



Research article

Postnatally induced metabolic and oxidative changes associated with maternal high-fat consumption were mildly affected by Quercetin-3-O-rutinoside treatment in rats



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ABSTRACT

Oxidative stress is usually associated with prolonged intake of high-fat diet (HFD). However, little is known about the impact of maternal HFD on endogenous modulation of antioxidant-defence-enzyme-network, its link to adverse fetal growth and overall effects of Quercetin-3-o-rutinoside (QR) supplementation. Sprague-Dawley rats were initially assigned to normal diet (ND) or HFD for 8 weeks and mated. Post-conception, rats were further divided into four groups, of which two groups had diets supplemented with QR while others continued with their respective diets until delivery. Measurements include food and water consumption, physical parameters (body weight, body mass index (BMI) and fur appearance), oral glucose tolerance, lipid profiles, and placental/liver oxidative changes. We observed that water consumption was significantly increased in dams fed HFD without marked differences in food intake, body weight, BMI and glucose tolerance. Surprisingly, offspring of HFD-fed dams had reduced body weight marked by delayed fur appearance compared to the ND offspring. In dams, there were alterations in lipid profile. Lipid peroxidation was increased in the placenta and liver of gestational day (GD) 19 HFD-fed dams and their postnatal day (PND) 21 male offspring. There was evidence of HFD-induced nitrosative stress in dams and PND28 female offspring. Adaptive defence indicate decreased placenta and liver superoxide dismutase (SOD) levels as well as differential changes in total antioxidant capacity (TAC) and catalase (CAT) activity in HFD treated dams and their progenies. Overall, the results indicate that intrauterine metabolic alterations associated with maternal high-fat consumption may induce oxidative challenge in the offspring accompanied by mild developmental consequences, while QR supplementation has little or no beneficial effects.

1. Introduction

Studies have established the link between certain phenotypic alterations that result from early adverse life exposures and intergenerational susceptibility to poor health outcomes [1, 2, 3]. Increasing evidence clearly indicate that modulation of phenotypic traits is also of direct relevance to maternal nutritional imbalance with profound implications involving transmissible imprints and early life programming that could provoke metabolic dysfunctional states in the progenies [4, 5, 6, 7]. Indeed, extensive experimental data suggests that maternal experience, such as chronic exposure to high-fat diet (HFD), may not only influence programmed effects in the fetus but also accompanied by dysregulated placenta development and renal functions [6], cardiovascular disorder

[8] and other related metabolic dysfunctions including type II diabetes mellitus [9] and fetal obesity [10, 11].

Prolonged intake of HFD is commonly associated with cellular oxidative stress. Recent study by Yu et al (2018) showed that HFD-induced oxidative stress impaired lipid homeostasis in mice by blocking the activity of hepatic nuclear factor 4 α , downregulated apolipoprotein B and reduced very-low density lipoprotein (VLDL) secretion [12]. These changes are linked to development of non-alcoholic fatty liver disease. On the other hand, rats fed on HFD and/or high-fructose diet can develop cluster of physiological abnormalities which include hyperglycaemia, hyperinsulinemia, glucose intolerance, hypercholesterolemia, hypertriglyceridemia and hypertension [13, 14, 15]. Other studies have shown that intrinsic antioxidant defence enzyme system, proteins and other essential cellular components are also compromised

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by poor and/or imbalanced maternal nutrition during gestation which leads to significant alterations in the balance between oxidative and antioxidant factors, coupled with excessive production of free radicals, leading to compromised fetal development [16, 17]. Although the precise association between fetal energy imbalance and the resulting metabolic disorder has been established, the mechanism linking oxidative stress, adverse fetal growth and later risk of developing metabolic syndrome is yet to be fully understood. An understanding of HFD-induced placental and/or *in-utero* oxidative and metabolic changes may help unravel the mechanisms involving maternal-foetal transmission.

Quercetin-3-O-rutinoside (QR) is a flavonoid glycoside popularly known for its antioxidant potential and has been used as nutritional supplements in the treatment of variety of diseases. Using voltammetric and flow cytometric methods, Zhang et al (2011) showed that quercetin increased the production of total antioxidant capacity (TAC) and decreased reactive oxygen species (ROS) and nitric oxide (NO) production in lipopolysaccharide-stimulated human THP-1 acute monocytic leukemia cells [18]. Another study demonstrated that daily consumption of monoglucosyl-rutin inhibited HFD-induced visceral fat accumulation and prevented excessive weight gain by suppressing gastric inhibitory polypeptide secretion in mice [19]. It is worth noting that QR data from animal studies at times contradicts reports from human studies [20], hence merits further investigation.

The current study aimed at investigating the impact of HFD-induced oxidative changes in the placenta and liver of Sprague Dawley (SD) rats [21] and addressed how *in utero* exposure to HFD influences offspring glucose homeostasis and lipid profiles, antioxidant enzyme network and fetal development. Overall effects of QR supplementation on HFD-induced metabolic and developmental alterations were also examined.

1.1. Materials and methods

This study was carried out in accordance with the approved protocol (AREC/005/018D) by Animal Research Ethics Committee of the University of KwaZulu-Natal (UKZN), South Africa in accordance with National Institute of Health (NIH) guidelines for the care and use of laboratory animals.

1.1.1. Animals and diets

Male and female SD rats (180–200g) used in this study were obtained from the Biomedical Resource Unit (UKZN). They were bred and housed under standard laboratory conditions (50–60% humidity, $23 \pm 2^\circ\text{C}$ room temperature, and 12h light/dark cycle with lights on at 06h00). Regular rat chow (ND) and modified diet containing energy from dietary lard-based fat were used in this study. The normal rat chow was composed of grain and grain by-products, forage products, plant protein products, animal protein products, oils and fats, minerals, vitamins and registered stock remedies, with approximately 18% protein, 2.5% fat, 6.0% fibre, 1.8% calcium, 0.7% phosphorus and 12% moisture (EPOL, South Africa). The pelleted lard-based diet which is hereafter referred to as HFD was composed of normal food and sugar containing approximately 45% fat, 20% protein and 35% carbohydrate (Tshwane University of Technology, South Africa) [22, 23]. Rats had free access to their diets and water, except where mentioned otherwise. Food and water intake were recorded during the first eight weeks of dietary treatments.

