



Sodium butyrate attenuates aortic lipotoxicity and impaired nitric oxide synthesis in female Wistar rats exposed to high-fructose drink

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Abstract

High fructose (HFr) intake results in dyslipidemia with consequent insulin resistance through metabolic derangements. Inadequate mobilization of excess lipid into lipid droplets causes lipid accumulation with subsequent lipotoxicity in non-adipose tissues, thus contributing to cardiovascular disease. Short-chain fatty acid butyrate protects against cardiometabolic disorder (CMD). This study aimed at investigating the effect of sodium butyrate (NaB) on selected markers of endothelial function and aortic lipotoxicity in female Wistar rats. Twenty female Wistar rats with body weight 120–125 g were divided after a week of acclimatization into 4 groups of 5 rats each: control (CTR; normal chow), HFr drink: 10% fructose (w/v), NaB (200 mg/kg; *po*), and NaB + HFr. The administration was carried out for 6 weeks. The animals were euthanized with ketamine hydrochloride and blood samples were collected by cardiac puncture. Aortic tissue ring was excised and homogenized for biochemical parameters using standard assay methods. The results showed that HFr drink decreased plasma and aortic NO, endothelial nitric oxide synthase (eNOS), vascular endothelial growth factor (VEGF), and high-density lipoprotein cholesterol (HDL), while it increased plasma and aortic triglyceride (TG), free fatty acid (FFA), uric acid (UA), and C-reactive protein (CRP) compared with the control group ($p < 0.05$). Interestingly, administration of NaB attenuated these metabolic-related perturbations in rats exposed to HFr. Therefore, the results of this study suggest that NaB protects against aortic lipotoxicity and endothelial dysfunction associated with HFr drink by attenuating aortic lipid deposition and improving VEGF.

Keywords Sodium butyrate · Fructose drink · Lipotoxicity · Endothelial dysfunction · Vascular endothelial growth factor · Cardiometabolic disease

Introduction

The complex multifaceted pathogenesis of cardiovascular disease contributes to the challenges associated with several drug-based therapies. Intake of high dietary fructose has been linked to increasing cardiovascular disease (Rippe and Angelopoulos 2015; Olaniyi and Olatunji 2019a). In addition, consumption of high fructose through corn syrup and as added sugar in soft drinks is globally on the rise

despite its link to metabolic syndrome, which includes obesity, dyslipidemia, hypertension, and T2D (Stanhope et al. 2016; Putakala et al. 2017). Similarly, a number of previous studies have demonstrated that high fructose consumption causes cardiovascular pathology that contributes to increased prevalence of cardiovascular disease (CVD) (Annandale et al. 2021; Handayani et al. 2021), which has consistently remained the leading cause of morbidity and mortality worldwide (Mensah et al. 2019; Roth et al. 2020). However, the pathophysiological mechanisms remain inconclusive. C-reactive protein (CRP) is an acute phase protein produced by the liver in response to macrophages-invaded hypertrophied adipocytes which are the features of visceral obesity and insulin resistance. C-reactive protein increases cardiovascular risk through increased vascular cell adhesion molecules, uptake of lipids by macrophage, vascular inflammatory cytokines infiltration, deposition of plaques on vessel wall, and impaired nitric oxide synthesis. Vascular endothelial growth factor (VEGF) is a potent growth

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factor that is critical in the formation of new blood vessels usually in response to hypoxia. Elevated VEGF has been observed in tumor growth, diabetic complications, and inflammatory diseases (Nakagawa et al. 2007), although the part played by VEGF in these pathological conditions is yet to be fully clear. Reports have suggested that VEGF upregulates NO production and plays key role in vascular homeostasis through induction of endothelial vasorelaxation (Carie et al. 2009). Another study shows that endothelial NO acts as a factor that determines the direction of the effects of VEGF; thus, NO bioavailability contributes to regulated angiogenesis (Zhao et al. 2002). Since metabolic syndrome is characterized by a low-grade inflammatory state and oxidative stress disrupts endothelial function, the role of VEGF modulation in high fructose-mediated vascular dysfunction is therefore worth understanding.

