



Sodium acetate ameliorated systemic and renal oxidative stress in high-fructose insulin-resistant pregnant Wistar rats

Adewumi Oluwafemi Oyabambi¹ · Olugbenga Samuel Michael² · Emmanuel Damilare Areola¹ · Salam Babatunde Saliu¹ · Lawrence Aderemi Olatunji¹

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Abstract

Pregnancy is an insulin-resistant condition especially at near term predisposing maternal kidneys to hyperinsulinemia-induced oxidative stress. The impact of fructose on renal metabolic dysregulation and oxidative stress in pregnancy requires elucidation. Short-chain fatty acids (SCFAs) are known for protective roles in oxidative stress conditions. Therefore, the study aimed at investigating fructose-induced glucose dysregulation and renal oxidative stress in pregnant and non-pregnant rats and the possible preventive role of SCFA, acetate. Thirty female Wistar rats were grouped ($n = 5/\text{group}$). Three groups were made pregnant (P); the other three remained non-pregnant (NP). Both pregnant and non-pregnant rats received drinking water (control), 10% fructose (w/v) (NP+F or P+F), and 10% (w/v) fructose plus sodium acetate (200 mg/kg) (NP+F+A or P+F+A) for 3 weeks. Renal and plasma glutathione antioxidant index (GSH/GSSG), G6PDH, and adenosine were significantly lower in NP+F and P+F groups compared with control while renal and plasma adenosine deaminase (ADA), xanthine oxidase (XO), uric acid (UA), lactate dehydrogenase (LDH), and malonaldehyde (MDA) were significantly elevated in NP+F and P+F groups compared with controls. HOMA-IR showed marked impairment in both NP+F and P+F groups. The P+F group revealed greater suppression in plasma and renal G6PDH-dependent antioxidant index, adenosine, and aggravation of LDH, MDA compared with the NP+F group ($p < 0.05$). Sodium acetate reduces plasma and renal surrogate oxidative stress markers, improved G6PD-dependent antioxidant index, and HOMA-IR in NP+F and P+F groups. Pregnancy exacerbates fructose-induced insulin resistance and renal oxidative stress whereas acetate ameliorated fructose-induced redox and glucose dysregulation in pregnant and non-pregnant rats.

Keywords Sodium acetate · Pregnancy · Oxidative stress · Fructose · Glucose dysregulation

Introduction

Last trimester of pregnancy is physiologically characterized by peripheral resistance to insulin action, mainly due to increased maternal adiposity and the production of diabetogenic hormones by the placenta (Napso et al. 2018). In normal pregnancy, the pancreatic β -cells increase insulin secretion to

compensate for this resistance, thereby regulating glycemic levels (Cerf 2013). There is evidence that maternal hyperinsulinemia is associated with increased oxidative stress (Réus et al. 2019), which may also be involved in the pathophysiology of maternal tissue and fetal complications (Fabiane et al. 2018). Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defense system. This status can cause damage and alter the functions of biomolecules, such as lipids, proteins, and DNA (Lobo et al. 2010). Maternal tissues like the kidney are likely to suffer if these metabolic alterations are aggravated especially through diet. For example, dietary fructose has been shown to be a major candidate in the onset of NAFLD (Choi et al. 2017) and fructose is a monosaccharide which is commonly used as a sweetener, e.g., in high-fructose corn syrups. Industrially, it is frequently found in soft drinks and pre-packaged foods (Zargaraan et al. 2016).

✉ Adewumi Oluwafemi Oyabambi
oyabambi.ao@unilorin.edu.ng

¹ Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, University of Ilorin, P.M.B. 1515, Ilorin 240001, Nigeria

² Cardiometabolic, Microbiome and Applied Physiology Laboratory, Physiology Program, College of Health Sciences, Bowen University, Iwo, Nigeria

Epidemiologic data suggest that there has been a significant rise in calories consumed from saturated fat and fructose-rich foods (Bray et al. 2004). Fructose consumption accounts for approximately 10.2% of all calories in our average diet in the USA (Vos et al. 2008). The involvement of high fructose intake in the prevalence of metabolic syndrome is accentuated by mechanisms such as increased uric acid production, increased production of triglycerides, reduced nitric oxide production, and increased oxidative stress.

Antioxidants are molecules that can convert ROS into less reactive products, thus neutralizing them. Antioxidants can be enzymes such as glutathione peroxidase (GPx), glutathione reductase, and glutathione (GSH) transferase, as well as non-enzymatic endogenous molecules, such as adenosine, glutathione, α -tocopherol, β -carotene, and ascorbic acid (Meghri et al. 2019). G6PD is not just the cytosolic enzyme for the synthesis of pentose phosphate but also contributes considerably to the anti-oxidant defenses through glutathione system, by converting glutathione disulfide (GSSG) to its reduced form, GSH by GPx (Volodymyr 2012).

Adenosine, an important regulatory nucleoside to which various protective functions have been attributed, has vasodilatory, anti-inflammatory, and anti-oxidant properties. Besides, its ability to mitigate renin-angiotensin system together with aforesaid roles confer a protective shield on renal and cardiometabolic functions (Xu et al. 2017; Zhang et al. 2005). Adenosine deaminase is involved with purine metabolism and is ubiquitously present in mammalian cells with prominence in immune cells, liver, and skeletal muscle. ADA activity has an undoubted correlation with inflammatory response by negatively regulating adenosine (Xu et al. 2017). Pregnancy disorders like preeclampsia and T2DM have been observed to involve elevated ADA activity when compared with normal pregnancies (Jadhav and Jain 2013). Increased ADA can lead to increased XO since ADA furnishes XO with substrate. Xanthine oxidase (XO) catalyzes the last two steps of purine metabolism leading to uric acid (UA) production (Mandal and Mount 2015). Enhanced XO activity releases reactive oxygen species and is agreeably a culprit in the pro-oxidant property of UA (Kelley et al. 2010).

End products from colonic microbiota fermentation of complex carbohydrates or resistant sugars are known as short-chain fatty acids (SCFA) (Wong et al. 2006). Acetate, propionate, and butyrate make up over 90% of the SCFAs present in the colon (Mortensen and Clausen 1996) of which acetate constitutes about 65% reaching micromolar concentrations in the systemic circulation after partially avoiding hepatic clearance (Frost et al. 2014). SCFAs have therapeutic impacts on inflammatory diseases, metabolic syndrome in diet-induced obese mice, colitis, airway disease, and kidney disease (Maslowki et al. 2009). Reports also showed that acetate decreased the lipopolysaccharide-induced production of intracellular reactive oxygen species (ROS) concomitant with an increase in glutathione (Moriyama et al. 2016).

The involvement of colonic microbiota dysregulation leading to deregulated SCFA production in fructose-induced metabolic derangement in view of the impact of pregnancy is the aim of this study.