1.1.2. Experimental design

After two weeks acclimatization, the female rats were randomized to two treatment groups ($n = 28$ per group) that either received ND or HFD for eight weeks and body weight changes were monitored. At estrus, the nulliparous female rats were paired with their male counterparts (previously fed ND) at a ratio of 2:1 (two females to one male per cage) for mating. Pregnancy was confirmed by the presence of spermatozoa in the vaginal smear when viewed under light microscope. Thereafter, the male

rats were removed, and the dams were housed individually. After fertilization, the pregnant rats continued with their respective diets (ND or HFD; $n = 7$ per group), of which half set had their diets supplemented with QR (150 mg/kg; ND/QR and HFD/QR; $n = 7$ per group) added to drinking water, until delivery (Figure 1). As expected, that rats weighing 250–300g would consume an average of 30–50ml of water per day, hence we first dissolved QR (dose calculated based on weight) in 20ml of water and presented to each rat in calibrated bottles early in the morning which was later topped up in the day. Average daily water consumption was carefully monitored and recorded. Half of the pregnant rats ($n = 7$ per group) were killed on gestation day 19 (GD19) and tissues were collected. The remaining female rats gave birth at approximately 21 days post-conception, littering was carefully monitored to ensure age matching while QR and HFD treatments were discontinued. Thereafter, the pups were weaned at three weeks of age, housed by gender and maintained on ND. After weaning, the postpartum (PP) 21 dams were killed and their offspring were sacrificed at postnatal day (PND) 21, 28 and 35.

1.2. Tissue collection

Rats were killed by inhalation of anaesthesia (Isofor) in an airtight chamber and both placentas (GD19 dams) and livers (GD19 dams, PP21 dams and all offspring) were collected, weighed and snap-frozen in liquid nitrogen for subsequent protein and biochemical analyses.

1.3. Oral glucose tolerance test (OGTT)

OGTT was performed before and after 8-week HFD treatment. After an overnight fast female rats ($n = 10$ per group) randomly selected from ND and HFD groups were mildly bled by puncturing the tail vein and blood glucose was determined using Accu-chek active glucometer (USA) at basal time, 15, 30, 60 and 120 min. Between the basal and the 15 min time interval, the rats were given oral glucose solution (2 g/kg bw) to induce spiking effect.

1.4. Quantification of plasma lipid content

Blood was collected into lithium heparinized or plain tubes by milking the punctured tails [24] of ND and HFD female rats ($n = 10$ per group). Due to the amount of blood required for lipogram analysis, blood samples were pulled ($n = 2$ into each sampling tube) before assaying for total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL) and low-density lipoprotein (LDL), performed by Global Clinical and Viral Laboratories, Durban.

1.5. Measurement of lipid peroxidation in liver and placenta

Lipid peroxidation in the rats was determined using a standard laboratory procedure to assay for the concentration of malondialdehyde (MDA). MDA levels was assayed using Thiobarbituric acid reactive substance (TBARS) colorimetric method [25, 26]. Tissues were homogenised with 0.2% phosphoric acid and centrifuged for 10 min at 10000rpm. Phosphoric acid (2% or 7%), butylated hydroxyl toluene and thiobarbituric acid (TBA) solution were added while the resultant solution was transferred to a water bath and heated at 100°C for 15 min. Thereafter, butanol was added, and the top phase was transferred to 96 well plate in triplicate. Absorbance was measured at 532nm and 600nm.

1.6. Quantification of antioxidant defence system in liver and placenta

1.6.1. Superoxide dismutase (SOD)

The tissue was homogenised and centrifuged in a cold centrifuge at 10000 rpm for 10 min to obtain the supernatant. Determination of SOD activity was based on the premise that hydrogen peroxide produced from the dismutation of superoxide ion by SOD oxidized 6-hydroxydopamine (6-HD) to produce a coloured product and 0.1mM

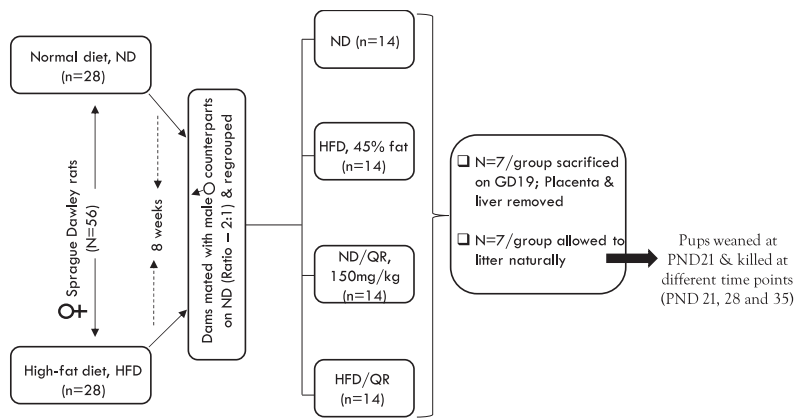


Figure 1. Schematic showing experimental design and timeline of the study. Female SD rats were randomly assigned to dietary treatment groups, ND-fed or HFD-fed groups ($n = 28/\text{group}$) for approximately 8 weeks. Thereafter, these groups of rats were mated with their male counterparts who received normal rat chow and regrouped after conception, based on the dietary treatments supplemented with or without QR. It is worth noting that dietary treatments continued throughout gestation. Half set of the pregnant rats ($n = 7/\text{group}$) were killed at GD19, their placenta and liver were harvested. The remaining pregnant rats were allowed to litter naturally and hereafter referred to as PP21 dams. Both PP21 dams and one-third of their progenies were killed at PND21 after weaning. The remaining two-third of the offspring were killed at PND 28 and 35. At all the killing time-points, liver was removed and preserved for subsequent biochemical analyses.

diethylenetriaminepentaacetic acid (DETAPAC) was used to inhibit aerobic autoxidation of 6-HD. 1.6mM 6-HD was prepared using MilliQ water and hydrochloric acid which was sonicated to remove air bubbles by negative pressure. The resulting 1.6mM 6-HD was wrapped in aluminium foil and stored on ice for immediate use. SOD assay buffer was used for blank. Absorbance at 490nm was recorded for 5 min in 1 min interval using at spectrophotometer 96 well plate reader [27, 28].

