



Suppression of uric acid and lactate production by sodium acetate ameliorates hepatic triglyceride accumulation in fructose-insulin resistant pregnant rats

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ABSTRACT

High fructose intake has been associated with perturbed lipid, uric acid and lactate homeostasis. However, consumption of fructose-sweetened beverages is not usually regulated during pregnancy. The effect of short-chain fatty acid (acetate) on the metabolic effects of high fructose intake during pregnancy is not known. We hypothesized that acetate prevents gestational fructose-induced hepatic triglyceride (TG) accumulation by suppressing uric acid and lactate production. Pregnant Wistar rats were randomly separated into three groups ($n = 6/\text{group}$) receiving drinking water (CON), 10 % (w/v) fructose drink (FRU) and 10 % (w/v) fructose with 200 mg/kg (w/w; *p.o.*) sodium acetate (FRU + ACE) daily for nineteen days. Fructose intake resulted in increased body weight gain, liver weight, fluid intake, visceral fat, insulin resistance, fasting blood glucose, insulin, plasma and hepatic TG, total cholesterol, free fatty acid, lipid peroxidation, adenosine deaminase, xanthine oxidase, uric acid, lactate, lactate dehydrogenase, and liver injury marker enzymes. However, gestational high fructose intake led to depressed plasma and hepatic glucose-6-phosphate dehydrogenase (G6PD)-dependent antioxidant barrier, adenosine and food intake. All these effects except water intake and food intake were abated by sodium acetate. These results demonstrate that maternal fructose-enriched drink would cause hepatic TG accumulation that is associated with perturbed glucose, uric acid, lactate homeostasis, and G6PD-dependent antioxidant barrier. These results also demonstrate that acetate protects the liver against gestational fructose-induced TG accumulation by inhibiting uric acid and lactate production. Thus, acetate may be useful in the treatment of hyperuricemia- and hyperlactatemia-related disorders.

1. Introduction

Obesity, diabetes and fatty liver have escalated to epidemic proportions worldwide including developing countries. The advent of metabolic syndrome or diabetes mellitus during pregnancy commonly called gestational diabetes predisposes the mother and fetus to a molecular insulin resistance milieu. Insulin resistance is considered as the key promoter of these disorders. The use of fructose ranging from food additive to sweetened beverage has increased progressively over the past three decades (Akar et al., 2012; Bray et al., 2004) and this has been suggested to contribute to the rise in prevalence of obesity, metabolic disorders and hepatic disease (Bantle, 2009). In addition, studies have shown that increased fructose intake is associated with cardiometabolic disorders including hypertension and non-alcoholic fatty liver disease (Bantle, 2009). It has also been demonstrated that high

fructose intake during pregnancy programmes fetus for insulin resistance, hyperglycemia and fatty liver in the adult life (Saad et al., 2016).

Unlike glucose, fructose seems to induce features of metabolic syndrome in humans or laboratory animals (van den Berghe et al., 1977). Of interest, the mechanism whereby fructose induces insulin resistance as well as fatty liver is independent of total energy intake. However, hepatic uric acid production has been reported to be elevated during high fructose intake (van den Berghe et al., 1977). Increased uric acid has been shown to induce hepatic triglyceride accumulation (Migue et al., 2012), inflammatory and oxidative stress-related tissue injury (Kanellis et al., 2003; Kang et al., 2005; Sautin et al., 2007; Corry et al., 2008; Yu et al., 2010).

The liver is a glucoregulatory organ that converts excess glucose uptake to glycogen in the fed state. At minimal fructose intake, pre-

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hepatic metabolism converts about 60 % of fructose to glucose (Douard and Ferraris, 2013). However, high fructose intake results in higher hepatic metabolism into glucose, lactate and even triglyceride (Sun and Empie, 2012). Moreso, fructose is considered to be lipogenic and rats fed on high fructose rapidly develops fatty liver (Sun and Empie, 2012), which is accompanied by ATP depletion resulting in uric acid generation. In addition, free fatty acids (FFAs) derived from various sources, including adipose tissue, de novo hepatic fatty acid synthesis, even FFA from dietary sources, are required as a major substrate for hepatic TG production, contributing to the development of fatty liver. The pathophysiology of fructose-induced fatty liver remains inconclusive, but one of the well-established mechanisms is the influx of triglycerides into hepatocytes leading to overproduction of reactive oxygen species (ROS) by β -oxidation, which causes a redox imbalance (Elliot and Vidal-Puig, 2004), and results in tissue inflammation, IR and mitochondrial dysfunction (Botezelli, 2012).

Adenosine deaminase (ADA), a polymorphic purine enzyme that is present in almost all the mammalian cells and the activity has been reported to be prominent in lymphoid/immune cells and some metabolic tissues including liver and skeletal muscle. The activity of ADA has been demonstrated to initiate inflammatory response via its ability to reduce adenosine (Xu et al., 2017; Ungerer et al., 1992). Reduced serum activity of ADA has been observed in normal pregnancy compared to non-pregnant women, whereas, increased activity was observed in disorders of pregnancies such as preeclampsia (Jadhav and Jain, 2013). Likewise, an increase in ADA activity was also noted in non-pregnant T2DM patients (Lee et al., 2011; Daniela et al., 2014; Xu et al., 2017; Zhang et al., 2005). Xanthine oxidase (XO) catalyzes the last two steps of purine metabolism which converts hypoxanthine to xanthine and subsequently to uric acid (Mandal and Mount, 2015). The increased XO activity has been shown to be responsible for the pro-oxidant property of uric acid and its negative impact on cardiometabolic disorders (Kelley et al., 2010).

Blood lactate quantifies the gap between energy expenditure and oxidative capacity and is largely produced by anaerobic glycolysis in muscle, adipose, and other tissues (Iossa et al., 2004; Petersen et al., 2003). Studies in humans have shown that lactate is closely related to insulin resistance, fasting glucose, fasting insulin, type 2 diabetes and hypertension (Thorburn et al., 1990; Woerle et al., 2006; Chen et al., 1993; Lovejoy et al., 1992; Reaven et al., 1988; Crawford et al., 2010; Juraschek et al., 2013). Elevated lactate may promote hepatic gluconeogenesis and interfere with glucose uptake and oxidation. Since reports have it that elevated FFAs do not only impair glucose uptake and oxidation but also interfered with glycogen synthesis via defective insulin signaling pathways (DeFronzo, 2010). Considering these and the afore-mentioned uric acid-led metabolic derangement, it is not surprising that inhibition of uric acid, lactate and FFA synthesis has been shown to improve IR induced by long-chain fatty acid, palmitate (Leam and Egnatchik, 2013).