Plausible beneficial effect of non-pharmacological nutritional supplements is currently gaining scientific attention. Research has highlighted that the diversity and composition of gut microbiota influence nutrient metabolism and can be affected by many factors including dietary intake. Sodium butyrate is a short-chain fatty acid (SCFA) produced by distal gut bacteria fermentation of mainly undigested dietary carbohydrates and fiber. SCFAs usually modulate biological responses of host's gut health by acting as a histone deacetylase (HDAC) inhibitor and signaling through several G protein-coupled receptors (GPCRs). From previous data, oral butyrate decreases adiposity, enhances glucose uptake, and protects from inflammatory and oxidative damage (Matt et al. 2018; Badejogbin et al. 2019). The current study was therefore designed to investigate whether sodium butyrate would protect against aortic lipotoxicity and endothelial dysfunction in female Wistar rats exposed to high-fructose drink (HFr). We also sought to understand the contribution of angiogenesis marker, VEGF, in metabolic disorder-related vascular dysfunction.

Materials and methods

Animals and protocol

The study was carried out in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the study protocol was approved by the University of Ilorin Ethical Review Committee. The animals were obtained and housed at the Animal Holding Unit of the University. The animals were fed ad libitum with standard rat chow. After a week of acclimatization, female Wistar rats with body weight between 120 and 125 g were randomly distributed into four group with $n = 5$ /group. Control group was treated with vehicle (distilled water, *po*), NaB group was treated with 200 mg/kg of sodium butyrate

(*po*), HFr group was exposed to 10% fructose drink (w/v), and HFr + NaB rats received a combination of NaB (*po*) and high-fructose drink (w/v) for 6 weeks.

Collection and preparation of samples

After overnight fast, the animals were euthanized with ketamine hydrochloride and blood samples were collected by cardiac puncture. Aortic tissue ring was excised and homogenized with phosphate buffer saline to assay for nitric oxide (NO), uric acid (UA), CRP, high-density lipoprotein cholesterol (HDL), triglycerides (TG), free fatty acids (FFA), VEGF, and endothelial nitric oxide synthase (eNOS).

Determination of lipid profile

Triglyceride, FFA, and HDL were determined by colorimetric assay methods using kits obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

Determination of VEGF, eNOS, and CRP

C-reactive protein, VEGF, and eNOS were determined by ELISA kits obtained from Elabscience Biotechnology Inc. (Wuhan, Hubei, P.R.C., China).

Determination of NO and uric acid

Nitric oxide was determined by measuring its stable degradation products, nitrate, and nitrite using kits from Oxford Biomedical Research Inc. (Oxford, UK). In addition, UA was determined by colorimetric assay method using kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

Statistical analysis

All values reported in the study were expressed as standard error of the mean (SEM). Statistical evaluations were done using GraphPad Prism version 5. Comparisons among the groups were made by one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. Statistical significance was considered at p value of less than 0.05 ($p < 0.05$).

Results

Effect of sodium butyrate and high-fructose drink on plasma and aortic lipid profile in female Wistar rats

Plasma and aortic TG increased in HFr group compared with control group, and administration of NaB significantly

decreased TG in HFr + NaB group when compared with HFr group (Fig. 1). In addition, plasma but not aortic tissue concentration of FFA significantly increased in HFr group compared with control group and significantly reduced following administration of NaB in HFr + NaB group when compared with HFr group. Likewise, plasma and aortic tissue concentration of HDL decreased in HFr group compared with control and this was attenuated by NaB administration in HFr + NaB group when compared with HFr group (Fig. 2).

