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High-Fat Diet-Induced Metabolic and Cardiovascular Alterations: Role of Vitamin C and E Supplementation in Modulating Lipid Profile and Blood Pressure

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ABSTRACT

Background: Obesity and its associated cardiovascular risks, including hypertension and dyslipidemia, are growing global health challenges. High-fat diet (HFD)-induced metabolic dysregulation exacerbates these conditions, necessitating effective interventions. Antioxidants such as vitamins C and E are known for their cardioprotective properties, but their impact on HFD-induced metabolic and autonomic dysfunction remains inadequately explored.

Objective: This study aimed to investigate the effects of vitamin C and E supplementation on body weight, lipid profile, blood glucose, blood pressure, and heart rate variability (HRV) in HFD-fed rats.

Methods: 32 Male Wistar rats were divided into four groups: (1) Control, (2) HFD, (3) HFD + Vitamin C, and (4) HFD + Vitamin E. Body weight was recorded biweekly, and blood samples were analyzed for fasting glucose and lipid profiles. Blood pressure was measured using a tail-cuff system. ECG recordings were obtained to assess HRV parameters. Statistical analyses were conducted using one-way and two-way ANOVA.

Results: HFD-fed rats exhibited significant weight gain compared to controls ($p < 0.05$), which was attenuated by vitamin C and E supplementation. HFD significantly elevated total cholesterol and triglyceride

levels ($p < 0.001$), while vitamin E supplementation effectively reduced cholesterol ($p < 0.001$). Both vitamins significantly lowered triglycerides compared to the HFD group ($p < 0.05$). No significant differences were observed in fasting glucose and HDL levels. HFD-induced hypertension was alleviated considerably by vitamin C and E supplementation, reducing systolic, diastolic, and mean arterial pressures ($p < 0.05$). HRV analysis revealed no significant differences in autonomic modulation between groups.

Conclusion: Vitamin C and E supplementation mitigated HFD-induced obesity, dyslipidemia, and hypertension, suggesting their potential as cardioprotective interventions. However, their effects on autonomic regulation warrant further investigation.

Keywords: *High-fat diet - Vitamin C - Vitamin E – Obesity – Dyslipidaemia - Blood Pressure - Heart Rate Variability.*

1. INTRODUCTION

Cardiovascular diseases (CVD) remain a leading cause of mortality worldwide, accounting for approximately 17.9 million deaths annually (Abbafati et al., 2020; Feigin et al., 2021; Ma et al., 2021; Roth et al., 2020). The primary contributors to CVD are long-term exposure to risk factors such as obesity, hypertension, hyperlipidemia, smoking, physical inactivity, and chronic stress. Excess caloric intake relative to energy expenditure leads to obesity and hyperlipidemia, which promote the abnormal accumulation of low-density lipoprotein (LDL) in blood vessels, ultimately resulting in atherosclerosis and hypertension (Elagizi et al., 2020; Koliaki et al., 2019; Ortega et al., 2016; Piché et al., 2020). Under normal physiological conditions, the cardiovascular system continuously generates reactive oxygen species (ROS), which are counteracted by endogenous antioxidant defensev mechanisms. However, in obesity, an imbalance between pro-oxidants and antioxidants

leads to excessive ROS production (Sharifi-Rad et al., 2020). When ROS levels exceed the capacity of antioxidants, oxidative damage occurs, affecting LDL, proteins, and endothelial cell DNA. This oxidative stress contributes to atherosclerosis, as the oxidized LDL hypothesis describes. The oxidation of LDL and lipid hydroperoxides disrupts lipid metabolism within endothelial cells, further exacerbating vascular dysfunction (Phaniendra et al., 2014). Cross-sectional studies have demonstrated that elevated ROS and lipid hydroperoxide levels are independent risk factors for adverse cardiovascular events. The assessment of antioxidant status through biomarkers such as transferrin, ceruloplasmin, uric acid, and albumin has revealed an inverse correlation with cardiovascular risk (Janciauskiene, 2020). Additionally, studies on antioxidant supplementation suggest a potential protective role against cardiovascular events. Key antioxidants, including β- carotene, vitamins A, C, and E, and glutathione, contribute to redox homeostasis. Vitamin C and E, in particular, play crucial roles in the antioxidant defense system, functioning synergistically with glutathione peroxidase to neutralize free radicals (Didier et al., 2023).

Low levels of vitamins C and E have been reported in individuals with obesity and cardiovascular disease (CVD), suggesting that supplementation with these antioxidants may be beneficial in mitigating cardiovascular risk. Numerous studies have demonstrated the protective effects of vitamin C and E supplementation on cardiovascular health. However, no study has directly compared the long-term effects of vitamins C and E on cardiovascular risk factors in a high-fat diet (HFD)-induced obese rat model. Therefore, we aimed to investigate the effects of vitamins C and E on cardiovascular risk factors in HFD-induced obese rats. We hypothesized that these two vitamins would affect different cardiovascular risk factors. To test this hypothesis, we first established an obesity model by feeding rats a 60% HFD for three months. Following the induction of obesity, rats were supplemented with either vitamin C or E for an additional three months. We assessed cardiovascular risk factors and antioxidant levels at the end of the supplementation period. Our findings revealed that three months of vitamin C and E supplementation significantly reduced body weight, triglyceride levels, and systolic blood pressure (SBP) in obese rats. Additionally, vitamin E supplementation further reduced total

cholesterol and diastolic blood pressure (DBP), suggesting a more pronounced effect of vitamin E in mitigating cardiovascular risk factors in HFD-induced obesity.

