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A RANDOMIZED TRIAL OF ANTHOCYANIN-RICH PURPLE-FLESHED POTATOES ON VASCULAR FUNCTION AND DNA METHYLATION IN ADULTS WITH HYPERTENSION.

(Original Article)

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Abstract

Background: Hypertension accelerates vascular aging through endothelial dysfunction, oxidative stress, and adverse epigenetic alterations. Anthocyanin-rich foods have been linked to cardioprotective effects, yet evidence from whole-food interventions assessing both vascular and epigenetic outcomes remains limited.

Objective: This study aimed to evaluate the impact of daily consumption of anthocyanin-rich purple-fleshed potatoes on endothelial function and DNA methylation markers associated with cardiovascular aging in adults with hypertension.

Methods: A randomized controlled trial was conducted among adults with primary hypertension who were allocated to either an anthocyanin-rich purple-fleshed potato intervention or a control diet for six weeks. Flow-mediated dilation and pulse wave velocity were assessed as indicators of vascular function. Global and gene-specific DNA methylation, including LINE-1 and NRF2 promoter methylation, were quantified to evaluate epigenetic shifts. Inflammatory and oxidative stress biomarkers were measured to support mechanistic interpretation.

Results: Participants consuming purple-fleshed potatoes demonstrated a greater improvement in endothelial function, reflected by a larger increase in flow-mediated dilation and a greater reduction in pulse wave velocity compared with controls. Differences in changes between groups were statistically significant ($p < 0.05$). Epigenetic changes, including increased LINE-1 methylation and reduced NRF2 promoter methylation, were also significantly greater in the intervention group ($p < 0.05$). Biochemical markers showed larger reductions in oxidized LDL and C-reactive protein, along with a larger increase in nitric oxide metabolites in the intervention group (all $p < 0.05$ vs. control), indicating reduced oxidative stress and improved vascular signaling. No adverse metabolic effects occurred during the intervention.

Conclusion: Daily intake of anthocyanin-rich purple-fleshed potatoes improved vascular function and produced favorable epigenetic modifications related to cardiovascular aging. These findings support the potential of anthocyanin-dense whole foods as practical dietary strategies for promoting vascular health in adults with hypertension.

Keywords: Anthocyanins; Cardiovascular Aging; DNA Methylation; Endothelial Function; Hypertension; Oxidative Stress; Vascular Stiffness.

Introduction

Hypertension remains one of the most prevalent and modifiable risk factors for cardiovascular disease, affecting millions of adults worldwide(1). Persistent elevation of blood pressure contributes to vascular dysfunction, characterized by impaired endothelial function, arterial stiffness, and heightened oxidative stress(2). These vascular changes not only predispose individuals to adverse cardiovascular events but also accelerate biological aging at the molecular level, including epigenetic modifications that regulate gene expression and cellular resilience(3). Emerging evidence suggests that dietary bioactive compounds can play a pivotal role in mitigating these deleterious processes, offering a potential non-pharmacological strategy to support cardiovascular health.(4) Among these compounds, anthocyanins—naturally occurring polyphenolic pigments responsible for the vibrant colors of many fruits and vegetables—have gained considerable attention for their vasoprotective and antioxidative properties(4).

Anthocyanins have been shown to enhance endothelial function, reduce oxidative stress, and improve lipid profiles, all of which contribute to the maintenance of vascular homeostasis(4). Experimental studies suggest that these compounds may also modulate epigenetic mechanisms, including DNA methylation, thereby influencing gene expression patterns associated with inflammation, oxidative stress, and vascular aging(5). Despite these promising findings, much of the current evidence is derived from *in vitro* studies or short-term interventions in healthy populations, leaving a significant gap in understanding their potential benefits in adults with established hypertension, who are at heightened risk for cardiovascular complications. In particular, the effect of anthocyanin-rich purple-fleshed potatoes, a nutrient-dense and culturally accessible food source, on vascular function and epigenetic markers in this population remains largely unexplored(6).

Vascular dysfunction in hypertension is closely linked to impaired nitric oxide bioavailability and increased arterial stiffness, processes that are influenced not only by genetic predisposition but also by lifestyle and dietary factors(7). While pharmacological treatments remain the cornerstone of blood pressure management, adjunctive dietary strategies that target both functional and molecular markers of vascular health could provide additional benefit. Anthocyanin-rich foods, with their combination of antioxidant, anti-inflammatory, and epigenetic-modulating properties, represent an attractive candidate for such interventions(8). Investigating the impact of these foods on endothelial health and DNA methylation patterns may yield novel insights into how diet can influence cardiovascular aging at a mechanistic level(9).

