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Preclinical trial of avocado pulp supplementation in an L-NAME model of cardiovascular injury

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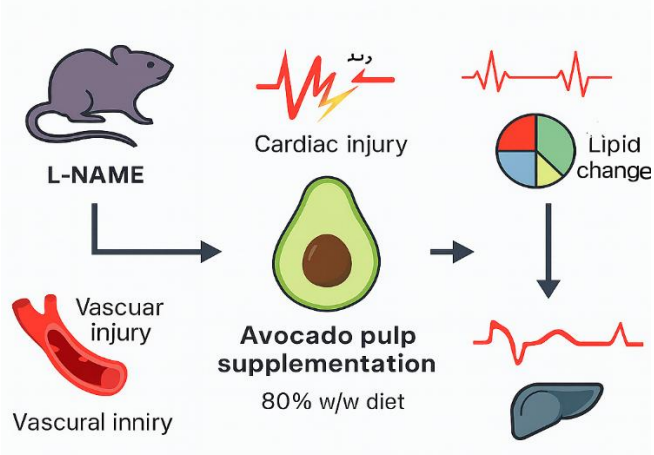
Abstract
Background: Endothelial dysfunction, dyslipidemia, and myocardial injury are major contributors to cardiovascular disease. Avocado (*Persea americana*), rich in monounsaturated fatty acids and phytochemicals, has shown lipid-lowering and anti-inflammatory properties, but its integrated effects on vascular injury remain unclear.
Objectives: The study evaluated the proof-of-concept evidence on the effect of avocado pulp supplementation on multi-system pathological phenotype (metabolic dysfunction, dyslipidemia, myocardial stress and vascular activation) in an L-NAME rat model of cardiovascular injury.
Methods: Male rats were randomized into six groups (n = 4 per group): control (normal chow, water only), avocado (normal chow + avocado + water only), L-NAME, L-NAME+drugs (metoprolol+losartan), L-NAME+avocado, and L-NAME+drugs+avocado (Groups 1 to 6, respectively). Morphometric indices, lipid profiles, cardiac injury enzymes, and vascular biomarkers were measured after treatment. One-way ANOVA with Tukey's test assessed group differences, while contour plots and correlation networks visualized biomarker interactions.
Results: L-NAME treatment to groups 3 to 6 induced a pathological phenotype characterized by reduced feed efficiency (–40%), weight gain (–80%), and BMI (–18%), together with dyslipidemia (LDL +120%, TG +55%, TC +42%, HDL –28%), myocardial stress (troponin +70%, CK +50%, LDH +35%), and vascular activation (endothelin +350%, VCAM-1 +55%, AngII +80%; all $p < 0.01$). Avocado supplementation mitigated these effects: BMI and feed efficiency returned to near-control levels, LDL, TG, and TC fell by 30–45%, and troponin, CK, and LDH decreased by ~25–30%. Endothelin, VCAM-1, and AngII were reduced by 40–55% relative to L-NAME. Network analysis revealed dense pathological correlations under L-NAME (density 0.42), simplified under avocado (0.17), and most normalized with avocado+drugs (0.09), indicating restoration of physiological biomarker independence.
Conclusion: Avocado supplementation attenuates L-NAME–induced vascular injury by improving metabolic efficiency, correcting dyslipidemia, reducing cardiac injury, and dampening endothelial activation, while proposing a potential capacity to shift the pathological biomarker network towards a more control-like organization in such a preclinical proof-of-concept- study.

Keywords: *Avocado, Cardiovascular, endothelial, dyslipidemia*

Graphical Abstract



Introduction

Cardiovascular diseases (CVDs) remain the leading cause of morbidity and mortality worldwide, accounting for an estimated 17.9 million deaths annually (1). Despite advances in pharmacological therapy, including antihypertensives, lipid-lowering drugs, and antiplatelet agents, the global burden of vascular dysfunction continues to rise. Endothelial dysfunction and vascular inflammation are central drivers of this burden, promoting hypertension, atherosclerosis, and organ damage through maladaptive interactions between lipid metabolism, neurohormonal signaling, and cardiac integrity. Identifying interventions that not only target isolated risk factors but also reprogram the interconnected biomarker networks that underpin cardiovascular pathology is therefore an urgent priority (2). Experimental models provide insight into the integrated pathophysiology of vascular injury.

Nω-Nitro-L-arginine methyl ester (L-NAME) is a strong suppressor of nitric oxide synthase (NOS) used for hypertension induction in animal models like rats. Chronic administration of Nω-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, induces hypertension, endothelial dysfunction, and cardiac stress, recapitulating key features of human vascular disease (3). L-NAME–treated animals exhibit dyslipidemia, increased circulating endothelin, and activation of the renin–angiotensin system, along with elevated cardiac enzymes and troponin, all of which reflect vascular inflammation and myocardial injury (4). Importantly, these disturbances do not occur in isolation; rather, they form dense, pathological correlation networks in which lipid abnormalities reinforce vascular activation and cardiac stress (5). This systems-level integration highlights the limitations of single-target therapies and underscores the need for multifaceted interventions capable of reshaping pathological biomarker interactions (6).

Dietary strategies represent a promising avenue for addressing this gap. Epidemiological and interventional studies increasingly support the role of plant-derived foods rich in unsaturated fatty acids, phytosterols, and antioxidants in lowering cardiovascular risk (7). Avocado (*Persea americana*) has emerged as a functional food with demonstrated benefits on lipid profiles, glycemic control, and vascular function (8). Avocado is uniquely rich in monounsaturated fatty acids, fibre, phytochemicals, and bioactive compounds with anti-inflammatory and antioxidant properties (8-11). Clinical studies have shown that avocado consumption can reduce LDL cholesterol, elevate HDL cholesterol, and improve endothelial reactivity (12-14). However, the extent to which avocado supplementation can counteract integrated vascular, cardiac, and metabolic injury in experimental models of endothelial dysfunction has not been comprehensively examined. The study illustrates a nutraceutical, food-based evaluation exploring whole food matrices that maximize synergistic effect compared to isolated extracts (avocado oil and seed) used in previous studies (15,16).

