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Sodium butyrate aggravates glucose dysregulation and dyslipidemia in high fat-fed Wistar rats

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ABSTRACT

Sodium butyrate (NaB), a short chain fatty acid (SCFA) has been shown to improve metabolic, glucose and lipid signaling. High fat diet elicits increased risk of cardiometabolic disease due to dysmetabolism, altered endothelial function and elevated oxidant activities. This study aims at evaluating the effect of NaB on high fat diet-fed female Wistar rats, and the possible role of vascular endothelial growth factor (VEGF). Twenty female Wistar rats with mean weight of 120 ± 5 g were divided randomly after one week of acclimatization into four groups: Control diet (CTR), High fat diet (HFD), NaB (200 mg/kg), and HFD + NaB. After six weeks of the experimental procedure, blood samples were collected by cardiac puncture. Data were analyzed and expressed in mean ± SEM and p-values <0.05 were accepted as significant. Data showed that HFD increased lactate dehydrogenase (LD) and free fatty acid (FFA), but not triglyceride (TG) and total cholesterol (TC). It also led to insulin resistance (elevated fasting blood glucose, insulin and homeostasis model assessment for insulin resistance). These effects of HFD were accompanied by increased lipid peroxidation (malondialdehyde and 4-hydroxynonenal). Sodium butyrate significantly decreased circulating nitric oxide (NO) and LD while increasing FFA, TG, insulin resistance, aggravated lipid peroxidation and increased VEGF in HFD rats ($P < 0.05$). We speculated therefore, that NaB aggravated glucose dysregulation and dyslipidemia, which is accompanied by increased VEGF.

1. Introduction

Disordered glucose and lipid metabolism remains a major metabolic syndrome phenotype that progresses to type 2 diabetes and cardiovascular disease [1]. Dietary risk factors such as intake calorie-rich diets have been established in human and animal studies to alter systemic and tissue metabolic milieu through glucose/insulin deregulation, lipotoxicity-induced low grade inflammation, cellular oxidative damage and/or lipid peroxidation [2,3]. Although the mechanisms by which these deleterious undertones engender cardiovascular problems are still not completely understood.

Elevation in levels of circulating lipids after dietary ingestion or from de novo synthesis can predispose vascular endothelial cells to oxidative stress, triggering atherosclerosis and other CVD features [4]. Lipid peroxidation, a process that is initiated by oxidants is a key contributor in the pathogenesis of endothelial dysfunction. Oxidation of circulating artherogenic lipid particle, low density lipoprotein (LDL) produces oxidized LDL (OxLDL) which can interact with β2-glycoprotein I (β2GPI) to form oxLDL/β2GPI complexes, leads to vascular endothelial injury and thrombotic events [5]. Increased malondialdehyde (MDA) and 4-hydroxynonenal (4HNE) in tissues and/or circulation correlate with impaired cellular oxidative capacity and peroxidation of lipid products [6,7].

Vascular endothelial growth factor (VEGF) is a protein of the platelet-derived growth factor subgroup responsible for the differentiation of endothelial cells and formation of new blood vessels. VEGF enhances transportation of fatty acids across vascular endothelial cells, promoting lipid deposition and local vascular inflammation [8]. Raised VEGF in circulation has been reportedly observed in diabetic complications and CVD patients [9]. It is also regarded as having an endothelial remodeling property and promoting new blood vessels formation having been originally known as a vascular permeability factor (VPF).

Short chain fatty acids (SCFAs) are fatty acids with carbon backbone constituting about 1–6 carbon atoms. They are produced from gut

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Table 1
Diet components and percentage calories.

	Normal chow (%/100g)	Calorie content (kcal/100 g)	High fat feed (%/100 g)	Calorie content (Kcal/100 g)
Protein	27.5%	110	16.2	64.8
carbohydrate	60.3%	24.1	30.28	121.2
Fat	10%	90	50.5	454.5
Vitamin	0.28%		0.28	
Minerals	2.6%		2.68	
Total (Kcal/ 100g)		224.1		640.5
Protein	g/kg		g/kg	
Casein	80.0		100.0	
Corn gluten meal	65.0		40.0	
Rice bran	50.78		90.0	
Corn bran	100.0		30.0	
Methionine	2.00		3.0	
CHO				
Corn starch	27.0		200.0	
Sucrose	91.38		85.0	
Oat meal	20.22		30.0	
Wheat	180		40.0	
Fat				
Corn oil	70.0		81.0	
Butter oil	30.0		100.38	
Soy bean oil	10.0		100.0	
Meat fat	30.0		100.0	
Vitamin				
Grower premix	0.10		0.03	
Vit C	0.18		0.01	
Mineral				
Oyster shell	1.1		0.25	
Bone shell	1.0		0.3	
Salt	0.5		0.03	