1.6.2. Catalase (CAT)

Assessment of CAT activity was based on the principle that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide, and perchromic acid (unstable) is formed as an intermediate compound. The CAT preparation could split hydrogen peroxide from different time. Chromate/acetic acid mixture was added to stop the reaction while the remaining hydrogen peroxide mixture was determined by measuring chromic acetate colorimetric after heating the reaction mixture for 10 min in boiling water. Standard concentrations at 10, 20, 30, 40, 60, 80 and 100 μ hydrogen peroxide was used. Absorbance was read at 570nm. A standard curve was plotted and catalase activity was extrapolated from the standard curve [27, 29].

1.6.3. Reduced glutathione (GSH) concentration

Reduced GSH assay was based on a previously modified method by Oyeboye et al. 2018 [27]. After precipitating with 10% trichloroacetic acid (TCA), supernatants were transferred to 96-well plate, 0.5mM DTNB (5, 5'-dithio-bis (2-nitrobenzoic acid)) and 0.2M sodium phosphate buffer (pH 7.8) were added and incubated for 15 min. Absorbance was read at 415nm.

1.6.4. Determination of nitric oxide (NO)

An indirect diazotization technique was used to assay for NO concentration in placenta and liver tissues. Briefly, the concentration of nitrites in the tissue homogenates were measured based on Griess reaction method, as previously described [30].

1.7. Total antioxidant capacity (TAC)

The concentration of total antioxidant molecules in placenta and liver tissues were determined using commercially obtained enzyme linked immunosorbent assay (ELISA) kit (Elabsience Biotechnology, USA). The kit uses the FRAP (ferric reducing antioxidant power) method for colorimetric quantification of antioxidant levels, such that Fe-TPTZ (2,4,6-tripyridyl-s-triazine) are reduced by antioxidants under acid conditions. TAC in the samples were detected at 593nm wavelength.

1.8. Statistical analysis

Results are presented as mean \pm SEM, and were statistically analysed using Student's t-test, one-way analysis of variance (ANOVA) or two-way

ANOVA, followed by Bonferroni post-hoc analysis, where appropriate. Values were considered statistically significant when $p < 0.05$. Statistical procedures were performed using GraphPad Prism software (version 5.0, USA).

2. Results

2.1. HFD-induced changes in food and water consumption, body weight composition and fur appearance

To confirm the hypothesis that QR treatment could protect against transgenerational homeostatic imbalance owing to maternal nutritional drift through exclusive high-fat intake, we initially exposed adult female Sprague-Dawley rats to ND or HFD for 8 weeks, mated and subsequently fed with QR supplemented diets. Figure 2a indicates no significant difference in the amount of food consumed by both ND (control) and HFD-treated rats during the first 8 weeks of dietary exposure ($p > 0.05$). Surprisingly, water consumption during this period was greatly increased in dams fed with HFD compared to the ND dams ($p = 0.0006$; Figure 2b). Moreover, we observed time-related progressive increases in body weight of both HFD and ND fed dams ($F_{(8, 96)} = 376.1, p = 0.001$; Figure 2d) without marked differences in their weight gain and body mass index (BMI) ($p > 0.05$; Figure 2d, c). Strikingly, two-way ANOVA detected significant interaction ($F_{(6, 40)} = 2.885, p = 0.0197$; Figure 2e) between effects of HFD and time on body weight gain in male offspring rats (HFD treatment, $F_{(3, 40)} = 15.07, p < 0.0001$; time, $F_{(2, 40)} = 607.2, p < 0.0001$; Figure 2e), but not in the females ($F_{(6, 40)} = 2.166, p = 0.0667$; Figure 2f). However, body weights of all male and female offspring of HFD-treated dams were significantly lower than offspring of ND dams (ND and ND/QR) when assessed post-weaning to early adulthood ($p < 0.05$; Figure 2e, f). No difference in body weight composition was observed between the offspring of HFD and HFD/QR-treated dams ($p > 0.05$; Figure 2e, f), suggesting that maternal QR treatment has no impact on HFD-induced weight changes in the progenies. Differences in diet composition of the offspring rats also coincide with changes in their fur appearance. After birth, almost all offspring of HFD or HFD/QR dams had delayed fur appearance that persisted till PND 28 (Figure 2gii), as opposed to normal fur appearance in offspring belonging to ND or ND/QR groups (Figure 2gi).

