THE ANATOLIAN JOURNAL OF CARDIOLOGY

Official journal of the TURKISH SOCIETY OF CARDIOLOGY

Modulation of Gut Microbiota Using VSL#3 and Its Impact on Aortic Parameters in a Rat Model

ABSTRACT

Background: The increase in aortic stiffness is a significant parameter of cardiovascular diseases (CVDs), posing a substantial global health challenge and economic burden. The gut microbiota and its homeostasis, directly and indirectly, influence CVD. This study investigated the extent to which alterations in the gut microbiota can affect aortic parameters in a rat model through the administration of VSL#3.

Methods: Twelve male Wistar rats were divided into VSL#3-treated and control groups. Cardiac function, aortic systolic, and diastolic values were assessed via echocardiography on day 0 and day 42, and fecal specimens were simultaneously collected from each rat. The formation and composition of the gut microbial flora were profiled using 16S rDNA gene sequencing.

Results: Differences in bacterial density, as indicated by Chao analysis, exhibited statistical significance ($P = .037$) between the 2 groups. Additionally, in the VSL#3-treated group, significant improvements were observed in aortic systolic and diastolic diameters, as well as in aortic strain parameters, compared to the control group.

Conclusion: This research highlights the potential of gut microbiome modulation, specifically through VSL#3 administration, as a promising strategy to improve aortic parameters, suggesting a novel avenue for cardiovascular health interventions.

Keywords: 16S rDNA, aortic stiffness, metagenome, probiotic

ORIGINAL INVESTIGATION

INTRODUCTION

The alteration in aortic diameter represents a critical clinical condition commonly observed in medical practice, with its pathogenesis attributed to various factors, including inflammation of the aortic wall, initiation of muscle cell apoptosis, breakdown of the matrix, formation of plaques, oxidative stress, and restructuring of the vasculature.[1,2] Aortic dimensions may provide important prognostic information for cardiovascular outcomes such as aortic elastic parameters.[3] In clinical practice, proximal aortic dilatation is an indicator of vascular organ damage.[4] Aortic strain analysis via various imaging methods, notably the simplified transthoracic echocardiography, is recommended as a valuable, noninvasive approach for assessing transverse ascending aortic strain and cardiac function.[5-7]

The main clinical term for the components of arteriosclerosis and atheromatosis is arterial stiffness (AS).[8] Arterial stiffness, indicative of large arteries' capacity to respond to pulse pressure by expanding, is affected by several established atherosclerotic risk elements like aging, smoking, high cholesterol levels (hypercholesterolemia), diabetes mellitus (DM), and hypertension (HT).[9-13] Increased AS or decreased distensibility indicates widespread atherosclerotic involvement in the vascular system.[14,15] In addition to being an indicator of overall mortality, AS also plays a significant role in vascular disorders like renal disease, stroke, dementia, heart failure (HF), and myocardial infarction.[12,14-17] Aortic compliance, AS index, and aortic distensibility are expressions used to measure aortic elasticity, and provide information about vascular stiffness.[18] Tonometry, echocardiography, and magnetic resonance imaging are used to assess AS.[19] The mammalian gut microbiota is composed of colonizing microorganisms in the gastrointestinal

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Received: November 21, 2024
Accepted: February 3, 2025
Available Online Date: March 19, 2025

Cite this article as: Altun M, Küçük U, Yıldırım N. Modulation of gut microbiota using VSL#3 and its impact on aortic parameters in a rat model. *Anatol J Cardiol.* 2025;29(6):282-290.

DOI:10.14744/AnatolJCardiol.2025.5048

tract, influenced by critical aspects such as nutrition, intestinal epithelial cell structure, immunity, and inflammation.[20] Microbiota is influenced by genetic, age, ambient factors, and diseases.[21,22] Imbalances in gut microbiota, called dysbiosis, play a role in the onset of conditions like atherosclerosis, HT, HF, arrhythmia, and cardiac tumors.[23] The gut microbiota and its homeostasis play a role in cardiovascular diseases (CVDs), with inflammation being a key factor in the severity and progression of the disease, while the relationship between gut microbiota, inflammation, and lipid metabolism disorders has been implicated as an underlying contributor to increased cardiovascular risk.[24] The potential effects of gut microbiota on CVDs have been demonstrated.[25,26] VSL#3 is a multi-strain probiotic, classified as a medical food, containing 8 bacterial species: *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus paracasei, Lactobacillus delbrueckii subsp. bulgaricus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, and Streptococcus thermophilus*. The bacterial content in 1 gram of VSL#3 ranges from 112.5 billion to 900 billion colony-forming units (CFU), depending on the product formulation. These strains act synergistically to offer therapeutic benefits by balancing the gut microbiota, enhancing intestinal barrier integrity, and modulating immune responses.[27] VSL#3 has demonstrated the ability to improve tight junction protein function, regulate cytokine expression, and exhibit protective and therapeutic effects in various systemic diseases, including atherosclerosis.[28] VSL#3 has been shown to have beneficial effects on atherosclerosis.[29]

We aimed to investigate the extent to which VSL#3, a probiotic mixture, can induce changes in the composition of the gut microbiota and its potential effects on aortic parameters in a rat model.