fermentation of indigestible food constituents by the gut microbial community [10]. Sodium butyrate (NaB) is a SCFA with well reported metabolic effects. Some studies have reported that NaB enriches intestinal cells with energy as well as alleviates metabolic syndrome features via histone code modification, particularly histone deacetylase (HDAC) inhibition, and G Protein-coupled receptors (GPCRs) downstream signaling [11]. Conversely, other studies show that NaB may instigate metabolic issues through induction of de novo lipogenesis and reactive oxygen species generation [12]. Moreover, another study revealed that high colonic SCFAs may induce inflammation through mitogen activated protein kinase (MAPK) downstream inflammatory signaling [13]. Also, high fiber diet, a rich source of SCFAs via gut microbial fermentation was shown to disrupt cellular response to insulin [14]. Therefore, the differential findings on the effects of SCFAs on immune/inflammatory mediators [15–18] and metabolic syndrome features [6] necessitates further investigation into the effects of SCFAs, butyrate on metabolic phenotypes in health and disease. Although, butyrate is known for its role in health and disease and to reduce the risk of inflammatory diseases, type 2 diabetes, obesity, heart disease and other conditions [19], but its role in cardiovascular-linked pathophysiology remains unclear. We therefore aimed to investigate the effect of NaB on glucose and lipid metabolism and the possible role of VEGF in high fat fed animal models.

2. Materials and method

2.1. Animals grouping

The study was carried out in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and was approved by the Ethical Review Committee, University of Ilorin, Ilorin, Nigeria. Effort was made to reduce the number and suffering of animals. Twenty adult female Wistar rats weighing between 120 ± 5 g were obtained from the animal house of the College of Health Sciences, University of Ilorin (Ilorin, Nigeria). The rats were fed ad libitum with standard rat chow and had unlimited access to tap water. After a week of acclimatization, the animals were randomly assigned to four groups (n = 5 rats/group). Group1 (Control) rats was fed with standard chow (CTR), group 2 was fed with High fat diet (HFD; 20% wt/wt fat) only Table 1, group 3 was fed NaB (200 mg/kg bw), and group 4 was fed HFD; 20% wt/wt fat + NaB; 200 mg/kg bw). High fat diet was prepared for the study as previously reported [20]. Rats were maintained in the animal house under environmental conditions of temperature (22–26 °C), relative humidity (50–60%) and 12-h dark/light cycle.

2.2. Treatment

Sodium butyrate (200 mg/kg;*p.o*; Science Company Lakewood, CO, USA) was used for the treatment and the treatment lasted for six weeks.

2.3. Collection of blood sample

At the end of six-week treatment, the animals were anesthetized with sodium pentobarbital (50 mg/kg *ip*). 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.

2.4. Biochemical assay

Plasma insulin was determined using ELISA kit from Ray Biotechnology, Inc. (Georgia, USA). The method used 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*)* was determined using the homeostasis model assessment for insulin resistance (HOMA-IR; (fasting insulin* fasting glucose)/22.5).

Total cholesterol (TC) and triglyceride (TG) were measured by standardized enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

Free fatty acid (FFA) was measured by standardized enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

Vascular endothelial growth factor (VEGF) and 4-hydroxynonenal (4-HNE) assay were done using the Sandwich-ELISA principle with a kit purchased from Elabscience®

Malondialdehyde (MDA) and Nitric oxide determination were done using standardized colorimetric methods and assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

Lactate dehydrogenase was done using standardized enzymatic colorimetric methods and assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

2.5. Statistical analysis

All data were expressed as means ± 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 pair wise comparisons of mean values among the groups. Statistically significant differences were accepted at $p < 0.05$.

