ORIGINAL ARTICLE

CHANGES ASSOCIATED WITH DIETARY BEETROOT EXTRACT ON DIET-INDUCED DAMAGE IN THE COMMON CAROTID ARTERY OF THE ALBINO RAT (*RATTUS NORVEGICUS)*

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ABSTRACT

The high-fat high-fructose diet (HFHFr) has become widespread globally corresponding to a rise in obesity and cardiovascular diseases. Traditionally, these diseases were managed by multiple-drug regimens whose adherence was limited by side effects, hence leading to consideration of natural compounds. Recent studies have exploited the phytochemical properties of numerous plant extracts in ameliorating the histological changes seen in common carotid artery (CCA) damage, however, hardly any studies have investigated the effect of beetroot extract. The CCA were obtained from a biobank of a study that induced HFHFr in 45 male rats. Rats in the control group had received standard rat chow and water, and the positive control group received HFHFr. Those in the experimental group received HFHFr and 200mg/kg beetroot extract. These rats were randomly selected, euthanized and perfused on weeks 4,8,10 and 16. Their CCA were harvested, processed and stereological techniques applied to determine the densities of different histological components of the CCA.The HFHFr fed experimental animals revealed medial vascular thickening with a resultant increase in carotid intima-media thickness, increased vascular smooth muscle density, reduced elastic fiber density, and increased collagen fiber density compared to controls. Beetroot co-administration was protective against most of these structural changes. Dietary administration of beetroot extract has been found to be ameliorative to structural changes on the CCA following administration of HFHFr. Therefore, dietary beetroot extract may be indicated to mitigate harmful changes following long term exposure to a high fat high fructose diet.

Keywords: Common Carotid Artery; Beta vulgaris

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INTRODUCTION

The common carotid artery (CCA), being the highway of arterial supply from the aorta to the head, has often faced the brunt of vascular damage due to poor dietary practices (Ratchford & Evans, 2014; Sigala et al., 2018). The high-fat high-fructose diet (HFHFr), in particular, has become widespread globally in recent times (WHO, 2021). This dietary shift has corresponded to a rise in obesity which, once considered a problem only in high income countries, has dramatically risen in low- and middle-income countries (Toklu et al., 2017). The rise has corresponded to an increase in numerous cardiovascular diseases including carotid artery damage, atherosclerosis and aortic stiffening (Hirsiger, 2010; Santana et al., 2014).

Cardiovascular diseases caused by a HFHFr have traditionally been managed by multiple-drug regimens whose adherence has been limited by side effects (Carrizzo et

al., 2020). This has brought attention to natural compounds in the recently expanding nutraceutical field which may be used as alternatives (Grand View Research, 2020; Statista, 2021). These natural substances are known to mitigate harmful effects with minimal side effects as compared to multiple-drug regimens. Recent studies have exploited the anti-oxidative and anti-inflammatory properties of phytochemicals in numerous plant extracts in ameliorating the histological changes seen in carotid artery damage (Kim & Shim, 2019).

The red beetroot is the edible root of the *Beta vulgaris* plant that is rich in inorganic nitrates and phytochemicals, such as belatins, that have anti-inflammatory, antioxidant and chemo-preventive activities (Chhikara et al., 2019; Mirmiran et al., 2020). Such properties make beetroot extract have the potential to influence multiple targets of the damaged CCA and hence an attractive therapeutic tool against the development and progress of vascular disease. However, few studies have explored the ability of beetroot to ameliorate the effects of HFHFr on the histology of the CCA. This study therefore, aimed to investigate the effects of beetroot extract on diet induced damage in the common carotid artery of the albino rat.

MATERIALS AND METHODS

This quasi-experimental study was conducted at the Department of Human Anatomy upon ethical approval from the Biosafety, Animal Use and Ethics Committee, Faculty of Veterinary Medicine. Rodents were used as animal models due to their genetic, physiological, and anatomical similarity to humans, ease of maintenance, short life cycle and their small size (Bryda, 2013). Only specimen from male rats was used since female rats are under the influence of changing hormonal profiles that may affect the common carotid artery.

A sample size of 45 was calculated using a formula from Charan and Biswas, 2013. Five study animals from the intervention and 5 from the positive control groups were euthanized during the 4 harvesting periods (weeks 4, 8, 12 and 16). The control group had 5 animals that were used for baseline results. Specimen was selected from the biobank from study animals from the following 3 groups: Group A rats on HFHFr diet; Group B rats on HFHFr diet + fresh beetroot extract while group C rats, the control groups fed on normal diet composed of standard rat pellets and drinking water ad libitum. The HFHFr diet was prepared as described by Macharia et al., 2019 using saturated fat (20% w/w) added to standard rat chow and containing protein (29.82%), fat (13.43%), carbohydrates (56.74%), fiber (5.3%) and vitamins and minerals in small quantities. Fructose (30%) was added to drinking water of animals under the HFHFr diet. The animals were fed ad libitum. Beetroot juice extraction was done as described by Carrillo et al., 2017 (Carrillo et al., 2017). A fresh beetroot sample (5 g) was placed in a tube and 5 ml of acidic methanol/water mixture (50:50, v/v, pH 2) added. The tube was shaken vigorously for one hour and centrifuged at 2500 g for 10 minutes. The supernatant was separated from the residue. Acetone/water mixture (70:30, v/v) was then added to the residue with a repeat of the shaking and centrifugation steps. The two extracts were then combined and stored at -40 °C. The extract was given every morning via oral gavage at a dose of 200mg/kg.

