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Research article

# Sildenafil ameliorates leptin resistance and normalizes lipid handling in the hypothalamic and adipose tissues of testosterone-exposed pregnant rats

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ABSTRACT

Leptin and hypothalamic-adipose lipid handling are relevant in determining the shift of metabolic activities. There are scanty findings connecting glucose dysregulation as a result of hyperandrogenism during gestation to hypothalamic-adipose axis and leptin resistance. Sildenafil has recently gained attention in the prevention of intra-uterine growth restriction. The present study aimed at demonstrating the effect of sildenafil on leptin resistance and hypothalamic-adipose lipid handling in testosterone-exposed pregnant rats. Three groups of pregnant Wistar rats (n = 5/group) received olive oil (Ctr, S.C.) or testosterone propionate (Tes, 3.0 mg/kg; sc)or testosterone propionate (3.0 mg/kg; sc) and sildenafil (Tes + PDE5, 50 mg/kg; po)from gestational day 14–19. Blood samples, hypothalamus and adipose tissue were excised for biochemical analysis on day 20. Adipose and body weights, plasma leptin and adiponectin, adipose and hypothalamic leptin and triglyceride, adipose uric acid, hypothalamic Nrf2, catalase and nitric oxide were reduced following gestational testosterone exposure. Also, fasting insulin, plasma triglyceride, uric acid, leptin-adiponectin ratio, hypothalamic free fatty acid, total cholesterol, uric acid, aspartate transaminase and cyclic guanine monophosphate were elevated by testosterone exposure to pregnant animals. Sildenafil ameliorated leptin resistance and normalized hypothalamic-adipose lipid handling. Therefore, sildenafil protects against testosterone-induced leptin resistance and adverse hypothalamic-adipose lipid handling in pregnant rats.

## 1. Introduction

Secreted as a hormone chiefly from the adipose tissue, leptin, a 16 kDa peptide plays essential roles in body weight homeostasis and energy balance and its plasma levels have a compelling correlation with obesity [1]. Leptin regulates body functions like thermogenesis, angiogenesis, and arterial pressure control among others [2, 3, 4, 5]. In relation to these functions, leptin is expressed by various organs like skeletal muscle and placenta [6]. Leptin has also been implicated in a range of functions that concerns reproduction: from ovarian function regulation to embryo development, implantation and placentation [7, 8]. However, leptin deficiency is regarded as risk factor for obesity or obesity-related diseases and its increase is associated with proinflammatory processes in metabolic syndrome. Also, dysregulation of leptin-adiponectin ratio is observed in metabolic disease and related to leptin resistance where leptin level is higher than the anti-inflammatory adiponectin.

However, it was found that leptin deficit does not usually underlie cases of obesity since many of the cases exhibited increased leptin levels which match their adipose tissue mass [9, 10, 11]. The question raised that stimulated further research endeavour in the field of leptin functions and roles in metabolic syndrome was that high levels of leptin should decrease feeding and prevent obesity. The findings from such endeavours resulted in the phenomenal physiological leptin resistance. Leptin resistance has to do with the failure of high levels of leptin to decrease feeding and adiposity and prevent obesity. Furthermore, research works had proposed a number of mechanisms responsible for the initiation of leptin resistance including altered leptin receptor signaling, compromised leptin transport across the blood brain barrier and changes in developmental programming among others [12, 13, 14, 15, 16].

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Aside via leptin, adipose tissue also communicates with the hypothalamus via lipid derivatives especially fatty acids (FAs) to initiate important hypothalamic inputs that regulate energy balance. Research findings show that FAs affect the central nervous system (CNS) for regulation of glucose metabolism and energy homeostasis [17, 18]. For instance, when long chain FAs were administered centrally, glucose production and food intake were inhibited [19, 20] with decrease in neuropeptide Y and glucose-6-phosphatase expression in the hypothalamus as seen also in overfeeding [20]. Therefore small increases in FA levels after feeding can be detected by the CNS as a signal for satiety [21]. Also, it was found that central triglyceride infusion into the brain initiated insulin resistance in the liver [22]. Therefore, in cases of starvation and obesity the adipose tissue can communicate to the hypothalamus via circulating lipids and also receive a hypothalamic lipid handling-dependent feedback. Hence, chronic mishandling of lipids by either or both hypothalamus and adipose tissue might progressively shift overall body metabolic activities towards pathological status.

Gestational diabetes mellitus is the pathological pregnancy version of cardiometabolic syndrome accompanied by complications like preeclampsia, intrauterine growth restriction (IUGR), steroidogenesis dysregulation and microvascular complications. Gestational cardiometabolic syndrome is of more social and scientific importance than non-gestational cardiometabolic syndrome because it impacts both maternal and fetal tissues leading to post-gestational maternal and offspring ill-health. Gestational dysregulated steroidogenesis could stem from pre-existing conditions like PCOS which is associated with persistent hyperandrogenism. Studies from our laboratory [23, 24] have elucidated the deleterious effects of gestational hyperandrogenemia on maternal lipid homeostasis, tissues and pregnancy outcomes. Despite limited existing studies on the link between lipids and pregnancy outcomes, maternal lipid homeostasis during pregnancy has been found to correlate with fetal growth as much as glucose [25].