3. Results

3.1. Effects of sodium butyrate on free fatty acids, lactate dehydrogenase, triglycerides, and total cholesterol in healthy- and high fat diet-fed rats

High fat diet increased plasma FFA and LDH but caused no change in

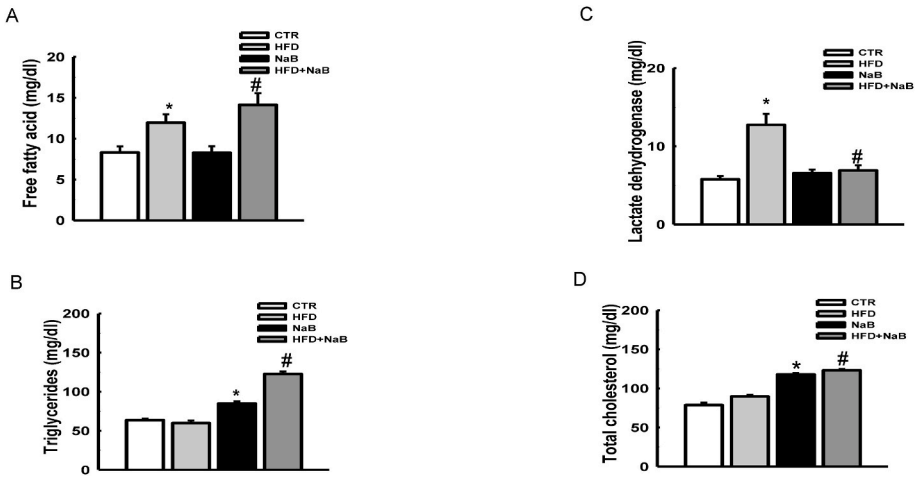

Fig. 1. Effects of NaB on free fatty acids, lactate dehydrogenase, triglycerides and total cholesterol in healthy- and high fat diet-fed rats. High fat diet caused increased plasma free fatty acids (FFA) and lactate dehydrogenase (LDH) (*P < 0.05 vs. CTR). NaB increased free fatty acids (a), triglycerides (b), and cholesterol (d) in high fat diet fed rats(#P < 0.05 vs HFD).Data were analyzed by one-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.

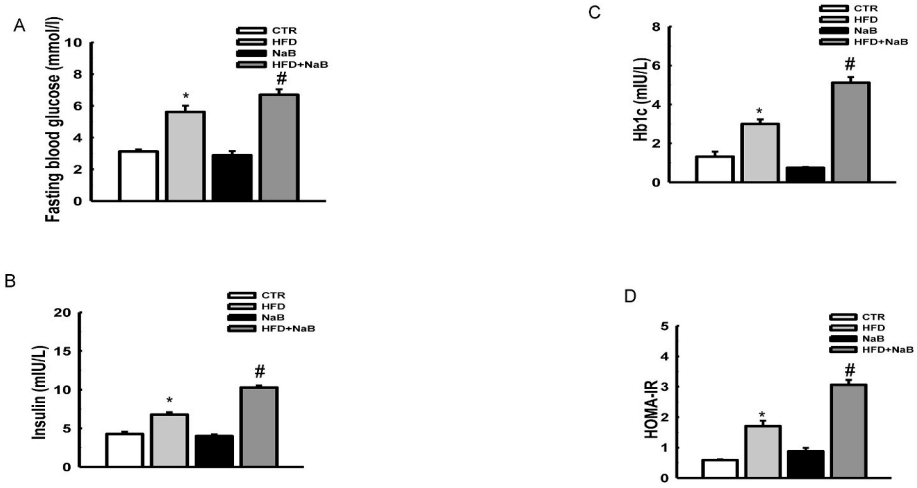

Fig. 2. Effect of NaB on glucoregulation and insulin sensitivity in healthy- and high fat diet-fed rats. NaB increased fasting blood glucose (a), insulin (b) and HOMA-IR: Homeostasis assessment of insulin resistance (d), but decreased hb1c: glycated hemoglobin (c) in healthy diet-fed female Wistar rats (*P < 0.05 vs. CTR). NaB increased all glucoregulatory and insulin sensitivity indices in high fat fed female Wistar rats (#P < 0.05 vs HFD). Data were analyzed by one-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.

