Heliyon 6 (2020) e05863

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Oral L-glutamine rescues fructose-induced poor fetal outcome by preventing placental triglyceride and uric acid accumulation in Wistar rats

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ARTICLE INFO

Keywords:
Fructose
Metabolism
Placenta
Pregnancy
Uric acid
Xanthine oxidase

ABSTRACT

Background: Metabolic adaptation of pregnant mothers is crucial for placental development and fetal growth/survival. However, evidence exists that indiscriminate consumption of fructose-enriched drink (FED) during pregnancy disrupts maternal-fetal metabolic tolerance with attendant adverse fetal outcomes. Glutamine supplementation (GLN) has been shown to exert a modulatory effect in metabolic disorders. Nevertheless, the effects of GLN on FED-induced poor fetal outcome, and in particular the impacts on placental uric acid/lipid accumulation are unknown. The present study was conducted to test the hypothesis that oral GLN improves fetal outcome by attenuating placental lipid accumulation and uric acid synthesis in pregnant rats exposed to FED.

Materials and methods: Pregnant Wistar rats (160–180 g) were randomly allotted to control, GLN, FED and FED + GLN groups (6 rats/group). The groups received vehicle by oral gavage, glutamine (1 g/kg) by oral gavage, fructose (10%; w/v) and fructose + glutamine, respectively, through gestation.

Results: Data showed that FED during pregnancy caused placental inefficiency, reduced fetal growth, and caused insulin resistance with correspondent increase in fasting blood glucose and plasma insulin. FED also resulted in an increased placental triglyceride, total cholesterol and *de novo* uric acid synthesis by activating adenosine deaminase and xanthine oxidase activities. Moreover, FED during pregnancy led to increased lipid peroxidation, lactate production with correspondent decreased adenosine and glucose-6-phosphate dehydrogenase-dependent antioxidant defense. These alterations were abrogated by GLN supplementation.

Conclusion: These findings implicate that high FED intake during pregnancy causes poor fetal outcome via defective placental uric acid/triglyceride-dependent mechanism. The findings also suggest that oral GLN improves fetal outcome by ameliorating placental defects through suppression of uric acid/triglyceride accumulation.

1. Introduction

Pregnancy is a dynamic state that involves multiple physiological adaptations including metabolic adjustments that are necessary to ensure a continuous supply of essential metabolites to support placental function, growth and development of the fetus [1]. Pregnant women that experience abnormal adaptations due to imbalanced nutrition or some pathological conditions have an increased risk of maternal or fetal morbidity and mortality. Studies in animals and humans revealed that hyperphagia and enhanced *de novo* lipogenesis are contributing factors to early gestational anabolism [1, 2]. Similarly, previous studies in rodents have demonstrated a progressive conversion of glucose to fatty acids and glycerol during the first and second trimester of pregnancy [3]. An increase in lipoprotein lipase may also promote lipid accumulation in humans as well as in experimental rodents during pregnancy. This triggers maternal insulin resistance (IR) especially in the third trimester of pregnancy in order to direct metabolic fuels to the developing fetuses and prevent fat deposition in the mother [1, 4]. However, chronic IR is a feature of metabolic disease and associated syndromes such as type 2 diabetes (T2D) and cardiovascular disease (CVD) and its occurrence in pregnancy has been linked to increased obstetric complications that include:- pre-eclampsia, gestational diabetes, defective placentation, and intrauterine growth restriction [1, 5].

Among various obstetric complications, defective placentation remains the leading cause of maternal-fetal morbidity and mortality worldwide. Considering the central role of the placenta as a metabolic and physical barrier in feto-placental unit, in such that several metabolic substrates cross the placenta and establish a physiological mechanism of

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https://doi.org/10.1016/j.heliyon.2020.e05863
Received 13 September 2020; Received in revised form 8 November 2020; Accepted 23 December 2020

communication between the mother and the fetus [6, 7, 8]. The impaired placental function and metabolism as a result of exposure to metabolically stressing gestational factors such as nutrition imbalance, hypoxia, maternal adiposity, and glucocorticoids, poses a threat to the survival of the mother and the fetus [9].

Besides, numerous experimental studies in rodents and humans have linked a number of pregnancy complications such as pre-eclampsia, gestational hypertension, preterm birth and intrauterine growth restriction to impaired placental function [10, 11]. It has also been documented that impaired placental function is a key player in the programming of newborn and adulthood disease [11]. However, the etiopathology of improper placentation is under investigation. Several maternal risk factors including IR-linked metabolic, immunological and cardiovascular disorders have been reported [10, 12]. Aside genetic and environmental factors, poor dietary lifestyle especially during pregnancy has been shown to increasingly contribute to the incidence and prevalence of abnormal placentation and adverse pregnancy outcomes [13]. Consistently, earlier studies have reported that nutritional and other environmental exposures during pregnancy can immensely affect homeostatic system and multiple organs' functions, thus impairing fetal output and increasing offspring risk of future diseases [14].

The consumption of high caloric diets and a sedentary lifestyle during pregnancy are risk factors for gestational metabolic disturbances which include dyslipidemia, obesity, hypertension and T2D. Excessive fructose consumption through high fructose corn syrup (HFCS) and as added sugar in soft drinks is globally on the rise despite its implication in the development of metabolic syndrome. In addition, several studies, including earlier studies from our laboratory animals have demonstrated that high fructose consumption even in pregnancy leads to IR and hyperuricemia [15, 16, 17], which are the strong predictors of metabolic syndrome and T2D [18]. In fact, indiscriminate fructose consumption during pregnancy has been reported to disrupt maternal metabolic homeostasis, thereby defecting placental function and fetal development [19], potentially increasing the offspring's risk for metabolic health mayhem later in life [19, 20]. The health effect of fructose is usually due to its metabolism, in such that the ingested fructose is extracted by the liver and converted to fructose-1-phosphate, which leads to cellular depletion of adenosine triphosphate (ATP) and activation of adenosine monophosphate deaminase (AMPD) and increased activities of enzymes that are involved in purine metabolism particularly adenosine deaminase and xanthine oxidase. Adenosine monophosphate deaminase converts AMP to xanthine, which is then converted to uric acid by xanthine oxidase, uric acid extracellularly acts as a potent antioxidant and in excess intracellularly triggers oxidative stress, causing cellular dysfunction [18, 19]. Similarly, excess uric acid can also provoke *de novo* lipogenesis thereby causing lipotoxicity with consequent promotion of oxidative stress and inflammatory response, which are features of IR-related disorders such as obesity, T2D, non-alcoholic fatty liver disease among others [15, 17, 20].

