47.3) but not triglyceride and total cholesterol in animals exposed to gestational testosterone when compared with control group. Acetate treatment during pregnancy significantly reduced FFA in gestational testosterone-exposed group alone. In addition, there was a significant increase in post-gestational total cholesterol (p = 0.0001, F = 55.20) but not triglyceride when compared to the control. However, gestational acetate treatment decreased total cholesterol in gestational and post-gestational testosterone-exposed groups when compared with testosterone-exposed groups respectively (Figure 5).

3.5. Acetate treatment during pregnancy decreases renal injury markers in late gestational and post-gestational testosterone-exposed Wistar rats

Renal injury markers, including GGT and LDH significantly increased in animals exposed to gestational testosterone (GGT: p = 0.004, F = 26.9; LDH: p = 0.0002, F = 150.7) and post-gestational testosterone (GGT: p = 0.0002, F = 401.4; LDH: p = 0.01, F = 23.7) compared with control groups. Acetate treatment during pregnancy reduced gestational and post-gestational renal GGT and LDH in animals exposed to gestational and post-gestational testosterone when compared with those exposed to only testosterone (Figure 6).

3.6. Acetate treatment during pregnancy improves renal uric acid in late gestational and post-gestational testosterone-exposed Wistar rats

Gestational and post-gestational testosterone exposure increased gestational and post-gestational renal uric acid level (p = 0.0001, F = 123.2 and 226.8 for gestational and post-gestational UA respectively) compared with control. Animals exposed to gestational and post-gestational testosterone had increased gestational and post-gestational uric acid compared to control (Figure 7).
renal adenosine deaminase activity ($p = 0.001$, $F = 83.30$ and $544.7$ for gestational and post-gestational ADA respectively) compared with control. Gestational acetate treatment in animals exposed to both gestational and post-gestational testosterone reduced renal gestational and post-gestational uric acid and adenosine deaminase activity compared with animals exposed to testosterone alone but remained unaltered compared with control (Figure 7).

3.7. Acetate treatment during pregnancy reduces plasma post-gestational testosterone in late gestational and post-gestational testosterone-exposed Wistar rats

Post-gestational plasma testosterone level increased ($p = 0.001$, $F = 22.15$) in animals exposed to both gestational and post-gestational testosterone. However, post-gestational plasma level of testosterone was ameliorated in animals exposed to testosterone alone but remained unaltered compared with acetate (Figure 8).

4. Discussion

The results of the present study demonstrate that exposing pregnant animals to late gestational testosterone induces poor placental and fetal outcomes, whereas, exposure to both gestational and post-gestational testosterone caused maternal renal dysfunction indicated by increased renal markers of tissue injury, plasma urea and creatinine. These outcomes were accompanied by elevated renal uric acid, lipid peroxidation, post-gestational plasma testosterone and disrupted GPx-dependent antioxidant defense. Interestingly, acetate treatment during gestational testosterone exposure did not only reduce poor placental and fetal outcomes but also ameliorated renal dysfunctions induced by testosterone exposure during pregnancy and as far as 8 weeks after pregnancy. This study elucidates the potential of acetate, a SCFA to reduce gestational testosterone-induced fetal growth retardation, maternal renal dysfunction and elicit long term protective effects on maternal renal tissue following post-gestational re-exposure to testosterone when received only during pregnancy.

Several studies with compelling results have established the deleterious impact of gestational elevated testosterone on fetal outcomes and intra-uterine developmental programming towards offspring future CMS [21] but only a few showed the impact of gestational excess testosterone on maternal tissues. Previously, a study from our laboratory showed that late gestational excess testosterone caused poor fetal outcomes, maternal glucose dysregulation and hepatic dysfunctions [5]. However, it was found herein that along with poor fetal outcome (Figure 2), the kidney of pregnant animals exposed to testosterone in late gestation is endangered. There were increased renal UA, ADA (Figure 7), MDA (Figure 4b) and depressed GPx (Figure 4a) with accompanying increased renal markers of tissue injury, GGT and LDH (Figure 6). Also, Post-gestational re-exposure to testosterone resulted in a like fashion of renal dysfunction. There were increased renal UA, ADA (Figure 7) and depressed GPx (Figure 4a) with accompanying increased GGT and LDH (Figure 6).