METHODS

Animal Experiment Design and Sample Colleciton

A total of 12 male Wistar albino rats (250-350 g) were supplied from the Experimental Research Center. The rats were kept in a controlled environment at 22°C, with a 12-hour light-darkness cycle and free availability of food and tap water. The rats were divided into 2 groups: a control group (n = 6) and an experimental group that received VSL#3 (ACTIAL Farmaceutica SRL, 450 billion CFU per sachet. Available in box of 10 sachets or 30 sachets) via the gavage method. VSL#3, which was dissolved in distilled water, contained the following bacterial species: *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei*, and *Lactobacillus delbrueckii subspecies bulgaricus; Bifidobacterium breve, Bifidobacterium longum*, and *Bifidobacterium infantis*; and *Streptococcus salivarius subspecies thermophilus*. The experimental group received a dose of 10 billion bacteria/kg/day for a duration of 6 weeks. The entire process was closely monitored.[30] The rationale for administering VSL#3 for a 6-week period is based on the fact that prolonged probiotic treatment has been shown to profoundly affect the gut microbiota, with potential beneficial impacts on cardiovascular and metabolic parameters.[31] Both groups of rats were anaesthetised using an intraperitoneal injection of a mixture of ketamine hydrochloride (70 mg/kg) and xylazine (8 mg/kg). At the beginning and the end (42nd day) of the experiment, the cardiac functions, aortic systolic and diastolic measurements of the rats in both groups were examined using a ultrasound system (Horten, Norway) and a GE 12S (5-11 MHz) transducer. M-Mode imaging and the Teicholtz method were used for measurements.[32] Calculated as aortic strain (%) = (aortic diameters at systole-aortic diameters at diastole) / aortic diameters at diastole × 100. The experiments were carried out at the Experimental Research Center under veterinary supervision and were approved by the Committee for Animal Experiments (date: July 29, 2022 and decision number: 2022/07-01). This study did not use artificial intelligence-enabled technologies (such as large language models, chatbots, or image generators). Along with the cardiac measurements, fecal samples of the rats were collected under sterile conditions and stored in sterile tubes at −80°C for genetic studies.

Metagenomic Analysis

DNA Isolation from Fecal Samples

DNA isolation was performed using the QuickGene extraction system (Kurabo, Japan) and the Tissue DNA extraction kit (KURABO, Japan). Initially, 25 mg of the fecal sample was placed in a sample homogenization tube containing 250 µL of model development test solution. To aid homogenization, 15 mg of 0.1 mm zirconium beads were added to the sample tubes, which were then spun down at 5000 rpm for 2 120-second intervals. Following homogenization, 25 µL of proteinase K solution was introduced into the tube and then heated at 56°C for 60 minutes. Subsequently, the samples were centrifuged at room temperature at 15 000 g for 10 minutes. 200 µL of the supernatant was transferred to a sterile 1.5 mL microcentrifuge tube. After adding 180 µL of lysis buffer, the tube was vortexed for 15 seconds and incubated at 70°C for 10 minutes, followed by the addition of 240 µL of 99% cold ethanol and another 15-second vortexing; the entire volume was then transferred to columns and washed 3 times with 750 µL of Washing/Desalting solution. Finally, 200 µL of elution buffer was added to the columns, and genomic DNA was collected in a sterile 1.5 mL microcentrifuge tube with a yield of 50-60 ng. The quality and purity of the DNA were assessed using the Colibri Titertek Berthold and Qubit fluorometer 2.0 (dsDNA HS Kit, ThermoFisher) devices.

HIGHLIGHTS

- This study investigates the effects of gut microbiota modulation on aortic parameters in a rat model.
- VSL#3 supplementation resulted in significant alterations in the gut microbial community composition.
- Statistically significant changes were observed in aortic systolic and diastolic diameters, as well as in aortic strain parameters following VSL#3 treatment.
- The findings support the potential of gut microbiome modulation as a novel approach for improving cardiovascular health.

16S Amplicon Sequencing

The extracted DNA was subjected to amplification using the 16S rDNA V3-V4 primer set (314F-860R). The 16S rDNA in prokaryotic cells' small ribosomal subunit has 10 conserved regions and 9 hypervariable regions. Conserved areas stay uniform across bacteria, while hypervariable sections are specific to genera or species. Hence, 16S rDNA acts as a unique genetic code for species identification and serves as the prime marker for bacterial phylogeny and classification. As a result, 16S rDNA amplicon sequencing is crucial for analyzing microbial communities in environmental samples.