Materials and methods

Animals

The study was carried out following the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of the University of Ilorin with approval number: UERC/ASN/2016/357 and every effort was made to reduce both the number of animals used and their suffering. Adult female Wistar rats weighing between 120 ± 4 g were obtained from the animal house of the College of Health Sciences, University of Ilorin (Ilorin, Nigeria). Rats had unlimited access to standard rat chow and tap water. After 1 week of acclimatization, the animals were randomly assigned to six groups ($n = 6/\text{group}$). Rats were maintained in the animal house under environmental conditions of temperature ($22\text{--}26^\circ\text{C}$), relative humidity (50–60%), and 12-h dark/light cycle.

Pregnancy confirmation

Three groups of rats ($n = 6/\text{group}$) were made pregnant (PR) after harmonizing their estrous cycles and pregnancy confirmed by a post-copulatory plug and vaginal smear detection. The three other groups remained non-pregnant (NP).

Treatment

Both pregnant (PR) and non-pregnant (NP) rats were given distilled water (vehicle; *p.o.*) daily, 10% fructose (w/v; Merck KGaA, Darmstadt, Germany) NP+FRU or PR+FRU, and 10% (w/v) fructose plus sodium acetate (200 mg/kg; *p.o.*; Science Company Lakewood, CO, USA) NP+FRU+ACE or PR+FRU+ACE.

Sample preparation

At the end of treatment, the rats were anesthetized with sodium pentobarbital (50 mg/kg; *intraperitoneally*). Blood was collected by cardiac puncture into heparinized tube and was centrifuged at 3000 rpm for 5 min at room temperature. Plasma was stored frozen until needed for biochemical assay.

Kidney weight (renal mass)

The kidneys were excised, cleared of adhering connective tissues, blotted and weighed immediately, and corrected to body weight to eliminate variability.

Assessment of visceral fat mass

After dissection, visceral fat mass was assessed as previously described (Schiavone et al. 2017) by isolating and weighing the total peri-renal, retroperitoneal, and abdominal fat pad and corrected to body weight to eliminate variability.

Preparation of renal tissue homogenates

After dissection, renal of the tissues was excised, cleared of adhering connective tissues, and blotted. Thereafter, 100 mg of renal tissue was carefully removed and homogenized with a glass homogenizer in cold phosphate buffer solution, centrifuged at 10,000 rpm for 10 min at 4 °C.

Oral glucose tolerance test

Oral glucose tolerance test was performed a week before the rats were euthanized. After 12-h overnight fasting, a solution of 50% glucose was given (2 g/kg b.w.; p.o.). Blood was obtained from the tail vein before glucose load and then sequentially after 30, 60, 90, and 120 min. Terminal fasting blood glucose (FBG) was determined in 12-h overnight fasted rats on the day of euthanasia. Blood glucose levels were determined with a glucometer (ONETOUCH®-LifeScan, Inc., Milpitas, CA, USA). Glucose tolerance was expressed as a function of the area under the oral glucose tolerance test (OGTT) curve (AUC).

Biochemical assay

Plasma insulin *Plasma insulin* was determined using ELISA kit from Ray Biotechnology, Inc. (GA, USA). This method was based on the direct sandwich technique in which two monoclonal antibodies were directed against separate antigenic determinants on the insulin molecule.

Insulin resistance (IR) *Insulin resistance* (IR) was determined using the homeostasis model assessment for insulin resistance (HOMA-IR; (fasting insulin* fasting glucose)/22.5).

Adenosine deaminase activity, xanthine oxidase activity, adenosine, and uric acid concentration From plasma and renal tissue homogenate, adenosine deaminase (ADA) and xanthine oxidase (XO) activities together with adenosine and uric acid concentration were determined using standard enzymatic and non-enzymatic colorimetric

methods respectively employing assay kits from Randox Laboratory Ltd. (Co. Antrim, UK) and in compliance with the manufacturers' guide.

Lactate dehydrogenase activity (LDH) This was determined in the plasma and renal tissue homogenate using standardized enzymatic colorimetric methods and assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK). The LDH activity detection was based on the oxidation of NAD to NADH by LDH, which was specifically detected by colorimetric (450 nm) assay (Lu et al. 2008).

Lipid peroxidation biomarker and antioxidant-dependent parameters Malondialdehyde (MDA) is a marker of lipid peroxidation and was determined from plasma and renal tissue homogenate by standard non-enzymatic spectrophotometric method using assay kits from Oxford Biomedical Research Inc. (Oxford, USA). This method involves the reaction of MDA in the sample with thiobarbituric acid (TBA) to generate a MDA-TBA adduct (Uchiyama and Mihars 1978), which was quantified spectrophotometrically, whereas glucose-6-phosphate dehydrogenase (G6PD) activity and glutathione (GSH) were determined using standard enzymatic and non-enzymatic spectrophotometric methods respectively with assay kits obtained from Oxford Biomedical Research Inc. (Oxford, USA). The G6PD activity spectrophotometric determination is based on the evaluation of absorbance at 340 nm given by NADPH formation; the quantitative evaluation is made by adding a precise amount of enzyme solution to an assay mixture containing the substrate (glucose-6-phosphate) and its cofactor NADP; the rate of NADPH generation is spectrophotometrically measured at wavelength of 340 nm (Beutler 1984), whereas GSH spectrophotometric determination was based on the oxidation of GSH in the sample by the sulfhydryl reagent 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) to form the yellow derivative 5'-thio-2-nitrobenzoic acid (TNB), measured at 412 nm. The glutathione disulfide (GSSG) formed was recycled to GSH by glutathione reductase in the presence of NADPH (Rahman et al. 2007).

Statistical analysis

All data were expressed as means $\pm$ SEM. Statistical group analysis was performed with SPSS statistical software. Two-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 pairwise comparisons of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

Table 1 Effect of fructose and acetate on mean body weight, food intake, and water intake

	NP	NP+F	NP+F+A	PR	PR+F	PR+F+A
Body weight (g)						
Initial	123.5 ± 1.9	122.7 ± 1.7	123.3 ± 2.0	122.5 ± 3.9	121.2 ± 0.8	122.2 ± 2.1
Final	156.2 ± 1.8	164.0 ± 2.6	156.5 ± 1.4	165.5 ± 4.3	175.5 ± 1.6	170.5 ± 0.9
%Change	26.4 ± 0.4	32.9 ± 0.2 *	27.1 ± 0.7 [#]	35.1 ± 0.1	44.9 ± 0.5 ^{&}	39.6 ± 0.3 ^{\$}
Food intake (g/kg/day)						
Initial	94.4 ± 4.3	94.4 ± 4.3	93.1 ± 3.9	92.7 ± 1.6	94.9 ± 2.7	93.1 ± 3.8
Final	89.9 ± 2.5	90.9 ± 1.5	96.1 ± 3.9	93.7 ± 3.6	91.4 ± 2.4	105.3 ± 2.7
%Change	- 4.8 ± 0.1	- 3.7 ± 0.2	3.2 ± 0.1 [#]	1.1 ± 0.1	- 3.7 ± 0.3 ^{&}	13.1 ± 0.8 ^{&}
Water intake (ml/kg/day)						
Initial	177.9 ± 1.5	175.6 ± 1.3	178.6 ± 7.5	176.4 ± 6.7	179.3 ± 7.4	177.3 ± 7.4
Final	240.7 ± 1.2	275.7 ± 9.9	244.3 ± 11.9	270 ± 1.9	292.9 ± 7.8	282.9 ± 7.8
%Change	35.3 ± 5.2	57.1 ± 3.1*	36.5 ± 2.3 [#]	52.9 ± 5.9	63.5 ± 7.7	59.6 ± 4.1

Data were presented as mean ± SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. * $P < 0.05$ vs. NP; [#] $P < 0.05$ vs. NP+F; [&] $P < 0.05$ vs. PR; ^{\$} $P < 0.05$ vs. PR+F. Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test

NP, non-pregnant; F, fructose; A, sodium acetate; PR, pregnant.