Specimen from five rats in the control group in the biobank was used for baseline results and thereafter from every five rats from each group that was sacrificed at weeks 4, 8, 12 and 16. The CCAs were harvested, processed and stained using Hematoxylin and Eosin, Weigert's Elastin and Masson's trichrome stains**.** Photomicrographs were then taken at X100 and X1000 using a Richter Optica (Model UX-1) photomicroscope then entered into Fiji Image J software for analysis.

At X100 magnification, four random points of the arterial wall were measured and their average calculated to obtain the intima-medial thickness (CIMT). Connective tissue volumetric density estimation was done

using the Cavalieri principle of point counting (Mandarim-de-Lacerda, 2003) and data was expressed as volumetric densities (%). An 80-testcross gird from Image Fiji was superimposed on the digital images on the monitor screen and smooth muscle nuclei and elastic fibers counted by identifying test crosses that fell on these structures. This followed the technique described by Gundersen et al (Gundersen et al., 1988). The volumetric densities (Vv) of the histological structures were evaluated while unaware of the source of the tissue samples and were calculated by the formula Vv = P*p*/P*t*, where Vv is the volume density, *p* the tissue component under consideration (smooth muscle, elastic fiber), P*p* the number of test points associated with *p,* and P*t* the total number of points of tunica media (Mandarim-de-Lacerda, 2003).

Data was analyzed using Statistical Package for Social Sciences software (version 25.0.0.0). Normality of the data was determined using histograms and box plots. Means and standard deviations were calculated. The Kruskall-wallis test was used to compare differences amongst the groups over time for the various variables. A p value of ≤ 0.05 was considered statistically significant at 95% confidence interval.

RESULTS

All animals recruited for this study had remarkable weight gain, especially in the HFHFr fed group (Figure 1). They displayed normal social behavior throughout the experiment. None of the animals was lost to either disease or death.

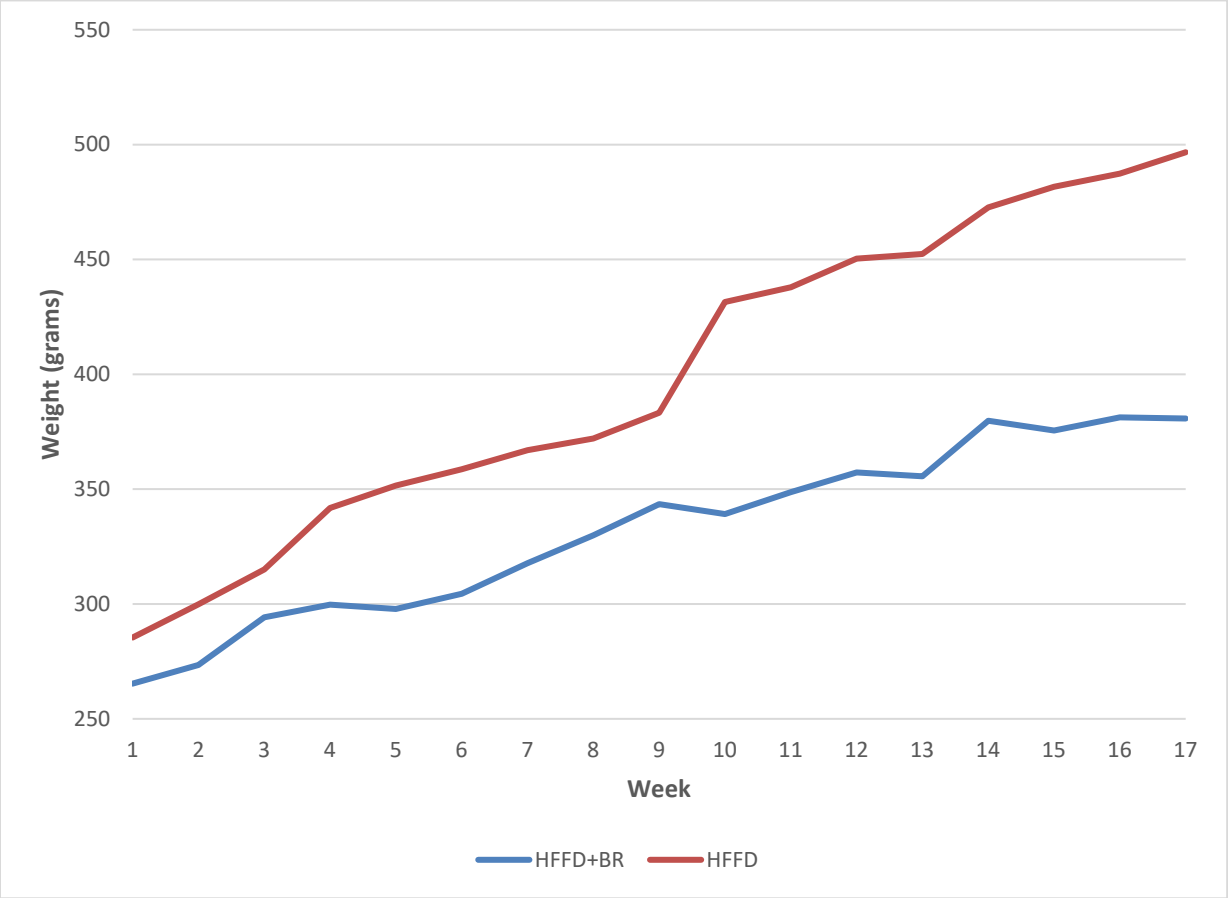


Figure 1. Trend of the average weight of the rats by group during the duration of the experiment

Changes in the Common Carotid Artery of Experimental Rats

Observations among the HFHFr fed experimental animals revealed medial

thickening with a resultant increase in carotid intima-media thickness, increased vascular smooth muscle density, reduced elastic fiber density, and increased collagen fiber density compared to controls. Beetroot co-administration was protective against most of these observed structural changes.