2.2. Effects of HFD on fasting blood glucose levels and plasma lipid profiles of dams

We also examined the impact of HFD consumption on glucose homeostasis and plasma lipid profiles. After 8 weeks of dietary treatments, fasted blood glucose levels in HFD-treated rats was similar to those of ND-fed rats, indicating that glucose tolerance was unaffected by 45% lard-based high-fat during the test period ($p > 0.05$, Figure 3a). Lipid analyses indicated that HFD-fed dams exhibited hypertriglyceridemia

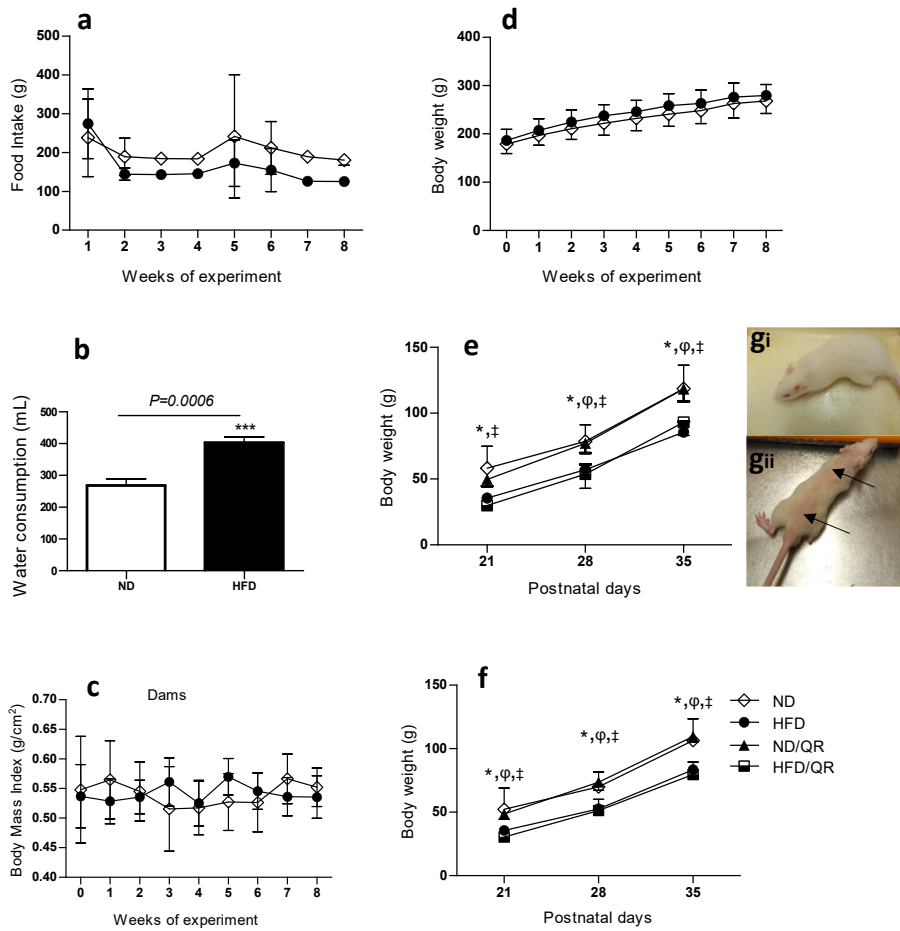


Figure 2. Indicate food intake (a) and total water consumed (b) by dams during 8 weeks of HFD exposure. Also, body weight (d) and BMI (c) of dams, and differences in body weight of male (e) and female (f) offspring rats were measured at PND 21, 28 and 35. Differences in fur appearance of offspring of control dams (ND or ND/QR; gi) and HFD-fed dams (HFD or HFD/QR; gii) observed at PND 28. Data shown represents mean \pm SEM; dams, n = 6 or 7 per group; offspring, n = 6 per group. * $P < 0.05$, ND vs. HFD, HFD/QR; $\phi P < 0.05$, HFD vs. ND/QR; $\ddagger P < 0.05$, ND/QR vs. HFD/QR; Student's t-test; Two-way ANOVA, followed by Bonferroni *post hoc* comparison test. Arrows indicate regions of late fur appearance.

accompanied by high plasma -level of LDL, when compared to ND-fed dams ($p < 0.05$; Figure 3c, e). Whereas, plasma TC and HDL levels were significantly lower in HFD-fed dams compared to ND-fed dams ($p < 0.05$; Figure 3b, d).

2.3. MDA levels in placenta and liver

MDA concentration was measured in GD19 dams' placenta and liver as well in the liver of PP21 dams and their offspring. Our findings show

that MDA concentration was significantly increased by HFD in placenta and liver of GD19 dams compared to ND-treated group ($p < 0.05$; Figure 4a, b). Also, liver and placenta MDA levels were significantly reduced in groups fed with QR supplemented diets compared to HFD-fed rats ($p < 0.05$; Figure 4a, b), whereas QR treatments did not reverse HFD-induced changes in these groups of rats ($p > 0.05$; Figure 4a, b). Moreover, both HFD and QR supplement had no effect on MDA concentration in the liver of PP21 dams ($p > 0.05$; Figure 4b). Strikingly, only PND 21 male offspring of HFD-fed dams exhibited significantly elevated hepatic

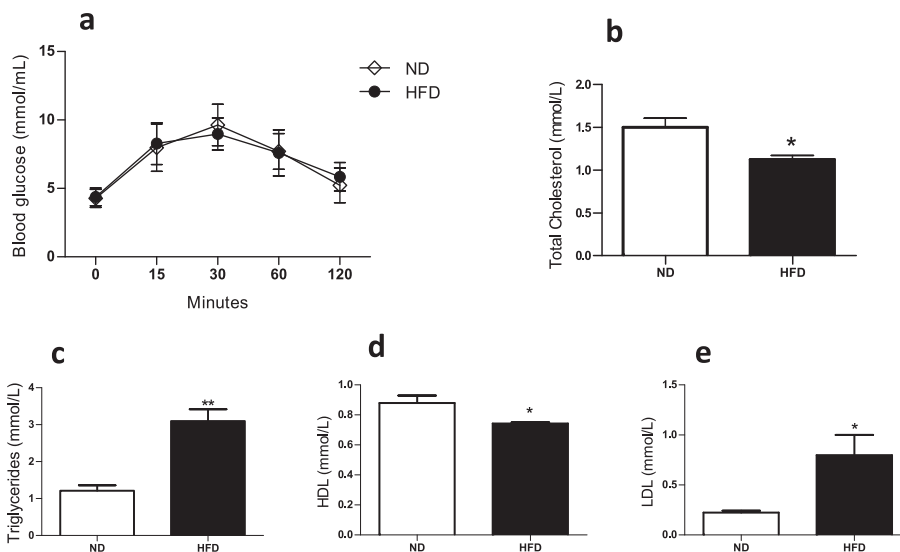


Figure 3. OGTT and lipogram results obtained from dams after 8 weeks of HFD consumption. Fasting blood glucose (a), TC (b), triglycerides (c), HDL (d) and LDL (e) levels in dam's blood post high fat meal. Data shown represents mean \pm SEM; OGTT, n = 10 per group; Lipids, n = 4 per group (please note that results from one sample was excluded in the statistics due to incomplete data). * $P < 0.05$, HFD versus ND; Student's t-test; Two-way ANOVA, followed by Bonferroni *post hoc* comparison test.