Short-chain fatty acids (SCFAs) particularly acetate is an end-product from the fermentation of complex carbohydrates and/or digestible fibers largely by the colonic microbiota, especially by anaerobic bacteria. Acetate, the most abundant SCFAs in the colon and it partially avoids hepatic clearance to reach micromolar concentrations in the systemic circulation (Frost et al., 2014). SCFA has been demonstrated to protect against colitis, airway disease, kidney disease and metabolic syndrome in diet-induced obese mice (Maslowski et al., 2009). Reports also have it that acetate decreased the lipopolysaccharide-induced production of intracellular reactive oxygen species (ROS) concomitant with an increase in glutathione (Moriyama et al., 2016). However, the effect of SCFAs particularly acetate on fructose-induced dysmetabolism is unknown. Therefore, this study hypothesized that sodium acetate would abate cardiometabolic derangements in high fructose-exposed pregnant rats by inhibition of uric acid and lactate production.

2. Materials and methods

2.1. Animals and treatment

Female Wistar rats weighing 140–170 g were obtained from the Animal House, College of Health Sciences, University of Ilorin, Ilorin, Nigeria. The rats were housed in a well-ventilated room kept under standard conditions of humidity, feeding, light and temperature with free access to standard rat chow and water. The investigation was conducted in line with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the study was approved by the Institutional Ethical Review committee of University of Ilorin, Ilorin, Nigeria. Efforts were made to minimize suffering and the number of animals used. After one week of acclimatization, the animals were time-mated. Pregnant rats were allotted into 3 groups ($n = 6/\text{group}$). Control rats received distilled water (Vehicle; *p.o.*), fructose group received 10 % (w/v) fructose drink and fructose plus acetate group received fructose drink with 200 mg/kg sodium acetate (*p.o.*) daily for nineteen days. Oral glucose tolerance test (OGTT) was performed on day 20 of gestation and the experiment was terminated on day 21. Body weight change, food intake and water intake were monitored.

2.2. Sample collection and handling

At the termination of the experiment, animals were anesthetized with sodium pentobarbital (50 mg/kg, *i.p.*) after an overnight 12-h fast. Blood was collected by cardiac puncture into heparinized bottles, centrifuged at 3000 rpm for 5 min; plasma was obtained and stored frozen until the period of biochemical assay.

The livers of the rats were excised and homogenized in phosphate buffer solution (PBS). The homogenized organs were centrifuged at 10,000 rpm for 10 min. the homogenates were decanted and stored frozen until the period of biochemical assay.

2.3. Quantification of visceral fat

Regional body fats were collected by opening up the rats' abdominal region after blood collection during the process of organ harvest. The fat pads collected were peri-renal, abdominal and retroperitoneal fats. The fat pads were corrected to body weight.

2.4. Insulin resistance indices

Oral glucose tolerance test (OGTT) was carried out by obtaining blood from the tail of each animal before glucose load (2 g/kg *bw*) (*p.o.*) and then sequentially after 30, 60, 90 and 120 min. Terminal fasting blood glucose (FBG) was determined after the rats had 12 h overnight fast on the day of the sacrifice. Blood glucose levels were determined using a glucometer (ONETOUCH®-LifeScans, Inc., Milpitas, CA, USA). Glucose tolerance was expressed as a function of the area under the OGTT curve (AUC). IR and pancreatic β -cell function were determined using the homeostasis model assessment for insulin resistance (HOMA-IR: (fasting insulin* fasting glucose)/22.5) and HOMA- β formula (20*fasting insulin/3.5-fasting glucose) respectively. Plasma insulin was determined using ELISA kit from Ray Biotechnology, Inc. (Georgia, USA).

2.5. Lipid profiles

Plasma and hepatic triglycerides (TG), high density lipoprotein (HDL), total cholesterol and free fatty acid (FFA) were estimated by standardized enzymatic colorimetric methods using reagents obtained from Randox Laboratory Ltd. (Co. Antrim, UK). TG/HDL and TC/HDL-cholesterol ratios were estimated.

2.6. Uric acid production

Plasma and hepatic adenosine deaminase (ADA), xanthine oxidase (XO) were determined by standard colorimetric method using reagents from Fortress diagnostics (Antrim, United Kingdom) while uric acid (UA) was determined using kits from Randox Laboratory Ltd. (Co. Antrim, UK).

2.7. Lactate production

Plasma and hepatic lactate and lactate dehydrogenase (LDH) were measured by standardized enzymatic colorimetric method using assay kit obtained from Fortress diagnostics (Antrim, United Kingdom)

2.8. Redox and inflammatory markers

Plasma and hepatic malondialdehyde (MDA), glucose-6-phosphate dehydrogenase (G6PD), oxidized glutathione (GSSG), reduced glutathione (GSH), glutathione peroxidase (GPx) were determined using colorimetric assay kits obtained from Oxford Biomedical Research Inc. (USA). Reduced to oxidized glutathione ratio (GSH/GSSG) was estimated as an indicator of antioxidant capacity. NO and adenosine were determined using non-enzymatic colorimetric assay kits (Oxford Biomedical Research Inc, Oxford, USA) and assay kit from Fortress diagnostics (Antrim, United Kingdom) respectively

2.9. Hepatic marker enzyme

Hepatic gamma-glutamyl transferase (GGT), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were determined using standard spectrophotometric method.

2.10. Statistical analysis

All data were expressed as means $\pm$ SEM. Statistical group analysis was performed with SPSS statistical software (Chicago, IL). One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

3. Results

3.1. Effect of sodium acetate on visceral fat, body weight, liver weight, food and water intake in fructose-administered pregnant rats

Fructose intake led to increased visceral fat, body weight, liver weight, water intake and decreased food intake when compared to the control group. However, treatment with sodium acetate ameliorated these alterations caused by fructose intake except water intake and food intake that were not significantly abated (Fig. 1).

3.2. Effect of sodium acetate on glucoregulatory parameters in fructose-administered pregnant rats

Fructose intake caused impaired glucose tolerance and insulin resistance and increased fasting blood glucose, insulin and reduced β -cell function when compared to the control group, which were attenuated by sodium acetate (Fig. 2).

3.3. Effect of sodium acetate on plasma and hepatic triglycerides, free fatty acid, TG/HDL-cholesterol and TG/TC ratio in fructose-administered pregnant rats

Fructose intake increased lipid accumulation with corresponding increase in plasma and hepatic TG, FFA, TG/HDL and TC/HDL ratio

when compared to the control group, which were ameliorated by sodium acetate (Fig. 3).