Effect of sodium butyrate and high-fructose drink on plasma and aortic CRP and UA in female Wistar rats

Plasma and aortic UA and CRP increased in HFr group compared with control group. Plasma and Aortic CRP and UA reduced in HFr + NaB compared to HFr group (Fig. 3).

Effect of sodium butyrate and high-fructose drink on plasma and aortic eNOS and NO in female Wistar rats

High-fructose drink reduced both plasma and aortic NO and eNOS when compared with the control group. However, plasma and aortic NO and eNOS increased in HFr + NaB group compared to HFr group (Fig. 4).

Effect of sodium butyrate and high-fructose drink on VEGF in female Wistar rats

Plasma and aortic VEGF was significantly reduced in HFr group when compared with control and increased in HFr + NaB compared to HFr group (Fig. 5).

Discussion

The findings of this study indicate that sodium butyrate decreased lipid profile and increased nitric oxide and eNOS, resulting in decreased aortic lipotoxicity and averting endothelial dysfunction. High-fructose diet increased lipid profile and reduced nitric oxide and vascular endothelial growth factor (VEGF) resulting in aortic lipotoxicity and impaired NO-dependent endothelial function. Study has shown that nitric oxide which is produced in the endothelium has a positive effect in the reduction of arterial cholesterol accumulation. Data revealed that exposure of female rats to high-fructose drink led to dyslipidemia and chronic systemic inflammation that is highlighted by elevated plasma CRP and hyperuricemia. In addition, high-fructose diet caused increased plasma free fatty acid, and in high-fructose diet plus sodium butyrate-administered rats, aortic free fatty acid is reduced. It shows that the effect of high-fructose diet in promoting aortic lipid deposition was ameliorated by sodium butyrate. This further implies that sodium butyrate protects against aortic lipotoxicity.

Studies have established that consumption of calorie-dense foods induces metabolic inflammation which can result in perturbed systemic and tissue environments, including major blood vessels. As derived from our study, high fructose led to elevated circulating FFA, CRP, and uric acid level in Wistar rats. Endothelial dysfunction is seen to be the peak of obesity and diabetes-related vascular dysfunction. Reduction in nitric oxide (NO) bioavailability is the central aspect of endothelial dysfunction. Hyperglycemia, oxidative stress, activation of renin angiotensin system as in the case of diet-induced obesity, and type 2 diabetes mellitus contribute to decrease in nitric oxide (Adam et al. 2012). Free fatty