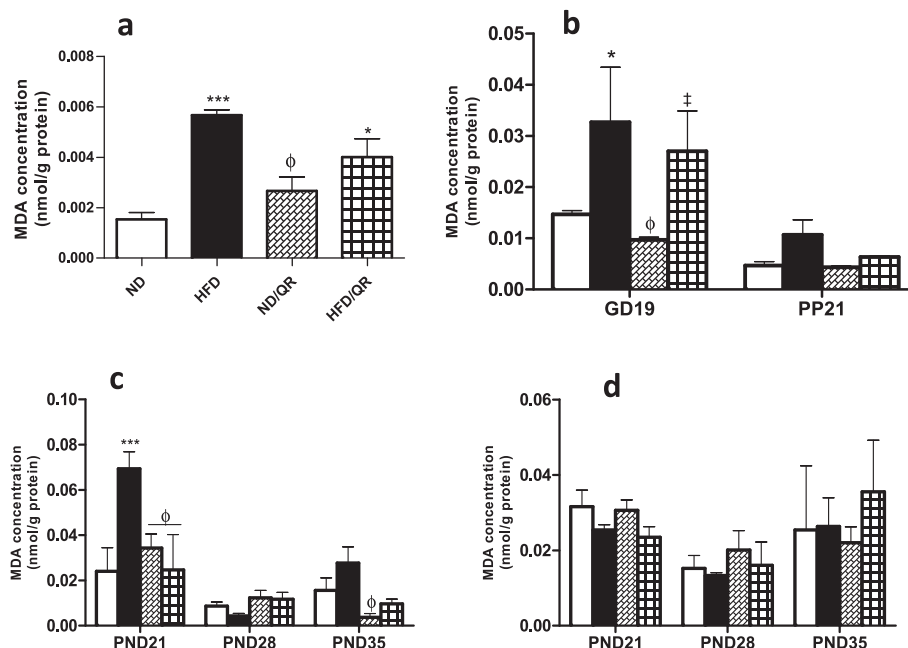


Figure 4. Lipid peroxidation profile indicating MDA concentration in (a) placenta, (b) liver tissues of GD19 and PP21 dams, (c) liver of male and (d) female offspring rats at PND 21, 28 and 35. Data shown represents mean \pm SEM; $n = 6$ per group. * $P < 0.05$, *** $P < 0.0001$ compared to ND; † $P < 0.05$, ND/QR vs. HFD/QR; One-Way or Two-way ANOVA, followed by Bonferroni *post hoc* comparison test.

MDA levels which was depressed in offspring of all QR-treated dams ($p < 0.0001$; Figure 4c). This variable was not altered in the female offspring ($p > 0.05$; Figure 4d).

2.4. Placenta and liver NO concentration in dams and offspring

Our data showed significant main effect of treatments on NO concentration in GD19 placenta ($F = 8.051$, $p < 0.001$; Figure 5a), while comparison test indicated only significant decrease in placenta NO of HFD/QR ($p < 0.05$; Figure 5a) and not HFD group compared to ND-fed rats ($p > 0.05$; Figure 5a). This suggest that placenta NO was unaffected by maternal HFD consumption. However, this was not the case in GD19 and PP21 dams' liver as HFD consumption provoked NO production which appears prevented by QR treatment ($p < 0.05$; Figure 5b). Overall, NO concentration was greater in GD19 liver compared to PP21 (Figure 5b). Similar to dams, hepatic NO was also significantly increased only in PND 28 female offspring of HFD-dams and decreased in offspring of QR-treated dams compared to ND ($p < 0.05$; Figure 5d). There was no change in liver NO of all groups of male offspring ($p > 0.05$; Figure 5c).

2.5. Correlation between MDA and NO in liver

(see Figure 6).

2.6. Antioxidant status of dams and offspring

As shown in Table 1, we observed significant decrease in placenta SOD concentration of all HFD and QR-treated GD19 dams compared to ND ($p < 0.05$), without any change in placenta CAT, GSH and TAC ($p > 0.05$). Also, GD19 liver SOD concentration was similar to the placenta profile, indicating decreased liver SOD in HFD and QR treated dams ($p < 0.05$, Table 2). In contrast, liver SOD was significantly increased in PP21 dams fed either HFD or diets supplemented with QR ($p < 0.05$, Table 2). In the offspring, liver SOD was significantly increased in PND 35 HFD females only compared to ND group ($p < 0.05$, Table 3), without significant change in other groups ($p > 0.05$, Table 3). We also observed that co-exposure to HFD and QR significantly increased liver CAT and TAC in

GD19 and PP21 dams, respectively, compared to ND-fed dams ($p < 0.05$, Table 2). Male offspring of HFD-fed dams had increased liver concentration of CAT and TAC at PND 21 and 28, respectively ($p < 0.05$, Table 3), while liver GSH was significantly increased in offspring of all HFD-fed dams only at PND 28 and 35 ($p < 0.05$, Table 3). Moreover, there was also a significant increase in liver GSH of PND 21 and 35 male offspring of dams that received normal diets supplemented with QR (ND/QR group) compared to ND group ($p < 0.05$, Table 3). In the female offspring, there was no significant alterations in liver CAT, GSH and TAC levels at PND 21, 28 and 35, except an observed significant increase in liver TAC concentration of PND 35 female offspring of HFD-fed dams ($p < 0.001$, Table 3).