Tissues that play central roles in regulation of body functions might elicit severe systemic malady when they are locally disturbed. Aside the important impact of controlling hormonal secretions which affects metabolic activities in organs, hypothalamus as a CNS tissue plays role in energy balance due to its various sensitive nuclei that regulate appetite and satiety. Intracellular hypothalamic metabolic sensors may be responsible for its regulatory functions on metabolic tissues through humoral or neural pathways. Oxidative stress and inflammation constitute cellular events that can dysregulate hypothalamic metabolic functions. An important mechanism stimulated by ROS which regulates their levels in the cell is the nuclear factor erythroid 2-related factor 2/antioxidant responsive element (Nrf2/ARE) pathway that is involved in transcription of antioxidant target genes in order to protect the cell from ROS-induced damage [26]. PhaseII enzymes like NADPH quinone oxidoreductase, glutathione S-transferase, heme oxygenase-1, and g-glutamylcysteine synthetase which lead to the production of cellular antioxidants: glutathione (GSH), superoxide dismutase (SOD), catalase help to clear excess ROS from the cell. Studies have shown that pharmacologic activation of Nrf2prevents oxidative stress and injury induced by hyperglycemia in micro vessels and kidneys [27]. Several studies have derived the direct cellular processes involved in Nrf2/ARE pathway [28], whereas, others have elucidated the impact of molecules like uric acid [29] and lactate on Nrf2 signaling.

Sildenafil is a phosphodiesterase type 5 (PDE5) inhibitor used mainly for male erectile dysfunction. It potentiates nitric oxide action through the sparing of cyclic guanine monophosphate (cGMP), leading to strong vasodilation [30]. This drug is therefore used in other conditions like pulmonary hypertension and in athletes at moderate altitude [31, 32]. However, sildenafil has recently gained reputation as a pharmacologic candidate for clinical trials in the prevention of IUGR especially in women prone to hyperandrogenism. This scientific venture is with the hope that the vasodilatory action of sildenafil might enhance blood flow in the feto-placental interface. However, studies on the effect of sildenafil on metabolic functions/tissues in gestational excess androgen will assist to isolate the advantage or disadvantage of sildenafil aside protecting the fetus. Therefore, the present study aimed at demonstrating the effect of sildenafil on leptin resistance and hypothalamic-adipose lipid handling in testosterone-exposed pregnant rats.

## 2. Material and methods

### 2.1. Animals

The process of the study was in accordance with the guidelines of the National Institutes of Health Guide for the Care of Laboratory Animals and approval was sought from the Ethical Review Committee, University of Ilorin, Ilorin, Nigeria. Suffering of the animals was prevented and number of animals was minimized. Female Wistar rats were acquired from College of Health Sciences Animal House, University of Ilorin, Ilorin, Nigeria. The animals were kept in a well ventilated house under standard temperature, humidity and light conditions. The animals were made pregnant and unrestrictedly fed with standard rat chow and tap water. Fifteen (15) pregnant rats allotted randomly to three groups of five (5) each received oliveoil (control), testosterone propionate and testosterone propionate with sildenafil respectively.

### 2.2. Treatment dose and route of administration

The control (Ctr) group received olive oil (sc). The testosterone (Tes) group received testosterone propionate (3.0 mg/kg; sc) and the testosterone with sildenafil (Tes + PDE5) group received testosterone propionate (3.0 mg/kg; sc) in combination with sildenafil (50.0 mg/kg; *po*)The treatments were administered from gestational days 14–19.

### 2.3. Body and organ weights

Body weight of animals was obtained every week of the experiment while hypothalamic and visceral adipose tissue weights were obtained at the end of the experiment period by a digital weighing scale.

### 2.4. Tissue and plasma sample preparation

After 12 h fast, on gestational day 20, animals were anesthetized with pentobarbital sodium (50 mg/kg, I.P.). Cardiac puncture was carried out with 5ml syringe to obtain blood and kept inheparinized bottles. Blood samples were centrifuged at 3000 rpm for 5 min and the plasma obtained from centrifuged blood was stored frozen until needed for biochemical assay. Hypothalamus and visceral adipose tissue from animals were homogenized and centrifuged at 3000 rpm for 15 min. Tissue homogenates were obtained and kept in sample bottles for biochemical analysis.

### 2.5. Biochemical assay

Testosterone,Nrf2, CGMP, leptin and adiponectin, were determined using ELISA kit from Elabscience (Wuhan, China). Uric acid, FFA, TG, TC, aconitase, lactate dehydrogenase (LDH), lactate, malondialdehyde (MDA), aspartate transaminase (AST), alanine amino transferase (ALT), superoxide dismutase-1 (SOD-1), superoxide dismutase-2 (SOD-2), catalase were determined by standard colorimetric methods. Nitric oxide (NO) and PDE5 activity were measured by non-enzymatic colorimetric assay obtained from Oxford Biomedical Research Inc. (USA).

### 2.6. Glucoregulatory and metabolic disturbance assessment

Glucoregulatory and lipid disturbance assessment was taken as a function of plasma glucose, insulin, triglyceride, total cholesterol and free fatty acid levels. Hypothalamic and adipose lipid handling was determined by hypothalamic and adipose triglyceride, total cholesterol and free fatty acid levels. Leptin resistance was determined as a function

of adipose, hypothalamic and plasma leptin levels and plasma leptin-adiponectin ratio.