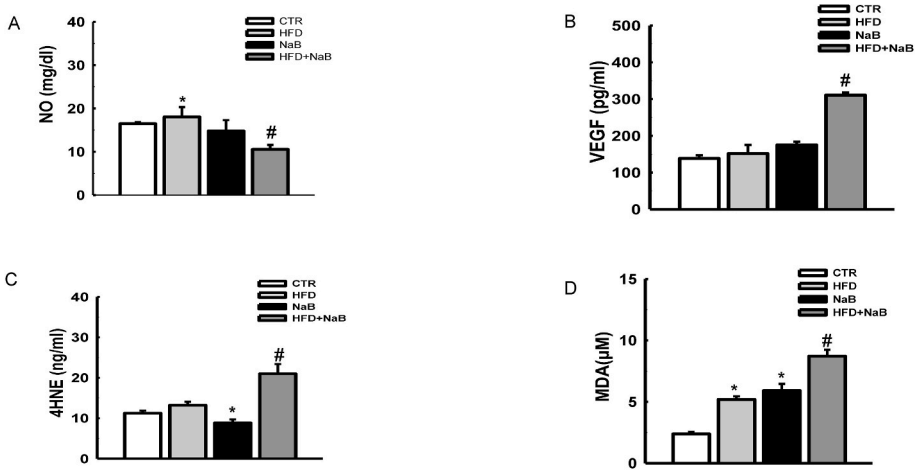

Fig. 3. Effects of NaB on NO, VEGF, and lipid peroxidative markers in healthy- and high fat diet-fed rats. NaB decreased plasma NO: nitric oxide (a), and increased VEGF: vascular endothelial growth factor (c), 4HNE: 4-hydroxynonenal (b) MDA: malonaldehyde (d), in high fat fed female Wistar rats (#P < 0.05 vs HFD). NaB increased malonaldehyde: MDA (d) (*P < 0.05 vs. CTR) and in healthy-diet fed rats. Data were analyzed by one-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.

plasma TG and cholesterol compared with control. Sodium butyrate caused no change in plasma free fatty acids and LDH in healthy diet-fed rats compared with control but increased plasma FFA, TG and TC in high fat-fed Wistar rats compared with high fat-fed diet alone (Fig. 1A and B and 1C).

3.2. Effects of sodium butyrate on glucoregulation and insulin sensitivity in healthy- and high fat diet-fed rats

High fat diet-fed rats but not NaB-exposed normal diet –fed rats caused glucose dysregulation with increased fasting blood glucose (Fig. 2A), Hb1c (Fig. 2C), fasting insulin (Fig. 1B) and HOMA-IR

(Fig. 2D). However, NaB worsened glucose dysregulation in HFD-fed rats with increase in fasting blood glucose, HB1c, fasting insulin and HOMA-IR compared with control and HFD-fed rats (Fig. 2).

3.3. The effects of sodium butyrate on vascular endothelial growth factor, nitric oxide and lipid peroxidation in healthy - and high fat diet-fed rats

High fat diet-fed rats increased malondialdehyde (MDA) (Fig. 3D) with no significant changes in the circulating nitric oxide when compared with the control. Sodium butyrate on the other hand, decreased nitric oxide production in HFD-fed rats while producing a double fold increase in lipid peroxidative makers (4HNE and MDA) and circulating vascular endothelial growth factor (VEGF) compared with HFD-fed rats alone (Fig. 3A and B and C) .

4. Discussion

The results of the present study showed that high fat diet increased body weight, circulating FFA, lactate dehydrogenase, but not triglyceride. These findings were accompanied by indications of insulin resistance in HFD-fed animals. High fat diet also had no significant changes in circulating NO despite the increase in MDA observed. Sodium butyrate on the other hand resulted in increased plasma lipids in both normal and HFD-fed animals. However, there was significant increase in lipid peroxidation, elevated NO and VEGF in butyrate-treated HFD-fed animals only. Normal diet-fed animals treated with NaB had no indication of IR but NaB aggravated IR in HFD fed animals. Also, treatment with NaB was associated with decreased plasma NO and elevated VEGF level and evidence of increased lipid peroxidation in HFD rats. This shows that NaB is associated with VEGF elevation which might lead to aggravation of IR in HFD. The interplay between decreased nitric oxide, a known endothelial growth factor, and an increased VEGF, an angiogenic marker, coupled with profound lipid peroxidation seen in HFD rats provides insights into possible elucidation of lipids induced abnormality of butyrate proliferation and endothelial dysfunction.