In this study, we used the L-NAME model of vascular injury to test whether avocado supplementation alone or in combination with standard pharmacological therapy could ameliorate cardiometabolic dysfunction. We evaluated morphometric indices, lipid profiles, cardiac enzymes, vascular biomarkers, and integrative outcomes using contour density plots and correlation network analyses. By complementing traditional statistical comparisons with systems-level visualization, we sought to determine not only whether avocado improves individual markers but also whether it remodels the pathological biomarker interactions that characterize vascular injury.

Methodology

Research Design

The study was conducted for nine weeks; one-week acclimatization, hypertension induction and confirmation for four weeks and avocado supplementation experiment lasted for four weeks.

Experimental Design and Animal Housing

Male Wistar rats (8–10 weeks old; 180–200 g) were procured from the Animal Facility of Imo State University and acclimatized for 7 days under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle, 50–60% humidity). Rats had free access to commercial chow (UAC Nigeria Grand Cereals, Jos, Nigeria) and water ad libitum. All procedures complied with the Principles of Laboratory Animal Care (NIH Publication No. 85–23, revised 1996).
Animals were randomized into six groups (n = 4 per group, total 24 rats):
Group1: Control (normal chow, water only without L-NAME)
Group 2: Avocado only (avocado pulp supplementation without L-NAME))
Group 3: L-NAME (vascular injury model without treatment)
Group 4: L-NAME + drugs (metoprolol 25 mg/kg + losartan 20 mg/kg)
Group 5: L-NAME + avocado (80% w/w diet inclusion)
Group 6: L-NAME + drugs (metoprolol 25 mg/kg + losartan 20 mg/kg) + avocado (80% w/w inclusion).

Induction of Vascular Injury and Interventions
Endothelial dysfunction was induced (group 3 - 6) by oral administration of Nω-nitro-L-arginine methyl ester (L-NAME; 40 mg/kg/day in drinking water) for 4 weeks. Rats with systolic BP (tail-cuff) of 170-190mmHg were recruited. Avocado pulp was freshly prepared daily, homogenized, and thoroughly incorporated into standard chow at 80% w/w to ensure uniform intake throughout the feeding period. Standard pharmacological therapy comprised oral metoprolol (25 mg/kg/day) and losartan (20 mg/kg/day). The treatment groups (groups 4-6) therefore received either an avocado-supplemented diet, drug therapy, or their combination alongside L-NAME exposure, while controls received unsupplemented chow and water only (group 1 and 3) and group 2 received avocado, rat chow and water.

Morphometric Assessments
Body weight, feed intake, and feed conversion efficiency (FCE) were measured weekly. Final body weight, body length, and body mass index (BMI; g/cm²) were recorded at sacrifice following 12h overnight fasting.

Body weight: The weight of the rats was determined before treatment and after treatment using an Ohaus Scout series (Model SKX2222) calibrated top-loading digital balance with 0.01 g readability. The rats were placed on the pan for 5-10 seconds until stabilization, with readings accurate to 0.1 g taken on a weekly basis.

Body length: The length of the rats (nose-rump) was measured using a standardized calibrated measuring board (50 cm long) with a metre rule and a moveable ruler headpiece. The rats were placed ventral side down, nose against zero mark, and the body was straightened gently without stretching. The headpiece was pressed lightly against the anal region for nose-rump length. The measurement was read to 0.1 cm.

Lee Index (BMI)
Lee Index = $\sqrt[3]{}$ (body weight g / nose-rump length³ cm) × 10
Normal range: 0.29-0.34 (Wistar rats)
Feed intake: Experimental feed was measured and served *ad libitum* every morning. The next morning, leftovers were weighed. Actual feed consumed was obtained by subtracting the diet served from the leftover.
Feed conversion efficiency was monitored weekly with the formula below:

$$FCE = \frac{\text{Total weight gain (g)}}{\text{Total feed consumed (g)}} \times 100$$

***Blood pressure*:** A tail-cuff system was used to determine blood pressure after a 14-16 h fast on a weekly basis in the morning by 9 AM, following 3 acclimatization sessions over 2 days' pre-measurement to minimize stress-induced variability. The assessor was blinded to group allocation using cage codes. An average of 6 valid readings per rat (20 cycles) was obtained after 5 – 10 minutes of warming and restraint (17). Systolic, diastolic, mean arterial, and pulse pressure were measured.

Induction of Vascular Injury
Hypertension was induced using Nω-nitro-L-arginine methyl ester (L-NAME, 40 mg/kg/day, oral gavage) for 28 days (18). L-NAME administration was selected to model chronic endothelial dysfunction and nitric oxide synthase inhibition, mimicking vascular injury (6,8,11).

Sample Collection
At study termination, animals were anesthetized with pentobarbital (40 mg/kg i.p.), and blood was collected by cardiac puncture. Serum was separated by centrifugation at 3,500 rpm for 15 min and stored at –20 °C until analysis.

Biochemical Analyses
Serum lipid profile was quantified enzymatically using commercial kits: total cholesterol (TC), triglycerides (TG), LDL, HDL, and VLDL (Randox Laboratories, UK). Cardiac integrity was assessed via troponin I ELISA (Eastbiopharm, Hangzhou, China), creatine kinase (CK) activity by the IFCC-recommended method, and lactate dehydrogenase (LDH) activity using a colorimetric kit (Sigma-Aldrich, MAK066). Vascular biomarkers were measured using ELISA kits: endothelin-1, vascular cell adhesion molecule-1 (VCAM-1), and angiotensin II (AngII) (Elabscience, Wuhan, China). These methods are consistent with previously validated protocols in our laboratory (19).