3.4. Effect of sodium acetate on plasma and hepatic oxidative stress capacity in fructose-administered pregnant rats

Plasma and hepatic lactate, LDH and MDA are markers of cellular oxidative capacity. Fructose increased plasma and hepatic lactate and LDH activity when compared with control group, which were attenuated by sodium acetate. In addition, plasma and hepatic MDA, an indication of lipid peroxidation was increased during fructose intake when compared with control which was attenuated by sodium acetate. However, plasma MDA was significantly higher in FRU + ACE-treated group compared to the control group (Fig. 4).

3.5. Effect of sodium acetate on uric acid production in fructose-administered pregnant rats

Fructose intake increased plasma and hepatic ADA and XO activities when compared to the control group. In addition, fructose intake also increased uric acid level when compared to the control group, which were significantly reduced by sodium acetate (Fig. 5)

3.6. Effect of sodium acetate on plasma and hepatic adenosine and NO in fructose-administered pregnant rats

Fructose intake led to a decrease in plasma and hepatic levels of adenosine and NO when compared with control which were significantly increased by sodium acetate (Fig. 6)

3.7. Effect of sodium acetate on plasma and hepatic glutathione-dependent antioxidants in fructose-administered pregnant rats

Fructose intake led to decrease in plasma and hepatic G6PD, GPx, GSH/GSSG-dependent antioxidants compared to the control group, which were ameliorated when fructose was combined with sodium acetate (Fig. 7).

3.8. Effect of sodium acetate on plasma and hepatic injury enzyme markers in fructose-administered pregnant rats

ALT, AST and ALP are important markers of liver enzyme functions. Fructose intake increased ALT, AST and ALP when compared with control group, which were attenuated when sodium acetate was combined with fructose intake. Plasma and hepatic AST was higher in FRU + ACE-treated group when compared to the control (Fig. 8).

4. Discussion

This study demonstrates that high fructose intake led to metabolic derangements characterized by increased body weight, liver weight, abdominal fat and fasting blood glucose with evidence of insulin resistance and hyperinsulinemia. This phenotypic and biochemical evidences of glucose deregulation is accompanied by evidence of hepatic lipotoxicity, oxidative stress, inflammatory response and hepatic TG accumulation. Unsurprisingly, the fructose-induced events had an attendant elevated uric acid and lactate production. However, acetate treatment to high fructose-exposed rats led to attenuation of fructose-induced dysmetabolism and abated uric acid and lactate production. Therefore, acetate by uric acid and lactate suppression played beneficial role in fructose-induced TG accumulation and dysmetabolism during pregnancy.

Insulin resistance is the hallmark of metabolic syndrome and is usually associated with obesity and type 2 diabetes. Increase visceral fat and body weight are risk factors for cardiometabolic diseases leading to glucose deregulation and consequent insulin resistant-

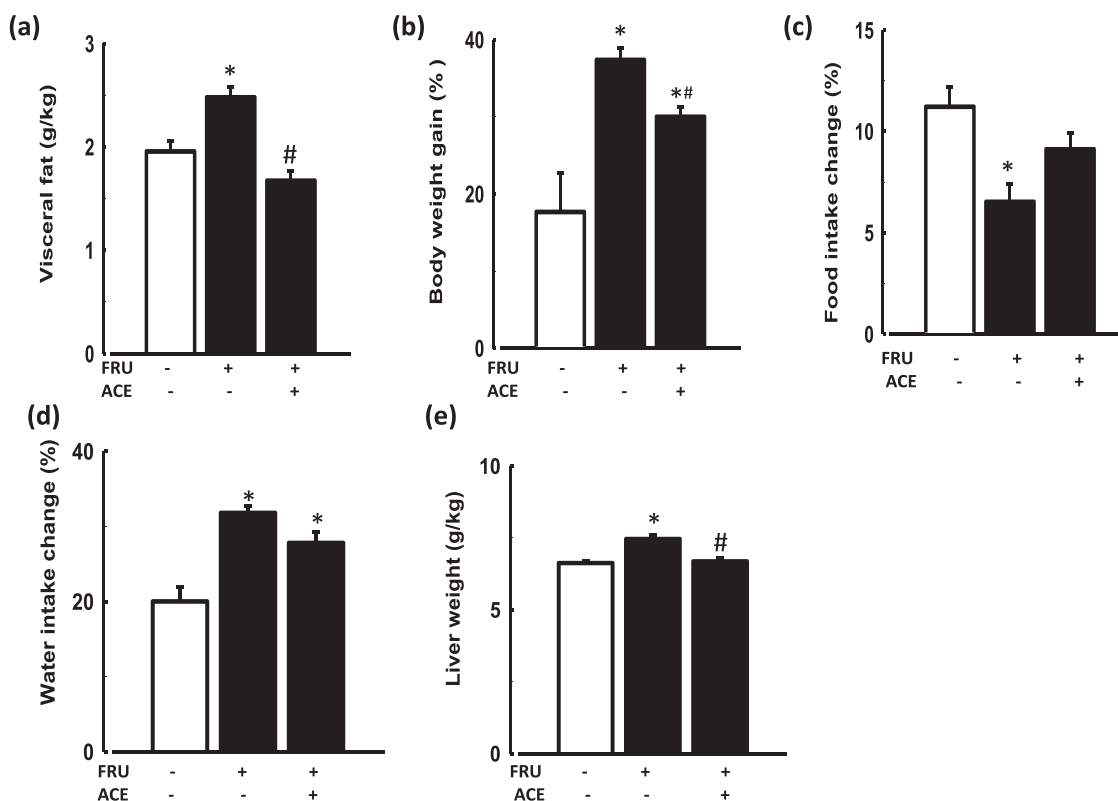


Fig. 1. Effect of sodium acetate (ACE) on visceral fat (a), percentage body weight gain (b), percentage change in food intake (c), percentage change in water intake (d) and liver weight (e) during high fructose (FRU) intake in pregnant rats. FRU intake increased abdominal fat, percentage body weight gain, food intake, water intake and liver weight, which were ameliorated by ACE except water and food intake. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

hyperinsulinemia. Fructose intake in excess of normal dietary requirement or threshold for intestinal metabolism can lead to the manifestation of CMD owing to hepatic fructolytic production of uric acid and liberation of carbon compounds (Fanny et al., 2014). This study demonstrated that high fructose intake led to increased body weight (Fig. 1), visceral fat mass and markers of glucose regulation such as insulin and HOMA-IR (Fig. 2). Increased body weight and visceral fat are risk factors for CMD due primarily to increased lipolysis, circulating FFA, FFA flux into non-adipose tissues, production of adipokines and mineralocorticoid receptor activation. These effects of fructose on glucoregulation especially during pregnancy can predispose mothers to gestational diabetes and the fetus to future CMD by creating a molecular insulin resistance milieu. The findings of this study however show that hepatic glucoregulatory function and insulin sensitivity is undoubtedly affected by 10 % fructose (w/v) in drinking water since increased hepatic fructolytic burden for the period of gestation led to increased hepatic weight and certainly intrahepatic metabolic changes.