Changes in Intimal-Medial Thickness of the Common Carotid Artery

Compared to control animals, the CCA in experimental animals fed a HFHFr diet showed a significant progressive increase in intimal thickening with the most significant difference being between the animals in the purely HFHFr diet compared with those co-administered with beetroot (p=0.004) (Figure 2). The median CIMT in control animals was 30.64µm. In the HFHFr fed group, the median CIMT were 32.27 µm, 34.84 µm, 38.18 µm, and 40.42 µm over the sacrifice periods (Figure 3). In the HFHFr+Beetroot group, the medians were more comparable with that of the controls at 31.39 µm, 30.88 µm, 30.54 µm and 31.41 µm over the same period (Figure 4).

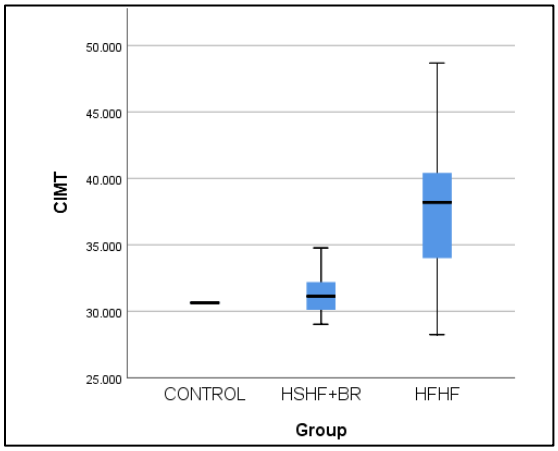


Figure 2. Box and whisker plot showing carotid intima-medial thickness in different animal groups

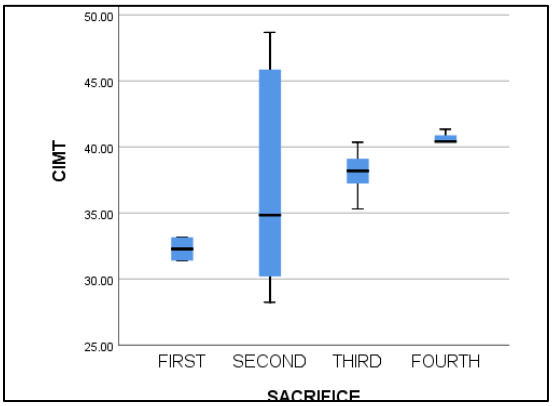


Figure 3. Box and whisker plot showing carotid intima-medial thickness in the HFHFr group at different sacrifice periods

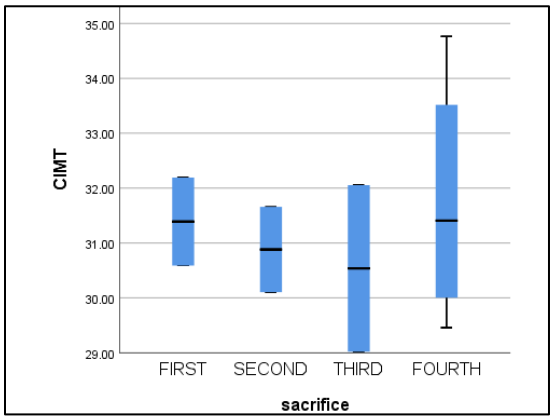


Figure 4. Box and whisker plot showing carotid intima-medial thickness in the HFHFr+ Beetroot group at different sacrifice period

Changes in Smooth Muscle Composition of the Common Carotid Artery

The CCA sections of HFHFr fed rats showed abundant smooth muscle cells localized in the tunica media and organized circumferentially but oriented more randomly around the vessel (Figure 5). Concurrently feeding the experimental animals with beetroot lessened the structural changes to levels more comparable to the normal histoarchitecture.