2. METHODOLOGY

Experimental animals

All experiments in this research employed adult male Wistar rats (Central Animal Facility, All India Institute of Medical Sciences, New Delhi, India) aged 8–10 weeks and weighed 230–280 g. Thirty-two male Wistar rats weighing 230–240 g was procured from the Central Animal Facility of the All India Institute of Medical Sciences (AIIMS), New Delhi. The rats were kept in filter top cages (four cage companions; floor area: 821 cm2, cat. no. SRC02, Orchid Scientific & Innovative India Pvt. Ltd., India). All the animals were provided optimal housing conditions (22±2 °C, 54–60% humidity, 12h cycle of light and dark). Before the commencement of the experiments, ethical approval was obtained from the Institutional Animal Ethics Committee (IAEC) of the All India Institute of Medical Sciences, New Delhi (Approval No. 15/IAEC-1/2017). All experimental procedures were conducted in strict accordance with the guidelines set by the Committee for Control and Supervision of Experiments on Animals (CPCSEA), Government of India, as well as the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1996). Every effort was made to minimize the number of animals used and to reduce their suffering. Food pellets (Lipton India Ltd., India) and water were provided ad libitum. The test animals were well-habituated with the experimenter before the behavioral paradigm and sample collection. All the behavioral experiments were carried out between 9.00 to 11.00 am. Before behavioral evaluation, the rats were tested for 2 weeks for any behavioral abnormalities like low growth rate and disorientation resulting in the animals being excluded from the study before being divided into groups. The study investigated the comparative role of vitamins C and E in reducing the CVD risk factors in obese rats. Rats were divided into 4 groups: control, high-fat diet (HFD), HFD + vitamin C, and HFD + vitamin E (n = 8 in each group). To induce obesity, the rats were fed a high-fat diet (purchased from Research Diet, USA, containing 20% carbohydrate, 20% protein, and 60% fat) for three months. While the control group was given a standard chow diet (Ashirwad Pvt. Ltd., India, containing protein: 24%, fat: 5%, fiber: 4%, carbohydrates: 55%, calcium: 0.6%, and phosphorus: 0.3%). In the HFD+ Vitamin C group, HFD with Vitamin C (orally with water; dose:100mg/kg) was given with HFD for 3 months. In the HFD+ Vitamin E group, HFD with Vitamin E (orally with water; dose 60mg/kg) was provided with HFD for 3 months. After 3 months, the rats were sacrificed, blood and tissues were collected and stored at -80°C.

Blood pressure recording

Non-invasive blood pressure measurements were obtained using the tail-cuff method based on the principle of volume pressure recording (VPR) with the CODA system (Kent Scientific Corporation, Torrington, USA). Before blood pressure recording, rats were acclimated to an appropriately sized restrainer for 15 minutes daily over five consecutive days. This was followed by an additional five-day acclimation period, during which rats were accustomed to the occlusion cuff and VPR cuff for 15 minutes daily. Thus, 10 days of acclimatization was provided before initiating blood pressure measurements. Blood pressure recordings were conducted over three consecutive days. Five measurements were taken each day, and the

daily average was recorded. On the day of recording, the restrainers were first placed on a thermal pad set at 22°C to maintain a stable temperature. The rats were then placed in the restrainer, ensuring proper positioning while taking special precautions to prevent any injury to the tail or genital area. After sufficient training, the rats voluntarily entered the restrainers. Once inside, the nose cone was adjusted toward the rear hatch to restrict movement. The rats were then allowed to rest for at least 10 minutes before placing the occlusion and VPR cuff on the tail. The occlusion cuff was positioned near the base of the tail, with the VPR cuff placed within 2 mm of it. After an additional 10-minute stabilization period, the occlusion cuff pressure was increased to 250 mmHg and then gradually released to zero. Blood pressure values were derived using the VPR cuff during this cycle based on the volume pressure recording principle. Five independent blood pressure measurements were recorded each day for three consecutive days to minimize movement artifacts. The parameters assessed included mean arterial blood pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean heart rate.

Biochemical tests

Blood samples (1.5 mL) were collected from the retro-orbital plexus of rats at baseline and at the end of the three-month study period to assess blood glucose and lipid profile. The collected blood was transferred into vials and centrifuged at 3000 rpm for 15 minutes at 25°C to separate serum and plasma. The obtained samples were then stored at −20°C until biochemical analysis. Plasma glucose concentrations were measured, while the lipid profile, including total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol, was analysed using serum. Lipid profile parameters were estimated using an enzymatic method on a semi-automated analyzer (Erba Chem-5).