Library preparation involved the use of the Nextera XT DNA Library Preparation Kit and specific Illumina indices. To ensure high-quality data, the generated libraries were cleaned by size selection according to the manufacturer's recommendations (AMPure XP, Beckman Coulter). After library preparation, sequencing was performed using the MiSeq system (Illumina).

Bioinformatics Analysis

Paired-end Illumina reads (2 × 250) were loaded into the Qiime2 system.[33] Initially, it was determined that all samples had a similar depth of coverage at approximately 100X, and no samples were excluded at this stage. Quality filtering and chimera detection were performed using the DADA2 algorithm in Qiime2 (via q2-dada2).[34] Regions with quality scores mostly below 30 were excluded, generating amplicon sequence variants (ASVs). The resulting ASVs were aligned with the GreenGenes (/greengenes.lbl.gov) database to generate taxonomic tables.[35,36] For data visualization and bioinformatics analysis, the files generated in Qiime2 were processed using the R programming language in RStudio.[37,38] Alpha diversity and beta diversity analyses, as well as within-group and between-group differences, were assessed using 3 different indices: Chao1, Shannon, and Simpson. Between-group *P*-values were calculated using the Kruskal–Wallis[39] (KW) test. Taxonomic distinctions in abundance between groups were determined using the DeSeq2 package.[40] The most significant differences between groups were identified using LEfSe (linear discriminant analysis effect size) analysis with a linear discriminant analysis (LDA) score threshold of 4 or higher.[41]

Statistical Analysis

For alpha diversity, KW analysis was employed, while PERMANOVA was utilized for beta diversity assessment using the adonis function in the vegan R package. Alpha diversity indices (Shannon, Simpson, and Chao1) were calculated using the phyloseq and microbiome packages in R. Beta diversity indices, including PCoA, were generated using the phyloseq package, while Adenism and Adenosis indices were calculated using the ade4 package in RStudio. Kruskal–Wallis and Wilcoxon tests, along with LDA, were applied for LEfSe analysis. The LEfSe analysis in this study was performed using the microbiomeMarker package in R with the following parameters: discriminant score threshold 2.0, *P*-value threshold for the KW test: .05, minimum relative abundance threshold: .01. Linear discriminant analysis effect size is an algorithm for high-dimensional biomarker discovery that identifies genomic features (genes, pathways, or taxa) characterizing the differences between 2 or more biological conditions. It emphasizes both statistical significance and biological relevance, allowing researchers to identify discriminative features that are statistically different among biological classes. Specifically, the non-parametric factorial KW sum-rank test is used to detect features with significant differential abundance with respect to the class of interest. As a last step, LEfSe uses Linear Discriminant Analysis to estimate the effect size of each differentially abundant feature and rank the features accordingly. Minimum relative abundance: 0.01. Relative abundance calculations for Abundance, Bar Plot, and Krona were performed using total sum scaling. Statistical data review was obtained using SPSS 19.0 (SPSS Inc, Chicago, IL, USA). The Shapiro–Wilk test assessed the distribution of continuous variables. Continuous variables resulting from the analysis are reported as mean ± SD. The *t*-test compared parameters fitting a normal distribution, respectively. *P*-values below .05 were considered statistically significant. The sample size was determined based on previous studies investigating the effects of gut microbiota on cardiovascular parameters in similar rat models. A power analysis was conducted to ensure adequate statistical power (80%) to detect significant differences in aortic parameters, assuming a medium effect size (Cohen's d = 0.5) with a significance level (α) of 0.05. This analysis indicated a minimum requirement of 6 rats per group, and thus 12 rats were included in the study.[42,43]

RESULTS

Cardiac Parameters

Significant changes were observed in the aortic diameters in the VSL#3 group compared to the control group on the 42nd day (Table 1). Specifically, aortic diastolic diameter showed a significant difference between the 2 groups. The control group exhibited an increase from 1.69 ± 0.13 mm to 1.93 ± 0.11 mm (*P* = .010), while the VSL#3 group showed a smaller increase from 1.58 ± 0.16 mm to 1.60 ± 0.20 mm. This difference between groups indicates that VSL#3 treatment may have a moderating effect on aortic diastolic diameter, potentially suggesting less vascular remodeling in the VSL#3 group compared to the control group.