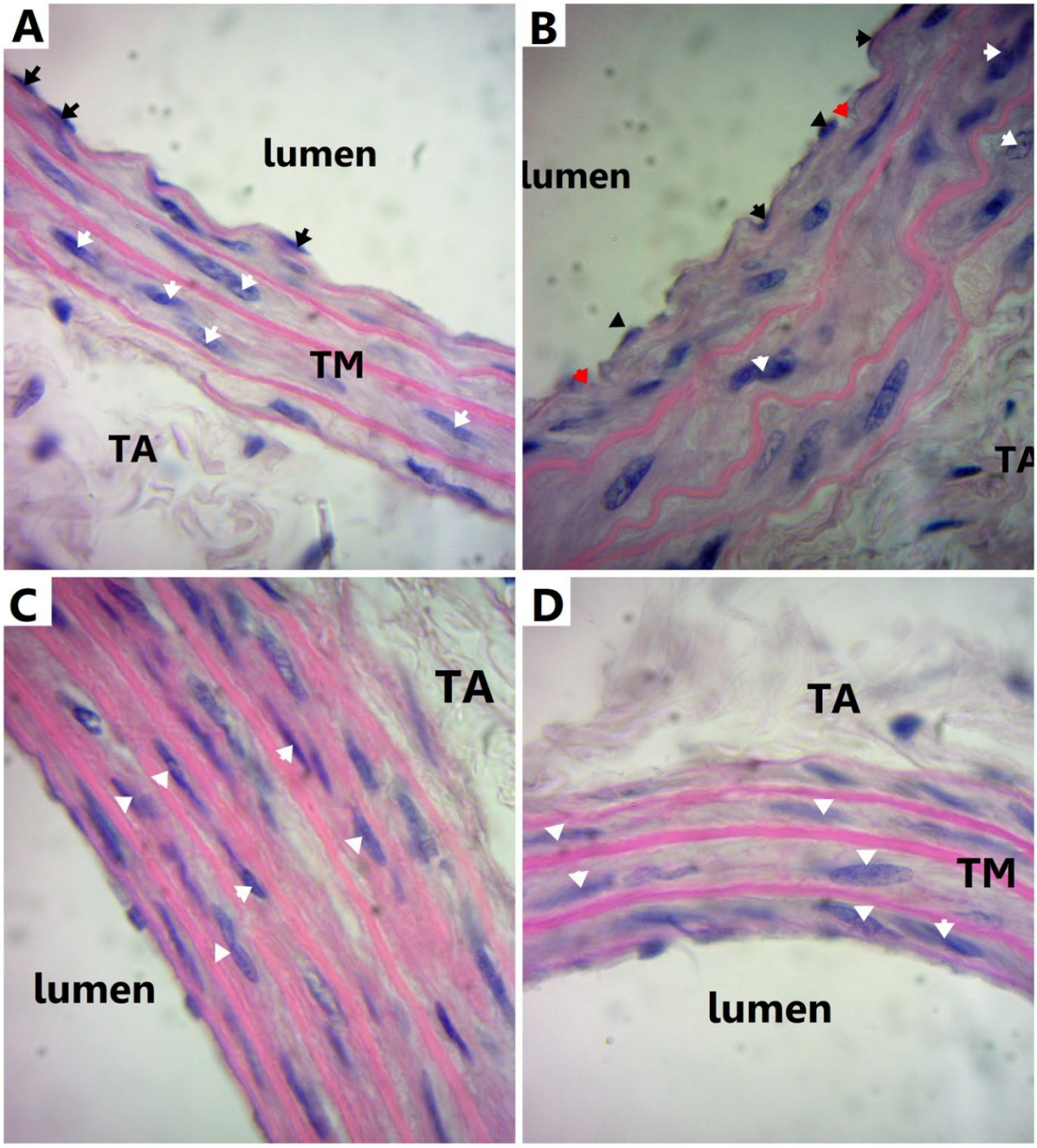


Figure 5: Photomicrographs showing the changes in vascular smooth muscles with intake of a high fat and fructose diet and the protective effects of beetroot on such changes **Figure 5A:** Photomicrograph showing the histological organization of the common carotid artery of a rat in the control group. Note that the squamous endothelial cells (black arrows) in the tunica intima. The fusiform vascular smooth muscle cells (white arrowheads) in the tunica media (TM) display a relatively regular arrangement. Note also fibrous tunica adventitia (TA). (Haematoxylin and Eosin, Magnification=X1000). **Figure 5B:** Photomicrograph showing the histological organization of the common carotid artery of a rat in the HFHFr fed experimental group at the second sacrifice. Note that the increased vascular smooth muscle cells (white arrowheads) in the tunica media (TM) compared to the control group and the HFHFr+Beetroot group. (Haematoxylin and Eosin, Magnification=X1000) (HFHFr: High Fat High Fructose).**Figure 5C:** Photomicrograph showing the histological organization of the common carotid artery of a rat in the HFHFr fed experimental group at the last sacrifice. There are more vascular smooth muscle cells (white arrowheads) in the tunica media (TM) compared to the other animal groups. Also, note the prominent thickening of the tunica media (Haematoxylin and Eosin, Magnification=X1000) (HFHFr: High Fat High Fructose).**Figure 5D:** Photomicrograph showing the histological organization of the common carotid artery of a rat in the HFHFr + Beetroot experimental group at the last sacrifice. Note that the number and organization of the smooth muscle cells in the tunica media

(white arrows) is more comparable to that of the controls (Haematoxylin and Eosin, Magnification=X1000) (HFHFr: High Fat High Fructose).

The average smooth muscle cell density (SMD) in the control group was 17.7%. The change in SMD was most evident in animals that had received an HFHFr diet for longer, with beetroot having a protective effect (Figure 6). In experimental animals, the median SMD was 20.1%, 22.2%, 23.5% and 24.9% in the first, second and third sacrifice of the HFHFr fed group, while it was 16.4%, 16.4%, 16.7%, and 17.1% at similar periods for the HFHFr+Beetroot fed group (Figures 7, 8). There was a statistically significant ($p<0.001$) difference in the group distributions, as showed by the Kruskal-Wallis test. Pairwise comparisons revealed the difference to be greatest between the HFHFr group and HFHFr+Beetroot group ($p<0.001$).

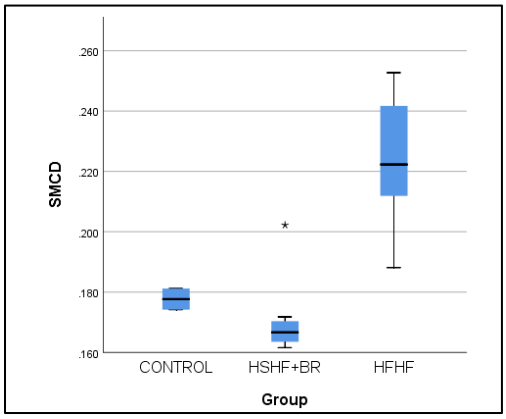


Figure 6. Box and whisker plot of vascular smooth muscle density in different animal groups

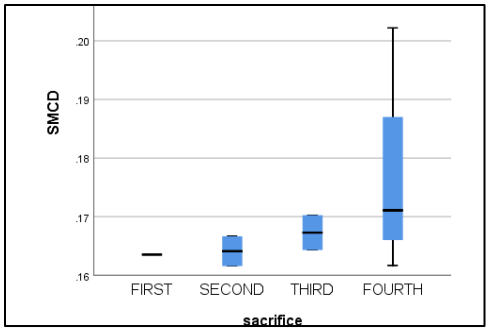


Figure 7. Box and whisker plot of vascular smooth muscle density in the HFHFr group at different sacrifice periods