Uric acid is a metabolically derived product from the activation of AMPD and increased activity of xanthine oxidase [18]. Increased synthesis of uric acid has been previously reported as underlying factor in fructose-induced placental insufficiency and consequent fetal impairment [19]. Likewise, studies in human and rodents have consistently demonstrated elevated circulating uric acid concentration in mothers with pregnancy complications such as pre-eclampsia, gestational diabetes and preterm birth [21, 22]. The uric acid effects might result in disruption of maternal metabolic homeostasis, initiation of oxidative stress and eliciting of inflammatory response, which subsequently precipitates tissue injury, including the placental tissue injury as previously observed in pregnant mothers with metabolic and related disorders such as obesity, T2D and hypertension [23, 24]. However, administration of allopurinol, a generic drug frequently prescribed to treat uric acid-linked syndromes such as gout and kidney stone was reported to significantly lower uric acid level and appeared to mitigate the negative maternal and fetal effects of high fructose without improving maternal glucose homeostasis, suggesting a direct effect of fructose-induced uric acid on placental function [19]. Importantly, the search for a novel nutritional agent for adequate preventive and therapeutic measures against the deleterious impacts of high fructose consumption during pregnancy would be imperative.

Glutamine (GLN) might be a novel nutritional/pharmacological intervention, especially in pregnancy. Glutamine is abundantly consumed by most cells in the body [25, 26]. It mediates insulin secretion, and acts as an alternative substrate for energy regulation in fasting tissue or T2D [27, 28]. Several studies in animals and humans have reported the benefits of GLN in metabolic disorders [17, 29, 30, 31, 32]. Interestingly, GLN is a modulator of cytoprotective proteins that are beneficial in oxidative insult [26], and it contributes majorly to the endogenous synthesis of glutathione (GSH) [28]. A previous report by Ren et al. showed that dietary GLN supplementation improved pregnancy outcome in virus infected pregnant mice [33]. However, the effects of GLN supplementation on placental uric acid/triglyceride accumulation and hampered fetal outcome induced by maternal fructose consumption are unknown. The present study was therefore conducted to test the hypothesis that GLN supplementation would improve fetal outcomes by attenuating placental triglyceride and uric acid production in pregnant rats exposed to fructose-enriched drink.

2. Materials and methods

2.1. Ethical statement

The investigation adhered to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the experimental protocol was approved by the University of Ilorin Ethical Review Committee Board with approval number UERC/ASN/2016/596. Minimal number of animals was used and the investigators minimized suffering/pain by anaesthetizing the animals with 50 mg/kg body weight of sodium pentobarbital (*ip*).

2.2. Animals and experimental protocol

Ten-week-old female Wistar rats were obtained from the animal house of the College of Health Sciences, University of Ilorin, (Ilorin, Nigeria). Rats were provided with unrestricted access to standard rat chow and tap water. The animals were acclimatized for one week and thereafter the animals with a known estrus phase were time-mated. Twenty-four (24) pregnant rats (160–180 g) were randomly allotted to 4 groups (n = 6 rats per group). Rats were maintained under standard environmental conditions of temperature (22–26 °C), relative humidity (50–60%) and 12-hour dark/light cycle.

Animals in the control group received distilled water (*po*) as vehicle, GLN group received (*po*) 1 g/kg body weight of GLN (LOBA Chemie PVT Ltd, India), fructose-enriched drink (FED) group received (w/v) 10% fructose (Merck KGaA, Darmstadt, Germany) and FED + GLN group received fructose plus glutamine. Glutamine (1 g/kg) was administered once daily through oral gavage and fructose (10%) was given through drinking water ad libitum and the treatment lasted for 19 days (Figure 1). Food and water intake as well as body wight were monitored.

2.3. Fasting blood glucose

The rats were fasted overnight prior to the termination of the experiment and fasting blood glucose was determined using a hand-held glucometer (ONETOUCH®-LifeScan, Inc., Milpitas, CA, USA). Homeostatic model assessment for IR (HOMA-IR) was used to determine insulin resistance. HOMA-IR is expressed as fasting glucose (mmol/l) * fasting insulin (μIU/l)/22.5 [34], and insulin sensitivity check index was estimated using QUICKI which is expressed as 1/[log (fasting insulin) + log (fasting glucose)] [35].

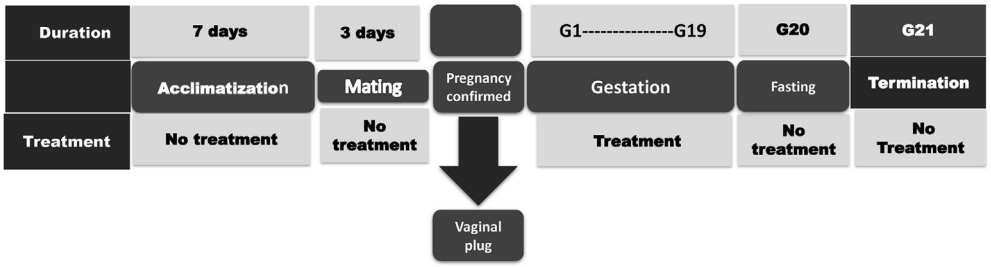

Figure 1. Experimental protocol.

2.4. Sample preparation

At gestational day 21, after anaesthetized the animals, blood was collected by cardiac puncture into heparinized tubes. The blood was centrifuged at 3000 rpm for 5 min at room temperature and the plasma was separated and stored frozen until required for biochemical assays.

The fetus and placenta were removed separately, blotted and weighed. The length of the fetus was also determined. After weighing placenta, 100 mg of the tissue was carefully removed and homogenized in phosphate buffer solution (PBS) with a glass homogenizer, centrifuged at 10000 rpm for 10 min at 4 °C.