Similarly, aortic systolic diameter was also significantly different between the groups on day 42. The control group

Table 1. The Aortic Systolic and Diastolic Measurements in the Control and VSL#3 Groups at Baseline and on Day 42

	Control	VSL#3 group	*P*
Aortic Diastolic Diameter (mm)			
Basal measurements	1.69 ± 0.13	1.58 ± 0.16	.110
Day 42 measurements	1.93 ± 0.11	1.60 ± 0.20	.010
Aortic Systolic Diameter (mm)			
Basal measurements	2.22 ± 0.10	2.21 ± 0.99	.750
Day 42 measurements	2.66 ± 0.22	2.33 ± 0.29	.020

Continuous variables are expressed as mean ± SD. A *t*-test was used for comparing normally distributed parameters.

showed an increase from 2.22 ± 0.10 mm to 2.66 ± 0.22 mm (P = .020), whereas the VSL#3 group showed a more modest increase from 2.21 ± 0.99 mm to 2.33 ± 0.29 mm (P = .750 for basal measurements, P = .020 for day 42). The smaller increase in the VSL#3 group suggests that VSL#3 treatment may help to limit systolic expansion, which could have beneficial implications for maintaining arterial function and reducing the risk of cardiovascular events.

These findings suggest that VSL#3 may influence vascular parameters, particularly by limiting the extent of changes in both aortic systolic and diastolic diameters. The changes in these parameters are critical, as they reflect the vascular remodeling process that can contribute to cardiovascular disease progression. VSL#3 may thus have a protective effect on vascular health, possibly reducing the risk of conditions such as hypertension and atherosclerosis.

Regarding aortic strain, a significant improvement was observed in the VSL#3 group compared to the control group after 42 days of treatment. At baseline, aortic strain was similar between the 2 groups (2.3 ± 0.3 in the control group and 2.7 ± 0.4 in the VSL#3 group, P = .130). However, by day 42, a significant difference emerged. The VSL#3 group exhibited a considerable increase in aortic strain from 2.7 ± 0.4 to 3.5 ± 0.5 (P = .010), while the control group showed a slight decrease in strain from 2.3 ± 0.3 to 2.1 ± 0.1 (P = .010) (Table 2). These results suggest that VSL#3 treatment may significantly enhance aortic elasticity, which is a crucial parameter for maintaining healthy arterial function and overall cardiovascular health.

The observed increase in aortic strain is particularly important because it reflects the arterial walls' ability to expand and contract in response to blood flow. Enhanced aortic strain is associated with better vascular health and lower cardiovascular disease risk. This result further supports the potential of VSL#3 in improving cardiovascular parameters, especially by promoting arterial elasticity and reducing the risk of conditions such as hypertension and atherosclerosis.

Table 2. The Aortic Strain Measurements in the Control and VSL#3 Groups at Baseline and on Day 42

Aortic Strain	Control	VSL#3 Group	*P*
Basal measurements	2.3 ± 0.3	2.7 ± 0.4	.130
Day 42 measurements	2.1 ± 0.1	3.5 ± 0.5	.010

Continuous variables are expressed as mean ± SD. A *t*-test was used for comparing normally distributed parameters.

Gut Microbiota Composition

DNA extraction was successfully carried out, yielding uniform DNA concentrations ranging from 18.5 to 212.5 ng/µL for all samples. The bacterial sequencing preparation (V4) was also successful, with read counts ranging from 31 574 to 221 386 after quality control and bioinformatic processing.

The gut microbiota structure was analyzed using the Illumina MiSeq PE250 sequencing platform. A total of 24 fecal samples were collected from the control and VSL#3 groups on days 0 and 42. Taxonomic composition at the phylum level revealed that *Bacteroidetes* (ranging from 54.66% to 65.37%), *Firmicutes* (ranging from 32.64% to 42.36%), and *Proteobacteria* (ranging from 1.14% to 2.22%) collectively accounted for over 80% of the total bacterial community in the gut (Figure 1). Figure 1 illustrates the taxonomic composition at the genus level, including *Prevotella* and *[Prevotella]* as well as *Ruminococcus* and *[Ruminococcus]*. To provide additional clarity on the taxonomic distinctions and their implications, an annotated version of Figure 1 is provided in Supplementary File 1.

A significant variation in bacterial density, as assessed by Chao analysis, was found between the 2 groups (P = .037), as shown in Figure 2A. Furthermore, beta diversity analysis using Adonis (unweighted; P = .052) indicated slight dissimilarities between the groups. Linear discriminant analysis effect size analysis further confirmed statistically significant differences between the groups on day 0 (P = .046) and day 42 (P = .015), as illustrated in Figure 2B with cladograms. The relative abundance (%) of bacterial genera and the differences in mean proportions (%) between groups with their corresponding LDA scores were also evaluated. At day 0, YRC22, *Clostridium*, and *Anaerovibrio* were significantly enriched in control group (LDA > 3; P < .05). At day 42, YRC22 and *Prevotella* were significantly enriched in control group (LDA