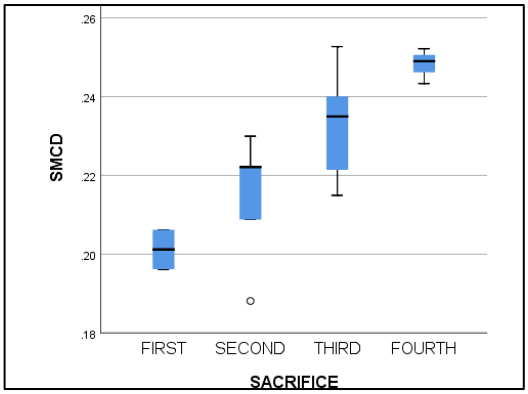


Figure 8. Box and whisker plot of vascular smooth muscle density in the HFHFr+Beetroot group at different sacrifice periods

Changes in Connective Tissue Fiber Density in the Common Carotid Artery

The tunica media of the HFHFr experimental animals displayed a progressive increase in collagen fiber density over time. The increase was most evident in animals that had been fed that diet longer (Figure 9). This increase in collagen fibers was also evident in the tunica adventitia. The beetroot fed experimental animals, on the other hand, showed arterial collagen organization more similar to the controls.

Elastic fibers appeared further interspersed in the HFHFr fed experimental animals compared to controls. The regular lamellar organization of the elastic fibers was also disrupted in this group. While elastic fibers were arranged in regular circumferential lamellae in controls, they showed fragmentation and branching coupled with reduced thickness in experimental animals. Beetroot seemed to have some protective effect against these structural changes.