2.5. Measurement of biochemical parameters

2.5.1. Plasma insulin

The ELISA kits obtained from Ray Biotech, Inc. (Georgia, USA) was used to determine the plasma insulin concentration. The assay method was based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the insulin molecule.

2.5.2. Lipid profile

The total lipid was extracted from the placental tissue homogenate as previously described by Hara and Radin [36] as well as Rodríguez-Sureda and Peinado-Onsurbe [37]. Standardized colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK) was used to determine the concentration of triglycerides (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDLc), low density lipoprotein-cholesterol (LDLc) and free fatty acid (FFA) However, the quantification of the tissue concentration of TG, TC, HDLc, LDLc and FFA (non-esterified) were determined by coupled enzyme assays which result in colorimetric (570 nm) products proportional to the TG, TC, HDLc, LDLc and FFA present respectively. Ratios of TG/HDLc and TC/HDLc were determined as atherogenic lipids [17, 38].

2.5.3. Lactate content and lactate dehydrogenase (LDH)

Standardized non-enzymatic and enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK) were used to estimate lactate concentration and LDH from placental tissue homogenates respectively.

2.5.4. Adenosine and nitric oxide

Adenosine and NO concentrations of placental tissue homogenate and plasma were determined using non-enzymatic kits from Randox Laboratory Ltd. (Co. Antrim, UK) and assay kits from Oxford Biomedical Research Inc. (Oxford, USA) respectively, and following the manufacturers' procedures.

2.5.5. Na^+/K^+-ATPase activity

Na^+/K^+-ATPase activity was determined in the plasma and placental tissue homogenate. Na^+/K^+-ATPase activity is an enzymatic assay and was estimated as previously described [39]. Protein concentration was estimated by Biuret method using assay kit from Randox Laboratory Ltd. (Antrim, UK).

2.5.6. Lipid peroxidation, glucose-6-phosphate dehydrogenase (G6PD) and glutathione

Malondialdehyde (MDA) and G6PD activity/glutathione (GSH) are markers of lipid peroxidation and cellular antioxidant capacity respectively. Malondialdehyde was determined from placental tissue homogenates by standard non-enzymatic spectrophotometric method using assay kits from Randox Laboratory Ltd. (Co. Antrim, UK). The method involves the reaction of thiobarbituric acid (TBA) with the MDA in the sample to generate MDA-TBA adduct [40], and quantified spectrophotometrically. Whereas, GSH was determined as previously described [41] using non-enzymatic spectrophotometric method with assay kits obtained from Oxford Biomedical Research Inc. (Oxford, USA). In addition, Standard spectrophotometric method using assay kits obtained from Oxford Biomedical Research Inc. (Oxford, USA) was used to determine G6PD from the placental tissue homogenates.

2.5.7. Adenosine deaminase (ADA), xanthine oxidase (XO) and uric acid

Standard enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK) were used to determine ADA and XO activities from the placental tissue homogenates and plasma, whereas placental and plasma uric acid (UA) concentrations were estimated by non-enzymatic colorimetric method using kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

2.6. Data analysis and statistics

Shapiro-Wilk test was used to determine the distribution of the data and the data were normally distributed. All data were presented using scattered plot and standard deviation as well as mean ± SD. Statistical group analysis was performed with GraphPad prism 5. One-way ANOVA was used to compare the mean values of variables among the groups. Bonferroni's test was used for *post hoc* analysis and at p less than 0.05, statistically significant differences were accepted.

3. Results

3.1. Effects of glutamine on food and water intake and body weight in pregnant rats that consumed high fructose-enriched drink

There was no significant change in food intake in all the experimental groups but water intake significantly increased in FED and FED + GLN groups when compared to the control group. In addition, GLN did not significantly reduced water intake. Similarly, body weight gain significantly increased in FED group compared to the control group. However, GLN significantly reduce the body weight gain in FED + GLN group (Table 1).

Table 1. Effects of glutamine on food and water intake and body weight in pregnant rats that consumed high fructose drink.

	CTL	GLN	FED	FED + GLN
Food intake (g/kg/day)	108.75 ± 3.63	109.53 ± 4.20	96.45 ± 2.59	103.34 ± 2.25
Water intake (ml/kg/day)	180.54 ± 6.02	176.41 ± 6.77	328.97 ± 6.62*	315.61 ± 8.20*
Body weight gain (g)	33.99 ± 1.62	36.64 ± 2.33	47.12 ± 1.25*	37.22 ± 1.69#

Data are expressed as mean ± SD. n = 6. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (*$p < 0.05$vs. CTL; #$p < 0.05$ vs. FED). Control (CTL), Fructose-enriched drink (FD).

3.2. Glutamine supplementation restored glucose homeostasis in pregnant rats that consumed high fructose-enriched drink

Consumption of high FED during pregnancy increased fasting glucose (Figure 2a) and plasma insulin (Figure 2b) when compared with control group. It also induced insulin resistance as measured by HOMA-IR (Figure 2c) with a correspondent decrease in insulin sensitivity as measured by QUICKI (Figure 2d). The metabolic phenotypes of the FED-treated group revealed a disrupted glucose homeostasis as a result of reduced insulin sensitivity. This led to insulin resistance and compensatory hyperinsulinemia, which characterize pre-diabetic condition [42, 43]. Nevertheless, GLN restored maternal glucose homeostasis with correspondent normalization of fasting glucose, plasma insulin and insulin sensitivity (Figure 2a, b, c & d).

3.3. Glutamine supplementation rescued placental defects and fetal growth restriction in pregnant rats that consumed high fructose-enriched drink

Maternal high fructose consumption significantly decreased fetal weight (Figure 3b) and length (Figure 3c) without significant change in fetal number (Figure 2d) when compared with control group. These observations suggest fetal growth restriction even with unaffected fetal number. Similarly, placental weights were significantly increased (Figure 3a) in FED-treated pregnant rats with corresponding decrease in fetal-placental weight ratio (Figure 3e) when compared with control group, suggesting that high fructose consumption during pregnancy led to placental defects. However, supplementation with GLN increased fetal weight and length with (Figure 3b & c) corresponding increase in fetal-placental weight ratio (Figure 3e) when compared with FRD-treated group, suggesting an improved fetal growth with efficient placentation.