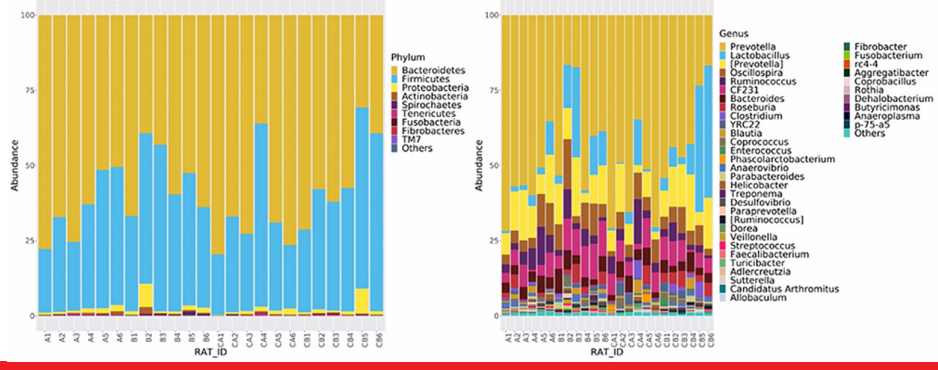

Figure 1. Relative abundance of total ASVs at the phylum and genus levels in fecal samples of rats. Groups include: A1-A6: VSL#3 treatment group on day 42; B1-B6: VSL#3 treatment group on day 0; C_{A1}-C_{A6}: Control rats on day 42; C_{B1}-C_{B6}: Control rats on day 0. Statistical analysis of the taxonomic composition was performed using the KW test (P < .05) to identify significant differences between groups.

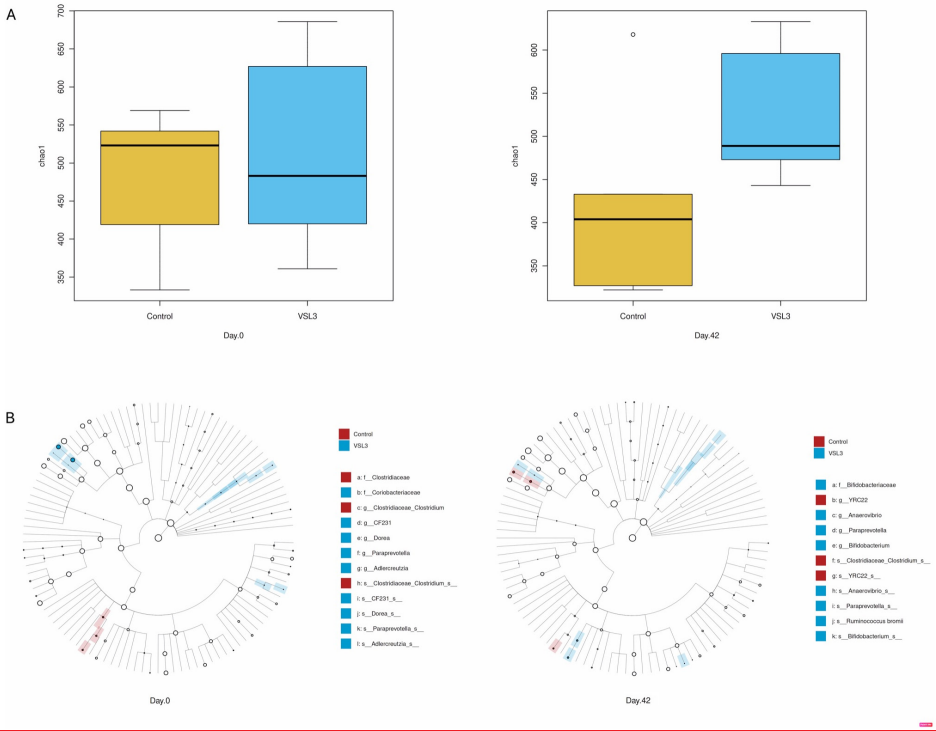

Figure 2. (A) Alpha diversity of each group at day 0 and day 42 revealed by Chao index. Statistical significance between groups was assessed using the KW test (*P* < .05). (B) Features that differentiate between the control and VSL3 treatment groups at both day 0 and day 42, as visualized in a cladogram using LEfSe analysis. Linear discriminant analysis effect size analysis identified significant taxa with an LDA score > 4 (*P* < .05).

> 4, *P* < .05). *Ruminococcus*, *Helicobacter*, and *Anaerovibrio* were significantly enriched in the VSL#3 group (LDA > 3, *P* < .05) (Figure 3).