The pathophysiology of insulin resistance-related disturbances has been associated with increased level of circulating lipids due to lipolysis in hypertrophied fat store. Elevated plasma free fatty acids promote subclinical inflammation by causing cytokine infiltration of tissues including the endothelium. In addition, raised FFAs and TG have been shown to bring about systemic perturbations through increasing uric acid production and cellular oxidative stress, which is altogether called lipotoxicity and can lead to dysfunction of endothelium of blood vessels [21]. In this study, HFD and sodium butyrate led to significant increases in plasma FFA as a dietary model of IR-led dyslipidemia. Elevated FFA here is also believed to be causative in the IR indicated in HFD-fed animals. Sodium butyrate increased TC and TG in normal diet-fed animals and a double fold increase in NaB treated rats which shows that despite causing increase in plasma lipid content in normal fed rats, its administration with HFD significantly aggravated lipid deposition. In HFD-fed animals, NaB increased FFA, TC and TG compared with their control (HFD only) and this showed that NaB aggravated hyperlipidemia in HFD-fed animals. The effect of NaB in HFD-fed animals shows that NaB may cause severe lipolysis in HFD-exposed individuals leading to aggravated hyperlipidemia which may predispose to atherosclerotic diseases and lipotoxicity in non-adipose tissues leading to peripheral insulin resistance and compensatory hyperinsulinemia.

Hyperinsulinemia, a known and predictable consequence of IR is shown to engender atherogenesis, atherothrombosis and oxidative injury and thus a key underlying cause of CVD [22]. Elevated plasma insulin can occur secondary to lipotoxicity especially in HFD but itself can disrupt lipid homeostasis (e.g. increase triglyceride and decrease high-density lipoprotein cholesterol) by raising catecholamines levels and by increasing the synthesis of very low-density lipoprotein. Here, HFD caused increase in markers of IR including insulin, fasting blood glucose, glycated haemoglobin levels and HOMA-IR. Clinical and experimental studies associate SCFAs with improved cardiometabolic status through enhancement of glucose uptake and fatty acid oxidation in insulin sensitive organs. These beneficial effects are mediated through the involvement of SCFAs in the epigenetic regulation of low-grade inflammation and atherosclerotic plaque formation [23]. In the present study, NaB maintained glucoregulation and did not cause insulin resistance in normal diet-fed animals. Nevertheless, insulin resistance and glucose dysregulation were aggravated in HFD-fed animals exposed to NaB. This outcome is consistent with the findings concerning circulating lipids in NaB-treated HFD-fed animals. Elevated FFA is capable of damaging insulin signaling in many ways. Some of the ways by which elevated circulating FFA in HFD and NaB-treated HFD-fed animals caused insulin resistance and hyperinsulinemia are the yield of fatty acid intermediates that promote the production of protein kinase C and consequent serine phosphorylation of insulin receptor substrate, interaction with toll-like receptor type 4 leading to inflammatory responses and inhibition of glucose utility. A study in which offspring of dams fed with butyrate diet displayed insulin resistance indicated by elevated HOMA-IR and serum glucose level, suggests that pure NaB diet caused IR via inhibition of PI3K/Akt pathway in the skeletal muscle [15]. This might also be the case for the effect of NaB in HFD-fed animals.

The present study also revealed that sodium butyrate treatment heightened circulating angiogenic marker, VEGF level and elevated lipid peroxidation markers (MDA and 4HNE) especially in HFD-fed animals. VEGF is an endothelium-derived cytokine with crucial role in angiogenesis [24]. Several processes including wound-repair, inflammation and hypoxia are major inducers of VEGF expression. In this study, we observed a significantly raised VEGF expression in high-fat diet-fed rats treated with sodium butyrate that is comparable among the groups. The pathological roles of VEGF in the development of diabetic vascular anomalies have been reported [9]. VEGF may increase permeability of the vascular endothelium and promote transendothelial activation and migration. Moreso, it stimulates free fatty acids transporters [9] and confer pro-atherogenic effects on lipoproteins. Impaired endothelial function in pre-eclamptic women was partly associated with increased plasma VEGF expression compared with healthy controls [25]. In another human study, VEGF and its receptors were highly expressed in atherosclerotic sites in coronary vasculature [26]. From our data, it seems that increase in plasma FFA and TG due to sodium butyrate in high-fat diet-fed rats compared with normal diet-fed rats is at least partly associated with elevated level of circulating VEGF.