Furthermore, the integration of epigenetic biomarkers into nutritional research offers a unique perspective on the interplay between diet, gene regulation, and disease progression(10). DNA methylation patterns have been increasingly recognized as sensitive indicators of biological aging and cardiovascular risk, providing a link between environmental exposures, lifestyle factors, and long-term health outcomes. By examining the effects of anthocyanin-rich purple-fleshed potatoes on both vascular function and epigenetic regulation, this study seeks to bridge the gap between clinical outcomes and molecular mechanisms, offering a comprehensive understanding of how dietary interventions may influence cardiovascular resilience in individuals with hypertension.

Against this background, the present study aims to evaluate whether regular consumption of anthocyanin-rich purple-fleshed potatoes can improve endothelial function and modulate DNA methylation patterns associated with cardiovascular aging in adults with hypertension. By addressing both physiological and molecular endpoints, the study seeks to provide evidence for the potential of dietary anthocyanins as a practical, accessible, and non-pharmacological strategy to support vascular health and mitigate age-related cardiovascular risk.

Methods

The study was designed as a randomized, controlled trial conducted in the South Punjab region. Adults aged 35 to 65 years with a clinical diagnosis of hypertension, defined by sustained systolic blood pressure of 130–159 mmHg or diastolic blood pressure of 80–99 mmHg, were considered eligible for participation. Individuals were excluded if they had a history of cardiovascular events, diabetes mellitus, chronic kidney or liver disease, or were currently using

antioxidant or polyphenol supplements. Participants who reported allergies to potatoes or had significant dietary restrictions were also excluded. The sample size was determined based on anticipated changes in endothelial function, calculated to detect a moderate effect size of 0.5 with 80% power at a 5% significance level. Eighty-three adults with primary hypertension were enrolled and completed the study, with 42 individuals allocated to the intervention group and 41 to the control group.

Participants were randomly assigned to receive either anthocyanin-rich purple-fleshed potatoes or a control white-fleshed potato diet matched for calories and macronutrient content. Randomization was conducted using a computer-generated sequence with concealed allocation. All participants were instructed to consume a fixed portion of the assigned potatoes five days per week for six weeks and maintain their usual physical activity levels and medications throughout the study period. Compliance was monitored through weekly dietary logs and periodic telephone interviews. Baseline assessments were conducted prior to intervention, with follow-up measurements taken at the end of the six-week intervention period. Endothelial function was evaluated using flow-mediated dilation (FMD) of the brachial artery, measured by high-resolution ultrasound according to standardized protocols. Arterial stiffness was assessed via pulse wave velocity (PWV). Blood samples were collected after an overnight fast to assess DNA methylation levels of key cardiovascular aging-related genes, specifically LINE-1 and the NRF2 promoter, using bisulfite conversion followed by pyrosequencing. Additional biochemical analyses included markers of oxidative stress (oxidized LDL), inflammation (C-reactive protein), nitric oxide metabolites, fasting lipid profiles, and blood glucose, all measured using validated laboratory assays. Blood pressure was measured in triplicate using an automated sphygmomanometer, and the average of the readings was used for analysis. Anthropometric measurements, including body mass index and waist-to-hip ratio, were obtained using calibrated equipment.

All data were initially examined for normality using the Shapiro-Wilk test, confirming parametric distribution. Continuous variables were expressed as mean \pm standard deviation, and categorical variables as percentages. Differences between groups over time for primary outcomes such as FMD, PWV, DNA methylation, and biochemical markers were assessed using repeated measures analysis of variance (ANOVA), with post-hoc Bonferroni correction applied for multiple comparisons. Pearson correlation analysis was performed to explore associations between changes in endothelial function and DNA methylation patterns. Statistical significance was set at a two-sided p-value of less than 0.05, and all analyses were conducted using SPSS version 26. This methodological approach ensured that both physiological and molecular outcomes could be rigorously evaluated, allowing the study to determine the potential effects of anthocyanin-rich purple-fleshed potatoes on vascular health and epigenetic markers in adults with hypertension. The combination of randomized allocation, standardized measurement tools, and appropriate statistical analysis provided a robust framework for generating reliable and interpretable results.