Heart rate variability

Before electrocardiogram (ECG) recording, rats were acclimated to the ECG leads and the restrainer for 30 minutes per day over three consecutive days. Heart rate variability (HRV) data were derived from lead II ECG recordings. For lead II ECG acquisition, adhesive disposable ECG electrodes were trimmed to fit the size of the rats' limbs. Two active electrodes were placed on the left forelimb and left hindlimb, while a grounding electrode was positioned on the right hindlimb. The electrodes were connected to an acquisition system (Power Lab, AD Instruments) via shielded lead wires. Rats were then restrained for 30 minutes for ECG recording. On the day of recording, rats were allowed to adapt to the restrainer for the first 10 minutes, followed by 10 minutes of artifact-free ECG acquisition. ECG signals were recorded using Lab Chart 8 software with a sampling rate of 1 kHz, a high-pass filter of 0.05 Hz, and a low-pass filter of 150 Hz (AD Instruments, Bella Vista, Australia). Following lead II ECG acquisition, HRV analysis was performed in both the time and frequency domains. Time-domain parameters included the R-R interval and the standard deviation of normal- to-normal intervals (SDNN). The frequency-domain analysis included low-frequency (LF: 0.2–0.75 Hz) and high-frequency (HF: 0.75–2.5 Hz) power, total power, and the LF/HF ratio.

3. RESULTS

Effect of Vitamin C and Vitamin E Supplementation on Body Weight in High-Fat Diet-Fed Rats:

In this study, we investigated the effect of vitamin C and E supplementation on body weights in rats fed a high-fat diet (HFD). Two-way ANOVA results showed a significant effect of time F (3, 20) = 7.401; P=0.0016 and dietary supplement on body weights F (1.530, 30.59) = 212.6; P<0.0001, figure 1A; n=5), with multiple comparisons revealing that rats in the HFD group had significantly body weights compared to the control group at 1.5 months (mean difference = -48.00g, P =0.0120; figure 1A; n=5) and at 3 months (mean difference = --48.67gm, P =0.0319; figure 1A; n=6). Vitamin C supplementation significantly reduced body weight in HFD-fed rats 1.5 months (mean difference = - 23.50, P =0.0301; figure 1A; n=5) and at 3 months (mean difference = 36.83gm, P =0.0046; figure 1A; n=6). Vitamin E supplementation significantly reduced body weight in HFD-fed rats 1.5 months (mean difference = -46.33gm, P = 0.0477; figure 1A; n=5) and at 3 months (mean difference = - 45.33gm, P = 0.0214 figure 1A; n=6).

(Figure: 1A)

Influence of Vitamin C and E Supplementation on Fasting Blood Glucose in High-Fat Diet-fed Rats.

Hyperglycaemia is one of the important cardiovascular risk factors, so we wanted to investigate the effect of vitamins C and E on blood glucose in HFD obese rats. We did not find any significant difference between the groups on fasting blood glucose levels. Control 72.166±13.97), HFD: 77.16± 13.6, HFD+C: 73.33±14.7 & HFD+E: 58.66±9.37, F (3, 20) = 2.269, P=0.1117

(Figure: 1B)

Effect of Vitamin C and Vitamin E Supplementation on Total Cholesterol Levels in High-Fat Diet-Fed Rats

In this study, we investigated the effect of vitamin C and E supplementation on total cholesterol levels in rats fed a high-fat diet (HFD). One-way ANOVA results showed a significant effect of

treatment on total cholesterol levels ($F(3,16) = 12.4$, $P < 0.001$, figure 1A; n=5), with multiple

comparisons revealing that rats in the HFD group had significantly higher cholesterol levels compared to the control group

(mean difference = -60.2 mg/dL, P < 0.001; figure 1A; n=5). Vitamin Esupplementation significantly reduced total cholesterol in HFD-fed rats (mean difference = 55.4 mg/dL, P < 0.001; figure 1A; n=5), while vitamin C supplementation showed a trend towardcholesterol reduction but did not reach statistical significance (mean difference = 30 mg/dL, P = 0.07; figure 1A; n=5). **(Figure: 2A)**

Impact of Dietary Vitamins on High-Density Lipoprotein Levels in Rats

Next, we assessed the effect of vitamins C and E on HLD levels in HFD rats. Statistical analysis revealed no significant difference in HDL levels among the treatment groups (One-way ANOVA: F(3, 16) = 3.67, p = 0.03; figure 1B; n=5), indicating that vitamin C and E did not significantly affect HDL levels. Tukey's post-hoc analysis demonstrated that HDL levels were significantly higher in the HFD group compared to the control (Mean Diff. = -13.6, 95% CI: -26.6 to -0.621, p = 0.04). The comparison of the control group to the HFD plus Vitamin C and E group showed no significance (Vitamin C; Mean Diff. = -12, 95% CI: -25.0 to 0.979, p = 0.08), (Vitamin E Mean Diff. = -6.6, 95% CI: -19.6 to 6.38, p = 0.49). Additionally, there was no significant difference between the HFD plus Vitamin C and HFD plus Vitamin E groups (Mean Diff. = 5.4, 95% CI: -7.58 to 18.4, p = 0.64). **(Figure: 2B)**

Effects of Vitamins C and E on Triglyceride Concentrations in High-Fat Diet-Exposed Rats