3.4. Glutamine supplementation attenuated excess lipid in the placentae of pregnant rats that consumed high fructose-enriched drink

High fructose consumption during pregnancy led to excess lipid in the placenta with correspondent increase in triglyceride (Figure 4a), total cholesterol (Figure 4b), low density lipoprotein-cholesterol (Figure 4c) and atherogenic lipid (Figure 4d & e) without alteration in free fatty acid (Figure 4f) when compared with control group, suggesting that maternal high fructose consumption increased placental lipid influx as a result of increased lipolysis that is associated with insulin resistance [15]. Accumulation of excess lipid in non-adipose cells results in oxidative stress and/or lipotoxicity [17, 20]. However, these alterations were attenuated when fructose consumption was supplemented with GLN (Figure 4a, b, c, d &e).

3.5. Glutamine supplementation attenuated placental lipid peroxidation, lactate synthesis and defective G6PD-dependent antioxidant defense in pregnant rats that consumed high fructose-enriched drink

Maternal high fructose consumption during pregnancy significantly increased placental concentration of malondialdehyde (Figure 5a), which reflects increased lipid peroxidation when compared with control group. Likewise, lactate synthesis was higher in FED-treated group with correspondent increase in LDH activity (Figure 5c) and lactate content (Figure 5b), suggesting a deviation between energy expenditure and supply, leading to tissue ischemia and a reflection of mitochondrial dysfunction in oxidative phosphorylation [44]. In addition, consumption of high fructose in pregnancy decreased placental G6PD activity (Figure 5d) and glutathione content (Figure 5e), suggesting a depletion in antioxidant capacity of placental tissue which increased placental susceptibility to oxidative stress. Notwithstanding, these placental effects were suppressed when supplemented with GLN (Figure 5a, b, c, d, e & f).

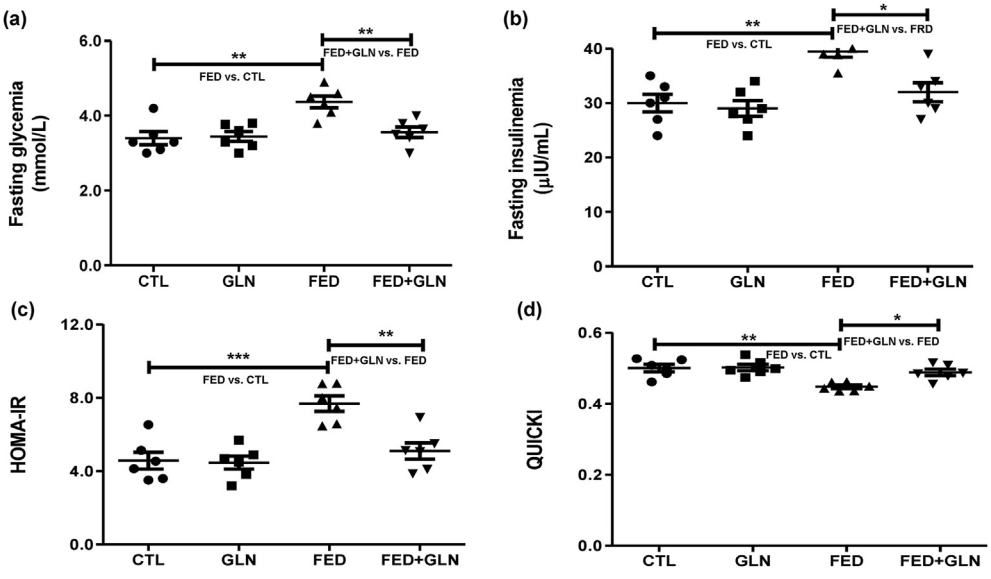

Figure 2. Effects of glutamine (GLN) on fasting glycemia (a), fasting insulinemia (b), homeostatic model assessment for insulin resistance (c) and insulin sensitivity (d) in pregnant rats that consumed high fructose drink (FED). FED increased fasting glycemia and insulinemia, induced insulin resistance (IR) and decreased insulin sensitivity (QUICKI). Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$). Homeostatic model assessment for insulin resistance (HOMA-IR).

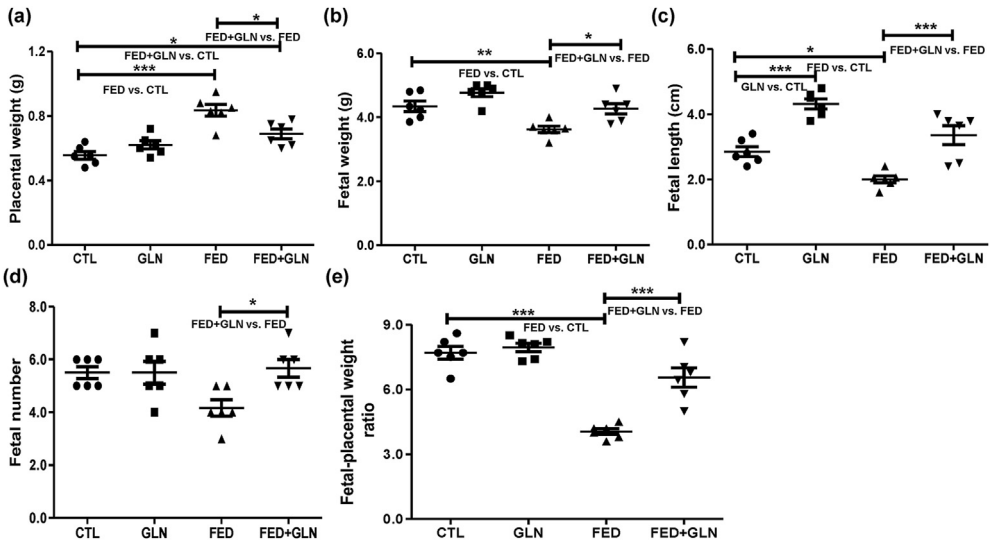

Figure 3. Effects of glutamine (GLN) on placental weight (a), fetal weight and length (b, c), fetal number (d) and fetal-placental weight (e) in pregnant rats that consumed high fructose drink (FED). FED increased placental weight and decreased fetal-placental weight and fetal weight/length, which were attenuated by glutamine supplementation. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$).