Results

Acetate attenuated mean body weight, food and water intake in pregnant and non-pregnant rats given a high-fructose drink

Fructose administration led to a significant increase in body weight and water intake in pregnant and non-pregnant animals, while a significant increase in food intake was observed in fructose-fed pregnant rats only. The percentage change in body weight and water intake of fructose-treated pregnant rats is higher compared to non-pregnant rats. Acetate, on the other hand, attenuated the increase in body weight, food intake, and water intake caused by fructose in both pregnant and non-pregnant rats (Table 1).

Effect of acetate on kidney, heart, and liver weight of pregnant and non-pregnant rats given a high-fructose drink

Fructose intake and acetate treatment in both pregnant and non-pregnant rats did not have any significant change on

kidney weight, heart weight, and liver weight in both pregnant and non-pregnant rats, although a slight decrease in kidney weight, heart weight, and liver weight was observed albeit insignificant following treatment with acetate (Table 2).

Acetate improves fetal outcome of pregnant rats given a high-fructose drink

Fructose intake led to a significant decrease in fetal weight and high resorption number of the fetus; interestingly, an increase in the fetal number was observed albeit insignificant. A significant reduction in the resorption number was however observed in the acetate-treated group. Also, acetate treatment led to a significant increase in fetal weight when compared to the fructose only treated (Table 3)

Acetate improves glucoregulation in pregnant and non-pregnant rats given a high-fructose drink

Fructose increased fasting blood glucose (FBG) and HOMA-IR in both pregnant and non-pregnant animals (Fig. 1a, e) and increased insulin in pregnant animals compared with control.

Table 2 Organ weights

	NP	NP+F	NP+F+A	PR	PR+F	PR+F+A
Kidney weight (g/kg body weight)	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	0.9 ± 0.0
Heart weight (g/kg body weight)	0.5 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	0.6 ± 0.0
Liver weight (g/kg body weight)	5.6 ± 0.6	5.5 ± 0.5	4.9 ± 0.6	7.0 ± 0.3	7.3 ± 0.2	7.0 ± 0.1

Table 3 Mean fetal parameters

	PR	PR+F	PR+F+A
Fetal weight (g/dam)	29.3 ± 0.6	24.8 ± 0.1 ^{&}	28.4 ± 0.6 [§]
Fetal number	8.4 ± 0.5	9.2 ± 0.6	9.6 ± 0.5
Fetal resorption (g/dam)	0.8 ± 0.2	2.8 ± 0.4 ^{&}	2.0 ± 0.3 [§]

Data were presented as mean ± SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. $P < 0.05$ vs. PR; $P < 0.05$ vs. PR+F. Data were analyzed by two-way ANOVA followed by Bonferroni's Post hoc test. F; fructose; A; sodium acetate; PR; pregnant

There was an increase in 90 min and 120 min post glucose load despite a peak in 1 h glucose in fructose and a significant increase in AUC of pregnant and non-pregnant animals following fructose administration compared with control. Fructose resulted in a double-fold increase in AUC in pregnant than non-pregnant animals. Fructose also increased visceral fat in pregnant and non-pregnant animals compared with control, and this was attenuated by acetate. The fructose-led changes in FBG, insulin, and HOMA-IR between fructose-exposed animals and respective controls were higher in pregnant animals compared with non-pregnant ones (Fig. 1a, d, e). Acetate led to a significant reduction in 1 h post glucose load, AUC, insulin, and HOMA-IR in fructose-exposed non-pregnant and pregnant rats compared with the fructose control group (Fig. 1).

Acetate augments plasma and renal glucose-6-phosphate dehydrogenase-dependent antioxidant defense in pregnant and non-pregnant rats given a high-fructose drink

Plasma but not renal glucose-6-phosphate dehydrogenase (G6PDH) was reduced significantly compared with control in both fructose-exposed pregnant and non-pregnant animals. Plasma- and renal-reduced glutathione-oxidized glutathione ratio (GSH/GSSG) was reduced significantly compared with control in both fructose-exposed pregnant and non-pregnant animals (Fig. 2c, d). However, acetate significantly increased plasma and renal GSH/GSSG and plasma G6PD in pregnant and non-pregnant exposed animals when compared with the control (Fig. 2).

Acetate augments plasma and renal adenosine and ameliorates adenosine deaminase in pregnant and non-pregnant rats given a high-fructose drink

Plasma and renal adenosine were reduced significantly in fructose-exposed pregnant and non-pregnant animals compared with control (Fig. 3a, b). Plasma adenosine deaminase (ADA) increased significantly in fructose-exposed pregnant animals compared with control (Fig. 3c), followed by a significant increase in renal adenosine deaminase (ADA) in