Advanced Analytical Approaches
To interrogate systems-level interactions, we generated contour density plots of pairwise biomarker relationships and constructed correlation networks (nodes = biomarkers; edges = Spearman correlations with $|\rho| \geq 0.7$, $p < 0.05$). Network topology metrics (density, clustering coefficient, average degree) were computed using NetworkX (Python).

Statistical Analysis
Data are presented as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's HSD test was used for group comparisons. Significance was set at $p < 0.05$.

Graphical analyses were performed with GraphPad Prism v10 and Python (Matplotlib, Seaborn).

Results

Morphometric responses to avocado supplementation and pharmacological treatment in an L-NAME–induced vascular injury model

To evaluate the effects of avocado supplementation on overall growth performance, we compared morphometric indices across treatment groups (Figure 1a–f). Feed intake (FI) varied significantly among groups (ANOVA $p = 0.0002$), with the L-NAME group (Group 3) exhibiting the lowest consumption, consistent with impaired metabolic activity. In contrast, avocado-treated groups (Groups 2, 5, and 6) maintained intake levels comparable to controls, suggesting preserved appetite and dietary utilization. Weight gain (WG) was markedly suppressed in L-NAME-treated rats compared with the control and avocado-supplemented groups ($p < 0.01$). In contrast, the combination of avocado with standard drugs (Group 6) partially restored WG to near-control levels. Feed conversion efficiency (FCE) was profoundly reduced by L-NAME (Group 3), indicating impaired nutrient assimilation. Avocado supplementation, alone or in combination with drugs, significantly improved FCE ($p < 0.01$ versus Group 3), underscoring its role in maintaining metabolic efficiency. Final body weight followed a similar pattern ($p < 0.0001$), with L-NAME rats showing the greatest loss, while avocado supplementation mitigated weight decline in both single and combination regimens. Length measurements did not differ significantly among groups ($p = 0.0566$), reflecting that treatment effects were primarily metabolic rather than structural. However, body mass index (BMI) was significantly reduced in L-NAME animals ($p = 0.0002$), with avocado supplementation restoring BMI to levels comparable with control and drug-treated groups. Collectively, these results demonstrate that L-NAME–induced vascular injury is associated with reduced feed intake, diminished weight gain, and impaired nutrient utilization. Dietary avocado supplementation appeared to attenuate these changes, improving FCE, weight, and BMI, with signals of additive benefits when combined with standard pharmacological therapy. In this proof-of-concept model, these findings are consistent with avocado acting as a dietary adjunct that supports morphometric and metabolic integrity under conditions of vascular stress.

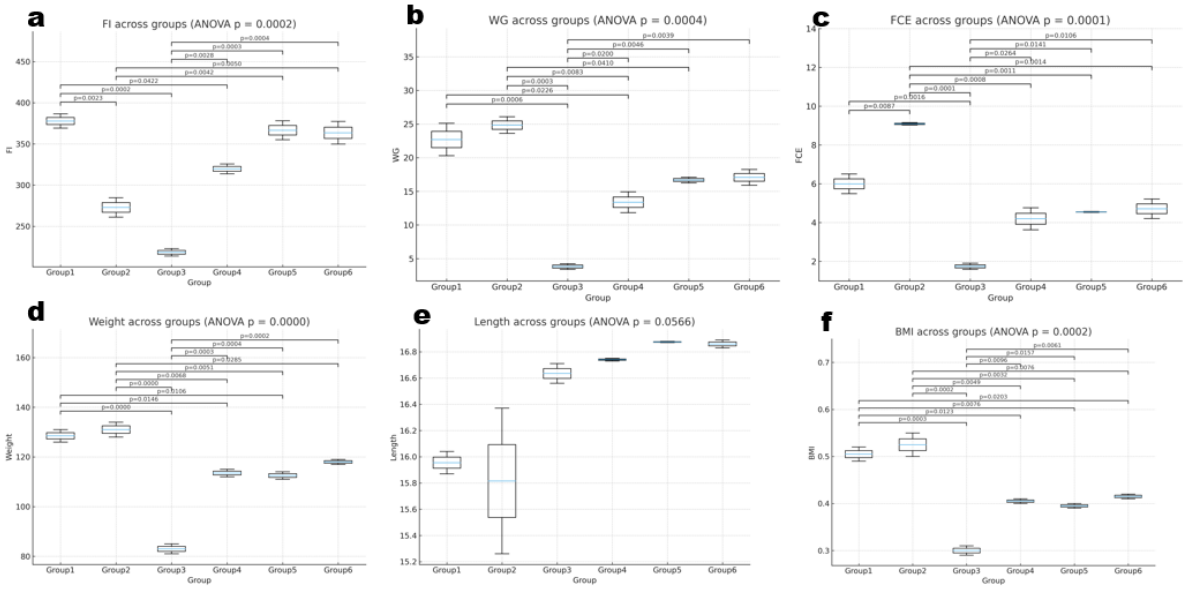


Figure 1. Effects of dietary avocado supplementation and pharmacological treatment on morphometric parameters in L-NAME–induced vascular injury model. Box plots show treatment group distributions (n = 4 per group) for (a) feed intake (FI), (b) weight gain (WG), (c) feed conversion efficiency (FCE), (d) final body weight, (e) body length, and (f) body mass index (BMI). Groups: Group 1 = Control (normal diet); Group 2 = Avocado only; Group 3 = L-NAME; Group 4 = L-NAME + drugs (metoprolol + losartan); Group 5 = L-NAME + avocado; Group 6 = L-NAME + drugs + avocado. Horizontal brackets indicate pairwise group comparisons with p-values derived from Tukey's HSD post-hoc test following one-way ANOVA. Significant group differences ($p < 0.05$) are annotated.