One-way ANOVA was conducted to assess the effect of treatment on triglyceride levels among the four treatment groups—Control, HFD, HFD + C, and HFD + E and observed significant differences (F (3, 16) = 9.98, p < 0.001; figure 1C; n=5). Tukey's post hoc comparisons revealed that the HFD group exhibited significantly higher triglyceride levels (mean 155 mg/dL) compared to the Control group (mean 56.6 mg/dL), with a mean difference of -98 mg/dL (95% CI: -161 to -34.8, p = 0.002). However, the HFD + C and HFD + E groups did not show significant differences from the Control group (mean differences of -17 mg/dL, p = 0.87; and 11 mg/dL, p = 0.96, respectively). Notably, significant low level of triglyceride levels were observed in the HFD + C and HFD + E groups compared to HFD, with mean differences of 81 mg/dL (p = 0.01) and 109 mg/dL (p < 0.001), respectively. **(Figure: 2C)**

Impact of Vitamin C and E Supplementation on Systolic Blood Pressure in High-Fat Diet-fed Rats

Results indicated a highly significant difference in SBP among the treatment groups (One-way

ANOVA: F(3, 23) = 31.4, p < 0.001; figure 2A; n=7), with Tukey's post-hoc analysis revealing that SBP was significantly elevated in the HFD group compared to the control group (Mean Diff. = -38.6, 95% CI: -49.9 to -27.2, p < 0.001). Additionally, the HFD plus Vitamin C group also showed a significant reduction in SBP compared to the control group (Mean Diff. = -13.3, 95% CI: -24.6 to -1.93, p = 0.02), as well as the HFD plus Vitamin E group (Mean Diff. = -23.4, 95% CI: -35.2 to -11.5, p < 0.001). In contrast, comparisons between the HFD group and both supplemented groups revealed significant increases in SBP (HFD vs. HFD + C: Mean Diff. = 25.3, p < 0.001; HFD vs. HFD + E: Mean Diff. = 15.2, p = 0.008). However, there was no significant difference between the HFD plus Vitamin C and HFD plus Vitamin E groups (Mean Diff. = -10.1, p = 0.11). **(Figure: 3A)**

Influence of Vitamin C and E Supplementation on Diastolic Blood Pressure in High-Fat Diet-fed Rats

The One-way ANOVA results showed a statistically significant effect of treatment on DBP (F(3, 23) = 4.82, p = 0.01; figure 2B; n=7). The post-hoc Tukey's multiple comparisons test indicated a significant difference between the control group and the high-fat diet (HFD) group, with the HFD group exhibiting a decrease in DBP by an average of 18.6 mmHg (95% CI: -33.0 to -4.17, adjusted p < 0.01). However, no significant differences were observed between the control and HFD plus vitamin C (HFD + C) groups (mean difference = -9.71, p = 0.27) or between control and HFD plus vitamin E (HFD + E) groups (mean difference = -3.52, p = 0.91). In contrast, the HFD group showed a significant increase in DBP compared to the HFD + E group (mean difference = 15 mmHg, p = 0.05). The R-squared value of 0.386 suggests that treatment accounts for a moderate portion of the variance in DBP. **(Figure: 3B)**

Influence of Vitamin C and E Supplementation on Mean Blood Pressure in High-Fat Diet-fed Rats

The analysis revealed a significant effect of treatment on MBP (F(3, 23) = 12.7, p < 0.001; figure 2C; n=7), with post-hoc Tukey's post-hoc tests indicating that the HFD group exhibited a significant increase in MBP by an average of 25.3 mmHg compared to the control group (95% CI: -36.8 to -13.7, adjusted p < 0.001). Dietary supplementation of vitamin C and E with high fat diet demonstrated significant low MBP compared to high fat diet group (HFD + C; mean difference = 14.6 mmHg, p = 0.01) and groups (HFD + E; mean difference = 17.2 mmHg, p = 0.003). However, no significant differences were observed between the control and HFD plus vitamin C (HFD + C) groups (mean difference = -10.7, p = 0.08) or between control and HFD plus vitamin E (HFD + E) groups (mean difference = -8.07, p = 0.27). **(Figure: 3C)**

Impact of Vitamin C and Vitamin E Supplementation on Heart Rate in High-Fat Diet-Fed Rats

The analysis using ANOVA revealed no significant differences in HR among the treatment groups (F(3, 21) = 2.18, p = 0.1202; figure 2D; n=7), suggesting that the HFD did not significantly affect HR when compared to the control and supplemented groups. Post-hoc Tukey's multiple comparisons showed that the control group had an HR of 334 beats per

minute (bpm) compared to 402 bpm in the HFD group (mean difference = -67.5; p = 0.2961), indicating a trend toward increased HR due to HFD but not reaching statistical significance. Furthermore, vitamin C and vitamin E supplementation did not yield significant differences in HR compared to the HFD group, with differences of -39.6 bpm (HFD + C vs. control, p = 0.7149) and 14.4 bpm (HFD + E vs. control, p = 0.9817). The R-squared value of 0.238 indicates that the treatment accounts for a modest portion of the variability in HR. **(Figure: 3D)**

Impact of Vitamin C and Vitamin E Supplementation on Heart Rate on Variability Parameters in **High-Fat Diet-Fed Rats.** The autonomic nervous system mainly controls the cardiovascular system. Most cardiovascular diseases have an autonomic imbalance between the sympathetic and parasympathetic systems. So, we also wanted to assess the effect of vitamins C and E on heart rate variability parameters representing sympathetic or parasympathetic dominance in rats. We assess the R-R interval, the standard deviation of RR, total power, and power of high frequency (HF) and low frequency (LF). We found no significant difference between the groups in RR Interval, SDRR, Total power, LF, HF, and LF/HF ratio. **(Table:1 and Figure:4)**