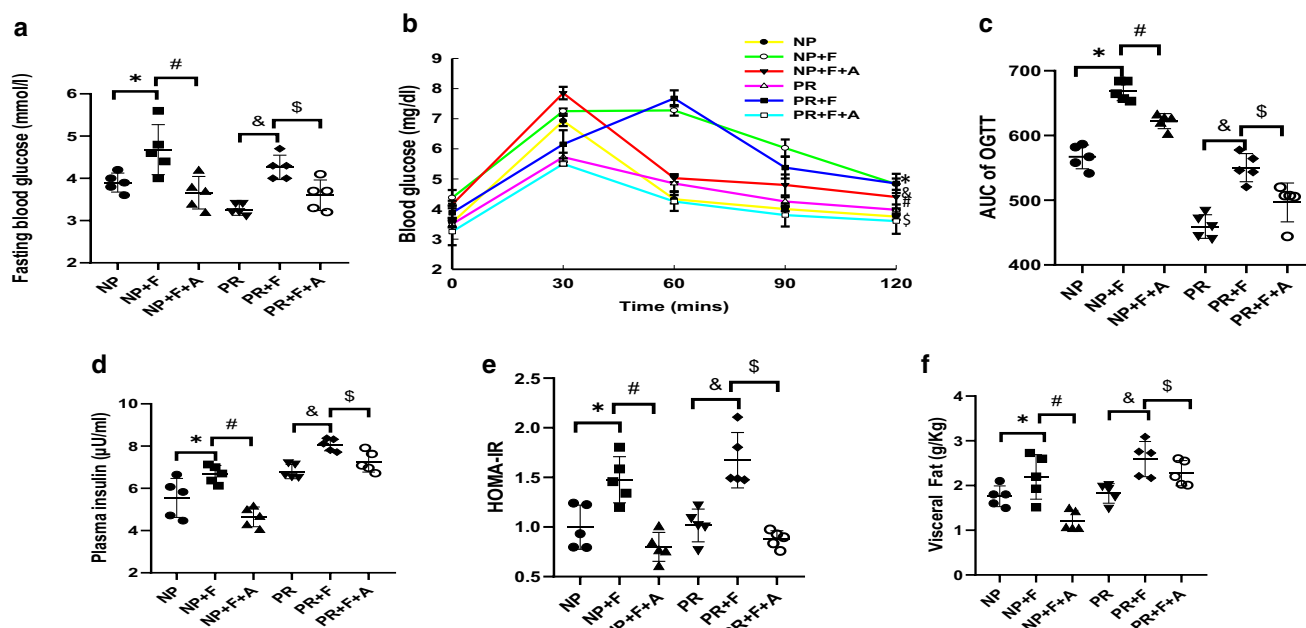


Fig. 1 Effect of acetate on glucoregulation in pregnant and non-pregnant Wistar rats exposed to oral fructose drink. Acetate reduced plasma fasting blood glucose (a), OGTT (b), AUC (c), insulin (d), HOMA-IR (e): Homeostatic model assessment of insulin resistance, and visceral fat (f) in fructose-exposed Wistar pregnant and non-pregnant rats. Fructose exposure in drinking water and oral acetate treatment was done for 3 weeks

gestation period of pregnant animals. Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test. Values are expressed as mean ± SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. * $P < 0.05$ vs. NP; # $P < 0.05$ vs. NP+F; & $P < 0.05$ vs. PR; § $P < 0.05$ vs. PR+F. NP, non-pregnant; F, fructose; A, sodium acetate; PR, pregnant

fructose-exposed pregnant and non-pregnant animals compared with control (Fig. 3c, d). Pregnancy caused an increase in plasma and renal adenosine deaminase in animals that were exposed to fructose. Acetate treatment to fructose-exposed animals attenuated plasma and renal adenosine in pregnant and non-pregnant rats compared to fructose treated only. Acetate treatment to fructose-exposed animals attenuated plasma and renal adenosine deaminase compared with the fructose treated only group. Pregnant animals without fructose exposure had increased renal ADA compared with non-pregnant animals without fructose (Fig. 3).

Acetate ameliorates plasma and renal xanthine oxidase and uric acid in pregnant and non-pregnant rats given a high-fructose drink

Fructose exposure in non-pregnant animals did not alter plasma and renal xanthine oxidase (XO) but increased plasma and renal uric acid (UA) compared with control (Fig. 4). Fructose exposure in pregnant animals increased plasma and renal XO and UA, respectively, compared with control. A double-fold increase in xanthine oxidase was observed in the plasma and renal

pregnant fructose-treated animals. We therefore deduce that high plasma and renal XO and ADA (Figs. 4 and 3c, d) led to measurable elevated UA levels and thereby contribute to redox unbalancing. Acetate treatment to fructose-exposed pregnant animals reduced plasma XO, UA, and renal XO in both pregnant and non-pregnant animals.

Acetate ameliorates plasma and renal lactate dehydrogenase and malondialdehyde in pregnant and non-pregnant rats given a high-fructose drink

Fructose increased plasma and renal lactate dehydrogenase (LDH) in both pregnant and non-pregnant rats compared with controls (Fig. 5a, b). Also, plasma and renal malonaldehyde (MDA) were increased in pregnant and non-pregnant animals compared with control (Fig. 5c, d). However, a double-fold increase was observed in the renal lactate dehydrogenase of pregnant animals treated with fructose. Acetate treatment to fructose-exposed pregnant and non-pregnant animals attenuated plasma and renal LDH and MDA compared with fructose treated only. A double- to triple-fold decrease in plasma and

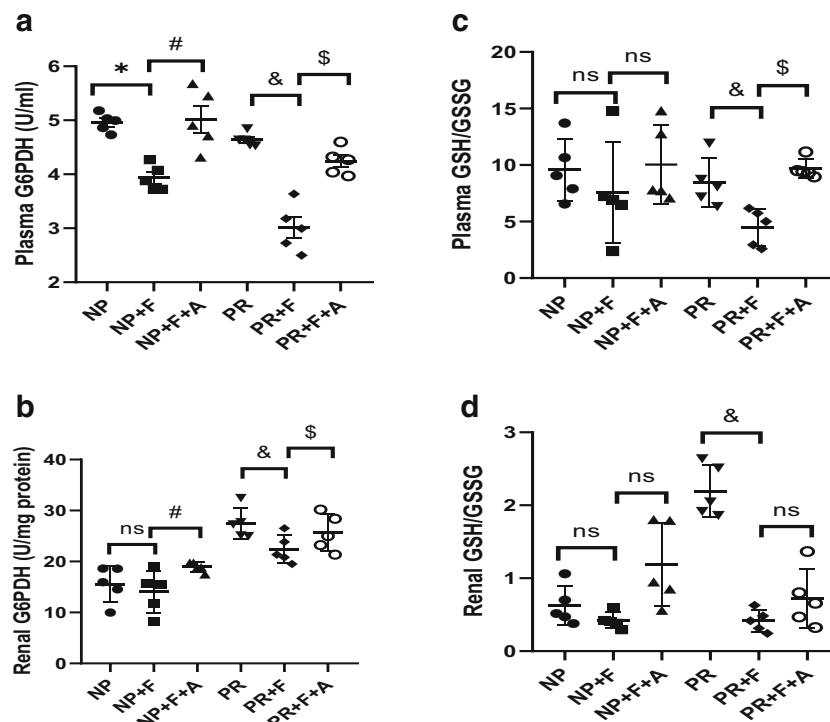


Fig. 2 Effect of acetate on systemic and renal redox status in pregnant and non-pregnant Wistar rats exposed to oral fructose drink. Acetate increased plasma G6PD: glucose-6-phosphate dehydrogenase (a), renal G6PD (b), plasma GSH/GSSG: reduced glutathione/oxidized glutathione ratio (c), and renal GSH/GSSG (d) in fructose-exposed Wistar pregnant and non-pregnant rats. Fructose exposure in drinking water and oral

acetate treatment was done for 3 weeks gestation period of pregnant animals. Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test. Values are expressed as mean ± SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. * $P < 0.05$ vs. NP; # $P < 0.05$ vs. NP+F; & $P < 0.05$ vs. PR; \$ $P < 0.05$ vs. PR+F. NP, non-pregnant; F, fructose; A, sodium acetate; PR, pregnant