Cardiovascular and lipid profile responses to avocado supplementation and pharmacological treatment in an L-NAME-induced vascular injury model

Heartbeat rate (HBR) was significantly altered across groups (ANOVA p = 0.0013). Rats treated with L-NAME (Group 3) exhibited the highest HBR, consistent with hypertension-induced tachycardia. Avocado supplementation, either alone (Group 2) or in combination with pharmacological therapy (Groups 5 and 6), normalized HBR to near-control levels, demonstrating a cardioprotective effect. Lipid profiles revealed marked dyslipidemia in the L-NAME group. LDL cholesterol was significantly elevated in Group 3 compared with controls ($p < 0.001$), while both avocado-supplemented and drug-treated groups displayed reduced LDL-c concentrations, indicating partial restoration of lipid homeostasis compared with Group 1 normal rats fed rat chow and water. Similarly, VLDL-c and triglyceride (TG) levels were highest in the L-NAME group ($p < 0.01$), with significant reductions observed in avocado-supplemented and drug-treated animals. Total cholesterol (TC) followed the same pattern, with L-NAME markedly elevating TC (p = 0.0038), whereas avocado and combination therapy normalized the values. HDL cholesterol, by contrast, was reduced in the L-NAME group, reflecting impaired reverse cholesterol transport. Although the ANOVA result approached significance (p = 0.0520), avocado supplementation was associated with modest HDL preservation, particularly in Group 6 (L-NAME + drugs + avocado), which showed the highest HDL levels among the treated groups. Overall, these findings indicate that L-NAME induces pronounced dyslipidemia characterized by elevated LDL, VLDL, TC, TG, and reduced HDL. Avocado supplementation effectively countered these disturbances, shifting toward lipid balance either alone or synergistically with pharmacological therapy. The improvement in HBR and lipid parameters towards normal ranges suggests the dual role of avocado in cardiovascular protection, combining hemodynamic stabilization with correction of lipid abnormalities.

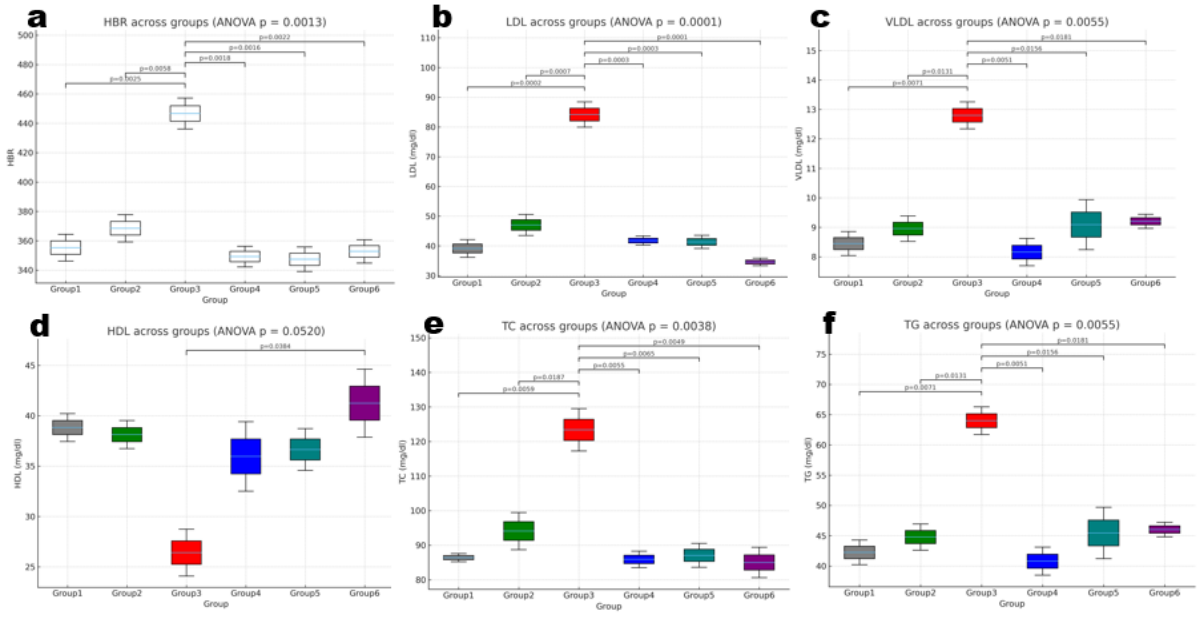


Figure 2. Effects of avocado supplementation and pharmacological treatment on cardiovascular and lipid profile parameters in L-NAME–induced vascular injury model. Box plots show treatment group distributions (n = 4 per group) for (a) heart beat rate (HBR), (b) low-density lipoprotein cholesterol (LDL), (c) very low-density lipoprotein cholesterol (VLDL), (d) high-density lipoprotein cholesterol (HDL), (e) total cholesterol (TC), and (f) triglycerides (TG). Groups: Group 1 = Control (normal diet); Group 2 = Avocado only; Group 3 = L-NAME; Group 4 = L-NAME + drugs (metoprolol + losartan); Group 5 = L-NAME + avocado; Group 6 = L-NAME + drugs + avocado. Horizontal brackets indicate pairwise group comparisons with p-values derived from Tukey's HSD post-hoc test following one-way ANOVA. Significant differences ($p < 0.05$) are annotated.