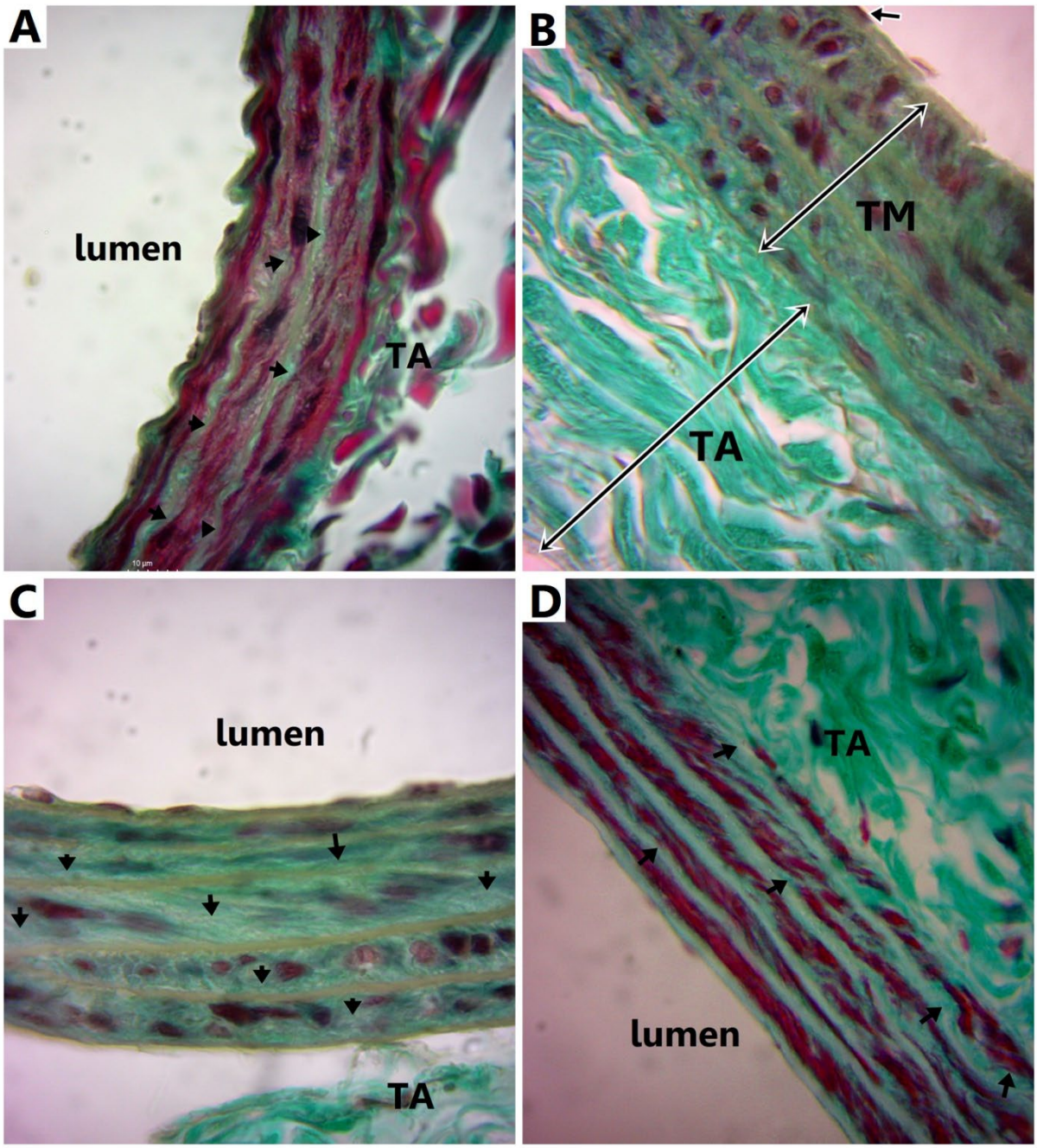


Figure 9: Photomicrographs showing the changes in collagen fiber composition and arrangement with intake of a high fat and fructose diet and the protective effects of beetroot on such changes **Figure 9A:** Photomicrograph showing the organization of collagen fibers in the common carotid artery of a rat in the control group. The collagen fibers within the tunica media (black arrows) are comparably less than those in the tunica adventitia (TA). The collagen fibers are regularly arranged around and similarly oriented as the elastic fiber lamellae (Masson's Trichrome, Magnification=X1000).**Figure 9B:** Photomicrograph showing the organization of collagen fibers in the common carotid artery of a rat in the HFHFr fed experimental group at the second sacrifice. Note the increased collagen fibers (arrowheads) within the Tunica Media (TM). The fibers are also disorganized with more random orientation and location. Also, note the prominent tunica adventitia (TA) displayed (Masson's Trichrome, Magnification=X1000) (HFHFr: High Fat High Fructose).**Figure 9C:** Photomicrograph showing the organization of collagen fibers in the common carotid artery of a rat in the HFHFr fed experimental group at the last sacrifice. Note the randomly oriented and increased collagen fibers (arrowheads) within the Tunica Media (TM) (Masson's Trichrome, Magnification=X1000) (HFHFr: High Fat High Fructose).**Figure 9D:** Photomicrograph showing the organization of collagen fibers in the common carotid artery of a rat in the HFHFr+Beetroot fed experimental group at the last sacrifice. Note the only slightly increased collagen fibers (arrowheads) within the Tunica Media (TM).

The organization and quantity of collagen fibers are comparable to that in the controls (Masson's Trichrome, Magnification=X1000) (HFHFr: High Fat High Fructose).

Collagen Fiber Density

The median collagen fiber density (CFD) in the control group was 48.1%. There was an increase in CFD in the HFHFr fed experimental group (Figure 10), with median CFD in the first, second, third and fourth sacrifices noted as 49.6%, 47.8%, 53.7% and 55.7%, respectively (Figure 11). The same median observations in the HFHFr+Br fed group were often reduced to 47.0%, 48.7%, 45% and 44.5%, respectively (Figure 12). The changes were not statistically significant between the three groups (p=0.061). However, a Mann-Whitney U test between the two experimental groups revealed a statistically significant difference between their distributions (p=0.019).

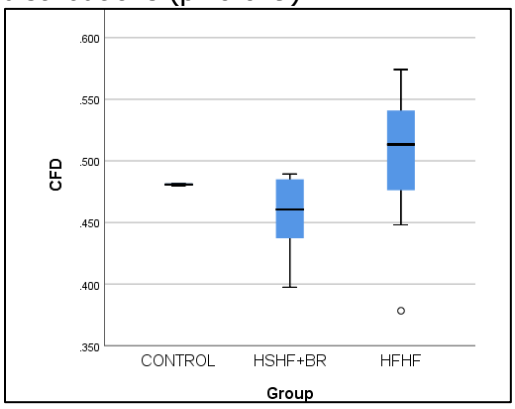


Figure 12. Box and whisker plot showing collagen fiber density in the HFHFr+ Beetroot group at different sacrifice period

Figure 10: Box and whisker plot showing collagen fiber density in different animal groups

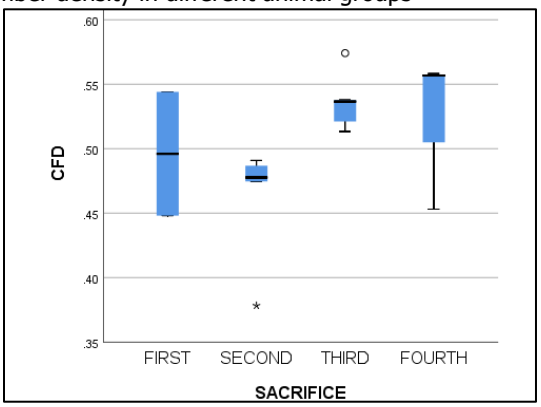
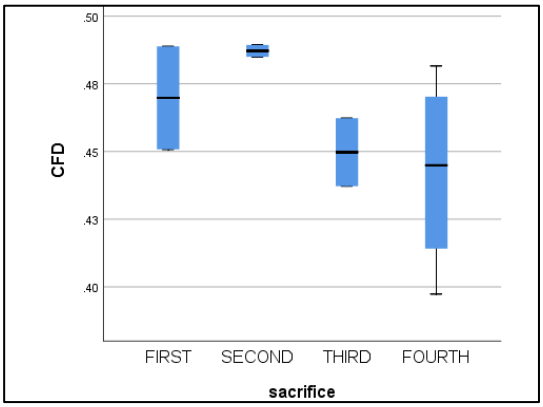


Figure 11. Box and whisker plot showing collagen fiber density in the HFHFr group at different sacrifice periods



Elastic Fiber Density

The median elastic fiber density (EFD) of the CCA was 49.5% in the control group. There was a reduction in EFD in the HFHFr fed experimental animals compared to the control group (Figure 13, 14). Median reported EFD was 48.4%, 46.9%, 45.7 and 45.0% for the first, second, third, and fourth sacrifice in these animals (Figure 15). Similarly reported medians for the HFHFr+Beetroot group deviated less from the controls at 49.2%, 48.4%, 47.5% and 47.3%, respectively (Figure 16). The changes were not statistically significant between the three groups (p=0.166).