## 2.7. Statistical analysis

Data were expressed as means $\pm$ SEM. One-way analysis of variance (ANOVA) and Bonferroni's Post hoc were employed for statistical analysis and identification of significance in pair wise comparison of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.
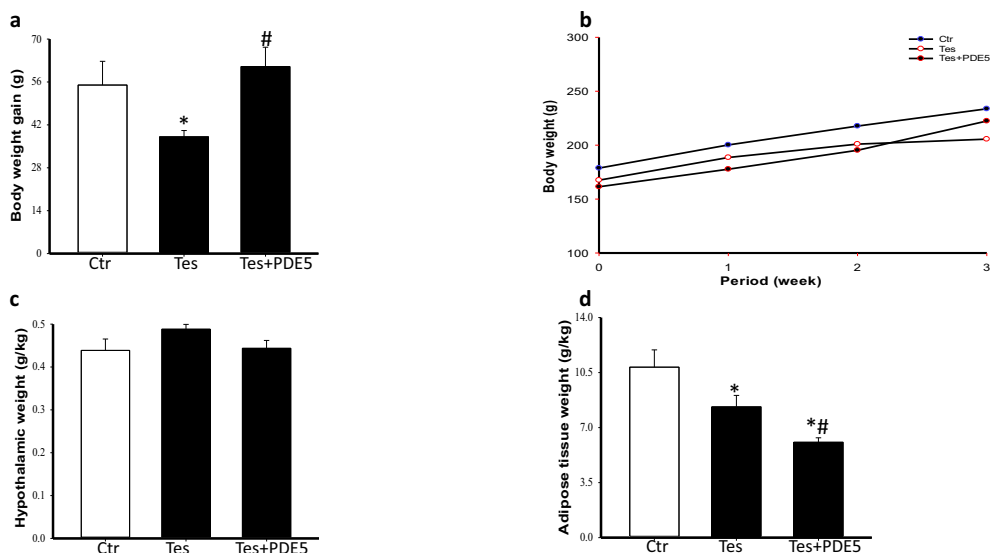
## 3. Result

### 3.1. Sildenafil increased body weight, reduced adipode mass and did not affect hypothalamic mass in testosterone-exposed pregnant Wistar rats

Exposure to testosterone during gestational days 14–19 reduced body weight gain compared with control animals (Figure 1(a)). This reduction occurred predominantly at the third week of pregnancy (Figure 1(b)) which corresponds to the days testosterone was administered to animals. Testosterone exposure did not significantly affect hypothalamic mass (Figure 1(c)) and adipose mass (Figure 1(d)). Sildenafil treatment to testosterone-exposed pregnant animals increased body weight change compared with testosterone-exposure without sildenafil but reduced adipose mass and did not affect hypothalamic mass compared with both control and testosterone-exposure without sildenafil (Figure 1).

### 3.2. Sildenafil improves glucose and lipid regulation in testosterone-exposed pregnant Wistar rats

Testosterone exposure during gestational days 14–19 did not affect fasting blood glucose and plasma free fatty acid but it increased fasting insulin and plasma triglyceride whereas it reduced plasma total cholesterol (Figure 2). Sildenafil treatment during testosterone exposure reduced fasting glucose compared with both control and testosterone-exposed animals without sildenafil treatment. Sildenafil treatment during testosterone exposure also increased fasting insulin (Figure 2(b)) reduced plasma triglyceride (Figure 2(c)) compared with control animals but not testosterone-exposed animals. However, sildenafil increased plasma total cholesterol compared with testosterone-exposed animals without sildenafil treatment but not control (Figure 2(d)).

### 3.3. Sildenafil averts leptin resistance in testosterone-exposed pregnant Wistar rats
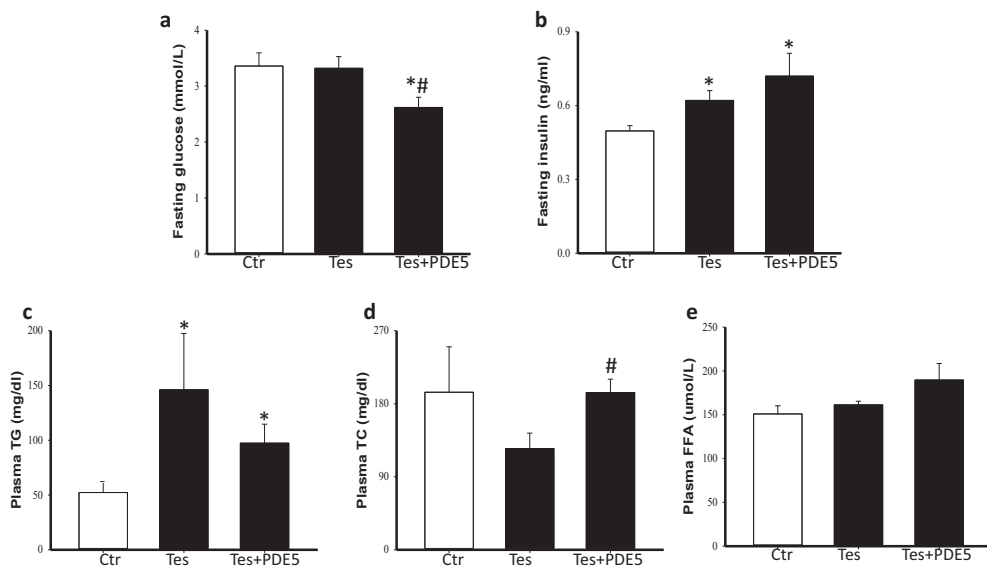
Testosterone-exposed pregnant animals had increased plasma leptin-adiponectin ratio (Figure 3(c)) and reduced plasma and adipose adiponectin and leptin when compared with control (Figure 3). Testosterone exposure also reduced hypothalamic leptin (Figure 3(f)) but did not affect hypothalamic adiponectin when compared with control (Figure 3(g)). Sildenafil treatment reduced plasma leptin compared with control (Figure 3(a)) and leptin-adiponectin ratio compared with both control and testosterone-exposed animals without sildenafil treatment (Figure(c)). Sildenafil treatment during gestational testosterone exposure increased hypothalamic adiponectin compared with control and testosterone-exposed pregnant rats without sildenafil treatment but did not affect both adipose and hypothalamic leptin and adipose adiponectin compared with control and testosterone exposure without sildenafil (Figure(3)).