Effects of avocado supplementation on cardiac integrity and vascular injury markers in an L-NAME–induced vascular injury model

Markers of cardiac damage were strongly elevated in L-NAME–treated rats. Troponin, a sensitive indicator of myocardial injury, was significantly higher in Group 3 compared with controls (p = 0.0017). Avocado

supplementation (Groups 2 and 5) and drug treatment (Group 4) reduced troponin levels, with the combination therapy group (Group 6) showing values approaching baseline. Similarly, creatine kinase (CRT-Kin) and lactate dehydrogenase (LDH) were elevated by L-NAME ($p = 0.0003$ and $p = 0.0064$, respectively), reflecting cardiomyocyte membrane leakage. Both avocado and drug treatments attenuated these increases, with the most pronounced reductions observed in the avocado + drug group. Vascular injury markers followed a comparable trend. Endothelin, a potent vasoconstrictor, was markedly elevated in L-NAME animals ($p < 0.0001$), confirming endothelial dysfunction. Avocado supplementation significantly reduced endothelin concentrations, indicating improved vascular tone. VCAM-1, a key adhesion molecule upregulated during vascular inflammation, was also elevated in L-NAME ($p = 0.0030$). Avocado treatment reduced VCAM-1 levels toward control values, suggesting mitigation of endothelial activation. Angiotensin II (AngII), central to the renin–angiotensin system, was highest in L-NAME–treated rats ($p = 0.0007$). Avocado supplementation, particularly in Groups 5 and 6, lowered AngII concentrations, indicating a dampening of vasoconstrictive and pro-inflammatory signalling. Taken together, these results demonstrate that avocado supplementation provides potential cardioprotective and vasculoprotective benefits. By reducing biomarkers of myocardial injury (troponin, CK, LDH) and attenuating vascular dysfunction (endothelin, VCAM-1, AngII), avocado not only improved markers of cardiac integrity but also reduced endothelial stress, supporting its potential as a dietary adjunct in the management of vascular injury.

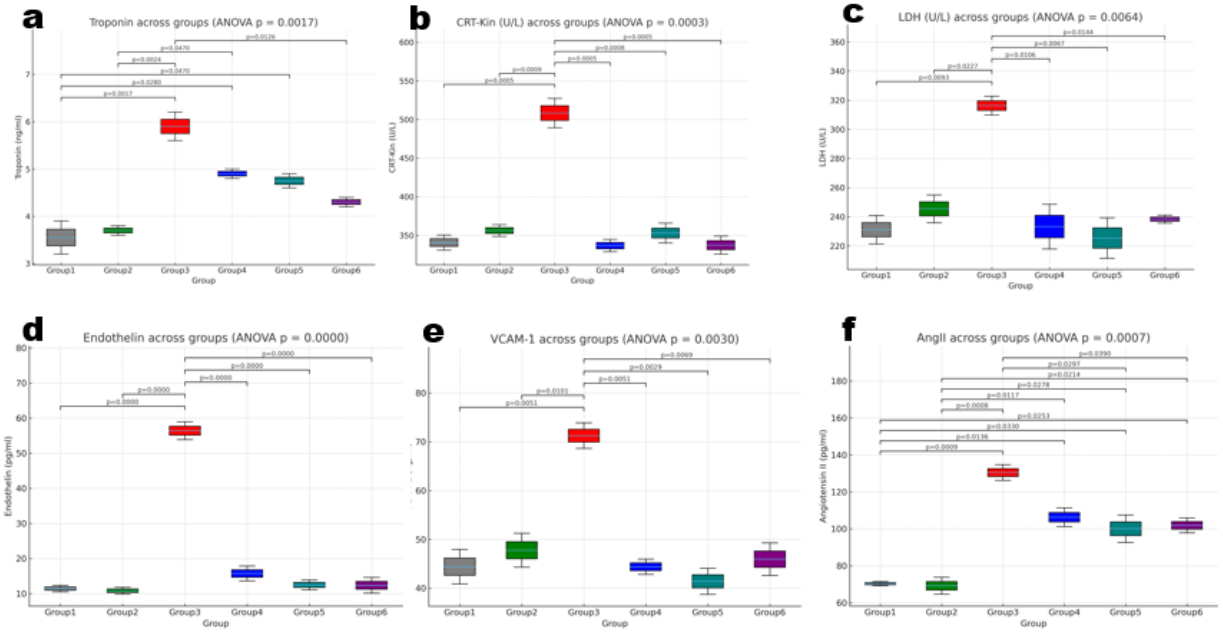


Figure 3. Effects of avocado supplementation and pharmacological treatment on cardiac integrity and vascular injury markers in L-NAME–induced vascular damage model. Box plots show treatment group distributions (n = 4 per group) for (a) troponin, (b) creatine kinase (CRT-Kin), (c) lactate dehydrogenase (LDH), (d) endothelin, (e) vascular cell adhesion molecule-1 (VCAM-1), and (f) angiotensin II (AngII). Groups: Group 1 = Control (normal diet); Group 2 = Avocado only; Group 3 = L-NAME; Group 4 = L-NAME + drugs (metoprolol + losartan); Group 5 = L-NAME + avocado; Group 6 = L-NAME + drugs + avocado. Horizontal brackets indicate pairwise group comparisons with p-values derived from Tukey's HSD post-hoc test following one-way ANOVA. Significant group differences ($p < 0.05$) are annotated.

Pairwise contour analysis of integrated biomarker relationships

To further interrogate how treatment regimens altered biomarker interactions, we applied contour-based visualization of pooled kernel density estimates (KDE) across groups (Figure 4a–d). LDL and TG exhibited a strong positive association, with the highest clustering observed in L-NAME–treated rats (Group 3), reflecting a dyslipidemic phenotype characterized by simultaneous hypercholesterolemia and hypertriglyceridemia. Avocado-supplemented groups (Groups 2, 5, and 6) shifted toward lower-density zones comparable to controls, indicating improvement in lipid parameters toward normal ranges. Heartbeat rate (HBR) versus endothelin demonstrated that L-NAME rats occupied the extreme upper quadrant, consistent with simultaneous tachycardia and endothelial dysfunction (Figure 4b). In contrast, avocado supplementation

reduced both HBR and endothelin levels, with biomarkers contours shifting toward those observed in controls. Cardiac stress biomarkers showed a clear strong co-elevation of troponin and angiotensin II in L-NAME animals (Figure 4c). This clustering was unique to the pathological state, with avocado or drug treatments attenuating the distribution toward lower-density zones. Improved troponin–AngII coupling toward near-control levels suggests potential cardioprotective effects of avocado. Finally, LDL and HDL exhibited an inverse relationship (Figure 4d). L-NAME rats clustered at high LDL and low HDL values, consistent with impaired reverse cholesterol transport. Avocado-containing groups showed a shift toward higher HDL and reduced LDL, again approximating control distributions. Together, these contour plots illustrate how L-NAME–induced dyslipidemia, endothelial dysfunction, and cardiac stress converge, and suggest a potential capacity of avocado supplementation to shift these pathological biomarker interactions toward healthier phenotypic patterns.