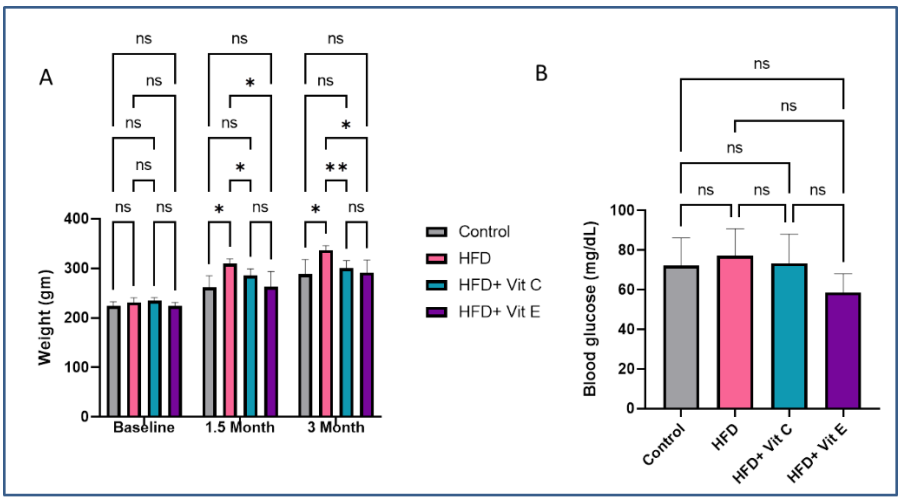

Figure 1. Vitamin C and E supplementation ameliorate high-fat diet-induced body weight and fasting blood sugar. A)

Statistical comparison of body weight: a significant effect of time P=0.0016 and dietary supplement on body weights P<0.001; n=6. B) No significant effect of HFD and dietary supplements on fasting blood glucose P=0.1117. All data are represented as mean±SD, analyzed by one-way ANOVA and Tukey's post hoc multiple comparison tests. *p<0.05; **p<0.01; ***p<0.001. HFD: High Fat Diet; HFD+C: High Fat Diet supplemented with Vitamin-C; HFD+E: High Fat Diet supplemented with Vitamin-E.

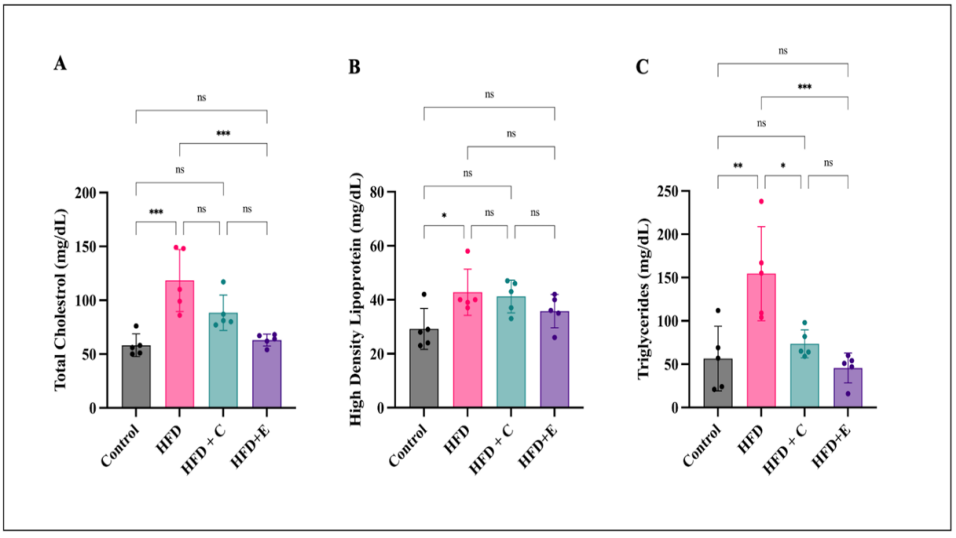

Figure 2. Vitamin C and E supplementation ameliorate High Fat Diet-induced dyslipidemia. A.

Statistical comparison of Total Cholesterol levels (mg/dL) ($p< 0.001$; n=5). **B.** Statistical comparison of HDL ($p=0.03$; n=5). **C.** Statistical comparison of Triglycerides levels (mg/dL) ($p < 0.001$; n=5) All data are represented as mean±SD, analyzed by one-way ANOVA and Tukey's post hoc multiple comparison test. *p<0.05; **p<0.01; ***p<0.001. HFD: High Fat Diet; HFD+C: High Fat Diet supplemented with Vitamin-C; HFD+E: High Fat Diet supplemented with Vitamin-E.