Hepatic handling of excess glucose differs from that of excess fructose. Aside increasing glycolytic flux, fructolysis is characterized by a higher degree of lipogenesis leading to TG accumulation. This hepatic TG accumulation in addition to increased hepatic total cholesterol, FFA and even lipid peroxidation was evident in the effects of high fructose intake in this study (Fig. 3). The fructose-induced increase in fatty acid and lipid content of the liver is known to cause oxidative stress, inflammatory response, insulin resistance (Samuel et al., 2004) and tissue damage. These various dysmetabolisms can coalesce to engender non-alcoholic fatty liver disease and hepatic steatosis (Fig. 9). The accepted mechanisms for FFA/TG-induced dysmetabolism include increased signaling through the activation of toll-like receptor-4 and increased production of inflammatory cytokines leading to serine instead of tyrosine phosphorylation of insulin receptor substrate-1/2 (IRS-1/2) with a resultant decrease in insulin sensitivity. IRS-1/2 tyrosine

phosphorylation by insulin receptor is the rate limiting step in insulin signaling through the phosphoinositol kinase-3 (PIK-3)-protein kinase B/Akt pathway (Bergman and Ader, 2000; Samuel et al., 2004). Another acquired event due to high FFA and lipid content of the cell is lipid peroxidation, which is usually marked by increased malonaldehyde (MDA) level and is also accepted as an indication of oxidative stress due to increased lipid peroxides. This study shows that fructose-induced dysmetabolism increased plasma and hepatic MDA (Figure). Various reports concede that oxidative stress worsens IR and promotes inflammatory responses, which leads to disruption of insulin signaling via the IRS-1/2-PIK-3-PKB/Akt pathway (DeFronzo, 2010). Existing reports also show that disrupted insulin signaling through the IRS-1/2-PIK-3-PKB/Akt pathway, suppresses nitric oxide synthesis and enhances insulin signaling via the mitogen activated protein kinase pathway, which leads to release of nuclear factors that have inflammatory cytokines and growth factors as target genes (DeFronzo, 2010).

ADA, an inflammatory mediator is also important in purine metabolism and plays pivotal role in the production of uric acid. Studies show that increased ADP/ATP ratio follows high fructose intake, which could provide more substrate for the immune mediating enzyme, ADA and thereby increase uric acid production. This study shows that excess fructose induced increase in hepatic uric acid production with increased ADA and XO activities. Taken together, fructose intake might increase ADA activity and resultant uric acid production by stimulation of oxidative stress/inflammation mechanisms and/or through the release of more purine metabolites due to increased ketohexokinase activities. However, the uric acid production along fructolysis also contributes to oxidative stress and reduction of nitric oxide (NO) synthesis (Jadhav and Jain, 2013). Uric acid has been shown to stimulate arginase a negative regulator of NO. In addition, XO during uric acid production supplies superoxides. The fructose-induced multi-factor oxidative stress

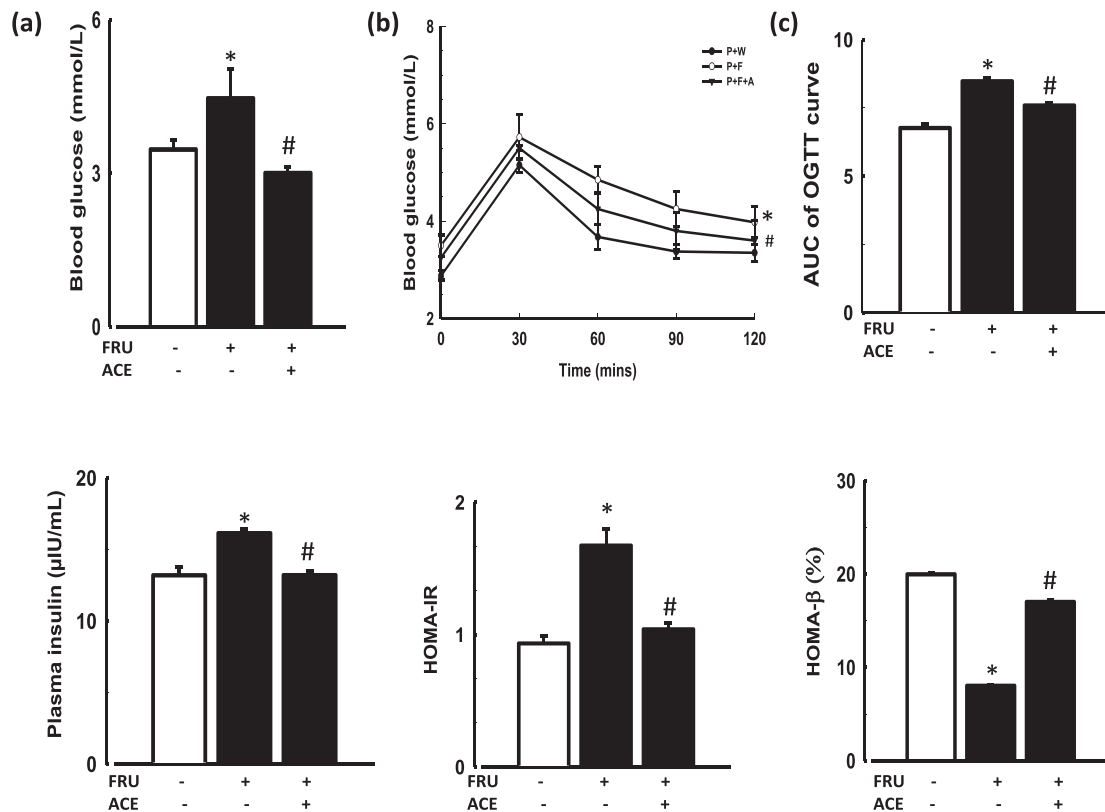


Fig. 2. Effect of sodium acetate (ACE) on fasting blood glucose (a), oral glucose tolerance (b, c), plasma insulin (d), HOMA-IR (e) and HOMA-β (f) during high fructose (FRU) intake in pregnant rats. FRU intake caused hyperglycemia, impaired glucose tolerance, hyperinsulinemia, insulin resistance and decreased pancreatic beta cell function, which were ameliorated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

deleteriously impacts mitochondrial function, which leads to lipogenesis as a result of mitochondrial citrate regurgitation. This elucidates the TG accumulation in the liver of fructose-exposed pregnant rats in this study. Adenosine, a nucleoside with anti-inflammatory property is usually cleaved by ADA in the UA production pathway. To corroborate the heightened hepatic ADA activity discovered in this study, high fructose intake led to markedly reduced adenosine, which reduces its anti-inflammatory effect. Nevertheless, the fact that uric acid production plays a central role in fructose-induced TG accumulation is not unknown. Studies have shown that increased uric acid production can lead to expression of the carbohydrate responsive element binding protein (ChREBP), which has fructolytic enzymes as target genes leading to more UA production (Lanaspa et al., 2012), lipogenesis and mitochondrial dysfunction.