3. Discussion

The current study comparatively examined HFD-induced oxidative changes in the placenta and liver of SD rats and addressed how *in utero* exposure to HFD influences offspring glucose homeostasis, lipid profiles, fetal growth and antioxidant enzyme network. Potential alleviation of HFD-induced metabolic and developmental alterations by QR was also investigated.

As documented in the literature, increased body weight gain and abdominal obesity are prominent features of metabolic syndrome (MS) generally associated with excess consumption of fat [31]. Here, we show that food consumption by SD rats that had exclusive access to 45% HFD for 8 weeks matched those of the control rats fed normal chow. These observations partly contrast with previous findings that SD rats fed lard-based HFD or diets rich in high-fat and high-sugar for either 4 or 8 weeks had lower food intake but consumed more calories, accumulated fat mass and exhibited increased body weight gain than rats fed with control diet [31]. 30–60% energy from saturated fat was previously recommended for use in animal models to induce obesity and/or metabolic alterations [32, 33]. In the current study, we observed that 45% fat did not provoke severe metabolic changes. However, many other studies that reported significant metabolic and weight changes used higher energy content and allowed prolonged consumption. There is also accumulating evidence that SD rats adapt to long-term feeding of

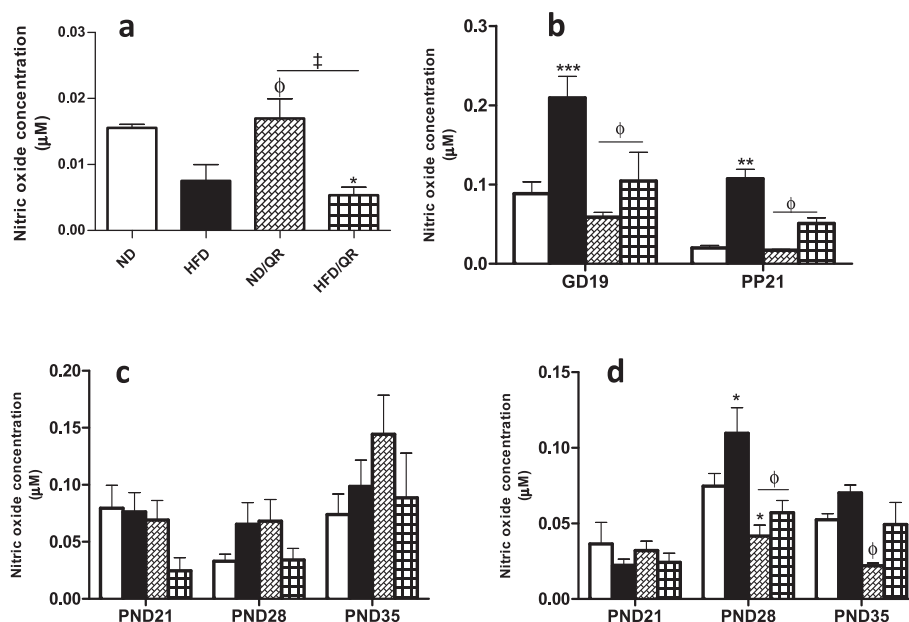


Figure 5. Concentration of NO in (a) placenta, (b) liver tissues of GD21 and PP21 dams, (c) liver of male and (d) female offspring rats at PND 21, 28 and 35. Data shown represents mean ± SEM; n = 6 per group. *P < 0.05, ***P < 0.001 compared to ND; ϕP < 0.05 compared to HFD; One-Way or Two-way ANOVA, followed by Bonferroni post hoc comparison test.

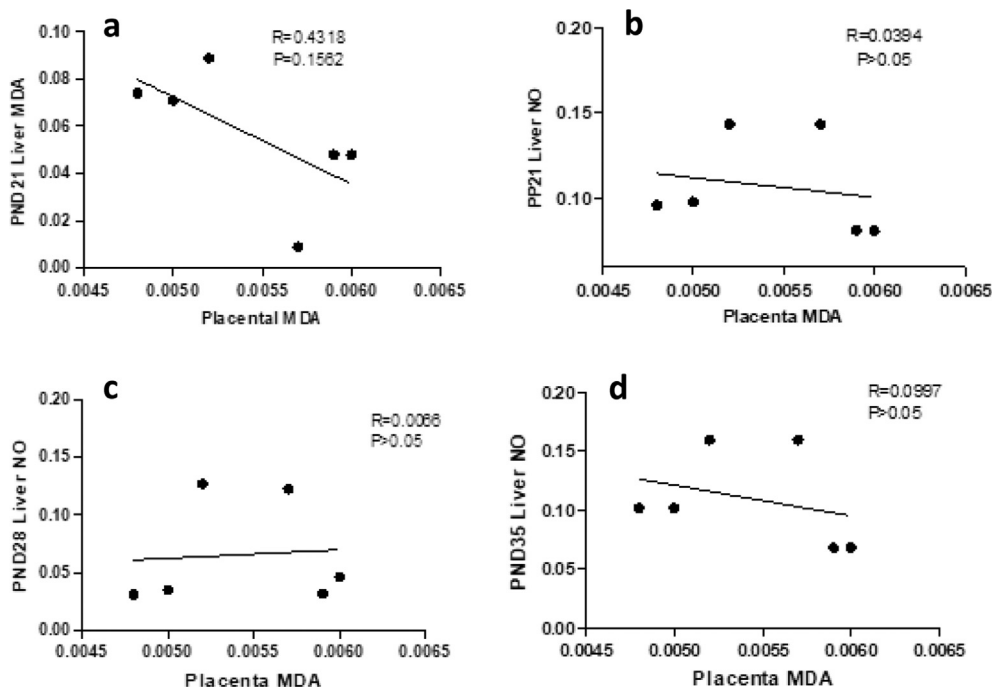


Figure 6. The above graphs indicate correlation between (a) MDA levels in PND21 offspring liver and placenta. Also, insignificant relationship between placenta MDA and liver NO production in (b) PP21 dams (c) PND 28 and (d) PND 35 offspring were observed.