VCAM-1 correlations with cardiac injury and lipid markers

We next examined vascular inflammation by analyzing VCAM-1 relationships with cardiac enzymes and lipid parameters (Figure 5a–d). VCAM-1 exhibited strong positive coupling with LDH (Figure 5a) and CK (Figure 5b), particularly in L-NAME–treated animals, consistent with endothelial activation paralleling cardiomyocyte injury. These associations were attenuated in avocado-supplemented groups, which clustered at lower LDH and CK levels, indicating reduced cardiac leakage. Similarly, VCAM-1 showed direct associations with triglycerides (Figure 5c) and total cholesterol (Figure 5d). L-NAME rats were uniquely clustered in zones of high VCAM-1 with elevated TG and TC, reflecting intertwined dyslipidemia and vascular inflammation. By contrast, avocado supplementation, alone or with drugs, shifted distributions toward lower VCAM-1 and lipid values, resembling control patterns. These findings highlight VCAM-1 as a central integrator linking vascular inflammation with both lipid dysregulation and cardiac injury in L-NAME pathology. Avocado supplementation appeared to decouple these pathological relationships, attenuating markers of endothelial activation and shifting biomarker interactions toward more physiological patterns.

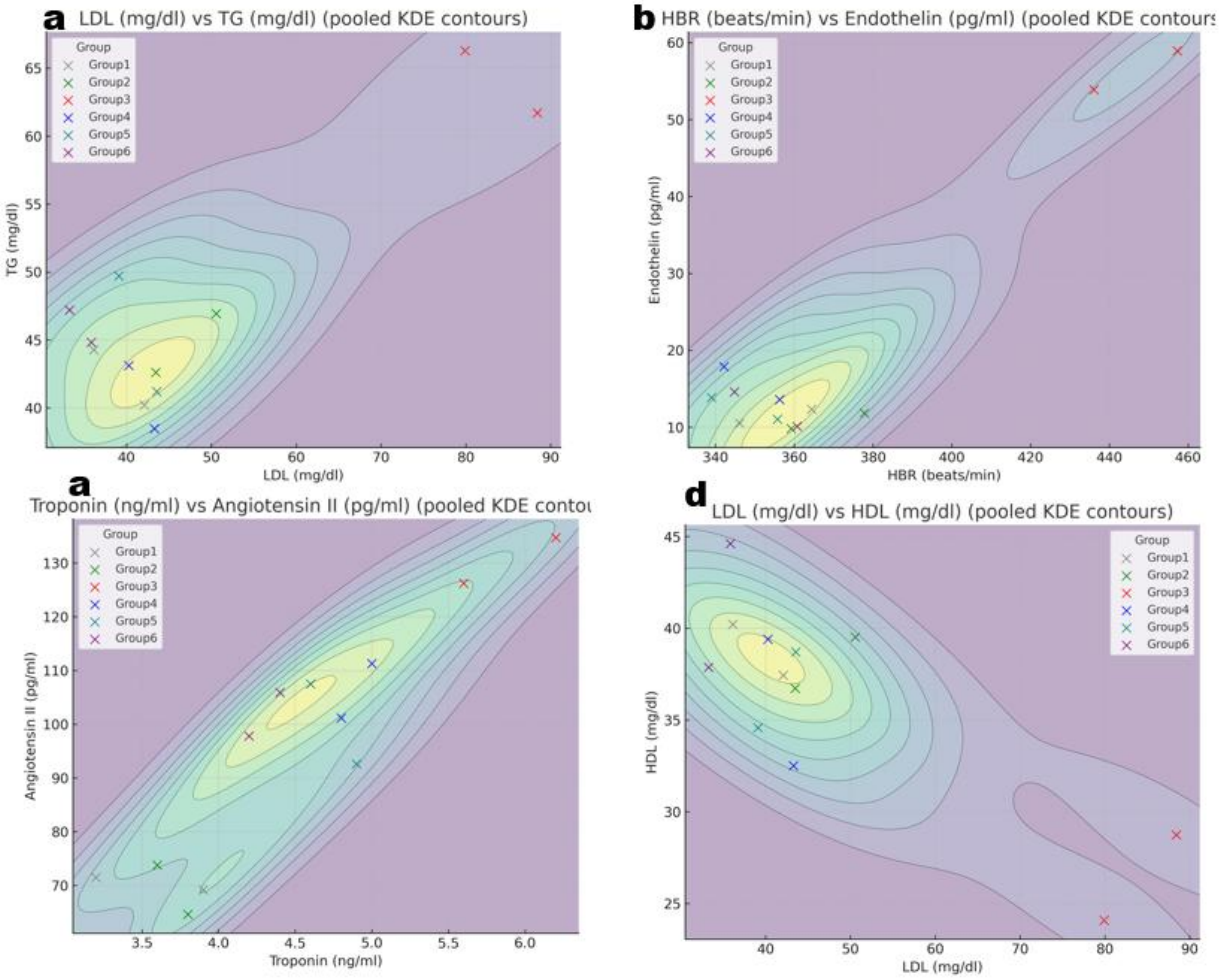


Figure 4. Contour density plots of pairwise biomarker relationships across treatment groups. Kernel density estimation (KDE) contour plots illustrate pooled distributions of selected biomarker pairs with individual group replicates overlaid (n = 4 per group). Panels show (a) LDL vs TG, (b) heart beat rate (HBR) vs endothelin, (c) troponin vs angiotensin II (AngII), and (d) LDL vs HDL. Contour shading represents increasing density of observations, while colored markers indicate group assignments: Group 1 = Control (gray); Group 2 = Avocado only (green); Group 3 = L-NAME (red); Group 4 = L-NAME + drugs (blue); Group 5 = L-NAME + avocado (teal); Group 6 = L-NAME + drugs + avocado (purple). These plots suggest that L-NAME treatment pushes animals into distinct pathological biomarker zones (e.g., high LDL and TG, high HBR with endothelin, elevated troponin with AngII, reduced HDL), whereas avocado supplementation and drug combinations shift the biomarker distributions toward regions overlapping with regions.