Another key finding in this study is that hepatic lactate production increased with high fructose intake (Fig. 7a) in consonance with Sun and Empie, 2012. Increased lactate production has been implicated in diabetes, IR (Juraschek et al., 2013) and tissue hypoxia or reduced mitochondrial oxidative capacity. The fructose-induced uric acid production and/or insulin resistance can disturb nitric oxide synthesis and compromise blood flow leading to tissue hypoxia and resultant lactate accumulation. Also, tissue hypoxia marked by lactate accumulation and lipogenesis can synergistically engender injury via initiation of apoptotic processes. Hepatic markers of tissue injury were raised in high fructose-exposed pregnant rats according to this study (Fig. 8) indicating that the 10 % (w/v) excess fructose exposure during pregnancy can initiate multiple processes leading to oxidative stress and hepatic injury. Hepatic G6PD-dependent anti-oxidant barrier was depressed with high fructose intake in this study (Fig. 6). This outcome indicates a fructose-induced overwhelming oxidative stress leading to depressed glutathione antioxidant barrier. The cellular defense against oxidative

stress is lost herein and it attests to the hepatic tissue susceptibility to dysfunction and injury.

SCFAs especially products of colonic microbiota degradation of fibers, acetate, butyrate and propionate are growingly gaining the focus of scientific studies in recent years. Studies have reported plausible findings regarding the beneficial roles of SCFAs in cardiometabolic disease conditions (Maslowski et al., 2009). It is well established that SCFAs inhibit histone deacetylase and glycogen synthase kinase-3. Therefore, the SCFAs as therapeutic agents in treatment of metabolic syndrome especially is promising and more studies are required to fully elucidate the mechanisms involved in SCFA actions. Interestingly, the present study is the first to show that the SCFA, acetate, attenuates the glucose dysregulation and hepatic TG accumulation associated with high fructose intake during pregnancy. These effects were accompanied by suppression of uric acid production and lactate accumulation showing that acetate can alleviate metabolic syndrome or delay the onset of cardiometabolic diseases by UA and/or lactate-dependent mechanisms.

Although the effects of sodium acetate on food and water intake were not significant when compared with fructose-treated group and that might be responsible for attenuation but not normalization of body weight when fructose with sodium acetate group was compared to the control group. Similarly, Zhao et al., recently reported that fructose consumption increased gut microbial-derived acetate that triggered hepatic *de novo* lipogenesis via hepatic Acyl-Co A synthetase short chain family member 2 (ACSS2), which converts acetate to acetyl Co A (Zhao et al., 2020), however, the study did not reveal if the observation would be similar with exogenous acetate. The observation contradicts the report of the study by Li et al., who observed a significant decrease in the concentrations of acetate, propionate, butyrate, and total SCFAs in fructose-fed mice than in control group and reported that fructose

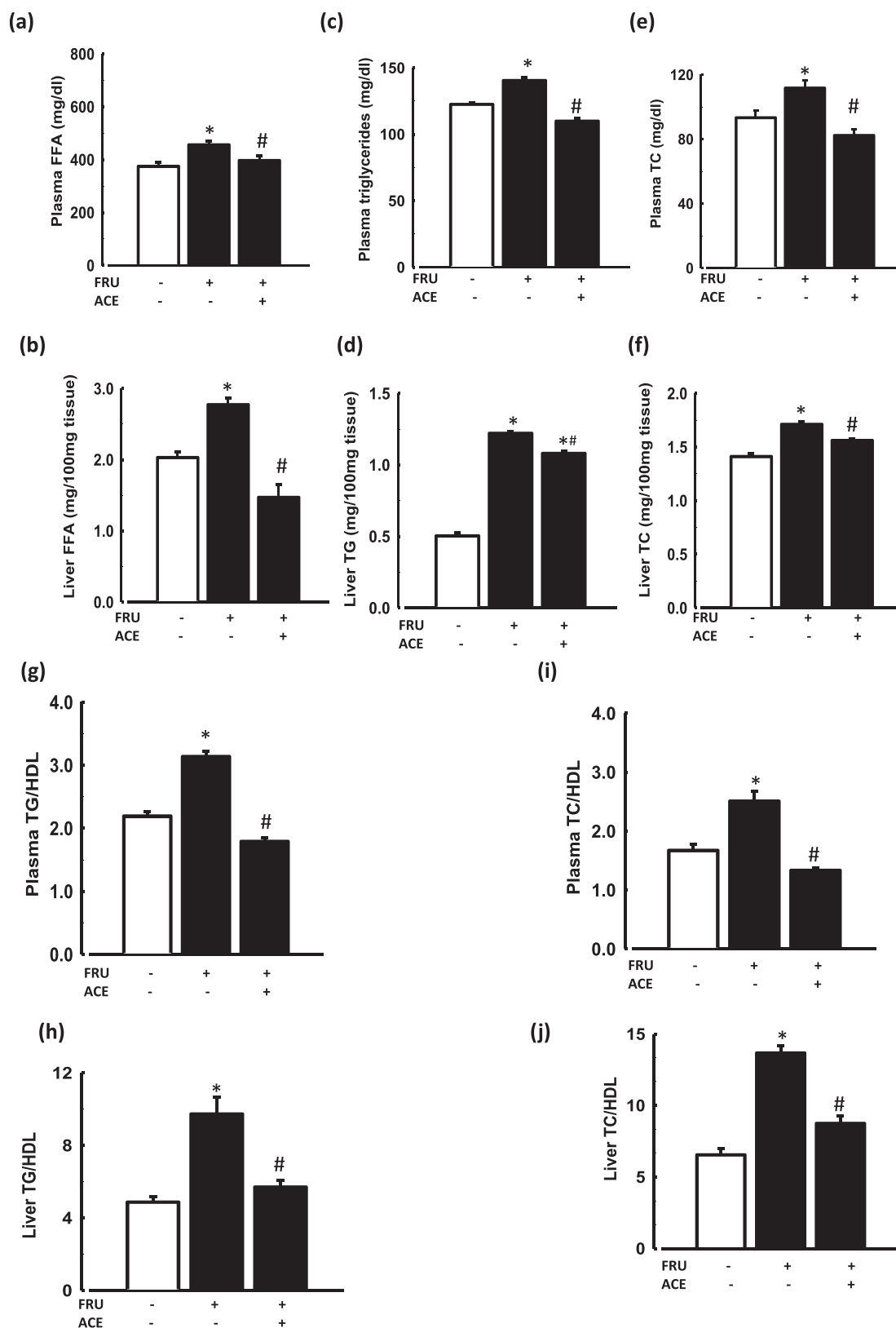


Fig. 3. Effect of sodium acetate (ACE) on plasma and liver free fatty acid (a, b), triglycerides (c, d), total cholesterol (e, f) TG/HDL-cholesterol (g, h) and TC/HDL-cholesterol (i, j) during high fructose (FRU) intake in pregnant rats. FRU increased plasma and hepatic FFA, TG, TC, TG/HDL-cholesterol and TC/HDL-cholesterol. These alterations were abated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