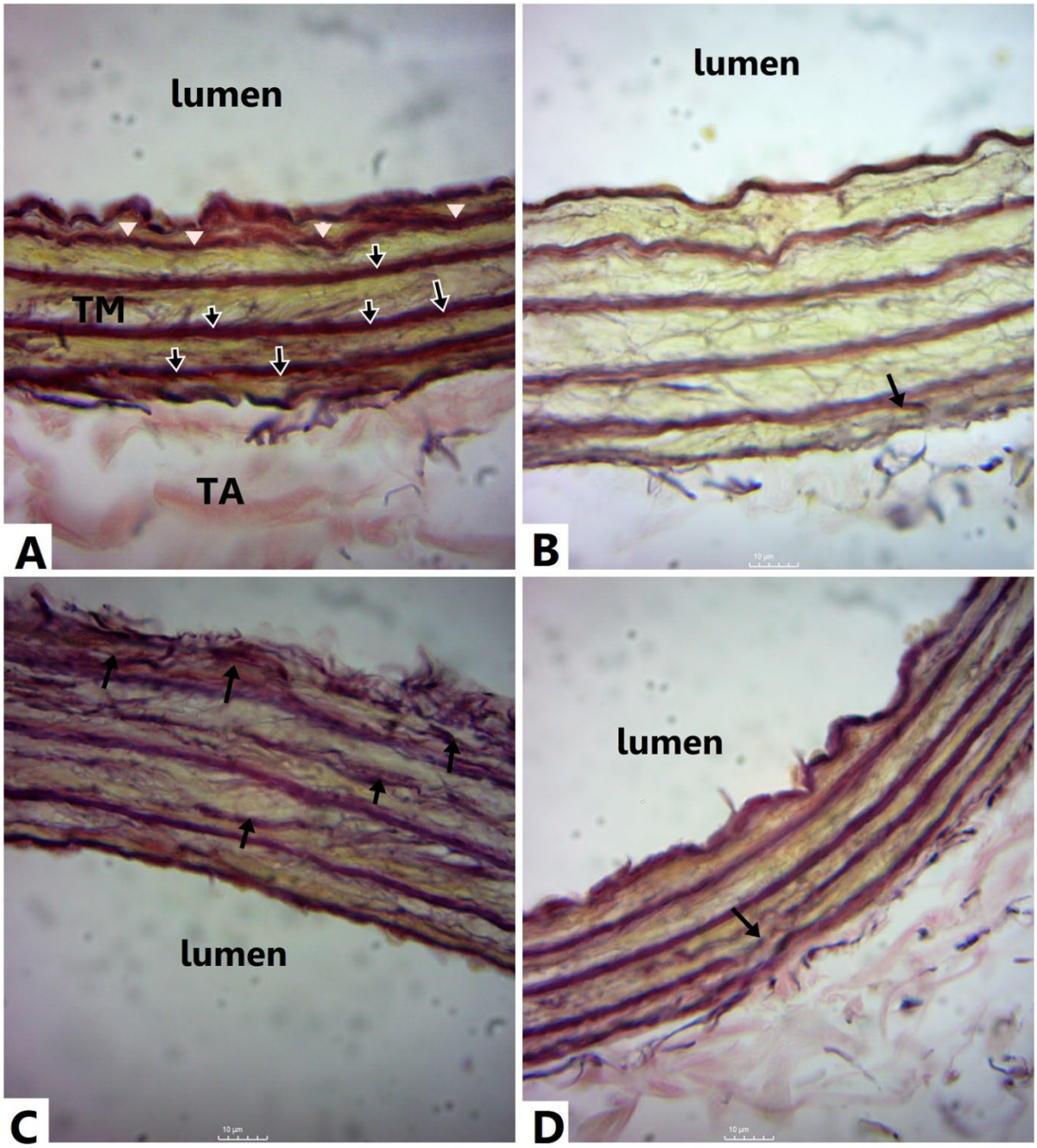


Figure 13: Photomicrographs showing the changes in elastic fibers in the common carotid artery following administration of high fat and fructose diet and the protective effects of beetroot on such changes

Figure 13A: Photomicrograph showing the organization elastic fibers in the common carotid artery in the control group. Note the continuous, regularly arranged, thick, dark-staining elastic bands. (Weigert's Elastin Stain, Magnification=X1000)

Figure 13B: Photomicrograph showing the organization elastic fibers in the common carotid artery of a rat in the HFHFr fed experimental group at the second sacrifice. Note the broader internals between the elastic bands. Note also the areas where the bands approximate (black arrow). (Weigert's Elastin Stain, Magnification=X1000).

Figure 13C: Photomicrograph showing the organization elastic fibers in the common carotid artery of a rat in the HFHFr fed experimental group at the last sacrifice. Note the wide intervals between the elastic bands. Also, note the more frayed appearance of the elastic lamellae with irregular kinks and fragmentation (black arrow). (Weigert's Elastin Stain, Magnification=X1000)

Figure 13D: Photomicrograph showing the organization elastic fibers in the common carotid artery of a rat in the HFHFr+Beetroot experimental group at the last sacrifice. Note a similar structure of the elastic lamellae with that of the control group. A slight discontinuity is, however, noticeable in some of the lamellae (black arrows) (Weigert's Elastin Stain, Magnification=X1000)

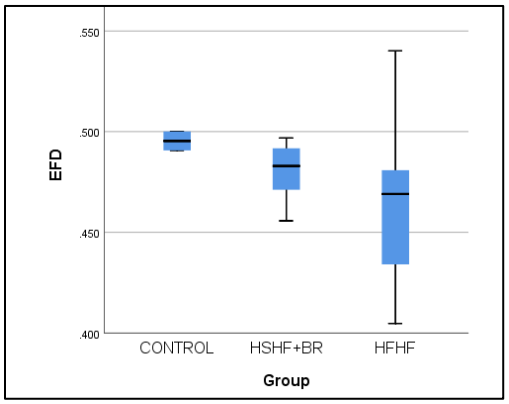
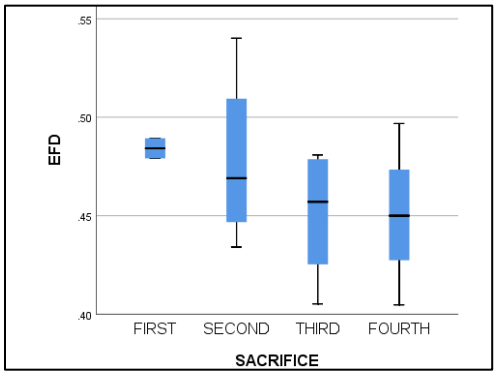