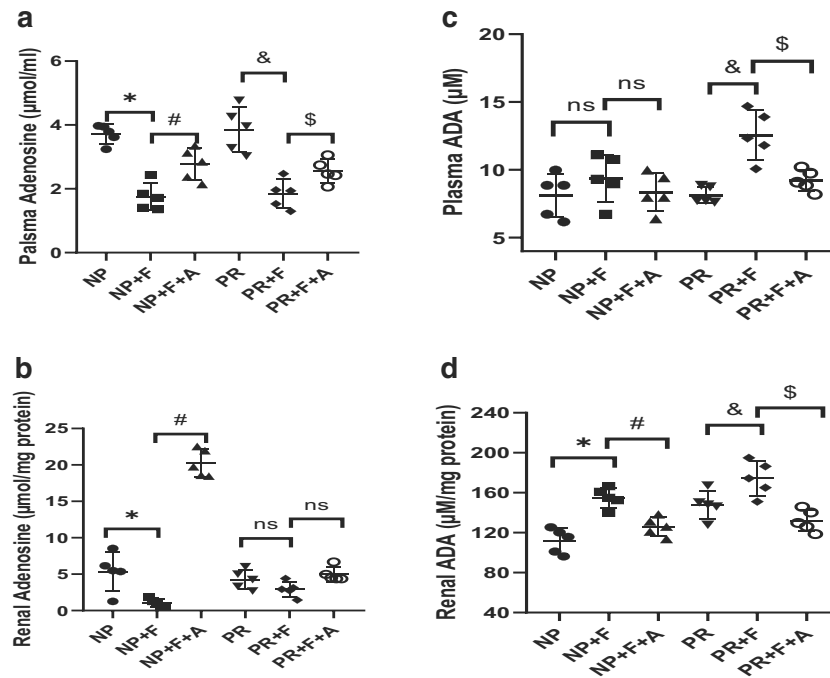


Fig. 3 Effect of acetate on systemic and renal adenosine and adenosine deaminase in pregnant and non-pregnant Wistar rats exposed to oral fructose drink. Acetate increased plasma adenosine (a), renal adenosine (b), reduced plasma ADA: adenosine deaminase (c), and renal ADA (d) in fructose-exposed Wistar pregnant and non-pregnant rats. Fructose exposure in drinking water and oral acetate treatment was done for 3 weeks

gestation period of pregnant animals. Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test. Values are expressed as mean $\pm$ SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. * $P < 0.05$ vs. NP; # $P < 0.05$ vs. NP+F; & $P < 0.05$ vs. PR; \$ $P < 0.05$ vs. PR+F. NP, non-pregnant; F, fructose; A, sodium acetate; PR, pregnant

renal MDA was observed following treatment with acetate in the non-pregnant group.

Discussion

The impaired insulin action indicated in this study following chronic fructose administration to pregnant and non-pregnant rats is accompanied by fructose-induced systemic and renal elevated ADA/XO/UA pathway activity (UA production), increased lipid peroxidation, and depressed glutathione antioxidant index (GSH/GSSG), which describe an oxidative stress status. Interestingly, pregnancy significantly affected renal G6PD-dependent antioxidant index without fructose exposure independent of ADA/XO/UA pathway. However, acetate, a product of gut microbiota degradation of dietary fibers, attenuated the fructose-induced oxidative stress and restored insulin action more significantly in non-pregnant than pregnant rats.

That fructose engenders oxidative stress through stimulation of UA production by causing increased purine breakdown is well known (Caliceti et al. 2017). Hence, the oxidative stress status realized in fructose-fed pregnant and non-pregnant rats in this study is not surprising: fructose led to increased ADA/XO/UA pathway activity by producing increased plasma levels of

elevated lipid peroxidation (MDA), LDH, and depressed GSH/GSSG and anti-inflammatory adenosine. XO has been described as a prooxidant ROS inducer (Zorov et al. 2014). It uses molecular oxygen as an electron acceptor while producing ROS and it is the basis for taking UA as a prooxidant molecule (Madlala et al. 2016). Adenosine deaminase also cleaves adenosine to inosine, a precursor for UA synthesis (Lee et al. 2019). Reduction in adenosine level helps withdraw its inhibitory effect on cellular inflammatory processes and this synergizes with XO-dependent ROS generation to induce deleterious events. Interestingly and for the first time, it is shown here that pregnancy led to an aggravated (Fig. 1) insulin resistance and response of the ADA/XO/UA pathway especially XO to chronic fructose intake. Pregnancy caused aggravated fructose-induced renal and systemic XO activity. This shows that pregnancy might aggravate fructose-induced XO-mediated oxidative stress response leading to plausible organ damage. Without fructose exposure, pregnancy seems to confer renoprotection by elevated renal G6PD-dependent antioxidant index (Fig. 2b, d) and reduced lipid peroxidation (Fig. 5d). Conversely, this effect can be viewed as compensatory and an indication of gestational renal vulnerability attributed to the increasing excretory burden imposed by the developing fetus, hyperinsulinemia (consequently leading to elevated renal glucose influx to furnish the pentose phosphate pathway that increases G6PD activity, which liberates NADPH for glutathione

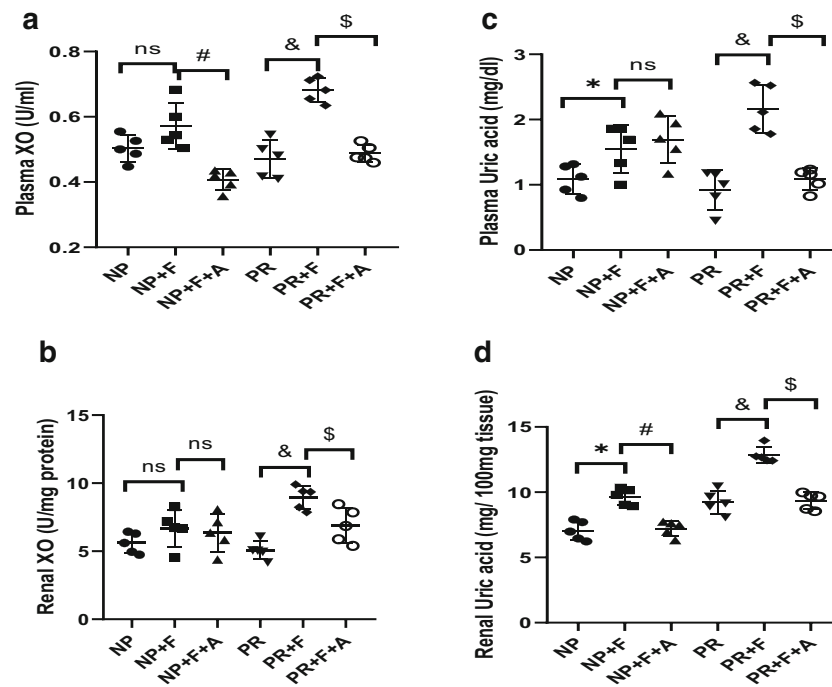


Fig. 4 Effect of acetate on systemic and renal xanthine oxidase and uric acid in pregnant and non-pregnant Wistar rats exposed to oral fructose drink. Acetate reduced plasma XO: xanthine oxidase (**a**), renal XO (**b**), plasma UA: uric acid (**c**), and renal UA (**d**) in fructose-exposed Wistar pregnant and non-pregnant rats. Fructose exposure in drinking water and oral acetate treatment was done for 3 weeks gestation period of pregnant

animals. Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test. Values are expressed as mean $\pm$ SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. * $P < 0.05$ vs. NP; # $P < 0.05$ vs. NP+F; & $P < 0.05$ vs. PR; \$ $P < 0.05$ vs. PR+F. NP, non-pregnant; F, fructose; A, sodium acetate; PR, pregnant

antioxidant system), and increased intrarenal XO activity and UA production (Fig. 4b, d). However, when there is unrestricted gestational exposure to fructose, an additional effect on XO activity and UA production may break the antioxidant barrier provided for the kidney during pregnancy. This can be because of pregnancy-led aggravated renal XO activity during fructose exposure producing increased ROS that plausibly overwhelms the renal antioxidant compensatory events during pregnancy leading to an oxidative stress status for the vulnerable organ.