### 3.4. Sildenafil improves hypothalamic-adipose lipid handling in testosterone-exposed pregnant Wistar rats

Gestational testosterone exposure increased hypothalamic free fatty acid (Figure 4b) reduced hypothalamic total cholesterol (Figure 4(c)) but did not significantly affect hypothalamic triglyceride (Figure 4(a)) when compared with control. Adipose triglyceride was reduced and adipose total cholesterol was increased whereas adipose free fatty acid was not affected by gestational testosterone when compared with control. Sildenafil treatment during gestational testosterone exposure increased hypothalamic triglyceride, reduced hypothalamic free fatty acid and did not affect hypothalamic total cholesterol when compared with both control and testosterone exposure without sildenafil. Adipose tissue triglyceride was increased compared with gestational testosterone only-exposed animals but not control whereas adipose free fatty acid and total cholesterol increased compared with both control and gestational testosterone only exposure (Figure 4).

### 3.5. Sildenafil reduced circulating and hypothalamic uric acid in testosterone-exposed pregnant Wistar rats

Gestational testosterone exposure increased plasma and hypothalamic uric acid but reduced adipose uric acid level compared with control animals (Figure 5). Sildenafil treatment during gestational testosterone
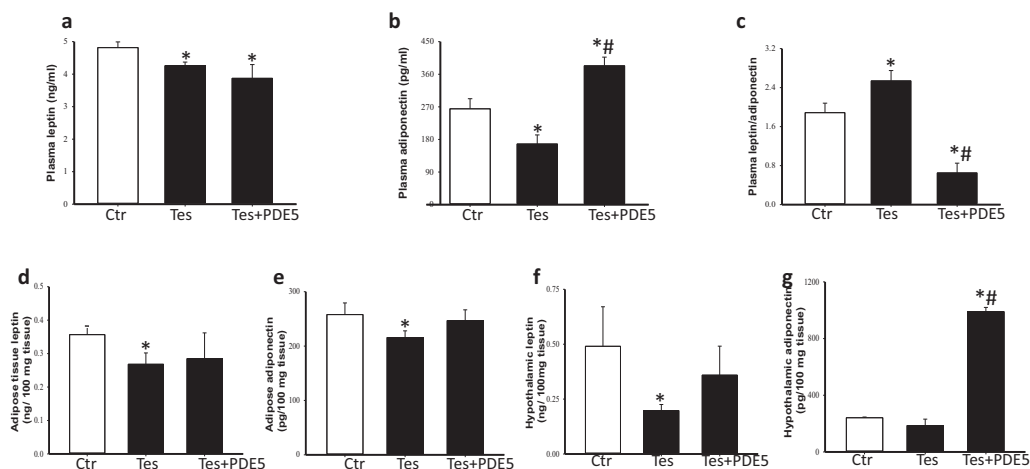


**Figure 1.** Effect of Sildenafil on body and organ weights in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil increased body weight gain (a), which was evident in third week of pregnancy (b), did not affect hypothalamic weight (c) but reduced adipose tissue weight (d) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.

**Figure 2.** Effect of Sildenafil on glucoregulation and circulating lipids in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil reduced fasting blood glucose (a), did not affect fasting insulin (b), plasma triglyceride; TG (c), sildenafil increased plasma total cholesterol; TC (d) and did not affect plasma free fatty acid; FFA (e) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.

exposure reduced plasma and hypothalamic uric acid but did not affect adipose tissue uric acid level compared with both control and testosterone exposure without sildenafil treatment (Figure 5).

### 3.6. Sildenafil did not improve Nrf2-dependent antioxidants in testosterone-exposed pregnant Wistar rats

Gestational testosterone exposure reduced hypothalamic Nrf2 and catalase but did not affect -1 and 2 compared with control animals (Figure 6). Sildenafil treatment during gestational testosterone exposure reduced hypothalamic Nrf2 compared with control but increased it compared with animals exposed to gestational testosterone alone (Figure 6(a)). Sildenafil treatment during gestational testosterone exposure reduced hypothalamic catalase compared with both control animals and animals exposed to gestational testosterone alone (Figure 6(d)). Sildenafil treatment during gestational testosterone exposure did not affect both hypothalamic SOD-1 and 2 compared with control and animals and animals exposed to gestational testosterone alone.

### 3.7. Sildenafil did not affect lipid peroxidation and markers of tissue injury in testosterone-exposed pregnant Wistar rats

Hypothalamic malondialdehyde, alanine transferase, aconitase and lactate were not affected across the groups (Figure 7). Hypothalamic aspartate transaminase was increased by gestational testosterone exposure compared with control but was not affected by sildenafil during testosterone exposure compared with both control and animals exposed to gestational testosterone only (Figure 7(c)).