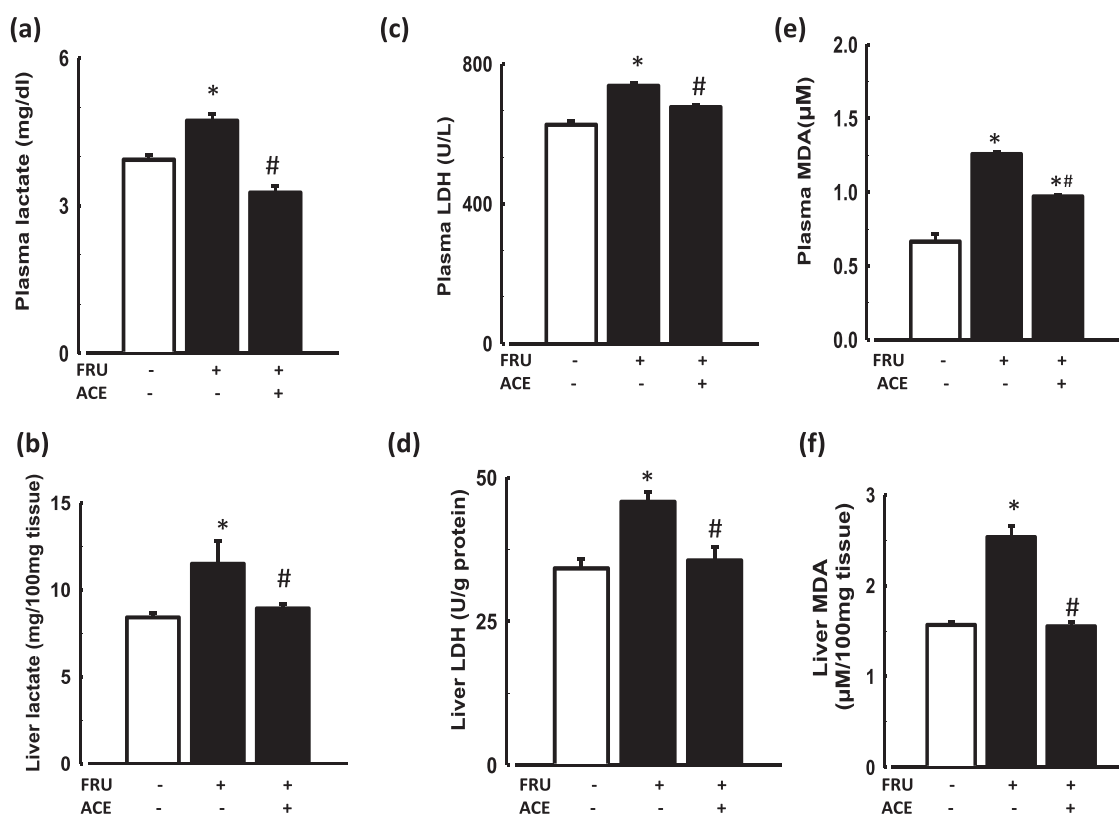


Fig. 4. Effect of sodium acetate (ACE) on plasma and liver lactate (a, b), lactate dehydrogenase (c, d), and malondialdehyde (e, f) during high fructose (FRU) intake in pregnant rats. FRU increased lactate production and MDA, which were ameliorated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

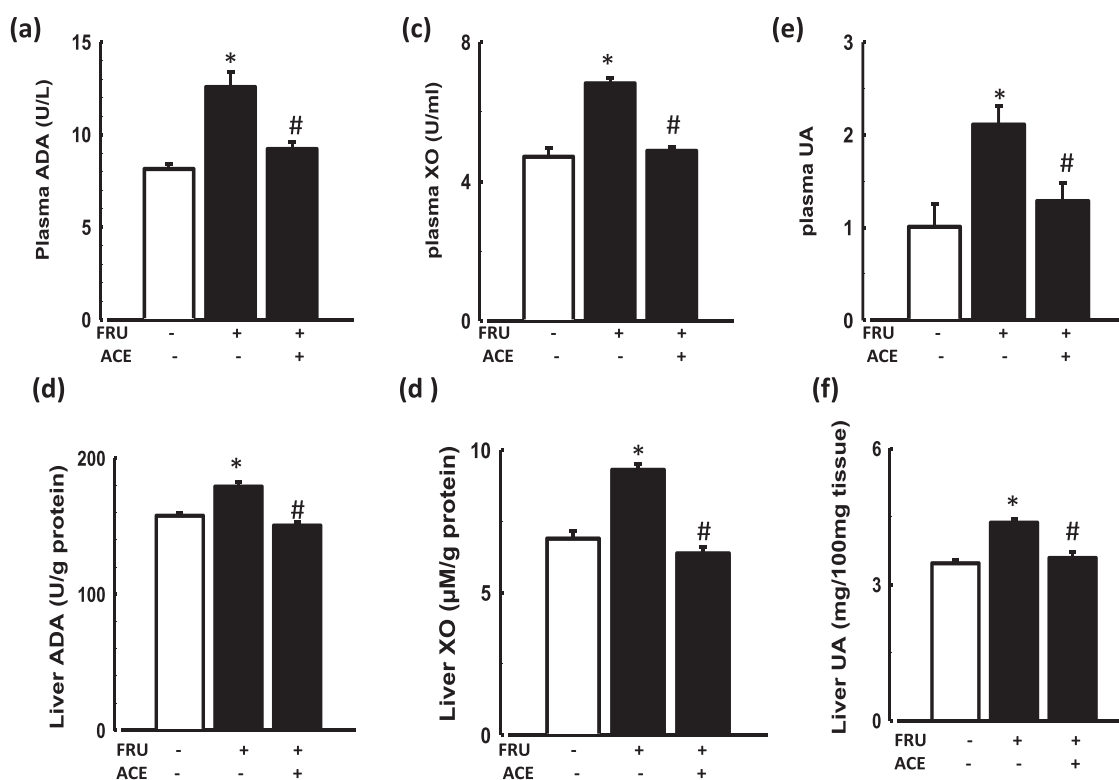


Fig. 5. Effect of sodium acetate (ACE) on plasma and liver adenosine deaminase (a, b), xanthine oxidase (c, d), and uric acid (e, f) during high fructose (FRU) intake in pregnant rats. FRU increased uric acid production, which was ameliorated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