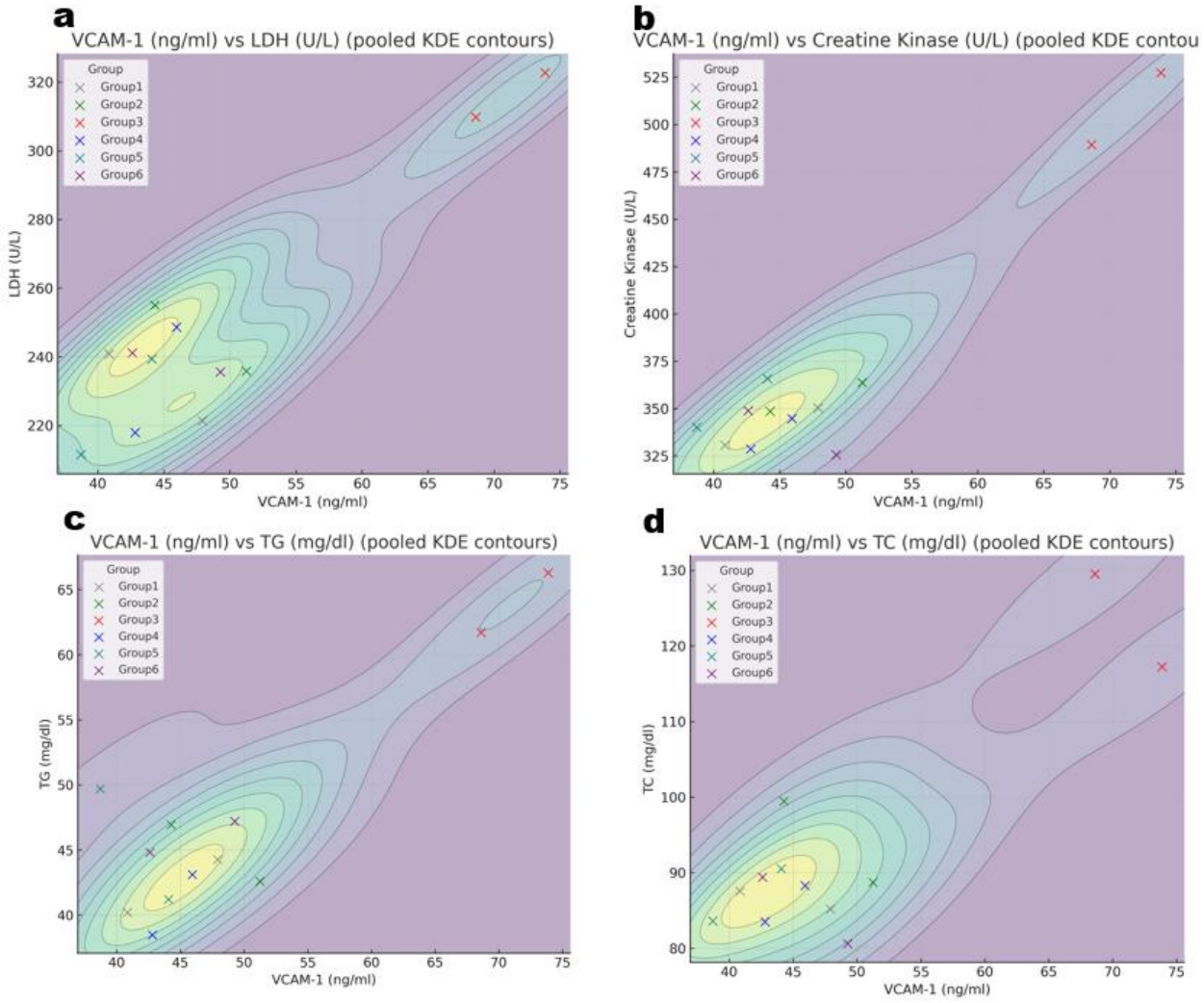


Figure 5. Contour density plots of VCAM-1 in relation to cardiac integrity and lipid markers across treatment groups. Kernel density estimation (KDE) contour plots show pooled distributions of VCAM-1 against selected cardiac and lipid biomarkers, with individual group replicates overlaid (n = 4 per group). Panels display (a) VCAM-1 vs LDH, (b) VCAM-1 vs creatine kinase (CK), (c) VCAM-1 vs triglycerides (TG), and (d) VCAM-1 vs total cholesterol (TC). Contour shading represents increasing density of observations, while colored points denote treatment groups: Group 1 = Control (gray); Group 2 = Avocado only (green); Group 3 = L-NAME (red); Group 4 = L-NAME + drugs (blue); Group 5 = L-NAME + avocado (teal); Group 6 = L-NAME + drugs + avocado (purple). These plots highlight distinct pathological clustering of L-NAME–treated rats (red) at high VCAM-1 levels alongside elevated LDH, CK, TG, and TC, consistent with vascular inflammation, cardiac damage, and dyslipidemia. Avocado supplementation, alone or in combination with drugs, shifted these distributions toward control-like patterns, reflecting attenuation of endothelial activation and improved cardiometabolic balance.