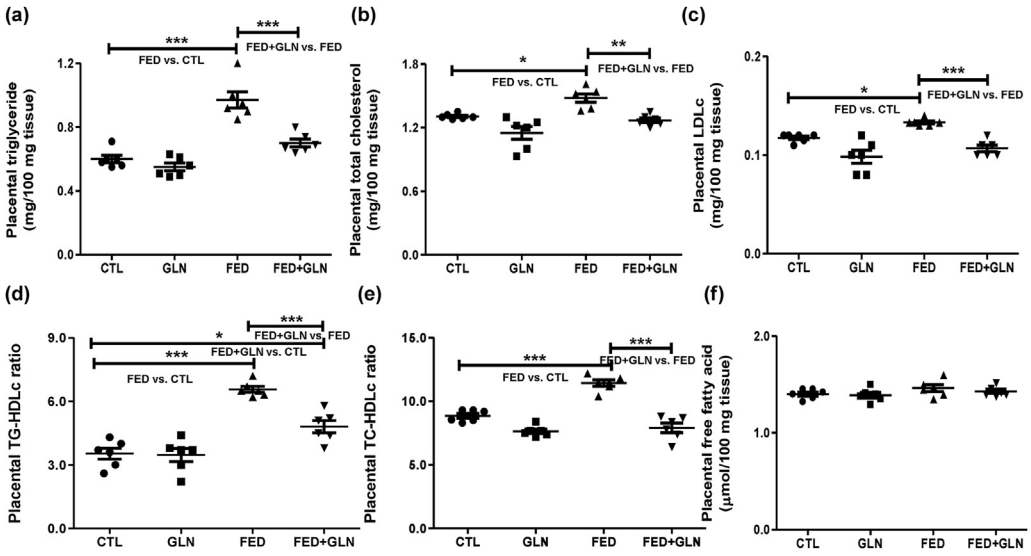

Figure 4. Effects of glutamine (GLN) on placental triglyceride (a), total cholesterol (b), low density lipoprotein-cholesterol (c), TG/HDLc ratio (d), TC/HDLc ratio (e) and free fatty acid (f) in pregnant rats that consumed high fructose drink (FED). FED increased placental triglyceride (TG), total cholesterol (TC), low density lipoprotein-cholesterol (LDLc), TG/high density lipoprotein-cholesterol (HDLc), TC/HDLc and free fatty acid (FFA). These changes were attenuated by glutamine supplementation. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$).

3.6. Glutamine supplementation improved nitric oxide and adenosine content in the placentae of pregnant rats that consumed high fructose-enriched drink

Consumption of high fructose drink during pregnancy significantly decreased placental and plasma nitric oxide (Figure 6a & b) and adenosine contents (Figure 6c & d) when compared with control group, suggesting a disruption of placental endothelial function that predisposed placenta tissue to ischemia and contributed to abnormal placentation. Nonetheless, GLN supplementation improved endothelial function with correspondent increase in nitric oxide and adenosine content when compared with FED-treated group (Figure 6a, b, c & d).

3.7. Glutamine supplementation improved plasma and placental Na^+/K^+-ATPase activity in pregnant rats that consumed high fructose-enriched drink

Consumption of high fructose drink during pregnancy significantly decreased placental and plasma Na^+/K^+-ATPase activities (Figure 7a & b) when compared with control group. However, GLN supplementation significantly increased the placental and plasma Na^+/K^+-ATPase activities compared with FED-treated group (Figure 7a & b).

3.8. Glutamine supplementation decreased uric acid synthesis in the placentae of pregnant rats that consumed high fructose-enriched drink

Maternal high fructose consumption during pregnancy significantly increased uric acid synthesis from the placenta (Figure 8c) with corresponding increase in ADA and XO activities (Figure 8a & b) when compared with control group, suggesting an increase in inflammatory response and oxidative stress in the placenta of pregnant rats that consumed high fructose. Similarly, a significant increase in circulating uric acid (Figure 8f) with correspondent increase in XO (Figure 8e) and ADA (Figure 8d) was observed. However, GLN supplementation mitigated these alterations (Figure 8a, b, c, d, e & f).

4. Discussion

The present study investigated the effects of glutamine supplementation on maternal glucose homeostasis and fetal-placental development

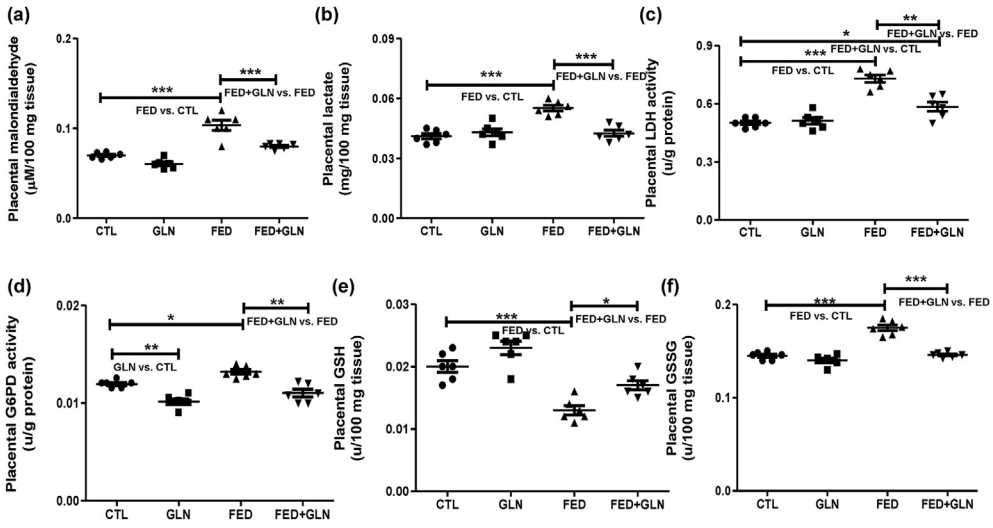

Figure 5. Effects of glutamine (GLN) on placental malondialdehyde (a), lactate concentration (b), lactate dehydrogenase activity (LDH, c), Glucose-6-phosphate dehydrogenase (G6PD; d) reduced glutathione (GSH; e) and oxidized glutathione (GSSG; f) in pregnant rats that consumed high fructose drink (FED). FED increased placental malondialdehyde, lactate synthesis, oxidized glutathione and reduced G6PD and reduced glutathione. These changes were attenuated by glutamine supplementation. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$).