high fat or high fructose diets without significant weight changes or prominent features of metabolic disorder [34], thus suggesting another possible reason for lack of morphometric changes in the current study. To further support this claim, several animal studies have indicated that rat strains vary widely in their propensity for diet-induced weight gain [14, 35], most especially outbred SD rats known to exhibit bimodal weight gain pattern [35]. Despite no significant differences in the physical parameters of dams, we observed that HFD offspring had delayed fur appearance and reduced weight gain which may suggest

transgenerational impact of maternal fat consumption on neurobiological development of the pups. In agreement with our findings, Santillan and colleagues (2010) have previously observed that female mice fed soy oil-enriched diet (SOD) or sunflower oil-enriched diet (SFOD) throughout gestation and lactation were not different in food consumption and body weight compared to mice fed commercial diet, however both SOD and SFOD offspring had shorter length and exhibited early simple reflexes while pubertal onset was delayed only in SFOD offspring [36].

Table 1. Antioxidant profile in Placenta of GD19 dams.

Parameters	ND	HFD	ND/QR	HFD/QR
CAT (H ₂ O ₂ /mg protein)	53.44 ± 1.15	52.57 ± 2.35	55.47 ± 0.41	56.24 ± 1.13
SOD (nmol/min/μg protein)	81.74 ± 9.60	41.26 ± 3.57*	43.41 ± 12.42*	16.50 ± 1.46**
GSH (mM)	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
TAC (μM)	0.57 ± 0.02	0.83 ± 0.13	0.69 ± 0.08	0.63 ± 0.08

Values are presented as mean ± SEM (n = 6 per group). *p < 0.05, **p < 0.01, ***p < 0.001 (vs. ND).

Table 2. Liver antioxidant profile of GD19 and PP21 dams.

Parameters	GD19				PP21			
	ND	HFD	ND/QR	HFD/QR	ND	HFD	ND/QR	HFD/QR
CAT (H ₂ O ₂ /mg protein)	37.07 ± 2.52	40.77 ± 0.95	33.97 ± 1.60	73.57 ± 7.49* [‡]	26.31 ± 1.67	41.32 ± 2.79*	34.08 ± 5.02	35.04 ± 3.21
SOD (nmol/min/μg protein)	763.8 ± 56.47	583.4 ± 25.49*	498.3 ± 36.02**	520.9 ± 25.08**	159.3 ± 32.01	449.1 ± 23.90*	562.3 ± 32.96**	245.2 ± 77.22 [‡]
GSH (mM)	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00 [‡]
TAC (μM)	1.37 ± 0.05	1.69 ± 0.04	1.21 ± 0.14	1.63 ± 0.07	0.85 ± 0.04	1.00 ± 0.06	0.62 ± 0.15	1.98 ± 0.18***

Values are presented as mean ± SEM (n = 6 per group). *p < 0.05, **p < 0.01, ***p < 0.001 (vs. ND); [‡]p < 0.05 (vs. HFD); [‡]p < 0.05 (ND/QR vs. HFD/QR).

Table 3. Liver antioxidant profile of male and female offspring rats.

Parameters	Male				Female			
	ND	HFD	ND/QR	HFD/QR	ND	HFD	ND/QR	HFD/QR
PND 21								
CAT (H ₂ O ₂ /mg protein)	31.61 ± 1.11	38.77 ± 4.64*	34.77 ± 2.23	31.11 ± 5.70	15.64 ± 3.92	24.31 ± 3.95	9.71 ± 1.29	19.91 ± 5.37
SOD (nmol/min/μg protein)	104.8 ± 17.02	101.9 ± 6.99	128.1 ± 15.26	126.8 ± 32.27	30.92 ± 5.64	31.86 ± 7.01	37.68 ± 1.76	24.23 ± 3.18
GSH (mM)	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00***	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.00	0.02 ± 0.00
TAC (μM)	0.71 ± 0.02	0.73 ± 0.07	1.09 ± 0.10	0.89 ± 0.03	0.13 ± 0.05	0.08 ± 0.04	0.45 ± 0.08	0.18 ± 0.05
PND 28								
CAT (H ₂ O ₂ /mg protein)	30.77 ± 1.90	30.04 ± 3.73	38.31 ± 2.75	27.54 ± 1.56	10.64 ± 0.19	9.57 ± 0.80	9.17 ± 0.30	8.17 ± 0.04
SOD (nmol/min/μg protein)	348.9 ± 87.28	449.9 ± 89.99	359.7 ± 34.62	322.1 ± 94.99	59.05 ± 10.48	65.50 ± 19.97	47.36 ± 8.43	58.86 ± 5.93
GSH (mM)	0.03 ± 0.00	0.03 ± 0.00**	0.03 ± 0.00	0.03 ± 0.00**	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
TAC (μM)	1.08 ± 0.04	1.16 ± 0.15	1.38 ± 0.02	1.11 ± 0.07	0.41 ± 0.05	0.55 ± 0.06	0.40 ± 0.01	0.50 ± 0.02
PND 35								
CAT (H ₂ O ₂ /mg protein)	54.61 ± 2.11	62.37 ± 10.46	48.11 ± 3.12	34.74 ± 1.82	9.04 ± 0.13	11.84 ± 0.88	10.24 ± 1.41	10.37 ± 1.11
SOD (nmol/min/μg protein)	718.9 ± 32.55	829.2 ± 18.57	629.8 ± 146.2	529.6 ± 83.65	45.87 ± 2.37	97.82 ± 9.81***	24.59 ± 1.09	33.33 ± 6.70
GSH (mM)	0.03 ± 0.00	0.03 ± 0.00*	0.03 ± 0.00*	0.03 ± 0.00*	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
TAC (μM)	1.66 ± 0.25	1.60 ± 0.08	1.18 ± 0.10	1.29 ± 0.04	0.50 ± 0.03	0.94 ± 0.06**	0.50 ± 0.03	0.49 ± 0.11

Values are presented as mean ± SEM (n = 6 per group). *p < 0.05, **p < 0.01, ***p < 0.001 (vs. ND).