In line with an established renal oxidative stress status in chronic fructose-exposed pregnant rats, lipid peroxidation, a known generator of hydrogen peroxide (H_2O_2) (Ayala et al. 2014), was significantly increased in pregnant rats exposed to fructose. MDA, the marker of lipid peroxidation, was reduced by pregnancy in the kidney without fructose exposure, showing that pregnancy loses its renoprotection against oxidative damage during fructose exposure. Oxidative stress and insulin resistance are key players in the mechanism involved in metabolic syndrome, and chronic fructose intake is a risk factor for this rather endemic disease and its co-morbid pathologies (Dharmalingam and Yamasandhi 2018). The elevated HOMA-IR in the present study indicated insulin resistance in both pregnant and non-pregnant rats exposed to chronic fructose drink. Also, visceral obesity plays an important role in the development of insulin

resistance. Increased visceral fat store and adipokine production are potent mediators of free radical release worsening oxidative. This study revealed that chronic fructose drink increased visceral adiposity more in pregnant than non-pregnant rats. Therefore, the additive impact of fructose and pregnancy on glucose regulation in this study is insightful: it is plausible to reckon that a high-fructose diet during pregnancy may engender deleterious metabolic conditions (insulin resistance and visceral fat dysmetabolism) leading to deteriorating effect on the organ (kidney) health of the mother and most assuredly the growing fetus during and after gestation. Aside from establishing that fructose-induced oxidative stress and glucose dysregulation are worsened by pregnancy, the therapeutic role of acetate a gut microbiota product of dietary fiber fermentation was investigated in this study. Previous studies have shown that there is an association between gut microflora and metabolic dysfunctions like type 2 diabetes mellitus (Adachi et al. 2019). Acetate has also been studied for its therapeutic/preventive role in the development of cardiometabolic diseases (Warmbrunn et al. 2020). Acetate treatment from our study attenuated oxidative stress in fructose-exposed pregnant and non-pregnant rats. It is noteworthy that the overall effect of acetate is comparable in pregnant and non-pregnant animals but the ameliorating influence of acetate on individual renal and systemic glucoregulatory and redox

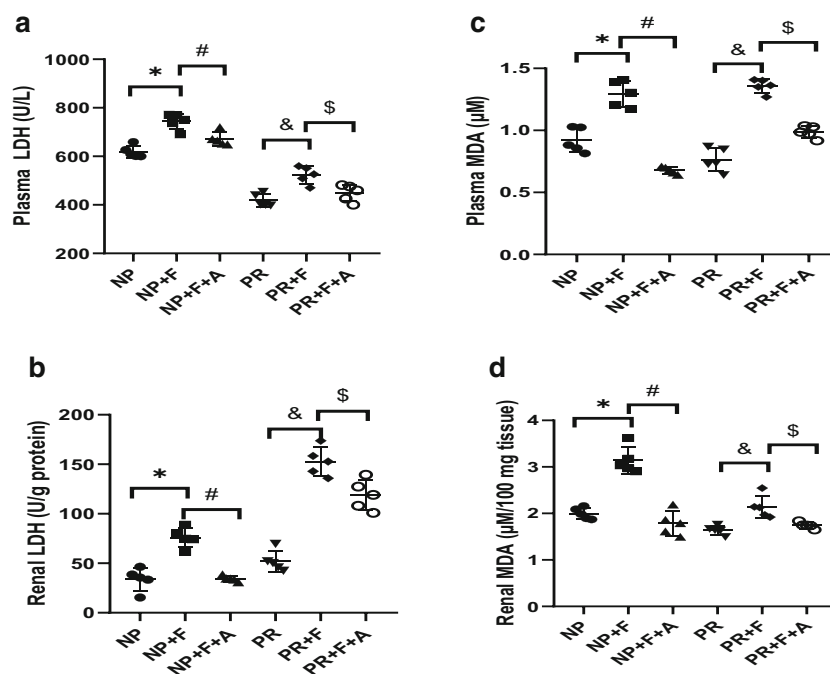


Fig. 5 Effect of acetate on systemic and renal lactate dehydrogenase and malondialdehyde in pregnant and non-pregnant Wistar rats exposed to oral fructose drink. Acetate reduced plasma LDH: lactate dehydrogenase (**a**), renal LDH (**b**), plasma MDA: malondialdehyde (**c**), and renal MDA (**d**) in fructose-exposed Wistar pregnant and non-pregnant rats. Fructose exposure in drinking water and oral acetate treatment was done for 3

weeks gestation period of pregnant animals. Data were analyzed by two-way ANOVA followed by Bonferroni's post hoc test. Values are expressed as mean $\pm$ SEM of 5 rats per group and $P < 0.05$ was taken as statistically significant. * $P < 0.05$ vs. NP; # $P < 0.05$ vs. NP+F; & $P < 0.05$ vs. PR; § $P < 0.05$ vs. PR+F. NP, non-pregnant; F, fructose; A, sodium acetate; PR, pregnant