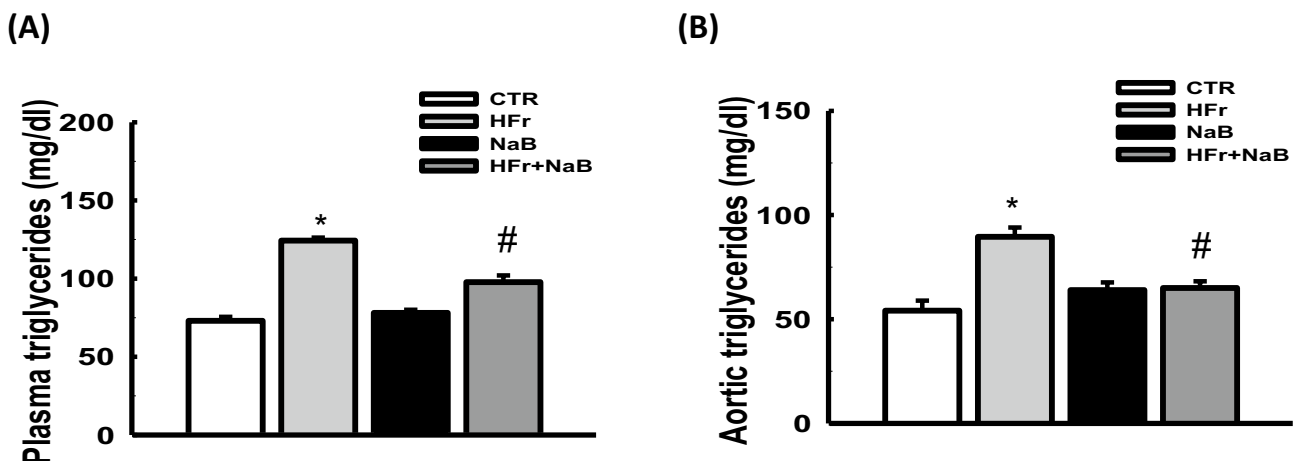


Fig. 1 Effect of sodium butyrate and high fructose on plasma and aortic triglyceride (A, B) in female Wistar rats. Data are expressed as mean $\pm$ S.E.M. $n=5$ rats per group; * $p < 0.05$ vs control; # $p < 0.05$ vs HFr