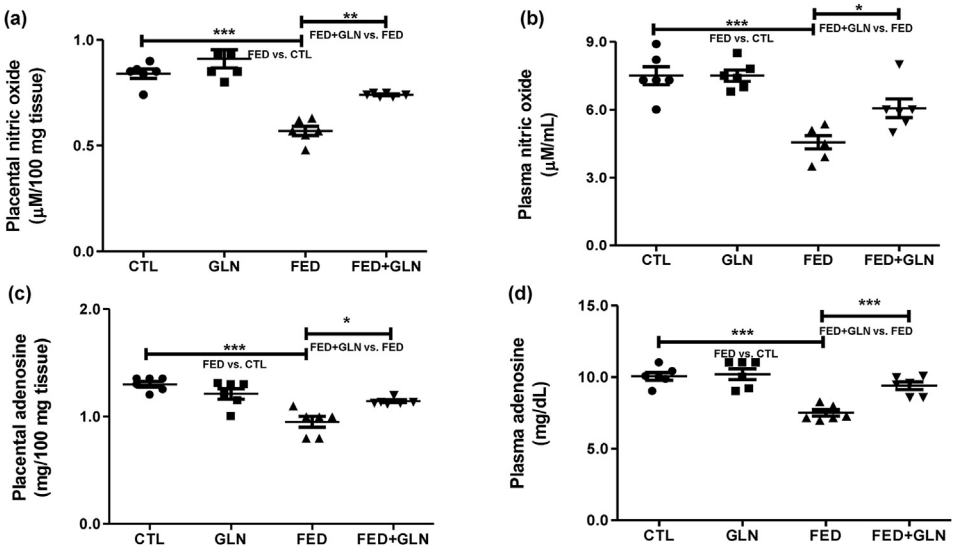

Figure 6. Effects of glutamine (GLN) on placental nitric oxide (a), plasma nitric oxide (b), placental adenosine (c) and plasma adenosine (d) in pregnant rats that consumed high fructose drink (FED). FED increased plasma and placental nitric oxide and adenosine, which were attenuated by glutamine supplementation. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$).

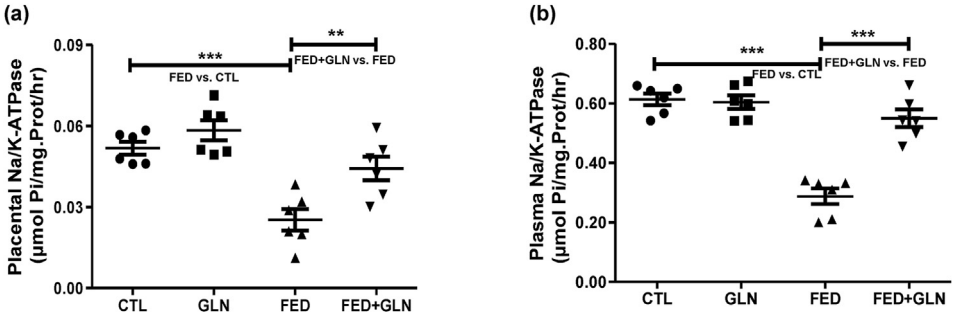

Figure 7. Effects of glutamine (GLN) on placental Na^+/K^+-ATPase activity (a) and plasma Na^+/K^+-ATPase activity (b) in pregnant rats that consumed high fructose drink (FED). FED decreased plasma and placental Na^+/K^+-ATPase activities, which were increased by glutamine supplementation. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$).

with corresponding fetal outcome in pregnant rats exposed to high FED. The key finding of the study demonstrates that glutamine supplementation ameliorates lipid-associated placental defects and adverse fetal outcome by suppression of placental ADA as well as XO, uric acid and restoration of glucose homeostasis in maternal rats exposed to high fructose during pregnancy. The results of the present study revealed that FED induced IR-driven placental defect, characterized by increased body weight gain, elevating lipid profile, lipid peroxidation, lactate production

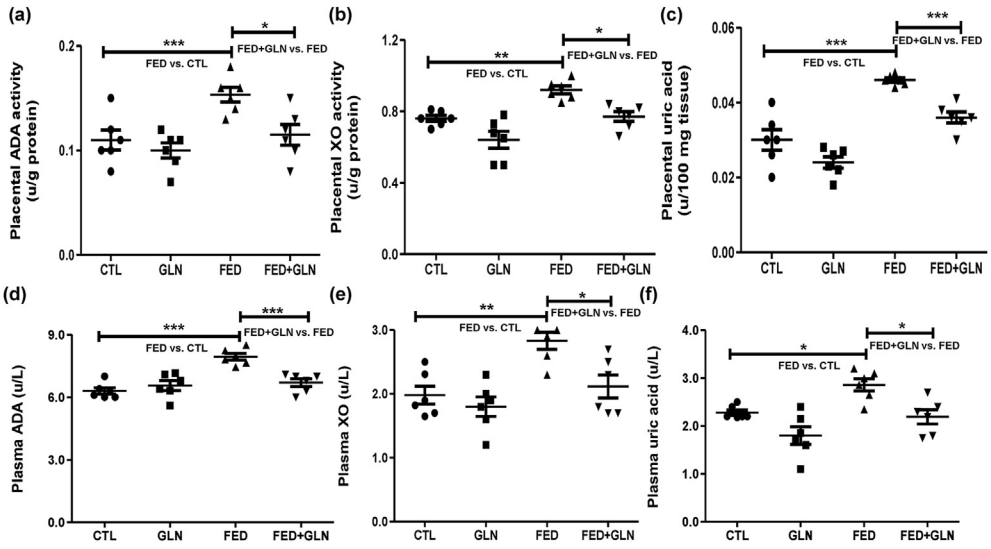

Figure 8. Effects of glutamine (GLN) on placental adenosine deaminase activity (ADA; a), xanthine oxidase activity (XO; b) and uric acid concentration (c) and plasma ADA (d), XO (e) and uric acid concentration (f) in pregnant rats that consumed high fructose drink (FED). FED increased plasma and placental ADA, XO activity and uric acid concentration, which were attenuated by glutamine supplementation. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc test.* (***$p < 0.0001$, **$p < 0.001$ and *$p < 0.01$).

and uric acid synthesis. In addition, FED consumption in pregnancy disrupted NO availability, Na^+/K^+-ATPase activity, G6PD- and glutathione-dependent antioxidant capacity. However, supplementation with glutamine abated placental defects including weight and biochemical changes and improved fetal outcomes (Figure 9).