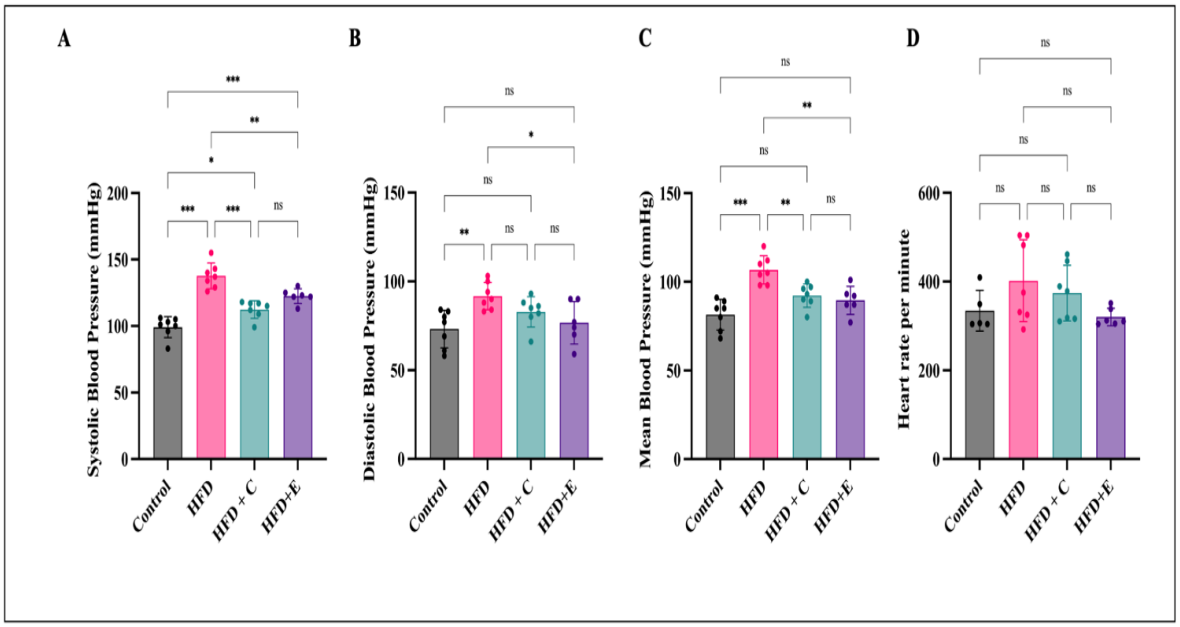

Figure 3. Vitamin C and E supplementation ameliorate High Fat Diet Cardiovascular dysregulations. A.

Statistical comparison of Systolic Blood Pressure ($p< 0.001$; n=7). **B.** Statistical comparison of Diastolic Blood Pressure ($p < 0.01$; n=7). **C.** Statistical comparison of Mean Blood Pressure ($p < 0.001$; n=7) **D.** Statistical comparison of Heart Rate ($p = 0.1202$; n=7). All data are represented as mean±SD, analyzed by one-way ANOVA and Tukey's post hoc multiple comparison tests. *p<0.05; **p<0.01; ***p<0.001. HFD: High Fat Diet; HFD+C: High Fat Diet supplemented with Vitamin-C; HFD+E: High Fat Diet supplemented with Vitamin-E.

HRV parameters	Control	HFD	HFD+ Vit C	HFD+ Vit E	P value
RR Interval (ms)	149.3 (138.8-185.2)	157.4 (150.8-175.8)	146.5 (132-161.2)	145 (134-156.8)	P=0.3759
SDRR (ms)	11.03 (8.7-12.4)	9.656 (4.9-13.01)	12.22 (7.643-16.64)	8.824 (4.841-13.41)	P=0.4037
Total Power (ms^2)	74.58 (33.9-203.3)	76.02 (5.234-269.1)	54.57 (33.64-187.4)	39.25 (24.13-113.8)	P=0.8999
LF (ms^2)	9.918 (7.19-13.45)	10.24 (5.199-13.69)	11.32 (7.751-17.66)	14.84 (10.42-16.76)	P=0.5202
HF (ms^2)	17.68 (12.8-33.47)	11.49 (7.805-23.88)	14.87(9.713-55.19)	30.84(22.06-38.04)	P=0.1733
LH/HF ratio	0.4763 (0.3709-0.7319)	1.062 (0.2043-1.691)	0.6119 (0.3022-1.119)	0.5731 (0.4956-0.6057)	P=0.7803

Table 1: Effect of Vitamin C and E supplementation on HRV parameters on HDF-induced obese rat model