Discussion

This study demonstrates that dietary avocado supplementation ameliorates multiple pathological features induced by chronic L-NAME administration, especially dyslipidemia, endothelial activation, myocardial stress, and altered hemodynamics. Rather than representing isolated biomarker shifts, the observed changes occurred across metabolic, vascular, and cardiac domains, suggesting coordinated physiological effects under nitric oxide synthase inhibition.

L-NAME is widely used to model endothelial dysfunction through inhibition of nitric oxide (NO) production, leading to vascular constriction, inflammation, and secondary metabolic disturbances (17). In this context, avocado supplementation was associated with improvements of lipid parameters, reduction in endothelial activation markers such as

VCAM-1 and endothelin, and attenuation of cardiac injury indicators including troponin and creatine kinase. The concurrent improvement across these domains suggests that avocado may interrupt interdependent processes linking dyslipidemia, endothelial inflammation, and myocardial stress (18). However, because measurements were performed at a single end-point and primarily reflect circulating biomarkers, these findings indicate association rather than confirmed mechanistic reversal of endothelial dysfunction.

The reduction in atherogenic lipoproteins observed with avocado supplementation is consistent with the known effects of monounsaturated fatty acids and phytosterols on lipid metabolism (19). Improved lipid profiles may, in turn, reduce endothelial activation, as oxidized lipoproteins promote VCAM-1 expression and leukocyte recruitment (19,20). The attenuation of angiotensin II and heart rate further suggests modulation of neurohormonal and hemodynamic stress pathways (20-22). While these coordinated improvements support a broad protective effect, we did not directly measure nitric oxide bioavailability, endothelial nitric oxide synthase activity, oxidative stress markers, or vascular reactivity at the molecular level. Therefore, the effect of avocado supplementation on nitric oxide, remains inferential. Future studies incorporating redox profiling and endothelial functional assays would be necessary to clarify these mechanisms.

Vascular inflammation appeared central to the L-NAME phenotype, as VCAM-1 correlated with lipid abnormalities and cardiac injury markers. Avocado supplementation weakened these associations and reduced absolute VCAM-1 levels. Network analyses showed that L-NAME increased correlation density and clustering among pathological markers, whereas avocado supplementation reduced this density and shifted distributions toward patterns observed in control animals. These analyses describe changes in biomarker interrelationships and do not establish causality; nevertheless, they suggest that dietary interventions may influence how pathological processes interact, not merely their individual magnitude. Importantly, this study was conducted using a controlled experimental model with a defined avocado dose. While the dose produced detectable biological effects, direct translation to human dietary intake requires caution. When scaled by body weight, the experimental intake may exceed typical human consumption levels. Despite these limitations, the data support the interpretation that avocado supplementation mitigates experimental vascular injury through coordinated improvements in lipid balance, endothelial activation markers, and cardiac stress indicators (23, 24). Rather than demonstrating definitive systems-level reprogramming, our findings suggest that avocado intake may influence the integrated physiological response to nitric oxide inhibition, reducing the convergence of metabolic, inflammatory, and hemodynamic stress signals (25, 26). In summary, dietary avocado supplementation attenuated multi-domain features of L-NAME-induced vascular dysfunction in this experimental model. These results provide a rationale for further mechanistic and translational studies examining the role of whole-food interventions in modulating endothelial injury and cardiovascular risk.

Conclusion

In summary, this proof-of-concept avocado supplementation trial attenuated several systemic consequences of L-NAME–induced vascular injury, including adverse morphometric changes, dyslipidemia, elevations in cardiac injury markers, and indices of endothelial activation. In addition to improving individual biomarkers, avocado intake was associated with altered interrelationships among metabolic, inflammatory, and cardiac markers, with partial normalization of correlation patterns toward those observed in control animals. These findings suggest that avocado may influence multiple interconnected domains of cardiovascular physiology under conditions of nitric oxide synthase inhibition. However, given the cross-sectional design, modest sample size, and absence of direct mechanistic assays assessing nitric oxide bioavailability or oxidative stress, the results are interpreted as functional associations rather than definitive evidence of systems-level reprogramming. While translational extrapolation requires caution, particularly regarding dietary dose equivalence, these data provide preclinical support for further investigation of avocado as a dietary component that may contribute to cardiometabolic risk modulation. Future studies in larger experimental cohorts and clinical settings will be necessary to clarify mechanisms, dose relevance, and therapeutic applicability.

Limitations and Future directions

This study used avocado inclusion level (80% w/w) that is high for dietary intervention model in humans. This is because the dose is exploratory and intended for proof-of-concept evaluation. Therefore, Clinical translation will require controlled dietary intervention trials in humans at risk for hypertension and dyslipidemia, testing whether avocado-enriched diets confer similar systems-level protection. However, the present findings establish avocado pulp as a potent dietary modulator of vascular and cardiac injury, several questions warrant further exploration. Larger preclinical cohorts are needed to validate these effects with greater statistical power and to delineate dose-response relationships. Mechanistic studies should focus on identifying the

bioactive compounds responsible for avocado's cardioprotective actions, particularly their influence on nitric oxide signaling, lipid metabolism, and inflammatory pathways. Integrative omics approaches-including transcriptomics, metabolomics, and lipidomics could reveal how avocado reprograms molecular networks underlying endothelial function and myocardial stress. Finally, comparative studies with other functional foods may define avocado's unique contributions within cardioprotective dietary patterns. Together, these directions will clarify avocado's potential as a nutraceutical adjunct in cardiovascular prevention and therapy.

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Data Availability Statement
Data are available from the corresponding author upon reasonable request and with permission from the ethical committee.

Ethical Approval
The study was approved by Imo State University Research Ethics Committee with approval number IMSU/ETS/HND/250441.

Conflict of interest
None to declare.

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