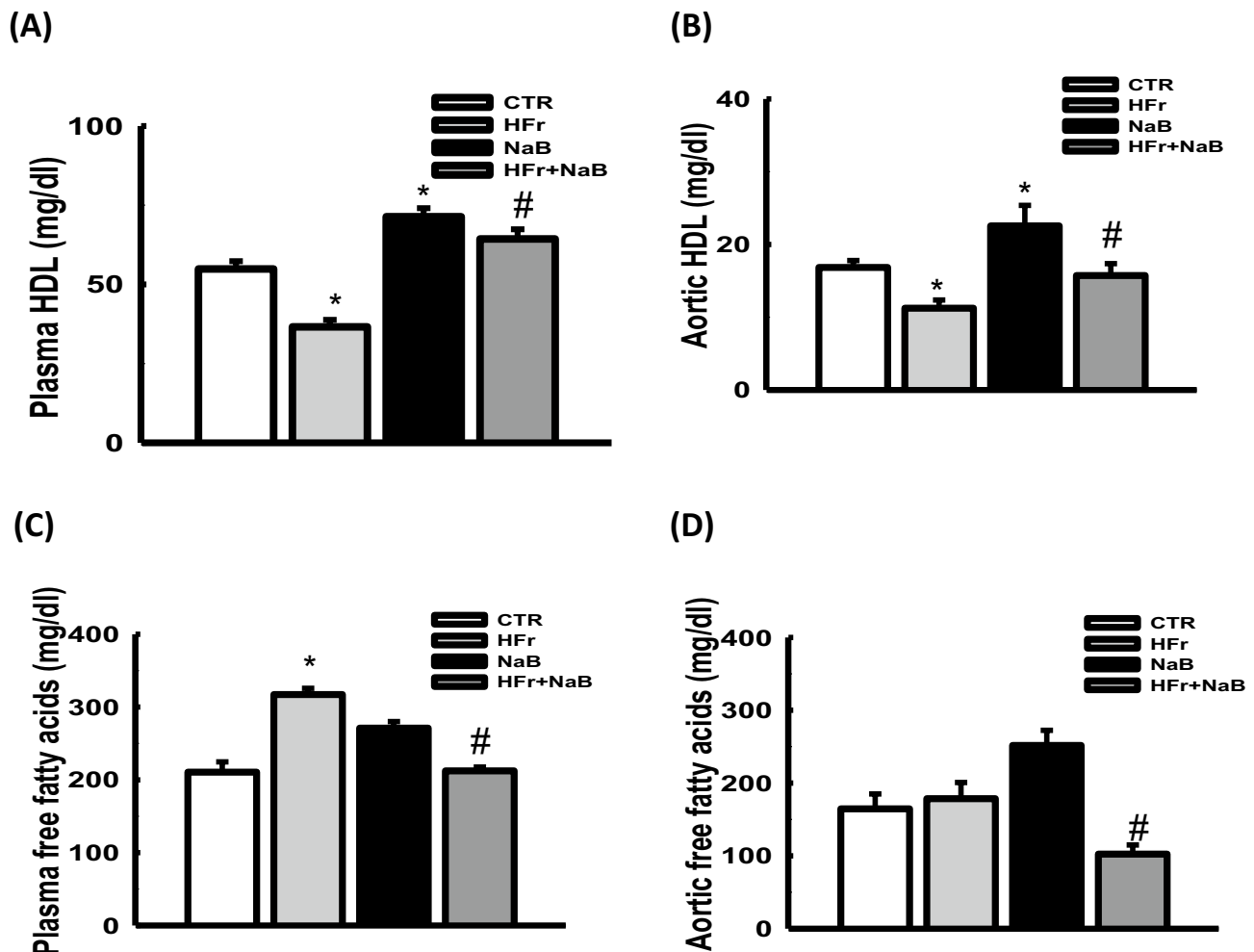


Fig. 2 Effect of sodium butyrate and high fructose on plasma and aortic HDL (A, B) and FFA (C, D) in female Wistar rats. Data are expressed as mean $\pm$ S.E.M. $n = 5$ rats per group; * $p < 0.05$ vs control; # $p < 0.05$ vs HFr). High-density lipoprotein cholesterol (HDL), free fatty acid (FFA)

acids in excess promotes de novo synthesis of diacylglycerol leading to activation of classic and novel protein kinase (C) isoforms (PKC). Activation of PKC inhibits insulin and vascular endothelial growth factor. Consistent with other studies, our data shows that high fructose content in drink elicits endothelial dysfunction (decreased level of VEGF/NO and eNOS) which are risk factors for CVD. Furthermore, our recent studies showed increased body weight in experimental animals exposed to high-fructose drink and this increase was accompanied with adipose hypertrophy in which the capacity of the adipose tissue to store accumulating fat was overwhelmed (Olaniyi and Olatunji 2019a, b), leading to release of FFA into circulation and consequent dyslipidemia as validated with the present results. Fatty acids modulate the expression of inflammatory biomarkers as observed in elevated plasma CRP in this study, which can further impair insulin signaling pathway.

Lipotoxicity has the potential to decrease endothelial NO synthase (eNOS) gene expression and eNOS catalytic activity to ultimately result in endothelial cell dysfunction via diverse mechanisms, which includes insulin resistance in the vasculature, inflammation, oxidative stress, or the accumulation of lipotoxic metabolites such as ceramide. Rats provided with butyrate in the diet exhibited significantly improved energy metabolism and decreased fat infiltration and aortic lipotoxicity caused by the high-fructose drink. Similar to our observation, previous studies have shown that butyrate can affect the expression of nine genes in the cholesterol biosynthesis pathway, which affects human metabolism by acting in concert with each other (Alvaro et al. 2008). Concomitantly, butyrate also regulates the release of nitric oxide, IL-6, IL-12, and other inflammatory mediators by inhibiting HDACs (Chang et al. 2014). Additionally, according to different functional states, macrophages can be divided