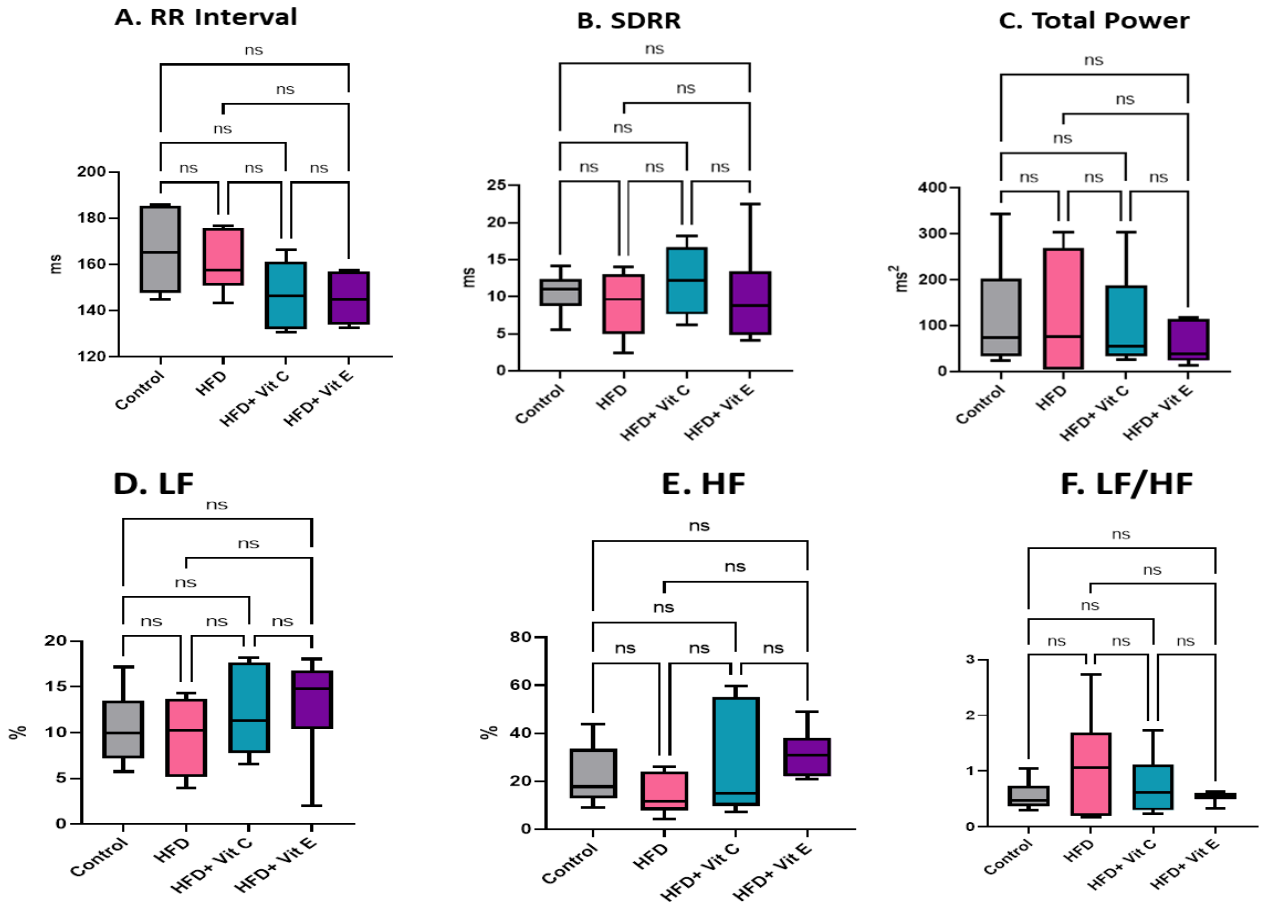

Figure 4. Vitamin C and E supplementation on heart rate variability parameters. A. Statistical comparison of R-R interval (P=0.3759; n=7). **B.** Statistical comparison of SDRR (P=0.4037; n=7). **C.** Statistical comparison of Total Power (P=0.8999; n=7) **D.** Statistical comparison of LF (One-wayANOVA; F (3, 20) = 0.6850, P=0.5202; n=7). **E.** Statistical comparison of HF (P=0.1733; n=7). **F.** Statistical comparison of LF/HF (One-way ANOVA; F (3, 20) = 1.280, P=0.7803; n=7). All data are represented as median (interquartile range), analyzed by the Kruskal-Wallis test and Dunn's multiple comparisons test. *p<0.05; **p<0.01; ***p<0.001. HFD: High Fat Diet; HFD+C: High Fat Diet supplemented with Vitamin-C; HFD+E: High Fat Diet supplemented with Vitamin-E.

4. DISCUSSION

Long-term consumption of a high-fat diet (HFD) is a well-established risk factor for the development of cardiovascular diseases, contributing to dyslipidemia, hypertension, and metabolic dysfunction. Antioxidants such as vitamin C and vitamin E play crucial roles in modulating lipid metabolism and mitigating oxidative stress, both of which are key contributors to hypercholesterolemia and atherosclerosis (Carr et al., 2000a; Cobley et al., 2015; Didier et al., 2023; Thomas & Stocker, 2000). In the present study, we investigated the impact of vitamin C and E supplementation on cardiovascular risk factors in an HFD-induced obese rat model. Elevated cholesterol levels are strongly associated with cardiovascular disease, particularly in populations consuming diets high in saturated fats (Siri-Tarino et al., 2010). Our findings revealed significantly increased total cholesterol levels in HFD-fed rats compared to controls, with vitamin E supplementation effectively lowering cholesterol levels ($p < 0.001$). This aligns with previous reports demonstrating that vitamin E inhibits lipid peroxidation and improves lipid profiles in hyperlipidaemic conditions (Carr et al., 2000b; Choi et al., 2012; Van Dam et al., 2003). Conversely, vitamin C exerted a more modest effect on cholesterol levels, consistent with prior studies suggesting that its primary role is preventing oxidative damage rather than directly influencing lipid metabolism (Dludla et al., 2022). The vitamin C and E treatment groups observed no statistically significant differences. Given that vitamin E is a lipid-soluble antioxidant capable of directly interacting with cholesterol-rich lipoproteins, its greater efficacy in reducing cholesterol may be attributed to this property (Arrol et al., 2000; Suzukawa et al., 1995, 1998; Wang et al., 2024).