The present finding that excessive fructose consumption in pregnancy altered maternal metabolic function with evidence of increased body weight gain (Table 1), reduced insulin sensitivity (Figure 2d) and correspondent high fasting glycemia (Figure 2a) and insulinemia (Figure 2b) is consistent with previous studies including our recent studies [17, 43, 44].These metabolic phenotypes that result from maternal fructose consumption are documented features of prediabetes, which might put the mother at risk of gestational diabetes and cardiovascular disease [1]. In addition, there was an increased in water intake without significant change in food intake (Table 1) and this increase in water consumption confirmed the increasing intake of fructose since it was administered through drinking water and this possibly contributed to increased body weight that was also associated with metabolic phenotypes observed in the FED group. Since disrupted metabolic homeostasis and/or reduced insulin sensitivity is known to drive impaired fetal-placental development [5, 19], the present findings that maternal fructose consumption led to placental defect and fetal growth restriction with correspondent decreased in fetal-placental weight ratio (Figure 3e) and reduced fetal weight (Figure 3b) were consistent with previous studies [45]. Though other authors have also reported altered placental weight with unchanged fetal weight [46, 47], while a number of authors observed altered placental weight and sex-specific reduction in fetal weight particularly in female fetus [48, 49]. However, our present findings imply that maternal fructose consumption induces insulin resistance early in pregnancy that drives placental inefficiency with corresponding fetal growth restriction, which may shed light on intrauterine growth restriction usually associated with obstetric complications in humans [10, 11]. On the other hand, the observed fetal weight reduction was contrary to some other studies who reported macrosomia in insulin resistant pregnancy [50, 51]. Nevertheless, macrosomia is common in hyperglycemia and insulin resistance that is associated with gestational diabetes [52]. Therefore, insulin resistant pregnancy without gestational diabetes might not present macrosomia as observed in this study. Intriguingly, supplementation with GLN restored body weight as well as metabolic and/or glucose homeostasis with a corresponding improvement in fetal-placental weight and fetal outcome. These metabolic and consequent effects of GLN on fetal-placental development may be due to its potential as alternative substrate for energy regulation in fasting tissue as earlier reported even in T2D individuals with or without pregnancy [27, 28]. The findings support the notion that gestating individuals require sufficient amount of amino acid particularly GLN for the optimal survival and growth of embryo and fetus [53].

It is unsurprising that fructose-induced metabolic perturbation led to elevated lipid concentration particularly in non-adipose tissue during pregnancy [16, 17, 18], but is of immense interest to observe elevated levels of triglyceride, total cholesterol and atherogenic lipid without alteration in free fatty acid level in the placenta tissue when compared with control group. Normal pregnancy is ideally characterized with de *novo* lipogenesis especially during early and mid-gestation in order to boost energy substrates and complement increased nutrient demand by developing fetus without significant fat deposit in the non-adipose tissue of the mother [1, 2]. However, disruption of this physiological process by high maternal fructose consumption in the present study led to excess placental lipid, which was likely due to increased maternal IR combined with peripheral adipose tissue lipolysis that further increased lipid influx into non-adipose tissue, including placental tissue. The increase in placental lipid content without alteration in free fatty acid suggests that excess placental lipid is independent of *de novo* lipogenesis but largely due to lipid influx into the placenta especially triglyceride. Lipid accumulation particularly in the non-adipose tissue has previously been reported to cause lipotoxicity and oxidative stress, which might lead to tissue injury [15, 20]. Consistent with this finding, the present data demonstrated that maternal fructose consumption increased placental lipid peroxidation, which is a reflection of increased oxidative stress as a result of depletion in placental G6PD/GSH-dependent antioxidant barrier. In addition, the present observation is associated with increased production of lactate that corroborate increased energy demand at the expense of mitochondrial oxidative capacity, a condition that promote tissue ischemia and may be accompanied by mitochondrial dysfunction [44]. Therefore, the present findings suggest that maternal fructose consumption during pregnancy induces lipid accumulation that is associated with placental defects and affects fetal outcome. Nevertheless, the placental oxidative stress or lipotoxicity resulting from maternal fructose consumption was attenuated when supplemented with GLN. Glutamine is a glutamate family of amino acids that is crucially demanded in the synthesis of nucleotides and glutathione [28, 54] and it possibly protects against placental lipid-associated defect through enhancement of G6PD/glutathione-dependent antioxidant defense.

Placenta is a highly vascularized organ and any impairment to its vasculature becomes a threat to placental efficiency and fetal outcome. Likewise, endothelial integrity is vital to placental vascularization [55].