### 3.8. Sildenafil did not improve phosphodiesterase-5 activity, nitric oxide and cyclic guanine monophosphate levels in testosterone-exposed pregnant Wistar rats

Gestational testosterone exposure reduced PDE5 activity and NO level but increased hypothalamic cGMP compared with control animals (Figure 8). Sildenafil treatment during gestational testosterone exposure reduced hypothalamic PDE5 activity and nitric oxide levels and increased hypothalamic cGMP compared with control but were not affected
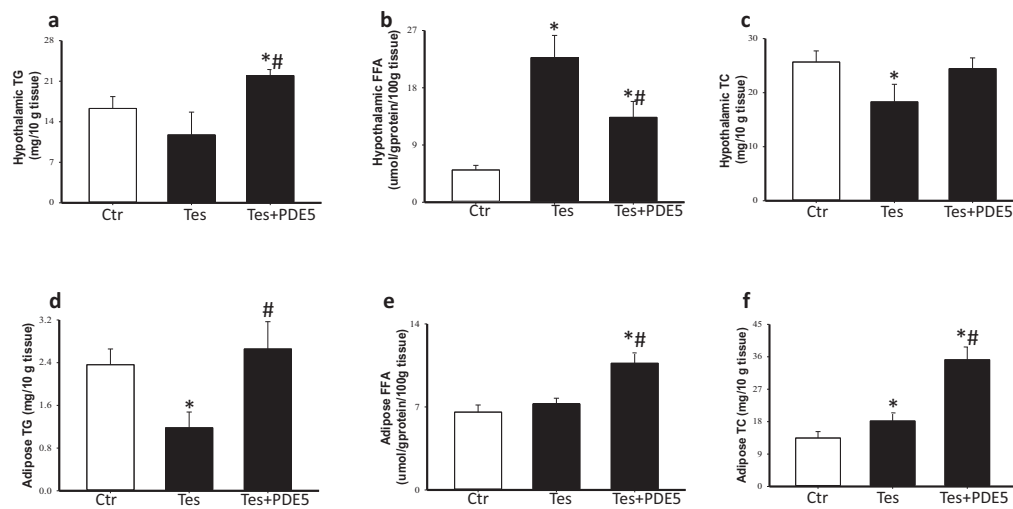


**Figure 3.** Effect of Sildenafil on leptin resistance in testosterone-exposed pregnant in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil did not affect plasma leptin (a), increased plasma adiponectin (b), reduced plasma leptin-adiponectin ratio (c) did not affect adipose tissue leptin (d), adiponectin (e), hypothalamic leptin (f) but increased hypothalamic adiponectin (g) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.
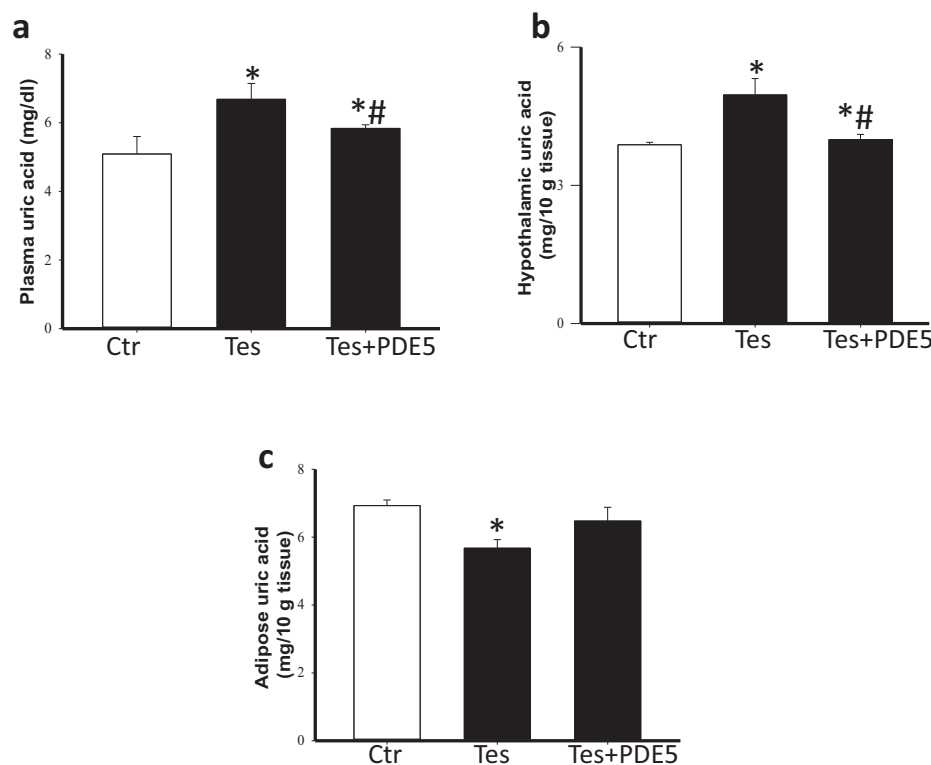
**Figure 4.** Effect of Sildenafil on hypothalamic-adipose lipid handling in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil increased hypothalamic triglyceride; TG (a), reduced hypothalamic free fatty acid; FFA (b), did not alter hypothalamic total cholesterol; TC (c), increased adipose TG (d), FFA (e), and TC (f) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.

compared with animals exposed to only gestational testosterone (Figure 8).