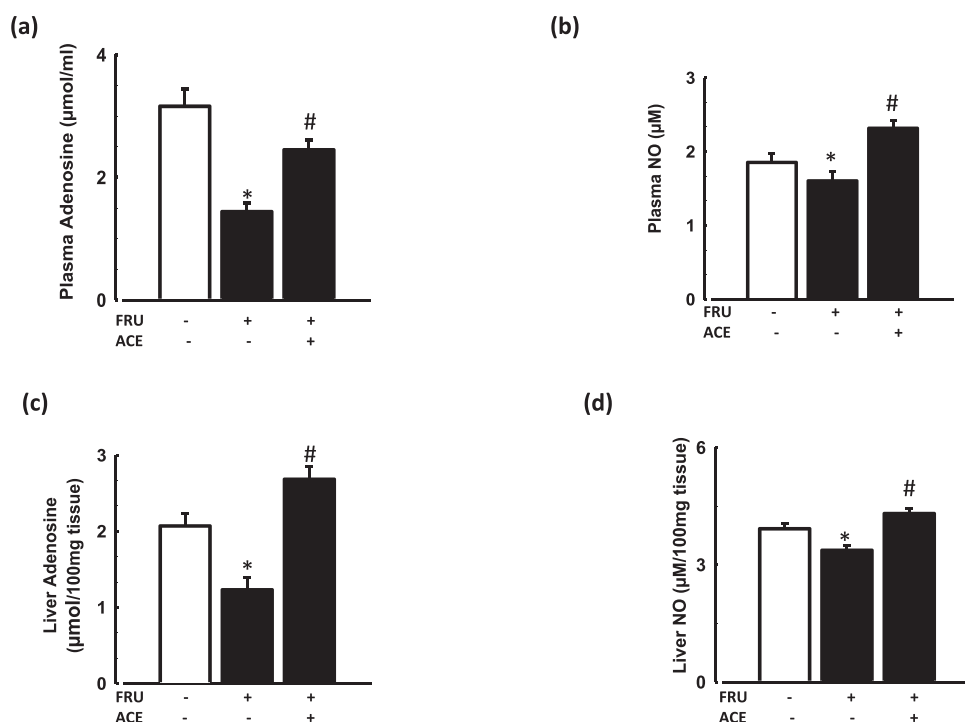


Fig. 6. Effect of sodium acetate (ACE) on plasma and liver adenosine (a, b) and nitric oxide (c, d) during high fructose (FRU) intake in pregnant rats. FRU increased adenosine and nitric oxide, which were ameliorated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

consumption causes gut microbial dysbiosis, which alters the SCFAs, including acetate and butyrate with consequent metabolic and inflammasome dysfunctions (Li et al., 2019). Further study is therefore required to fix the possible imbalance because SCFAs, including acetate

has been extensively demonstrated to be protective in CMD (Maslowski et al., 2009; Kim, 2018; Olaniyi et al., 2020). Nevertheless, in the present study, since acetate suppressed uric acid production with decrease in all the components of the pathway, ADA, XO, and UA,

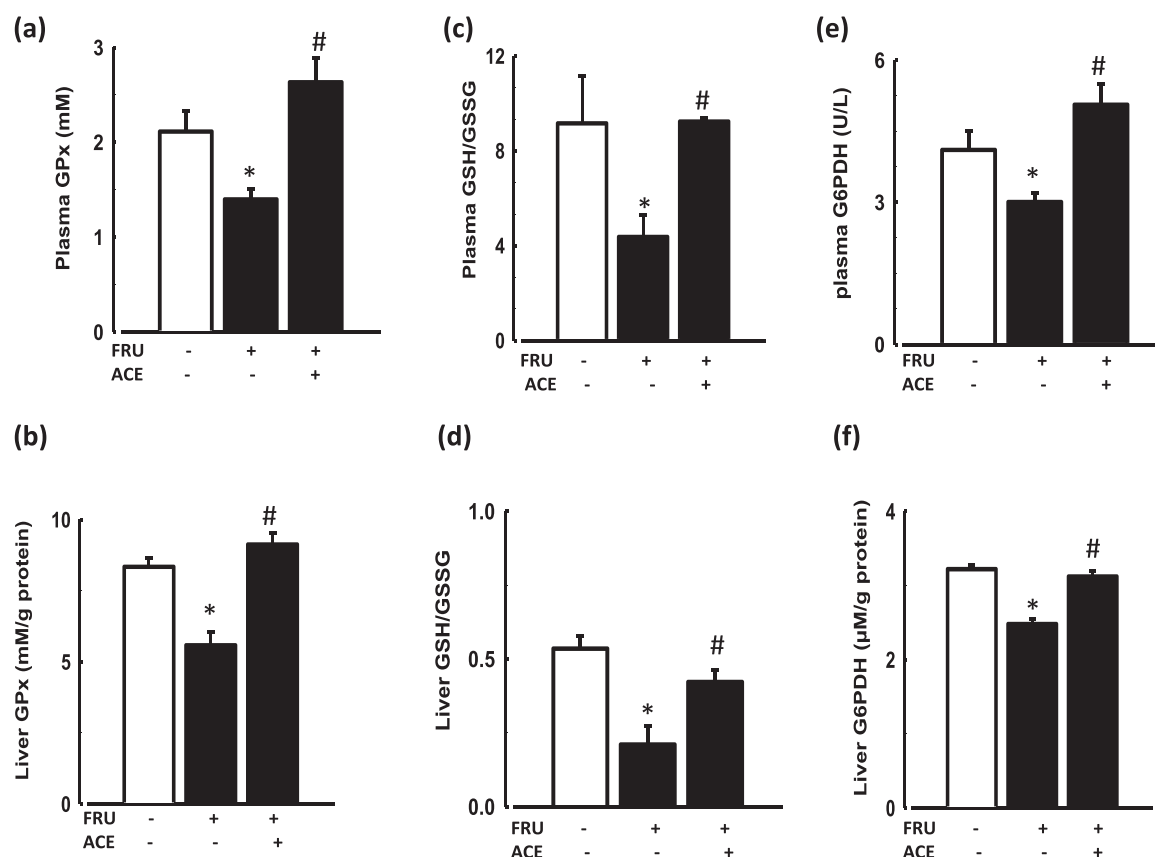


Fig. 7. Effect of sodium acetate (ACE) on plasma and liver GPx (a, b) GSH/GSSG ratio (c, d) and Glucose-6-dehydrogenase phosphate during high fructose (FRU) intake in pregnant rats. FRU decreased GPx, GSH/GSSG ratio and G6PD, which were ameliorated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