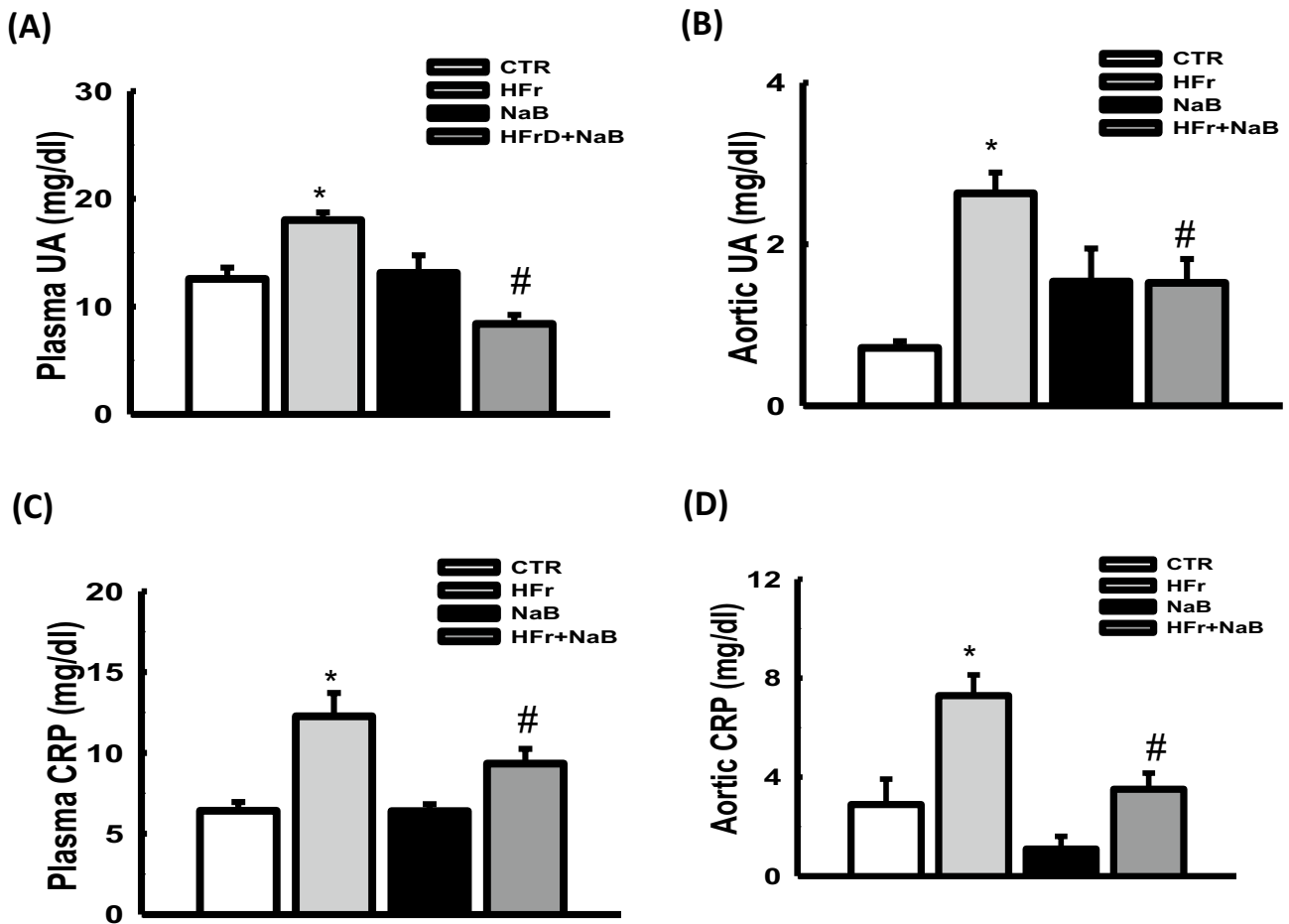


Fig. 3 Effect of sodium butyrate and high fructose on plasma and aortic UA (**A, B**) and CRP (**C, D**) in female Wistar rats. Data are expressed as mean $\pm$ S.E.M. $n = 5$ rats per group; * $p < 0.05$ vs control; # $p < 0.05$ vs HFr. Uric acid (UA), C-reactive protein (CRP)

into pro-inflammatory M1-type macrophages and anti-inflammatory M2-type macrophages. Butyrate can inhibit M1-type macrophages and increase the M2-type macrophage polarization, thereby playing a role in improving inflammation (Wang et al. 2020) as observed in the current study with significant reduction of CRP in HFr + NaB group compared with HFr group alone.