Most earlier studies have reported the adverse effect of consumption of high-fat and/or high sugar diets on glucose metabolism and lipid profiles. For example, Huang et al (2004) fed SD rats with 20% lard-based high-fat or 60% high-fructose diets for 8 weeks and observed increased plasma glucose concentrations in both groups of mice from the seventh week. While HFD increased plasma cholesterol level, group fed high-fructose diet showed higher fasting insulin and triglyceride concentrations [15]. Another study reported that C57BL/6 mice fed 58% HFD for 20 weeks had increased fasting blood glucose, pronounced glucose intolerance, and were more insulin resistant than mice fed 11% low fat-diet [37]. Unexpectedly, our OGTT result showed that rats fed HFD for 8 weeks did not develop glucose intolerance but exhibited decreased plasma cholesterol and HDL accompanied by hypertriglyceridemia and increased LDL. Undisturbed glucose homeostasis in this study may suggest intact functioning of the pancreatic β-cells and non-resistance of the liver and other peripheral tissues to insulin signal following chronic high-fat consumption. Our data also suggest that critical threshold

concentration of fat needed to induce adiposity in peripheral tissues may not have been reached by consumption of 45% high-fat for 8 weeks. HFD-induced changes in blood triglyceride, HDL and LDL are clear indications of underlying mild pathological and/or metabolic alterations.

Long-lasting metabolic perturbations induced by high-fat feeding also implicate cellular dynamics involving changes in tissue oxidative state and mitochondria bioenergetics [38, 39]. Indeed, high levels of oxidative damage markers, most especially lipid peroxidation, and decreased antioxidant defences are often associated with oxidative imbalance in animal models of HFD. Milagro et al (2012) previously demonstrated that liver MDA concentrations were increased in male Wistar rats fed HFD for 56 days (8 weeks), and that the MDA levels correlated positively with body weight gain, serum leptin and homeostasis model assessment [40]. Similarly, we show that consumption of HFD for more than 8 weeks induced lipid peroxidation (increased MDA levels) in the placenta and liver of GD19 dams but without significant impact on body weight. Insignificant changes in PP21 liver MDA levels further support previous

observation that SD rats adapt to prolonged high fat feeding [34]. However, increased liver MDA concentration in HFD offspring clearly suggest persistent *in-utero* HFD-induced oxidative changes. This further indicates that lipid deposition in the placenta and hepatic fatty infiltration may contribute to harmful prenatal/perinatal changes, as previously posited [41]. HFD-induced increases in liver NO concentration of GD19 and PP21 dams agree with a previous study which supports that increased plasma and hepatic NO levels contribute to nitrosative stress in animal models of HFD [42]. While NO flux at PND 28 in female HFD offspring may be linked to developmental and/or pubertal changes, future studies should address sexual dimorphic effects of nitric oxide synthase activity in HFD models of MS. Consistent with previous studies [43], the observed increase in oxidative stress parameters (MDA and NO) might be associated with decreased placenta and liver SOD and increased TAC/CAT activity in HFD treated dams and their progenies. Differentially increased SOD activity in the PP21 dams may also indicate normalisation of hepatic response to the high-fat challenge subject to adaptive changes. Like SOD, GSH is another crucial antioxidant produced naturally to reduce oxidative stress. We thought that GSH activity would be decreased by chronic consumption of HFD, as previously reported, and reversed by QR treatment. Contrary to our expectations, the current data show that GSH activity was not altered by HFD in direct consumers, however, the observed increases in liver GSH activities in the offspring of dams fed HFD and those supplemented with QR may be due to an activated biochemical response to prevent HFD-induced cellular damage and associated pathologies in the offspring rats. The stability of GSH has been correlated to these changes and partly provide additional support for possible adaptive responses to prolonged high-fat consumption.

Several studies have reported the beneficial effects of quercetin in animal models of HFD, these include modulation of gut microbiota to prevent development of non-alcoholic fatty liver disease and obesity [44, 45], amelioration of steatohepatitis [46], reduction of HFD-induced fat accumulation, inflammation and oxidative stress [47, 48, 49]. Despite this, the current study indicates that QR has little or no effect on HFD-induced changes in physical parameters and tissue homeostasis. These observations agree with the previous report of McNulty et al (2008) that chronic quercetin ingestion does not protect trained athletes from exercise-induced oxidative stress and inflammation [20]. Although, strain and dose differences can be attributed to response changes in our study compared to human and other *in vivo* experiments.

In conclusion, this investigation provides evidence that chronic consumption of HFD may not induce prominent obesity and/or MS-related features in SD rats, however maternal HFD exposure engages metabolic and pathological alterations *in-utero* leading to oxidative changes accompanied by mild developmental consequences. Whereas QR supplementation in this study has very little or no beneficial effects on HFD-induced metabolic and/or oxidative changes. Future studies should consider using a higher percentage of high fat with prolonged duration of consumption while examining varying doses of QR to fully understand the underpinned biochemical alterations and therapeutic intervention. Critical examination of the gonads for impact of HFD-induced molecular and biochemical changes may further improve knowledge on sex-linked differences highlighted in the current study.

Declarations

Author contribution statement

Toluwalope E. Adeyemi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Duyilemi C. Ajonijebu: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mahendra L. Channa, Anand Nadar: Conceived and designed the experiments.

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Data availability statement

Data included in article/supplementary material/referenced in article.

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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