## Discussion

The present study shows that gestational testosterone exposure reduced maternal body weight phenotypes concomitant with reduced adipose tissue weight. Although fasting blood glucose was not elevated, there was evidence of glucose dysregulation: hyperinsulinemia, hypertriglyceridemia, hyperuricemia and leptin resistance taken as elevated leptin-adiponectin ratio which indicates possible alterations in lipid handling by either or both hypothalamus and adipose tissue. In relation

to this, intramural events in the adipose tissue exhibited lipolysis and reduction in leptin and adiponectin following gestational testosterone exposure. The lipolytic action affirms overt insulin resistance and informs the increase in circulating triglyceride. However, hypothalamic lipid handling responses to gestational testosterone exposure in this study showed that free fatty acid was elevated whereas hypothalamic triglyceride and cholesterol accumulation was attenuated. This hypothalamic lipid status was also associated with elevated uric acid, reduced Nrf2 and PDE5 activity. Sildenafil however, attenuated gestational testosterone-induced glucose dysregulation, leptin resistance, restored hypothalamic-adipose lipid mishandling and bodyweight phenotype.



**Figure 5.** Effect of Sildenafil on circulating and tissue uric acid levels in testosterone (Tes)-exposed pregnant animals Wistar rats. Sildenafil reduced plasma uric acid (a), hypothalamic uric acid (b), did not alter adipose uric acid (c) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.
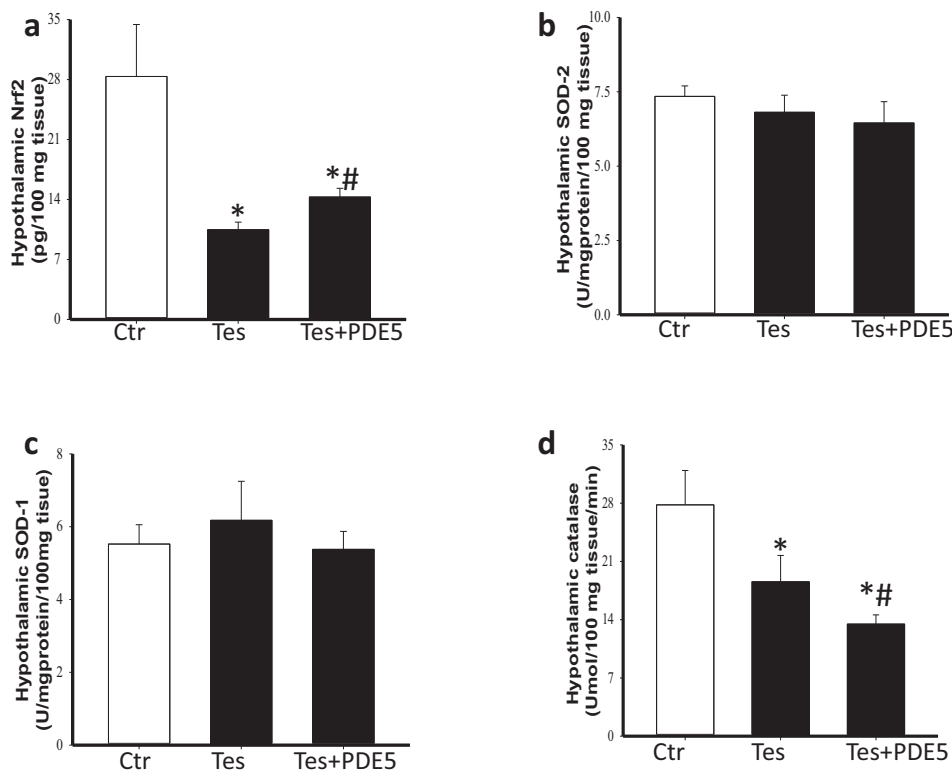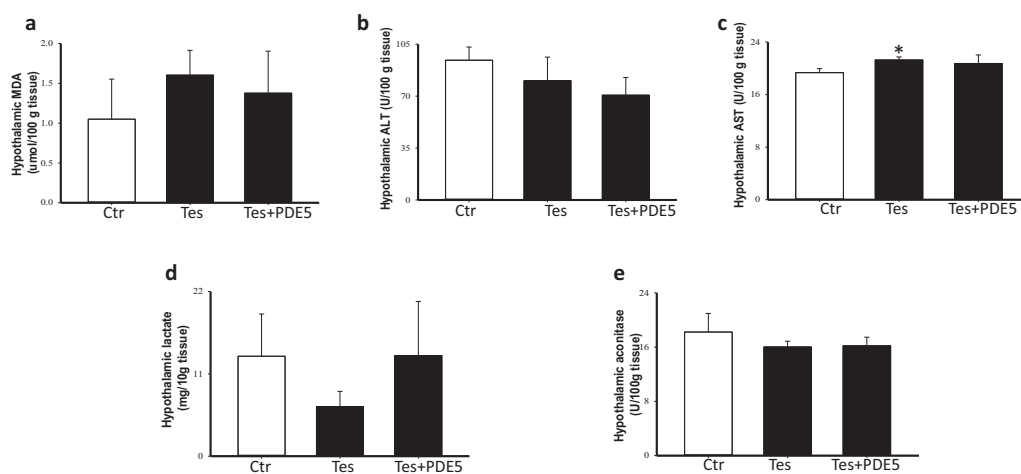
**Figure 6.** Effect of Sildenafil on Hypothalamic Nrf2-dependent antioxidants in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil increased hypothalamic nuclear factor erythroid 2-related factor 2; Nrf2 (a), did not affect hypothalamic superoxide dismutase-2; SOD-2 (b), superoxide dismutase-1; SOD-1 (c) but hypothalamic catalase (d) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.