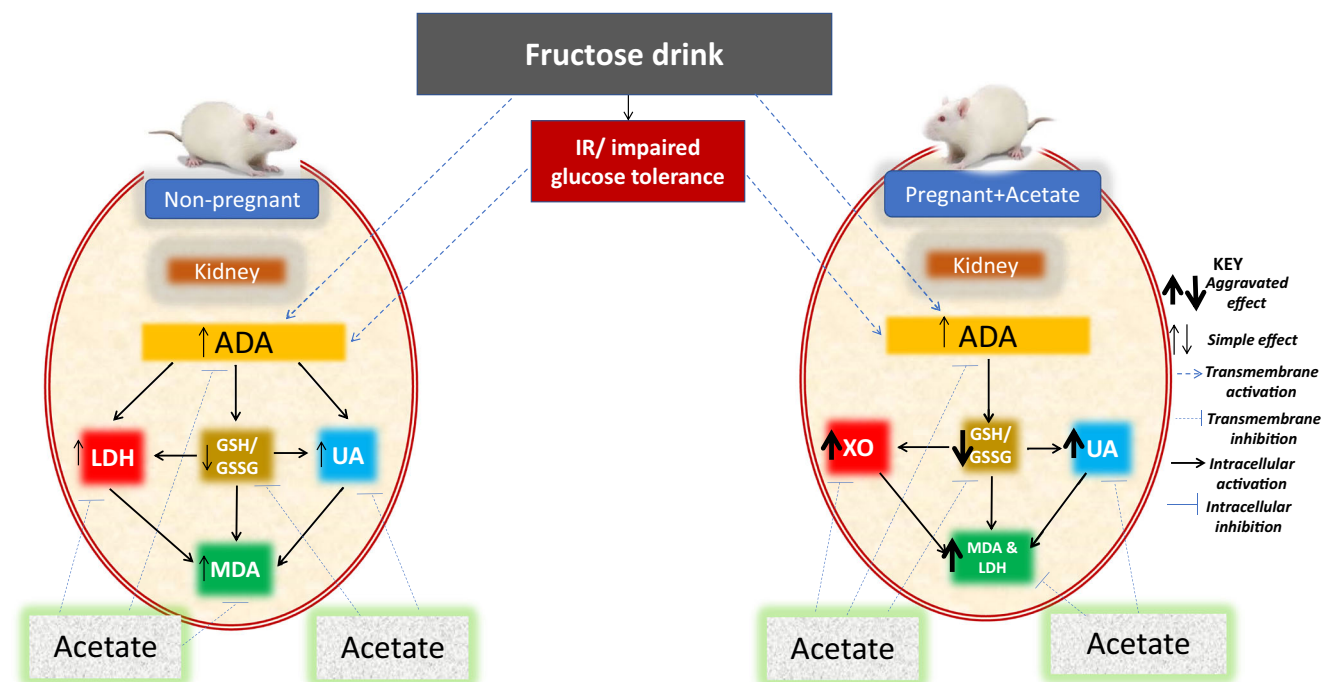


Fig. 6 Schematic diagram illustrating the likely pathway through which sodium acetate protects against renal tissue from damage in high-fructose pregnant and non-pregnant rats

parameters in animals exposed to fructose were more pronounced in non-pregnant groups than the pregnant one. This showed that pregnancy may modulate physiologic responses of the body to pharmacologic or dietary molecules. The restoration of insulin action in pregnant and non-pregnant rats by acetate is supported by evidence in literature that acetate or acetate yielding resistant sugars enhances insulin-mediated pathway of phosphoinositol kinase-3/protein kinase B pathway leading to increased Glut 4 translocation and glucose uptake (Zhao et al. 2019). Also, acetate through histone deacetylase inhibition might also promote expression of insulin-sensitive target genes like the pyruvate dehydrogenase complex leading to increased glucose utility and uptake (Ye 2013). Nevertheless, acetate also ameliorated elevated systemic and renal ADA/XO/UA pathway in fructose-exposed pregnant and non-pregnant rats. Therefore, acetate may play a preventive role in glucoregulatory dysfunctions by improving insulin action and suppressing oxidant-producing processes like the ADA/XO/UA pathway and lipid peroxidation, among others (Fig. 6).

Conclusion

The result of the study shows that high fructose intake in pregnant and non-pregnant rats impaired glucose metabolism and renal redox balance leading to high levels of renal lipid peroxidation. Also, despite conferring renoprotection via compensatory elevated G6PD-dependent antioxidant index without fructose exposure, pregnancy had a potentiating effect on fructose-induced deleterious impacts on the kidney through a XO-dependent pathway. However, acetate ameliorated fructose-induced impaired glucose regulation and renal oxidative stress status in fructose-exposed pregnant and non-pregnant rats with greater effect in the latter. These “acetogenic” effects are accompanied by improved G6PD-dependent antioxidant capacity and reduced lipid peroxidation. Therefore, normal pregnancy may elicit renoprotective antioxidant capacity but worsens fructose-induced renal deleterious effects. Hence, indiscriminate fructose intake in foods or drinks can be contraindicated in pregnancy due to renal vulnerability among other ill-effects. Nevertheless, in circumstances where a fructose diet is unavoidable, acetate-yielding diet or supplements can plausibly prevent renal damage.

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Author contribution AOO, OSM, and LAO conceived and designed the research. AOO, EDA, and SBS conducted the experiments. AOO, OSM,

EDA, SBS, and LAO contributed to the new reagents and analytical kits. AOO, OSM, EDA, and LAO analyzed and interpreted the data. AOO, OSM, EDA, SBS, and LAO drafted the manuscript. AOO, OSM, EDA, SBS, and LAO. All authors read and approved the manuscript, and all data were generated in-house and that no paper mill was used.

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Data availability Data for the research work were made available at submission.

Declarations

Ethics approval and consent to participate Ethical approval for the work was obtained from the University of Ilorin Ethical Review Committee with protocol number UERC/ASN/2016/357, having passed the Faculty of Basic Medical Sciences, University of Ilorin Ethical Review Committee. The investigation was conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of the University of Ilorin, with protocol identification number UERC/ASN/2016/357, and every effort was made to minimize both the number of animals used and their sufferings.

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Adewumi Oluwafemi Oyabambi, oyabambi.ao@unilorin.edu.ng
Olugbenga Samuel Micheal, michaelolugbenga2@gmail.com
Emmanuel Damilare Areola, areola577@gmail.com
Salam Babatunde Saliu, salambat50@gmail.com
Lawrence Aderemi Olatunji, tunjilaw@unilorin.edu.ng

Consent for publication The following underlisted authors agreed that the manuscript titled “Sodium acetate ameliorated systemic and renal oxidative stress in high-fructose insulin-resistant pregnant Wistar rats.” be published within your Journal, *Naunyn-Schmiedeberg's Archives of Pharmacology*.

Adewumi Oluwafemi Oyabambi, oyabambi.ao@unilorin.edu.ng
Olugbenga Samuel Micheal, michaelolugbenga2@gmail.com
Emmanuel Damilare Areola, areola577@gmail.com
Salam Babatunde Saliu, salambat50@gmail.com
Lawrence Aderemi Olatunji, tunjilaw@unilorin.edu.ng

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References

- Adachi K, Sugiyama T, Yamaguchi Y, Tamura Y, Izawa S, Hijikata Y, Ebi M, Funaki Y, Ogasawara N, Goto C et al (2019) Gut microbiota disorders cause type 2 diabetes mellitus and homeostatic disturbances in gut-related metabolism in Japanese subjects. *J Clin Biochem Nutr* 64:231–238
- Ayala A, Munoz MF, Arguelles S (2014) Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Med Cell Longev* 2014:360438
- Beutler E (1984) *Red Cell Metabolism: A Manual of Biochemical Methods*, 3rd edn. Grune & Stratton, New York