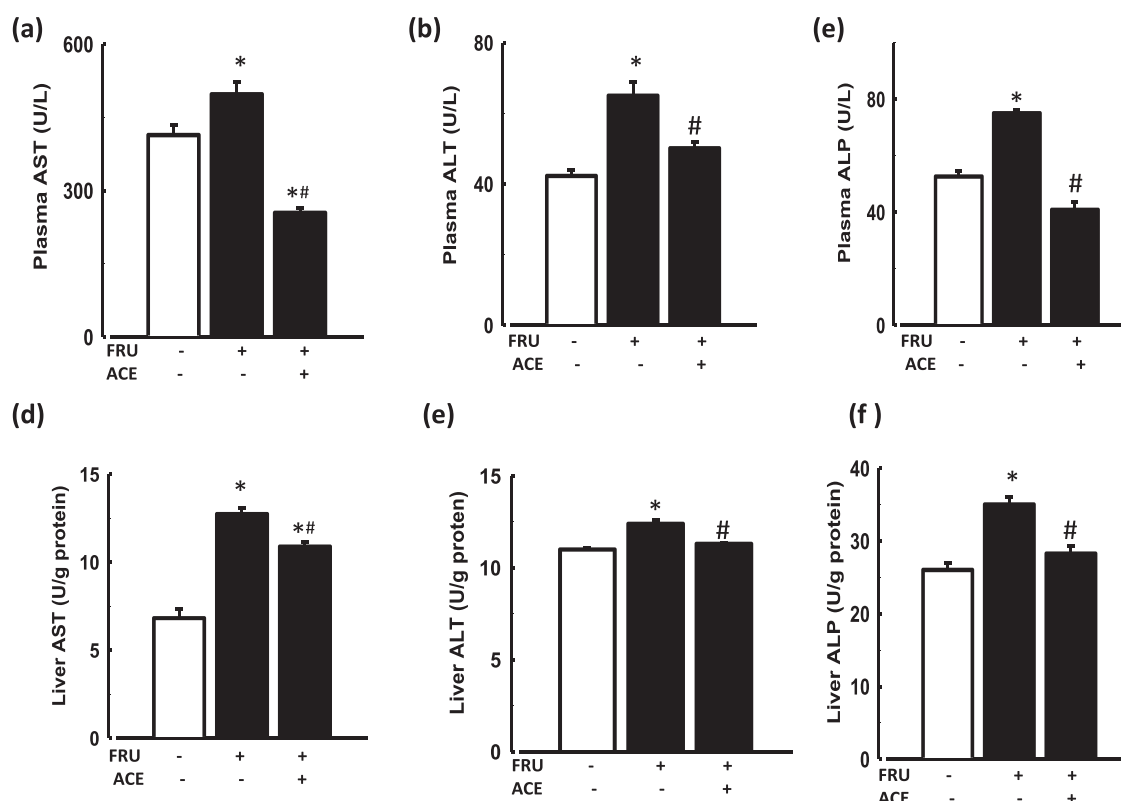


Fig. 8. Effect of sodium acetate (ACE) on plasma and liver aspartate transaminase (a, b) alanine aminotransferase (c, d) and alkaline phosphatase during high fructose (FRU) intake in pregnant rats. FRU increased plasma and liver AST, ALT and ALP, which were ameliorated by ACE. Data are expressed as mean $\pm$ S.E.M. $n = 6$. Data were analysed by one-way ANOVA followed by Bonferroni *post hoc* test. (* $p < 0.05$ vs control; # $p < 0.05$ vs FRU).

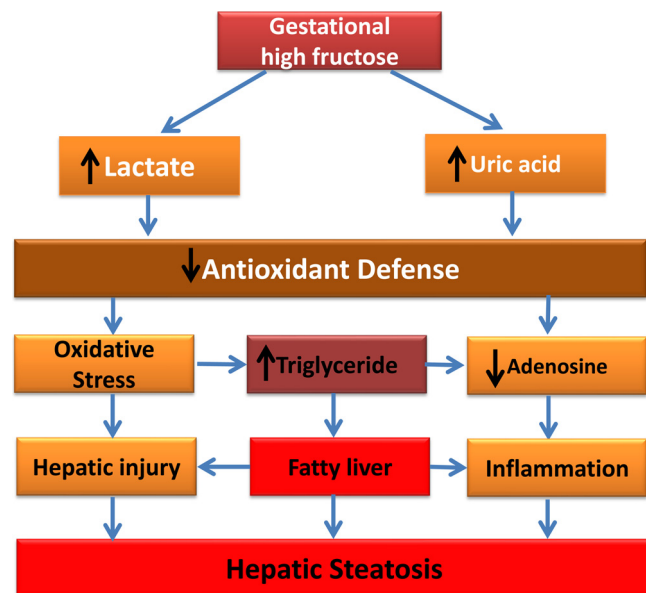


Fig. 9. Schematic diagram showing the metabolic pathways involved in the attenuation of fructose-induced hepatic triglyceride accumulation via inhibition of uric acid and lactate production.

accompanied by augmentation of adenosine shows that acetate is a suitable therapeutic agent especially for fructose-induced metabolic derangements since high fructose-led tissue insult is mediated ultimately by elevated uric acid production.

In conclusion, this study demonstrates that increased uric acid and lactate production are involved in fructose-induced glucose deregulation and hepatic TG accumulation during pregnancy. However, acetate

averted glucose deregulation and attenuated hepatic TG accumulation through mechanisms associated with UA and lactate suppression. Therefore, suppression of UA and lactate could be a promising procedure in the treatment of fructose-induced dysmetabolism and acetate is shown in this study to possess a potent UA and lactate suppressing properties.

Author contribution

AOO, AOS and LAO conceived and designed the research. AOO conducted the experiments. LAO contributed to the new reagents and analytical kits. AOO, KSO and LAO analyzed and interpreted the data. AOO, KSO and LAO drafted the manuscript. AOO, KSO, AOS and LAO read and approved the final manuscript.

Declaration of Competing Interest

The authors declare no conflict of interest.

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