DISCUSSION

The microbiota residing in the gut significantly influence host physiology, contribute to pathological conditions, and help maintain the delicate balance of the intestinal immune system.[44] In this study, alterations in the structure and composition of the intestinal microbial community due to VSL#3 probiotic treatment in a rat model were determined using 16S rDNA genomic sequencing. Furthermore, the impact of these changes on aortic elasticity parameters was investigated.

The primary composition of the gut microbiota community involves 5 principal bacterial phyla: *Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia*.[45] The prevalent bacterial phyla in this study were found to be *Bacteroidetes, Firmicutes, Proteobacteria*, and *Actinobacteria*, in that order. In these experimental results, no statistically significant change was observed in the bacterial phylum ratios between VSL#3 and the control group. The fecal microbiota structure of rats was analyzed using the Illumina MiSeq PE250 sequencing platform. Statistically, there were no significant differences observed between the control and VSL#3 treated cohort in terms of bacterial diversity and abundance in alpha diversity indices such as Shannon and Simpson, as well as in beta diversity indices assessed through PCoA, PCA, Adenism, and Adenosis indices. On the other hand, a significant difference in bacterial abundance between the 2 groups was detected in the Chao index. It is possible that VSL#3 treatment increased the richness of the gut microbiota without significantly altering the diversity or abundance of individual bacterial populations. Nevertheless, it is crucial to recognize the constraints of this study. A potential avenue for addressing these limitations could be the administration of higher doses of VSL#3 probiotics to rats for an extended duration.

The notion that increasing beneficial bacteria in the host's gut can yield favorable outcomes has led to the application of probiotics. Following probiotic administration, a meta-analysis of 14 studies published between 2002 and 2019, involving 846 cases diagnosed with hypertension, noted decreases in arterial pressure and blood sugar levels.[44] Another study conducted on spontaneously hypertensive rats demonstrated that probiotics such as *Bifidobacterium breve* and *Lactobacillus fermentum*, administered as lyophilized powders suspended in water via oral gavage at a daily dose of 109 CFU for 8 weeks, were effective in preventing hypertension and endothelial dysfunction. This protective effect was attributed to the regulation of gut microbiota balance, highlighting the role of probiotics in improving vascular health.[46]

In patients with Type 2 DM, it has been found that probiotic and synbiotic supplements have beneficial effects on systolic and diastolic blood pressure as cardiovascular health factors.[47] A study suggests that probiotic supplementation,

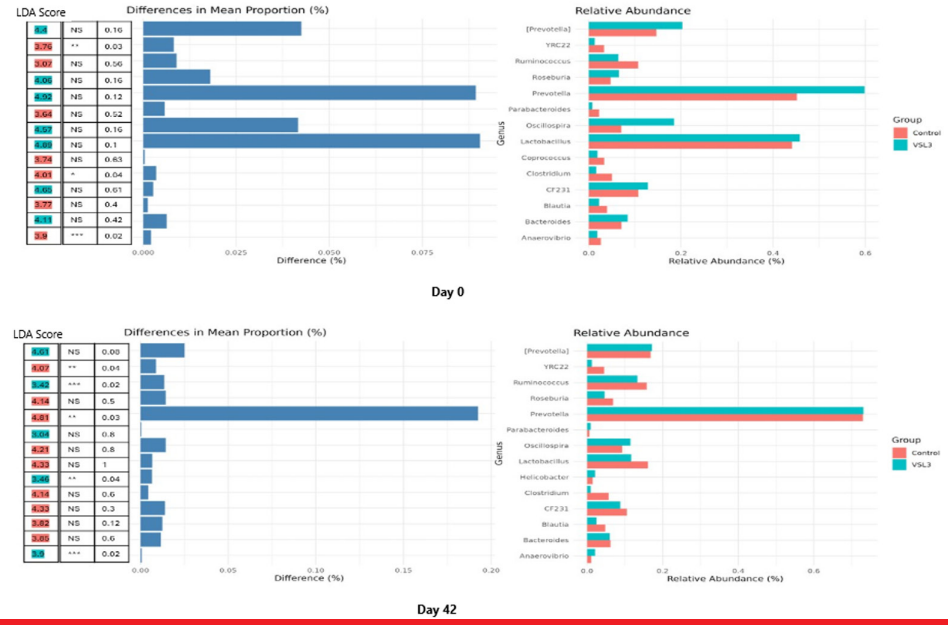

Figure 3. Linear discriminant analysis effect size analysis of the LDA histogram (A) day 0 (B) day 42. Significant taxa with an LDA score > 3. Statistical significance between groups was assessed by the KW test. Significance levels are indicated as follows: **P* = .05-.04; ***P* = .03-.04; ****P* < .03; NS: non-significant.