We also observed significantly elevated triglyceride levels in the HFD group, which were markedly reduced following vitamin C and E supplementation ($p < 0.05$). These findings underscore the substantial impact of an HFD on triglyceride levels and suggest that while vitamins C and E effectively ameliorate triglyceride elevations, they differ in their mechanisms

of action and overall impact. However, neither antioxidant significantly influenced HDL levels in HFD-fed rats. Given the well- established role of HDL in cholesterol metabolism and cardiovascular protection, the absence of an effect suggests that short-term antioxidant supplementation may not be sufficient to enhance HDL concentrations. This finding aligns with prior research, including a meta-analysis of 14 randomized controlled trials in hypercholesterolemic patients, which reported that at least four weeks of vitamin C supplementation at doses of 500 mg/day significantly reduced total cholesterol and LDL levels but had no impact on HDL levels (Dibaba, 2019; Luo et al., 2021; McRae, 2008; Sahebkar, 2014). Similarly, a cross-sectional analysis of NHANES 2007–2018 data demonstrated a significant negative association between higher vitamin E intake and total cholesterol and LDL but not HDL (Wang et al., 2024). Taken together, our results suggest that vitamins C and E play a role in lipid metabolism but may not directly enhance HDL levels within the timeframe of this study.

In addition to their effects on lipid metabolism, vitamins C and E influenced blood pressure regulation in HFD-fed rats. We observed significantly higher systolic blood pressure (SBP) and mean arterial pressure (MAP) in the HFD group compared to controls, both of which were significantly reduced following antioxidant supplementation ($p < 0.05$). Notably, vitamin E exhibited comparable antihypertensive effects to vitamin C. Similarly, diastolic blood pressure (DBP) was significantly elevated in the HFD group, with vitamin E exerting a more pronounced effect in reducing DBP than vitamin C. These findings agree with previous studies reporting the antihypertensive effects of vitamin E in high-salt-fed and fructose-induced hypertensive rats (Vasdev et al., 2002, 2003, 2005b, 2005a). Although vitamin C did not significantly alter DBP, its role in reducing SBP and MAP underscores the potential of dietary antioxidants in blood pressure regulation. Collectively, vitamin C and E supplementation may serve as effective strategies to mitigate HFD-induced hypertension, emphasizing their potential application in dietary interventions for cardiovascular health.

The pathophysiology of hypertension is multifactorial, involving genetic predisposition, environmental factors, and dietary habits. One key component of cardiovascular regulation is sympathovagal balance, which is often disrupted in obesity. Increased adiposity is associated with heightened sympathetic activity and reduced parasympathetic tone, contributing to autonomic dysfunction. In our study, we observed a trend toward increased low-frequency (LF) and decreased high-frequency (HF) heart rate variability (HRV) in HFD-fed rats, indicative of heightened sympathetic dominance. Although vitamin C and E supplementation improved HRV parameters, these changes did not reach statistical significance compared to control rats. These findings contrast with a study by (Kanthe et al., 2022), which reported significant sympathovagal imbalance in HFD-fed rats after three weeks of dietary intervention. Given the well-established link between high-fat diets, insulin resistance, and systemic inflammation in the development of hypertension (Duan et al., 2018), our results suggest that more extended intervention periods or alternative dosing strategies may be required to improve autonomic function significantly. Previous studies have demonstrated that dietary antioxidants can exert cardioprotective effects by reducing oxidative stress and enhancing endothelial function, potentially contributing to regulating blood pressure and HRV (Młynarska et al., 2024). Future research should optimize the dosages and duration of vitamin C and E supplementation to determine their maximal efficacy in lipid metabolism and blood pressure regulation. Investigating the potential synergistic effects of these antioxidants with other bioactive compounds could further elucidate their mechanisms of action. Additionally, exploring the impact of different dietary antioxidants and their combined effects with vitamins C and E may provide a more comprehensive understanding of nutritional interventions for hypertension and cardiovascular disease prevention.

5. CONCLUSION

This study highlights the beneficial effects of vitamin C and E supplementation in attenuating HFD- induced dyslipidemia and hypertension. Vitamin E demonstrated a more substantial cholesterol- lowering effect, likely due to its lipid solubility, while both vitamins significantly reduced triglyceride levels. Moreover, both antioxidants contributed to the reduction of SBP and MAP, with vitamin E exerting a more significant effect on DBP. Although HRV parameters suggested a trend toward autonomic improvement, these changes did not reach statistical significance. Taken together, our findings support the potential role of dietary antioxidants as adjunctive strategies for mitigating cardiovascular risks associated with high-fat diets, underscoring the need for further investigations into their long-term impact and optimal therapeutic application.

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Authors Contributions

P.K.Y.: Conceptualization; Data Curation; Formal Analysis; Methodology; Supervision; Validation; Project Administration; Original Draft; Review and Editing.

R.K.N.: Conceptualization; Data Curation; Formal Analysis; Methodology; Supervision; Validation; Original Draft; Review and Editing.

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Conflicts of Interest disclosure

The authors declare that they have no conflicts of interest.

Ethics Approval

Ethical approval for all animal experiments was given by the Institutional Animal Ethics Committee Board (IAEC) and the Committee for the Purpose of Control and Supervision on Experiments on Animals (CCSEA), India **(Approval Number: 327/IAEC-1/2021).**

Consent for Publication

Not applicable

Availability of Data and materials

Large datasets will be available to the corresponding author upon request.

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