VEGF primarily exerts its effect through the production of vasodilatory mediators. VEGF signaling through VEGFR increases nitric oxide (NO) production. Hence, there is enhanced vascular permeability and endothelial cell survival due to the increase in NO production by sodium butyrate. NO also diffuses to adjacent vascular

smooth muscle cells and mediates endothelium-dependent vasodilation. Therefore, the present results suggest that sodium butyrate protects against endothelial dysfunction, possibly by improving VEGF and NO synthesis. Although the present study is not without limitations in such that the molecular mechanisms underlying the beneficial effects of sodium butyrate against fructose-induced aortic lipotoxicity and endothelial dysfunction were not investigated, nevertheless, the present observations provide a rationale for future molecular study. The present data in addition provide insights for prevention and management of arterial cardiovascular risk factors and its attendant complications accompanying high fructose consumption.

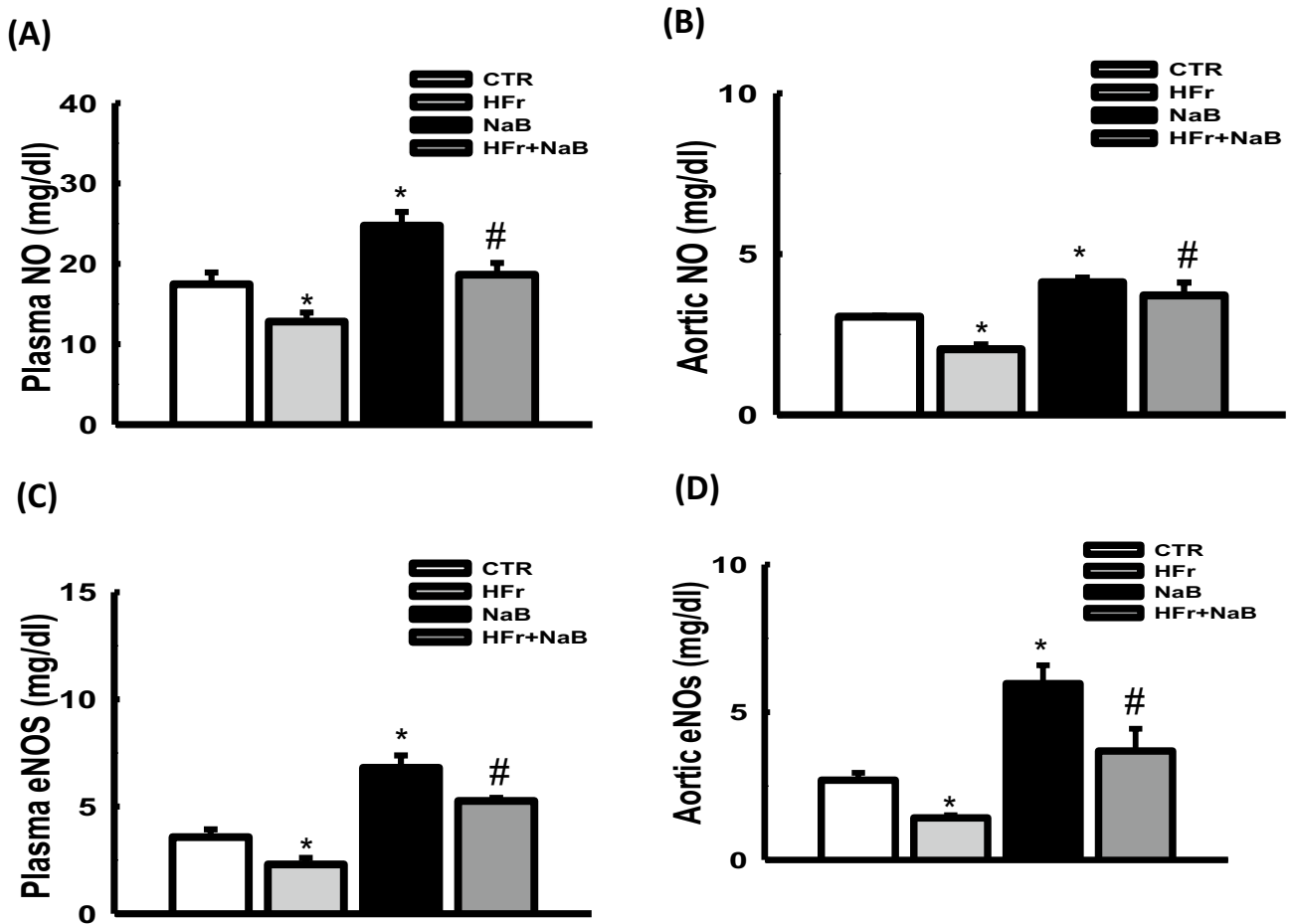


Fig. 4 Effect of sodium butyrate and high fructose on plasma and aortic NO (A, B) and eNOs (C, D) in female Wistar rats. Data are expressed as mean $\pm$ S.E.M. $n=5$ rats per group; * $p<0.05$ vs control; # $p<0.05$ vs HFr). Nitric oxide (NO), endothelial nitric oxide synthase (eNOs)