Abnormal accumulation and/or deregulated fat signaling is associated with the oxidative stress and lipid peroxidation process and are major features in fatty vasculature [2]. We found that plasma MDA level in HFD-fed rats was increased compared with control. Also, NaB increased lipid peroxidation in both normal diet- and HFD-fed animals. However, there was an aggravation in HFD-fed animals treated with NaB. First, since normal and HFD-fed animals had elevated lipid peroxidation, it is agreeable that with or without HFD, NaB can initiate the lipid peroxidation process which may limit its prophylactic effect in cardiovascular disease. Secondly, HFD with NaB exposure aggravated lipid peroxidation which is a risk factor for damage of vascular integrity. This shows that HFD-fed individuals may be at risk of expanded lipid peroxidation due to synergy of HFD and NaB exposure. The link between lipid peroxidation and VEGF has been discussed in various studies. Studies have shown that a reciprocal relationship exists between dyslipidemia-led oxidative stress and angiogenesis, with VEGF expression playing a central role [27–29]. Furthermore, it was shown that high VEGF level could increase ROS production by activating NADPH oxidase in vascular endothelial cells [28,30]. Increased VEGF expression was accompanied by significantly increased concentration of lipid peroxidation in human aortic endothelial cells [31] and in women having pre-eclampsia [26]. Observation in this study revealed that sodium butyrate may contribute to lipid peroxidation by increasing the mobilization of FFA, atherogenic lipid and free radicals in circulation through VEGF, in HFD-fed rats treated with NaB.

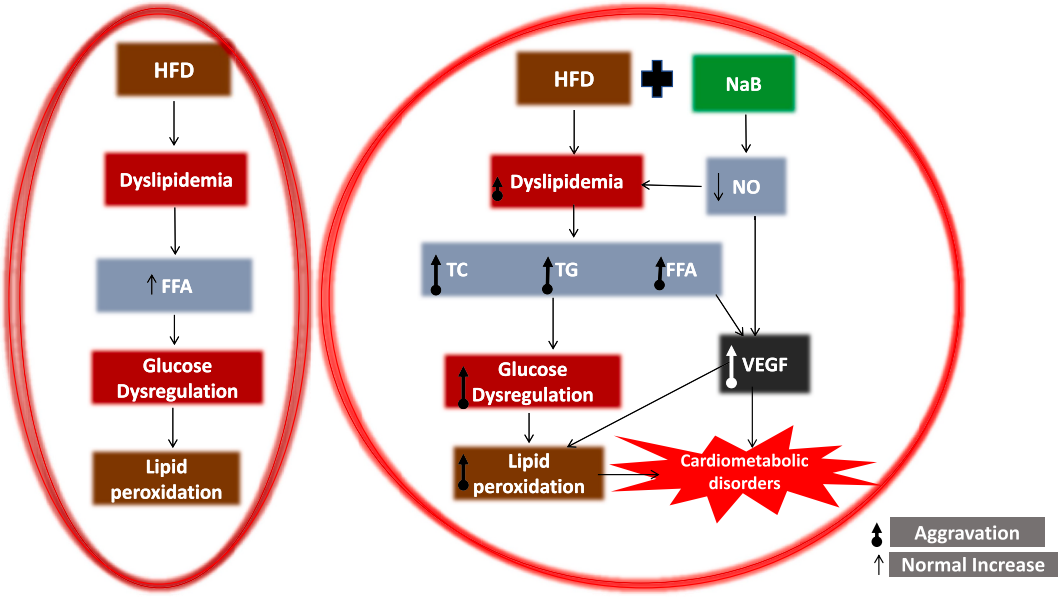

Fig. 4. Graphical abstract showing the cross link associated with butyrate enhanced high fat diet-induced cardiometabolic disorders.

5. Conclusion

The present results indicate that HFD alter metabolic status, particularly glucose and lipid handling. In addition, we observed that sodium butyrate worsens metabolic signaling by significantly elevating VEGF in high fat diet-fed animals while inducing IR in healthy-diet fed rats (Fig. 4). We recommend further investigation using qualitative analytical techniques to assay protein expression, to fully establish possible mechanisms involved in sodium butyrate-induced VEGF elevation and associated alteration in lipid homeostasis. Nonetheless, the findings indicate that sodium butyrate altered cardiovascular risk factors in the study and diet-rich in butyrate must be taken with caution in high fat-fed or overtly obese individuals.

Authors' declaration

The authors declared that there is no conflict of interest.

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CRediT authorship contribution statement

Adewumi Oluwafemi Oyabambi: Formal analysis, Writing – original draft, conceived, designed the research and conducted the experiments, contributed to the new reagents and analytical kits, analyzed and interpreted the data, drafted the manuscript, read and approved the manuscript. **Kehinde Samuel Olaniyi:** Formal analysis, Writing – original draft, contributed to the new reagents and analytical kits, analyzed and interpreted the data, drafted the manuscript, read and approved the manuscript.

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