- Bray GA, Nielsen SJ, Popkin BM (2004) Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *Am J Nutr* 79:537–543
- Caliceti C, Calabria D, Roda A, Cicero AF (2017) Fructose Intake, Serum Uric Acid, and cardiometabolic Disorders: A Critical Review. *Nutrients* 9:395. <https://doi.org/10.3390/nu9040395> [Google Scholar]
- Cerf ME (2013) Beta cell dysfunction and insulin resistance. *Front Endocrinol* 4:37 1–12
- Choi Y, Abdelmegeed M, Song BJ (2017) Diet high in fructose promotes liver steatosis and hepatocyte apoptosis in C57BL/6J female mice: role of disturbed lipid homeostasis and increased oxidative stress. *Food Chem Toxicol* 103:111–121
- Dharmalingam M, Yamasandhi PG (2018) Nonalcoholic fatty liver disease and type 2 diabetes mellitus. *Indian J Endocrinol Metabolism* 22:421–428
- Fabiane R, Leidiane D, Walter SN, Thissiane D (2018) Influence of gestational diabetes on the activity of δ -aminolevulinic acid dehydratase and oxidative stress biomarkers. *Redox Rep* 23(1):63–67. <https://doi.org/10.1080/13510002.2017.1402981>
- Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, Anastasovska J, Ghourab S, Hankir M, Zhang S, Carling D, Swann JR, Gibson G, Viardot A, Morrison D, Louise Thomas E, Bell JD (2014) The shortchain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* 5:3611
- Jadhav AA, Jain A (2013) Adenosine deaminase activity in normal pregnancy and pregnancy associated disorders. *Arch Physiol Biochem* 119:88–91
- Kelley EE, Khoo NK, Hundley NJ, Malik UZ, Freeman BA, Tarpey MM (2010) Hydrogen peroxide is the major oxidant product of xanthine oxidase. *Free Radic Biol Med* 48(4):493–498
- Lee S, Park H, Ki Y, Lee H, Han MS (2019) Development of a Simple Assay Method for Adenosine Deaminase via Enzymatic Formation of an Inosine-Tb³⁺ Complex. *Sensors (Basel)* 19(12):E2728. <https://doi.org/10.3390/s19122728>
- Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev* 4(8):118–126. <https://doi.org/10.4103/0973-7847.70902>
- Lu F, Chauhan AK, Fernandes SM, Walsh MT, Wagner DD, Davis AE III (2008) The effect of C1 inhibitor on intestinal ischemia and reperfusion injury. *Am J Physiol Gastrointest Liver Physiol* 295(5):G1042–G1049
- Madlala HP, Maarman GJ, Ojuka E (2016) Uric acid and transforming growth factor in fructose-induced production of reactive oxygen species in skeletal muscle. *Nutr Rev* 74:259–266
- Mandal AK, Mount DB (2015) The molecular physiology of uric acid homeostasis. *Annu Rev Physiol* 77:323–345
- Maslowki KM, Vieira AT, Ng A, Kranich J, Sierro F, Yu D et al (2009) Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature*. 461:1282–1286
- Meghri K, Maria F, Penelope DH (2019) Approaches and Methods to Measure Oxidative Stress in Clinical Samples: Research Applications in the Cancer Field. *Oxidative Med Cell Longev* 1279250:29. <https://doi.org/10.1155/2019/1279250>
- Moriyama M, Kurebayashi R, Kawabe K, Takano K, Nakamura Y (2016) Acetate attenuates lipopolysaccharide-induced nitric oxide production through an anti-oxidative mechanism in cultured primary rat astrocytes. *Neurochem Res* 41:3138–3146
- Mortensen PB, Clausen MR (1996) Short-chain fatty acids in the human colon: relation to gastrointestinal health and disease. *Scand J Gastroenterol Suppl* 216:132–148
- Napso T, Yong HE, Lopez-Tello J, Sferuzzi-Perri AN (2018) The Role of Placental Hormones in Mediating Maternal Adaptations to Support Pregnancy and Lactation. *Front Physiol* 9:1091
- Rahman I, Kode A, Biswas SK (2007) Assay for quantitative determination of glutathione and glutathione disulfide levels using enzymatic recycling method. *Nat Protoc* 1(6):3159–3165
- Réus GZ, Carlessi AS, Silva RH, Ceretta LB, Quevedo J (2019) Relationship of oxidative stress as a link between diabetes mellitus and major depressive disorder. *Oxidative Med Cell Longev* 2019:1–6. <https://doi.org/10.1155/2019/8637970>
- Schiavone S, Camerino GM, Mhillaj E, Zotti M, Colaianna M, De Giorgi A, Trotta A, Cantatore FP, Conte E, Bove M et al (2017) Visceral fat dysfunctions in the rat social isolation model of psychosis. *Front Pharmacol* 8:87
- Uchiyama M, Mihars M (1978) Determination of malondialdehyde precursor in tissues by thiobarbituric acid. *Ann Biochem* 86:271–278
- Volodymyr IL (2012) Glutathione homeostasis and functions: potential targets for medical interventions. *J Amino Acids* 736837:26. <https://doi.org/10.1155/2012/736837>
- Vos MB, Kimmons JE, Gillespie C, Welsh J, Blanck HM (2008) Dietary fructose consumption among US children and adults: The Third National Health and Nutrition Examination Survey. *Medscape J Med* 10:160
- Warmbrunn MV, Herrema H, Aron-Wisnewsky J, Soeters MR, Van Raalte DH, Nieuwdorp M (2020) Gut microbiota: a promising target against cardiometabolic diseases. *Expert Rev Endocrinol Metab* 15(1):13–27. <https://doi.org/10.1080/17446651.2020.1720511>
- Wong JM, de Souza R, Kendall WC, Emam A, Jenkins DJ (2006) Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 40(3):235–243. <https://doi.org/10.1097/00004836-200603000-00015>
- Xu Y, Wang Y, Yan S, Yang Q, Zhou Y, Zeng X et al (2017) Regulation of endothelial intracellular adenosine via adenosine kinase epigenetically modulates vascular inflammation. *Nat Commun* 16:8–943
- Ye J (2013) Improving insulin sensitivity with HDAC inhibitor. *Diabetes* 62:685–687
- Zargaraan A, Kamaliroosta L, Yaghoubi AS, Mirmoghataie L (2016) Effect of substitution of sugar by high fructose corn syrup on the physicochemical properties of bakery and dairy products: a review. *Nutr Food Sci Res* 3(4):3–11
- Zhang Y, Handy DE, Loscalzo J (2005) Adenosine-dependent induction of glutathione peroxidase 1 in human primary endothelial cells and protection against oxidative stress. *Circ Res* 96:83
- Zhao F, Liu Q, Cao J, Xu Y, Pei Z, Fan H, Yuan Y, Shen X, Li C (2019) A sea cucumber (*Holothurialeucospilota*) polysaccharide improves the gut microbiome to alleviate the symptoms of type 2 diabetes mellitus in Goto-Kakizaki rats. *Food Chem Toxicol* 135:110886. <https://doi.org/10.1016/j.fct.2019.110886>
- Zorov DB, Juhaszova M, Sollott SJ (2014) Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. *Physiological Reviews*. 94(3):909–950