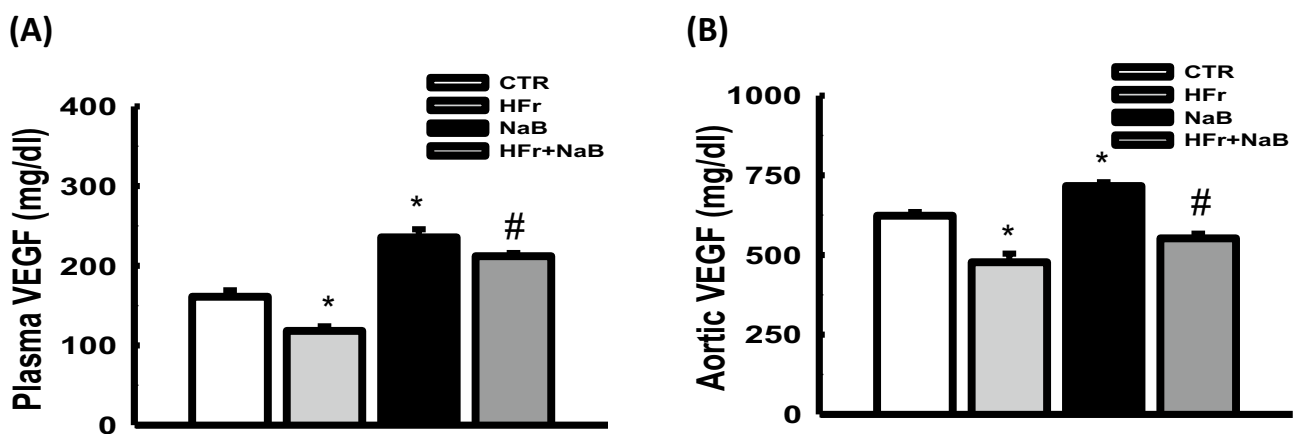


Fig. 5 Effect of sodium butyrate and high fructose on plasma and aortic VEGF (A, B) in female Wistar rats. Data are expressed as mean $\pm$ S.E.M. $n=5$ rats per group; * $p<0.05$ vs control; # $p<0.05$ vs HFr). Vascular endothelial growth factor (VEGF)

Conclusion

The study demonstrates that sodium butyrate protects against aortic lipotoxicity and endothelial dysfunction by attenuating aortic lipotoxicity and improving VEGF and NO synthesis.

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Author contribution All the authors contributed and approved the final manuscript for submission.

Data availability The data supporting the manuscript would be made available on request from the corresponding author.

Compliance with ethical standards

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Conflict of interest There is no conflict of interest to declare.

Ethical approval The study was carried out in accordance with the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the study protocol was approved by the University of Ilorin Ethical Review Committee. Consent to participate is not applicable.

Informed consent Not applicable.

Consent for publication All authors agreed to the submission and publication of the study.

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