including *Lactobacillus para casei LPC-37*, *Lactobacillus rhamnosus HN001*, *Lactobacillus acidophilus NCFM*, and *Bifidobacterium lactis HN019*, could serve as a safe complementary strategy to enhance cardiometabolic parameters in hypertensive women. Among hypertensive women, supplementing with probiotics led to a minor decrease of around 5 mm Hg in systolic blood pressure and approximately 2 mm Hg in diastolic blood pressure, although these changes were not statistically significant.[48] In the light of all these data, atherosclerotic burden and endothelial dysfunctions are subject to change as a result of various factors. In this study, aortic systolic and diastolic parameters in the VSL#3 group showed positive remodeling over time, and it would be a correct hypothesis to say that VSL#3 has a positive effect on this remodelling. The administration of VSL#3 (50 billion bacteria/kg body weight per day) for 7 weeks was found to prevent endothelial dysfunction in the mesenteric arteries of common bile duct ligation rats. This effect is likely associated with the improvement of vascular oxidative stress, which may result from the reduction of bacterial translocation and modulation of the local angiotensin system.[49] In a study conducted on ApoE−/− mice, a model of genetic dyslipidemia, low-grade inflammation was induced in the intestine and mesenteric adipose tissues using a low concentration of dextran sulfate sodium. The efficacy of VSL#3 probiotics, administered at a volume of 25 × 10^8 CFU/mouse/day for 12 weeks (6 days per week), was evaluated. The findings reported that VSL#3 hindered the development of histological features of mesenteric adipose tissue inflammation, inhibited steatohepatitis, and reduced the size of aortic plaques.[29] In another study, the administration of VSL#3 probiotic (2.78 × 1011 CFU/day) to ApoE−/− mice fed a high-fat diet for 12 weeks, by adding it to their drinking water, resulted in significant therapeutic effects, including the reduction of proinflammatory adhesion molecules, plaque rupture risk factors, vascular inflammation, and atherosclerosis. These effects were found to be comparable to those of the positive control, telmisartan (1 mg/kg/day), a well-established drug known for its beneficial impact on cardiovascular health.[28] In the study performed by Salim and colleagues, acute intestinal ischemia/reperfusion injury (AII/R) was induced in mice using superior mesenteric artery occlusion. VSL#3 probiotics (3 mg/mL in 100 µL PBS, 1.35 × 10^3 CFU/day) were administered via gavage. The results showed that VSL#3 significantly reduced AII/R-induced tissue inflammation and damage. A 2-week course was more effective than a 3-day treatment. These findings suggest that VSL#3 probiotics have protective effects against AII/R, with treatment duration playing a key role in efficacy. These results highlight the potential of probiotics in managing ischemia/reperfusion injury.[50]

It has been reported that long-term kefir supplementation leads to improvement in the gut, resulting in the alleviation of high blood pressure.[51] Furthermore, in apoE-/- mice fed a fat-rich diet, supplementation with *Lactobacillus rhamnosus GR-1* was found to reduce oxidative stress and inflammation, leading to a decrease in atherosclerotic lesion size.[52] Pathogenic bacterial strains in the gut produce various harmful substances, including trimethylamine-N-oxide (TMAO) and endotoxin (LPS [lipopolysaccharide]). Lipopolysaccharide produced by the gut microbiome has been closely linked to aortic stenosis, stimulation of interstitial valve cells, inflammation, and the immune response.[53] The root mechanisms through that VSL#3 exerts its effects likely involve the modulation of gut microbiota composition. By promoting beneficial bacterial strains and inhibiting Trimethylamine (TMA) producing bacteria, VSL#3 may reduced the precursor molecules available for TMAO

production. In the study conducted by O'Morain et al,[54] the probiotic bacterium *Lactobacillus plantarum CUL66 (Lab4P)* was observed to attenuate several processes associated with atherosclerosis. The administration of VSL#3 to mice induced colitis by exposure to sodium dextran sulfate resulted in reduced levels of COX_2, iNOS, TNF-α, and IL-6 in the colon.[55] Scientific studies have demonstrated the effectiveness of VSL#3 supplementation in mitigating inflammation and symptoms.[56,57]

Through genomic sequencing and metagenomic analysis methods, the potential effects of the gut microbiota on CVDs are being revealed.[25,26] In a study conducted by Liu et al,[58] differences were observed in the beta diversity of gut microbiomes between patients with cardiac valve calcification (CVC) and coronary artery disease (CAD). Within the CVC group, *Veillonella dispar, Bacteroides plebeius*, and *Fusobacterium* increased, while *Collinsella aerofaciens, Megamonas, Enterococcus, Megasphaera, Dorea*, and *Blautia* decreased. Furthermore, in correlation with dyslipidemia, 7 operational taxonomic units (*Parabacteroides distasonis, Megamonas, Fusobacterium, Bacteroides sp., Bacteroides plebeius, Lactobacillus,* and *Prevotella copri*) were identified as potential contributors to CAD.