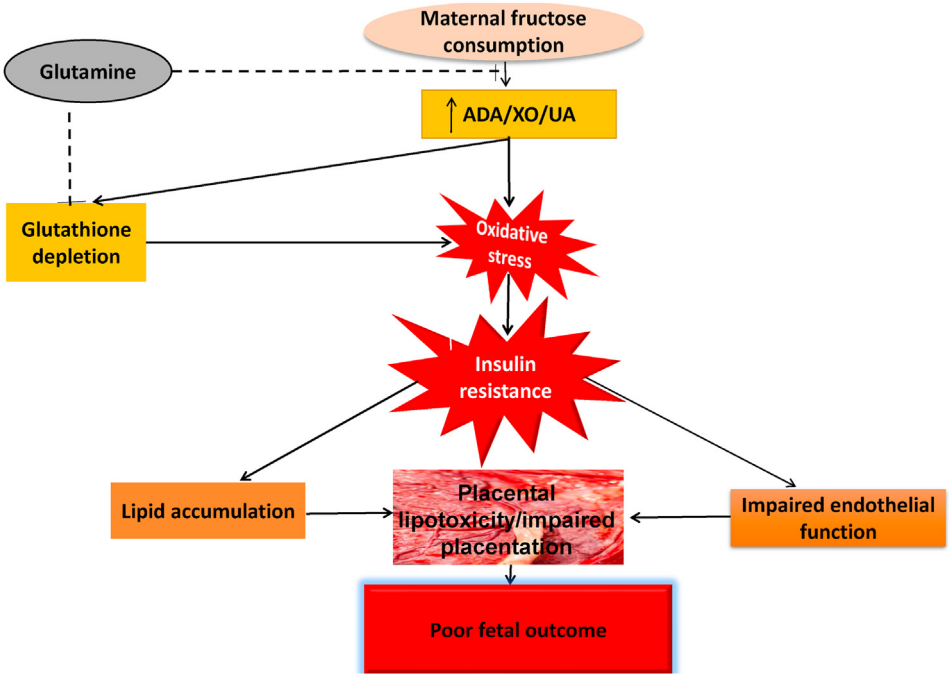

Figure 9. Schematic diagram depicting the metabolic pathways involved in maternal gestational high fructose-induced placental lipid/uric acid-related defect and consequent poor fetal outcome and interaction with glutamine molecule via enhancement of glutathione and suppression of ADA (Adenosine deaminase), XO (Xanthine oxidase) and UA (Uric acid).

However, maternal fructose consumption caused a significant decrease in circulating and placental nitric oxide concentration, and reduction in NO bioavailability is a very early and pervasive characteristics of endothelial dysfunction. Impaired NO bioavailability also correlated with decreased adenosine concentration following maternal fructose consumption. These are collectively known to reduce placental tissue perfusion, and thereby precipitate tissue hypoxia [56], which is associated with increased lactate and LDH activity in this study. Besides, increased activity of xanthine oxide and atherogenic lipid may also contribute to disruption of maternal NO-dependent endothelial integrity with consequent placental defect and reduced fetal weight. Reduction in fetal weight or intrauterine growth restriction has earlier been associated with impaired Na^+/K^+-ATPase activity, especially in pregnancy complications such as pre-eclampsia and gestational hypertension among others [57, 58]. Na^+/K^+-ATPase is important in maintaining the electrochemical gradient of Na^+, that drives Na^+-couple transport of nutrients and is affected by several factors, including oxidative, ATP depletion and NO deficiency, which might be the reason for reduction in plasma and placental Na^+/K^+-ATPase activity in animals that consumed high fructose. Importantly, supplementation with GLN attenuates nitric oxide deficiency, adenosine depletion and impaired Na^+/K^+-ATPase activity, thus restoring placental efficiency and improving fetal outcome. Glutamine is usually involved in *de novo* synthesis of arginine, a precursor of NO, and this process in brief involves conversion of glutamine to citrulline, which occurs mostly in the mitochondria of enterocytes, then citrulline is taken up from circulation by different cell types including endothelial to produce arginine [53]. Therefore, GLN supplementation may possibly protect endothelial function by stimulating nitric oxide production perhaps through *de novo* arginine synthesis.

Moreover, the present finding that maternal fructose consumption during pregnancy increased placental uric acid synthesis due to increase in ADA and XO activities was consistent with a number of earlier studies [19, 22]. Excess uric acid has also been reported to have direct effect on placenta thus disrupting placentation with consequent obstetric complications including adverse fetal outcome. It promotes *de novo* lipogenesis thereby causing lipotoxicity and contributing to oxidative stress that is biochemically revealed by elevated lipid peroxidation (MDA), and in addition this is known to cause NO depletion through increased XO activity as observed in this study. Altogether, contributes to placental defect and consequent poor fetal outcome. These observations were in consonance with previous studies which demonstrated that increased placental oxidative stress led to diverse deleterious effects, such as placental insufficiency and adverse intrauterine exposure with consequent defective fetal outcome [59, 60, 61]. Interestingly, supplementation with GLN normalized uric acid concentration with corresponding improvement in placental efficiency and fetal outcome. Similarly, earlier studies including study from our non-pregnant laboratory animals have demonstrated the beneficial effects of GLN in disorders such as prediabetes, type 2 diabetes, cardiac hypertrophy, liver disease among others, and attributed its impacts to anti-inflammatory and anti-oxidant properties [30, 31, 32, 62]. However, to the best of our knowledge, this is the first study to report the ameliorative effect of GLN on placental lipid-associated defects and adverse fetal outcome by suppression of ADA, XO with consequent decrease in uric acid during maternal fructose consumption. Although the present study has a mechanistic limitation especially the causative link of uric acid with other biochemical parameters and molecular basis of interaction of GLN with gestational metabolic disorder, however the present data would possibly be of public health impacts considering the increased risk of maternal and fetal morbidity and mortality worldwide.

5. Conclusion

The present study demonstrates that excessive consumption of FED during pregnancy causes placental lipid/uric acid-related defects with consequent fetal growth restriction. The findings also suggest that GLN supplementation improves fetal outcome by ameliorating placental defects through suppression of placental triglyceride/uric acid accumulation as well as enhancement of G6PD/GSH-dependent antioxidant defense. Similarly, the study also suggests that GLN supplementation in pregnancy could also be useful as a reprogramming intervention to prevent impaired placental-fetal development and subsequent newborn and adulthood metabolic-related disorders.

6. Future studies

The cause relationship effects of glutamine and the possible mechanisms were not investigated and should be explored in future studies. The molecular basis underpinning the glutamine interaction with metabolic derangements also requires investigation, especially in pregnancy.

Declarations

Author contribution statement

Kehinde Samuel Olaniyi: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Isaiah Woru Sabinari: Performed the experiments; Wrote the paper.

Lawrence Aderemi Olatunji: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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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