This occurred independent of insulin level and hypothalamic PDE5 activity.

A previous study from our laboratory with rats had shown that gestational testosterone exposure is associated with reduced body weight change and adiposity without alteration in food intake showing that the animals ate more than their weights [23]. The present study also acquired similar body weight and adiposity findings from data with the same experimental model employed by Usman et al. The mechanism of testosterone-induced changes in body weight and adiposity independent of food intake during pregnancy requires elucidation. Body weight, adiposity and feeding are phenotypic and behavioural indicators of energy homeostasis anchored mainly by the hypothalamus-adipose axis. In

addition, important drivers or signals of energy status like glucose, lipids and glucoregulatory hormones (insulin, leptin, adiponectin etc) levels are worthy of considerable attention putting their status in circulation and hypothalamus-adipose handling in perspective in order to elucidate the phenomenal maternal weight phenotype and feeding behaviour during gestational testosterone exposure.

Gestational cardiometabolic syndrome has significant association with hyperandrogenic milieu as proposed by Olatunji et al. [24]. The present study showed that gestational exposure to testosterone caused glucoregulatory disturbances that regardless of normal glycemia outcome (Figure 2(a)). Normal blood glucose can be obtained in insulin resistant conditions due to increased insulin secretion. Here, despite euglycemia,



**Figure 7.** Effect of Sildenafil on hypothalamic lipid peroxidation and markers of tissue injury in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil did not affect hypothalamic lipid peroxidation; MDA (a), alanine transferase; ALT (b), aspartate transaminase; AST (c), lactate (d) and aconitase (e) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.
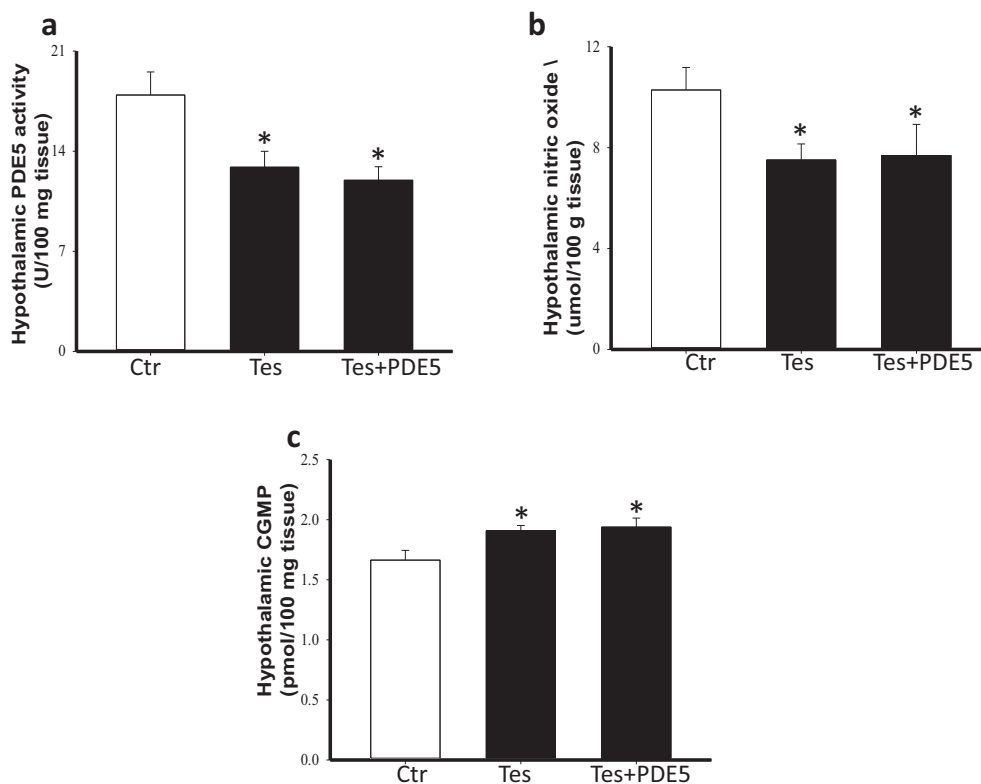
**Figure 8.** Effect of Sildenafil on hypothalamic PDE-5 activity, NO and cyclic guanine monophosphate levels in testosterone (Tes)-exposed pregnant Wistar rats. Sildenafil did not affect hypothalamic phosphodiesterase-5 activity; PDE5 activity (a), nitric oxide (b) and hypothalamic cyclic guanine monophosphate; cGMP (c) in gestational testosterone-exposed Wistar rats. Gestational testosterone exposure and sildenafil treatment were carried out in days 14–19 of gestation. Ctr: animals treated with olive oil subcutaneously and distilled water orally; Tes: animals treated with testosterone propionate subcutaneously; Tes + PDE5: animals treated with sildenafil during testosterone exposure. 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 Tes. Data were analyzed by one-way ANOVA and Bonferroni Post-hoc test.

there were hyperinsulinemia (Figure 2(b)), hypertriglyceridemia (Figure 2(c)) and hyperuricemia (Figure 5(a)) in testosterone-exposed pregnant rats. These outcomes are indicators of cardiometabolic syndrome. In support of this, adipose tissue triglyceride was reduced indicating insulin resistance-induced lipolysis which causes FFA spill over and eventual reconstitution in the circulation and ectopic deposition in non-adipose tissue [33]. Adipose tissue lipid handling, leptin and adiponectin secretion are signals to the hypothalamus for central regulation of body weight and feeding. It is however explicable that the observed reduction in adiposity is responsible for the reduction in circulating and adipose tissue leptin and adiponectin levels following gestational testosterone exposure (Figure 3(a)). In order to elucidate hypothalamic responses to the signals engendered by gestational testosterone exposure, levels of triglyceride, FFA, leptin, adiponectin, Nrf2-dependent antioxidant markers, uric acid, lactate and PDE5 activity were assessed in the hypothalamus of testosterone-exposed pregnant animals.