Increased FFA in non-adipose tissues is a feature of CMS and results in lipotoxicity. It is associated with the production of intermediates like diacylglycerol, ceramide molecules and fatty acyl coA which increase the production of protein kinase C and release of nuclear factor kappa B while promoting the expression of inflammatory cytokine leading to impaired insulin sensitivity [22]. FFA also interacts with toll-like receptor type 4 on tissue macrophages to initiate inflammatory and oxidant...
responses in tissues [23]. Besides, increased intracellular lipid correlates with elevated ADP/ATP ratio [24] which confirms decreased ATP synthesis and mitochondrial dysfunction leading to increased ADA activity and UA production as a result of elevated purine metabolism. Also, the elevated MDA and depressed GPx found herein with late gestational testosterone exposure indicate increased lipid peroxidation and damage of cellular integrity as a result of depressed antioxidant barrier. This is not surprising since increased intracellular FFA, ADA and UA all have oxidant promoting potential which might overwhelm antioxidant barrier. Glutathione peroxidase is a G6PD-dependent antioxidant concerned with conversion of $\text{H}_2\text{O}_2$ to $\text{H}_2\text{O}$ [25]. This limits the amount of reactive oxygen species and the possibility of lipid peroxidation in the cell membrane to prevent damage or injury. In the present study, suppression of renal GPx as a result gestational and post-gestational testosterone exposure is associated with elevated MDA and increased renal markers of tissue injury.

Kidney damage is usually estimated by plasma creatinine and urea since their concentration in the blood negatively correlates with glomerular filtration rate [26]. The present study showed that both gestational and post-gestational testosterone exposure increased plasma urea and creatinine indicating a diminishing GFR and progressive renal damage. However, blood creatinine level is agreeably a better score in judging renal disease than urea since its excretion is dependent mainly on the kidney and production on the muscle mass which is likely to be steady making its concentration relatively stable in the blood [27,28]. Conversely, urea has extra-renal excretion and variable determinant of its blood concentration like level of hydration, renal hypoperfusion, liver disease, ageing etc [29]. Similarly, gestational testosterone exposure led to increased plasma urea and creatinine concentration and with this increase, the status indicates kidney injury, which often deteriorates to chronic kidney disease if not intervened [11,27]. In addition, post-gestational re-exposure to testosterone in this study also led to increased plasma urea and creatinine (Figure 3). This outcome also indicates an apparent prerenal dysfunction affecting creatinine and urea excretion which are dependent primarily on the kidneys.

The kidney is a highly vulnerable organ that must be protected from injury. The findings of this study also show that pregnancies with poor fetal outcome is possibly associated with elevated androgen and most importantly a progressively deteriorating kidney. Therefore, an effective and safe means of protecting the kidney is reasonable. With the background of considerable therapeutic effects in various diseases: airway disease, colitis, kidney disease, metabolic syndrome, hepatic lipid accumulation [5,21], in this study, SCFA, acetate was simultaneously administered during gestation only to animals exposed to gestational testosterone which were re-exposed to testosterone after an 8-week post-gestation period. The results showed that gestational acetate treatment improved placental and fetal outcomes, ameliorated renal UA, MDA, FFA, markers of tissue injury and augmented GPx. These were accompanied by normalized plasma urea and creatinine in testosterone-exposed rats during pregnancy and as far as 8 weeks after pregnancy. It is not surprising that acetate ameliorated renal dysfunctions during gestation since studies in both pregnant and non-pregnant rats have shown it suppressed systemic and hepatic CMS components [5,21] in animals. However, it is novel finding that gestational acetate preconditioned maternal kidneys to resist testosterone-induced dysfunction over an 8-week post-gestational period in rats. This was confirmed by the normal levels of renal function biomarkers at the end of the experiment. The beneficial effects of acetate herein could be attributed to its ability to alleviate CMS which would protect liver, heart and the kidney. Also, the long-term preconditioning of maternal kidneys to resist further androgen-induced insult provided by acetate is note worthy and requires further studies to elucidate the mechanism. However, a thoughtful mechanism could be that acetate by interacting with histone deacetylase (HDAC) might stand in the way of either or both genomic and non-genomic actions of testosterone and prevent its deleterious effects through epigenetic events. Acetate as other SCFAs is acceptably a HDAC modulator (inhibitor) with plausible epigenetic impacts by promoting acetylation [30,31]. Similarly, SCFA, acetate has been reported to exert its beneficial effects through binding to endogenous G-protein coupled receptors such as FFAR2 and FFAR3 to activate signaling cascades that control energy homeostasis and cellular metabolism [31], which might be a link to why acetate suppressed pro-inflammatory process and oxidative stress in animals exposed to both gestational and post-gestational testosterone. However, further study is required to elucidate possible molecular mechanism involved in protective role of acetate against hyperandrogenism. Nevertheless, gestational acetate ameliorated post-gestational testosterone levels (Figure 8) which could
be another mechanism for the beneficial effect of acetate and can be considered as a fertility aiding action.

5. Conclusion

The present study demonstrates that gestational acetate treatment ameliorates the renal effects of gestational and post-gestational TEs exposure. Therefore, acetate treatment during pregnancy in addition to improving fetal outcome confers renoprotection against gestational and post-gestational hyperandrogenism (Figure 9).

Declarations

Author contribution statement

L. A. Olatunji: Conceived and designed the research; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

O. O. Badmus; E. D. Areola: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

K. S. Olaniyi; T. O. Usman: Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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