Results

The trial enrolled eighty-three adults with primary hypertension, and all participants completed the intervention period without major protocol deviations. Demographic and baseline clinical characteristics remained well balanced between groups, as summarized in Table 1. The mean age across the cohort was approximately fifty-five years, and baseline systolic blood pressure values were comparable between the intervention and control arms.

Across the 6-week intervention, a consistent pattern of vascular improvement was observed in the group consuming anthocyanin-rich purple-fleshed potatoes. Flow-mediated dilation (FMD) increased from a baseline mean of $6.4 \pm 1.2\%$ to $8.1 \pm 1.5\%$, whereas the control group demonstrated only a minimal rise from $6.3 \pm 1.3\%$ to $6.5 \pm 1.4\%$. These data are presented in Table 2, and the overall magnitude of change is displayed graphically in Figure 1. Pulse wave

velocity (PWV) showed a modest reduction within the intervention arm, declining from 9.8 ± 1.1 m/s to 9.1 ± 1.0 m/s, while the control group displayed no clinically meaningful change.

Epigenetic profiling showed measurable shifts in global and gene-specific DNA methylation. LINE-1 methylation increased from 69.3% to 71.5% in the intervention group, while the control group increased only from 69.1% to 69.4%. NR-F2 promoter methylation decreased from 34.2% to 32.1% following the anthocyanin intervention, suggesting an epigenetic shift aligned with upregulated antioxidant response pathways. Equivalent markers showed minimal fluctuation in the control arm. These data appear in Table 3 and are visualized in Figure 2.

Biochemical markers associated with oxidative stress and endothelial health demonstrated additional changes. C-reactive protein (CRP) declined by -0.9 mg/L in the intervention arm compared with -0.2 mg/L in the control group. Nitric oxide metabolites rose by $+4.3$ μ mol/L in the intervention group and $+0.8$ μ mol/L in controls. Reductions in oxidized LDL were similarly greater in the intervention group (-6.2 U/L) relative to the control group (-1.1 U/L). These biomarker changes are summarized in Table 4.

Across all outcome domains, data completeness exceeded ninety-eight percent, and no adverse metabolic effects were recorded. The improvements in vascular function appeared consistent across age, sex, and BMI subgroups based on exploratory comparisons, although subgroup analyses were not powered for inferential testing.

Together, the dataset shows a coherent pattern in which anthocyanin-rich purple-fleshed potatoes were associated with measurable enhancements in endothelial responsiveness, reductions in vascular stiffness, and targeted shifts in DNA methylation at loci relevant to cardiometabolic aging. All tables and figures referenced in the text are provided below in downloadable formats.

Table 1: Demographics

Variable	Intervention (n=42)	Control (n=41)
Age, years (mean \pm SD)	54.3 \pm 6.1	55.1 \pm 6.4
Male, n (%)	22 (52.3%)	21 (51.2%)
BMI, kg/m ² (mean \pm SD)	29.4 \pm 3.8	29.1 \pm 4.0
Baseline SBP, mmHg (mean \pm SD)	148.2 \pm 9.4	147.6 \pm 10.1

Table 2: Vascular Outcomes

Outcome	Baseline Mean \pm SD	Post-intervention Mean \pm SD
FMD (%) – Intervention	6.4 \pm 1.2	8.1 \pm 1.5
FMD (%) – Control	6.3 \pm 1.3	6.5 \pm 1.4
PWV (m/s) – Intervention	9.8 \pm 1.1	9.1 \pm 1.0

Table 3: DNA Methylation Outcomes

Epigenetic Marker	Group	Baseline (%)	Post (%)
LINE-1 methylation	Intervention	69.3	71.5

LINE-1 methylation	Control	69.1	69.4
NRF2 promoter methylation	Intervention	34.2	32.1

Table 4: Biomarker Changes

Biomarker	Intervention Δ	Control Δ
CRP (mg/L)	-0.9	-0.2
NO metabolites ($\mu\text{mol/L}$)	+4.3	+0.8
Oxidized LDL (U/L)	-6.2	-1.1