Hypertriglyceridemia due to insulin resistance has been shown to prevent leptin transport across blood brain barrier leading to leptin resistance [34] and non-suppression of feeding. Here, gestational testosterone exposure caused reduction in hypothalamic leptin but adiponectin remained unaltered (Figure 3). In this study, the hypothalamus was homogenized with cerebrospinal fluid which contains leptin and adiponectin that were able to cross the blood brain barrier. The results here indicate that less of leptin crossed the blood brain barrier owing plausibly to elevated circulating triglyceride (resulting in elevated circulating leptin-adiponectin ratio) and consequently leptin might have been prevented from maximal inhibition of hypothalamic orexigenic activities. Hypothalamic lipid handling also showed lipolytic events in which hypothalamic triglyceride was not significantly changed but tended towards reduction and FFA was elevated (Figure 4(a)). Elevated hypothalamic FFA can engender certain metabolic events that include inhibition of glucose utility, increased b-oxidation, increased uric acid production and lipid peroxidation which set the stage for inflammatory responses. To confirm these possibilities, the present study showed that

gestational testosterone exposure caused increased hypothalamic uric acid (Figure 5(b)), reduced Nrf2 (Figure 6(a)) and catalase (Figure 6(d)). The Keap1/Nrf2/antioxidant responsive element pathway regulates expression of various antioxidant genes [26]. Downregulation of Nrf2 signaling is implicated in conditions with oxidative stress which are improved by Nrf2 activators [27]. Hence, Nrf2 activators may be beneficial in gestational hyperandrogenemia. This outcome was however accompanied by reduced hypothalamic PDE5 activity elevated cGMP with reduction in nitric oxide. Hence, testosterone does not induce PDE5 activity in the hypothalamus and will therefore not affect nitric oxide signaling. But the reduction in NO levels shows that hypothalamic nitric oxide synthesis was affected by testosterone. This may compromise blood flow to the hypothalamus and reduce glucose supply leading to firing of orexigenic glucose-inhibited neurons.

In the hypothalamus, some aspects of the arcuate nucleus prefer FFA as fuel especially those that release proopiomelanocortin which are glucose-excited neurons in charge of anorexic signals whereas, the orexigenic neurons releasing neuropeptide Y prefer glucose and are glucose-inhibited [35]. The limitation of the present study is that specific hypothalamic neurons were not studied but the metabolic state of the whole hypothalamic tissue diffusely reveals a status that favours FFA and not glucose metabolism (Figure 8(c)). Therefore, it is plausible that the hypothalamic metabolic status described here following gestational testosterone exposure shows glucose-inhibited neurons that increase food intake may be firing and glucose-excited neurons may be inhibited. It is proposed here that gestational excess testosterone decreases hypothalamic glucose flux via FA-associated inhibition of glucose transport and glycolysis to engender orexigenic responses. Taken together, leptin resistance, hypothalamic-adipose lipid mishandling and metabolic obesity but not phenotypic obesity characterizes the metabolic outlook of excess testosterone in IUGR and related maternal illnesses.

Studies have not focused on the role of sildenafil in central energy homeostasis, whereas, deregulation of energy balance is prevalently associated with conditions that culminate in intra uterine growth

restriction. Although lack of data on fetal outcomes remains a limitation for this study, it was found here that sildenafil oral administration to testosterone-exposed pregnant rats reduced hypothalamic PDE5 activity but the reduction was not significantly different from what was obtained with testosterone alone which shows that the effects engendered by sildenafil herein are independent of PDE5 activity. Sildenafil improved body weight phenotype but did not correct adiposity, hypertriglyceridemia and hyperinsulinemia caused by gestational testosterone. The ability of sildenafil to increase insulin secretion might be responsible for the sustained hyperinsulinemia. But preventing lipolysis in adipose tissue (Figure 4d) showed that sildenafil might also improve insulin sensitivity. However hyperuricemia was corrected and circulating adiponectin were improved. Elevated circulating uric acid has been shown to reduce adiponectin production [36]. Correcting hyperuricemia might be the mechanism by which sildenafil upregulated adiponectin and relatively corrected testosterone-induced hypothalamic inflammation-related events (uric acid and Nrf2) and leptin resistance independent of hypertriglyceridemia. To affirm this, sildenafil increased hypothalamic adiponectin to approximately 5-folds. Also, sildenafil corrected testosterone-induced hypothalamic lipolysis and by reducing hypothalamic FFA it may avert the glycolysis inhibition and inflammatory responses associated with elevated FFA.

In conclusion, sildenafil treatment during gestational testosterone exposure can independent of PDE5 activity or nitric oxide action avert leptin resistance and hypothalamic-adipose lipid mishandling accompanied with augmented Nrf2 and attenuated uric acid in the hypothalamus of testosterone-exposed pregnant rats.

## Declarations

### Author contribution statement

Emmanuel Damilare Areolaa: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Isaiah Woru Sabinaria: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Faith Ifeoluwa Abayomia: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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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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