Study Limitations

This study was conducted in a rat model, which provides valuable insights into the potential role of gut microbiota modulation in cardiovascular health. However, inherent species differences between rats and humans limit the direct translatability of these findings to clinical practice. While the results offer preliminary evidence, additional investigations are required to confirm their applicability in human populations. Future studies with larger cohorts would help to establish more robust conclusions. Moreover, this study focused primarily on aortic diameter and strain values, without evaluating other critical cardiovascular parameters such as heart rate, vascular resistance, or left ventricular function. These aspects are important for a comprehensive understanding of the effects of VSL#3 on cardiovascular physiology. Lastly, the study was conducted in a healthy rat model, without incorporating a disease model that mimics human cardiovascular pathologies. Exploring the impact of VSL#3 in disease conditions such as HT, DM, or atherosclerosis would provide a deeper understanding of its therapeutic potential. Future research addressing these limitations will be essential to fully elucidate the role of gut microbiota modulation in cardiovascular health and its clinical implications.

CONCLUSION

Cardiovascular diseases continue to represent a significant threat to public health, with rising prevalence and mortality rates contributing to a substantial economic burden. In this study, the effect of VSL#3 probiotic intervention on gut microbiota composition in a rat model was investigated, aiming to reduce inflammation and enhance cardiovascular health. Although many probiotic studies report no significant changes in bacterial diversity or abundance, these findings emphasize the intricate and dynamic nature of the gut microbiota, where even small changes in specific bacterial populations can trigger cascading effects that impact the broader microbial ecosystem. Despite the absence of direct inflammatory marker analyses in this study, the observed changes in aortic parameters, particularly AS, suggest that gut microbiota modulation via probiotics may have a beneficial impact on cardiovascular health. This conclusion aligns with existing research suggesting that gut microbiota, through its effects on inflammation, may be linked to cardiovascular disease progression.

This study is original in its approach, as there is restricted comprehensive analysis addressing the effects of probiotic intervention on cardiovascular parameters mediated by gut microbiota in a mammalian model. These results provide novel insights into the positive impact of VSL#3 on aortic parameters, which are key indicators of cardiovascular health. By demonstrating that the reconfiguration of the gut microbiota through probiotic treatment can influence these parameters, it was suggested that probiotic interventions could play a role in preventing or mitigating the risk of cardiovascular diseases. The findings of this study contribute valuable data to the emerging field of gut microbiome research, providing a foundation for future investigations into gut microbiota modulation as a potential therapeutic target for cardiovascular diseases. Further research, particularly with larger sample sizes, disease models, and direct inflammatory assessments, is essential to fully understand the clinical implications of these findings and their potential for human application.

Ethics Committee Approval: This study was approved by the Local Ethics Committee for Animal Experiments (date: July 29, 2022 and decision no.: 2022/07-01).

Informed Consent: Animal experiments were conducted in accordance with international ethical standards and were approved by the Institutional Animal Care and Use Committee (date: July 29, 2022 and decision no.: 2022/07-01).

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – M.A., U.K., N.Y.; Design - M.A., U.K., N.Y.; Supervision - M.A., U.K., N.Y.; Resource - M.A., U.K., N.Y.; Materials - M.A., U.K., N.Y.; Data Collection and/or Processing - M.A., U.K., N.Y.; Analysis and/or Interpretation - M.A., U.K., N.Y.; Literature Search - M.A., U.K., N.Y.; Writing - M.A., U.K., N.Y.; Critical Reviews - M.A., U.K., N.Y.

Acknowledgements and Fundings: This study was supported by the Çanakkale Onsekiz Mart University Scientific Research Projects Unit (Project ID: TSA-2022-4196).

Declaration of Interests: The authors have no conflicts of interest to declare.

Use of AI for Writing Assistance: The authors declare that AI-assisted technologies were not used in this study.

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SUPPLEMENTARY MATERIAL

The use of parentheses indicates that the bacterial profile nomenclature has not yet received full approval, and the classification remains provisional. These notations suggest that the organisms were previously associated with a particular genus, but as research advances, they may be reclassified or accepted under a different genus. This reflects the evolving and dynamic nature of microbial taxonomy. Similarly, the use of square brackets around genera such as *Prevotella* and *Ruminococcus* denotes temporary taxonomic assignments. Microbiota studies often undergo reclassification processes based on new genetic and phylogenetic data. Square brackets refer to previous nomenclature, indicating that these organisms were once assigned to a specific genus, but the currently accepted genus classification may differ. Therefore, the use of square brackets signals terminological uncertainty and reflects the research community's ongoing efforts to reassign these genera to different genera.

https://www.smartgene.com/customer-area/faqs/280-what-do-square-brackets-around-an-organism-name-mean

https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=2764325