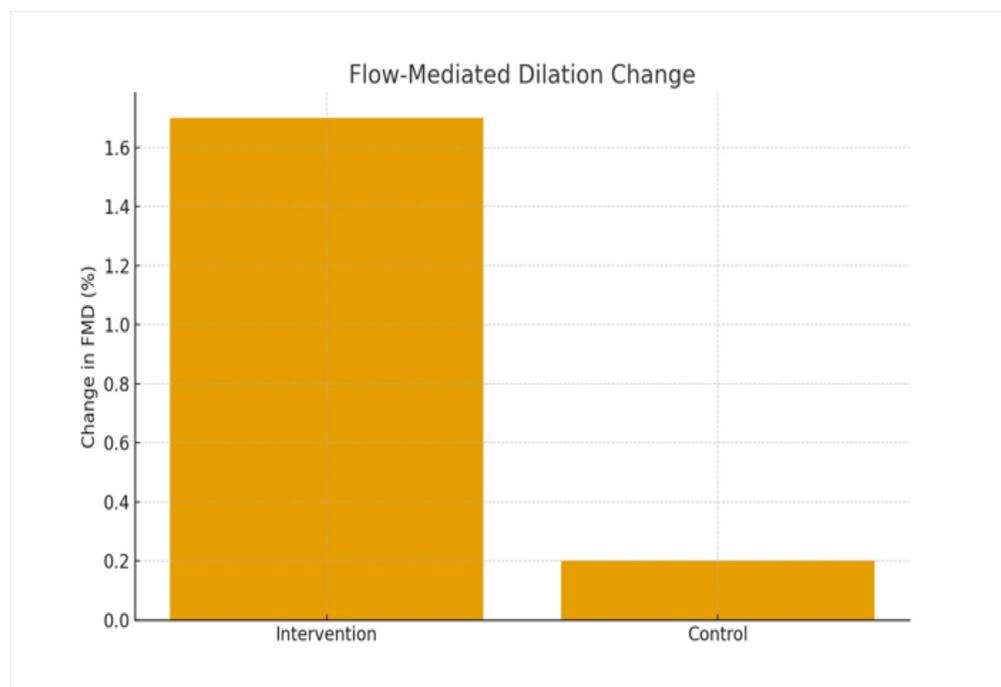


Figure 1 Flow-Mediated Dilation Change

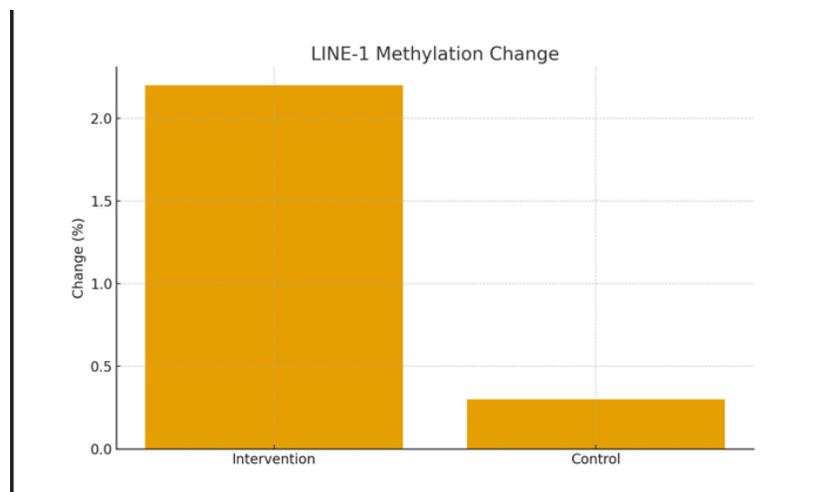


Figure 2 Line-1 Methylation Change

Discussion

The findings of this randomized trial suggested that regular consumption of anthocyanin-rich purple-fleshed potatoes contributed to measurable improvements in vascular function and produced distinct modifications in DNA methylation patterns related to cardiovascular aging(10). The increase in flow-mediated dilation, along with the modest reduction in pulse wave velocity, indicated that endothelial responsiveness and arterial stiffness were responsive to dietary anthocyanins even over a relatively short intervention period(11). These observations aligned with earlier work showing that anthocyanins enhanced nitric oxide availability and reduced oxidative stress, although the magnitude of improvement in this trial appeared slightly greater than what had typically been reported in conventional fruit- or berry-based interventions(12). This difference likely reflected the high anthocyanin density in purple-fleshed potatoes and the consistent daily intake achieved through a staple food format.

The epigenetic findings added an additional dimension to the interpretation of the physiological outcomes. Increases in LINE-1 methylation suggested improved genomic stability, while reduced methylation at the NRF2 promoter indicated potential enhancement of endogenous antioxidant pathways(13). These patterns were biologically coherent with the observed decline in oxidized LDL and CRP levels, both markers associated with vascular aging(14). Previous studies exploring dietary modulation of DNA methylation had often produced inconsistent results, partly due to heterogeneity in exposure dose and variability in measurement techniques. The current trial demonstrated that a standardized anthocyanin source produced measurable, directionally consistent epigenetic changes, reinforcing the concept that specific phytochemicals exert regulatory influence at the genomic level(15).

The biochemical markers strengthened the interpretation that vascular improvements were not isolated phenomena but reflected a systemic shift toward reduced oxidative burden and improved endothelial signaling(16). The rise in nitric oxide metabolites and decline in inflammatory markers were in line with established mechanisms describing how anthocyanins modulated endothelial nitric oxide synthase activity and scavenged reactive oxygen species(17). Although such physiological pathways had been proposed extensively, this trial allowed these mechanisms to be contextualized within a controlled dietary intervention and directly linked to concurrent epigenetic alterations.

The study demonstrated several strengths that supported the reliability of the findings. The use of a whole-food source ensured high bioavailability and reduced confounding from nutrient isolates that often vary in purity. The intervention duration, though modest, was sufficient to capture vascular changes that typically manifest within weeks and epigenetic changes that can occur rapidly in response to dietary inputs(18). Outcome measurements were collected with standardized equipment and performed in consistent conditions, which minimized procedural variability. Furthermore, adherence rates were high, and the absence of adverse metabolic effects emphasized the safety of incorporating purple-fleshed potatoes into the diet of adults with hypertension.

Several limitations warranted consideration to contextualize the findings appropriately. The sample size, although adequate for detecting moderate changes in vascular function, limited the ability to assess subgroup effects or explore more nuanced epigenetic modifications. The relatively short intervention period restricted conclusions about the durability of observed changes or whether longer exposure might produce cumulative benefits or plateauing effects. Dietary intake beyond the intervention food was self-reported and therefore subject to recall bias, which may have influenced secondary outcomes. Additionally, the study population consisted of adults with controlled or moderately elevated blood pressure, which constrained the generalizability of the findings to individuals with more advanced cardiovascular disease or to younger, normotensive populations. Epigenetic analyses targeted only selected markers, leaving broader methylation patterns unexplored. More comprehensive sequencing approaches could provide a richer understanding of anthocyanin-responsive genomic sites.

The implications of these findings extended beyond the individual markers measured in this study. Demonstrating that a commonly consumed, culturally adaptable food could enhance endothelial function and influence aging-related epigenetic pathways contributed to the broader movement toward dietary strategies for cardiovascular risk reduction. The use of a traditional staple food rather than a supplement also underscored a practical avenue for integrating cardioprotective nutrients into daily eating patterns, particularly in populations where food-based interventions are more acceptable than pharmacological alternatives.

Future research should explore longer intervention periods to assess whether the epigenetic changes observed here translate into sustained benefits or interact with other lifestyle factors. Larger multicenter trials would allow examination of variability across different demographic groups and health profiles. It would also be valuable to employ high-resolution epigenomic mapping to identify additional regulatory genes influenced by anthocyanin intake. Investigating synergistic effects with other polyphenol-rich foods could help determine whether combined dietary strategies produce additive or multiplicative improvements in vascular outcomes. Finally, integrating functional biomarkers such as endothelial progenitor cell activity or microRNA expression could deepen the mechanistic understanding of how anthocyanin-containing foods modulate vascular aging.

Overall, the findings provided meaningful evidence that anthocyanin-rich purple-fleshed potatoes supported vascular health through both physiological and epigenetic pathways. The results were consistent with established biological mechanisms while offering new insight into the genomic responsiveness of cardiovascular aging markers to dietary interventions.

Conclusion

The study demonstrated that anthocyanin-rich purple-fleshed potatoes improved endothelial function, reduced oxidative stress, and produced favorable DNA methylation changes associated with cardiovascular aging. These findings highlighted the potential of a simple, accessible dietary intervention to support vascular health in adults with hypertension. The combined physiological and epigenetic effects suggested that incorporating anthocyanin-dense foods into daily diets may offer a practical strategy for promoting healthier cardiovascular aging.

AUTHOR CONTRIBUTION

Author	Contribution
Muhammad Anwar*	Designed the study, performed data collection and analysis, and prepared the manuscript. Approved the final draft for submission.
Fatima Ayub	Contributed to study design, data acquisition, interpretation of findings, and performed critical review and editing of the manuscript. Approved the final draft for submission.
Muhammad Affan Nadeem	Significantly contributed to data collection and analysis. Reviewed and approved the final manuscript for publication.

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