Figure 14. Box and whisker plot showing elastic fiber density in different animal groups

Figure 15. Box and whisker plot showing elastic fiber density in the HFHFr group at different sacrifice periods



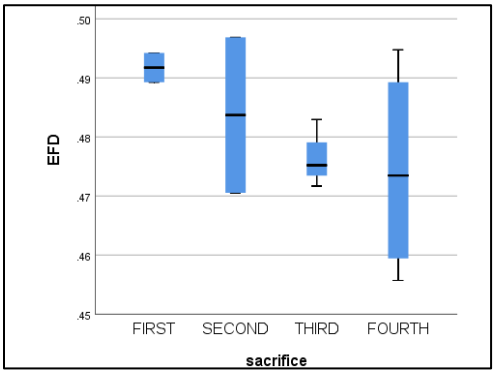


Figure 16. Box and whisker plot showing elastic fiber density in the HFHFr+ Beetroot group at different sacrifice period.

Adventitial Thickness

Median adventitial thickness in the control group was 42.56 (Figure 17). The median AT in the HFHFr experimental animals was 43.27 µm, 46.36 µm, 52.65 µm and 55.61 µm in the first, second, third and fourth sacrifice respectively (Figure 18), compared with 41.47 µm,42.67 µm, 45.57 µm and 46.57 µm in the HFHFr+Br group over the same periods (Figure 19). However, the differences in distribution between the three groups was not statistically significant (p= 0.070).

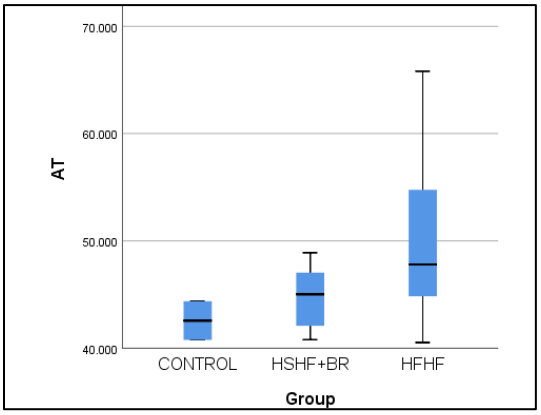


Figure 17. Box and whisker plot showing adventitial thickness in different animal groups

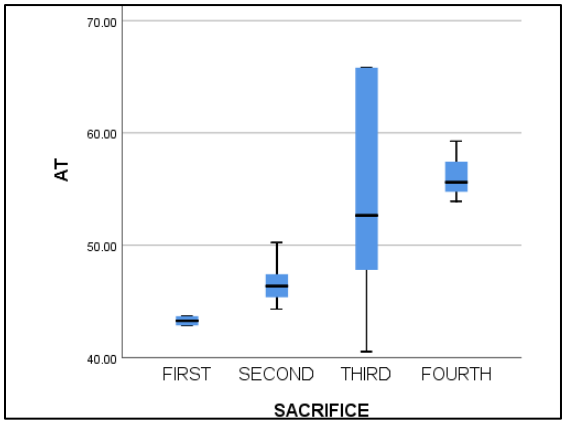


Figure 18. Box and whisker plot showing adventitial thickness in the HFHFr group at different sacrifice periods

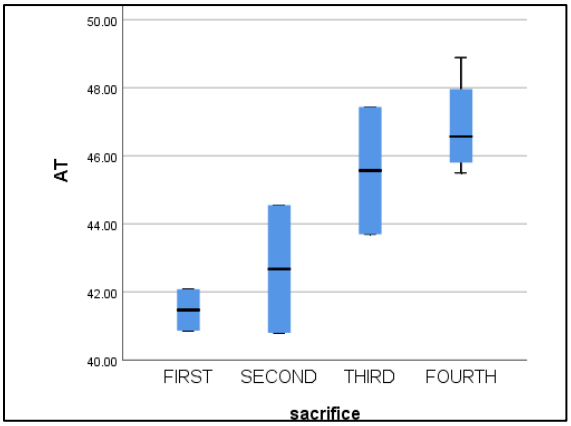


Figure 19. Box and whisker plot showing adventitial thickness in the HFHFr+ Beetroot group at different sacrifice period

DISCUSSION

Carotid Intima-Media Thickness

There was an increase in the carotid intima-media thickness in the group fed on the high fat high fructose diet. In the HFHFr+Beetroot group, the median thickness was more comparable to that of the controls, suggesting a positive influence of beetroot extract. These findings are consistent with those of McClintock et al., (2016), Qu and Qu, (2015) and Oren et al., (2003) that have shown an increase in IMT following administration of a high fat diet, and Arumbakti, (2019) following a high fructose diet. The mechanism of intima-media thickening on these diets is due to endothelial dysfunction and immune modulation following an increase in low density lipoproteins, apolipoproteins and pro-inflammatory moleclues (Qu & Qu, 2015).

Treatment strategies that have been set in place to regulate carotid intima media thickness, which is a known risk factor for atherosclerosis, have extended from dietary practices, with some encouraging Mediterranean diets, to clinical practices including use of drugs such as atorvastatin (Fang et al., 2015), rosuvastatin (Yokoi et al., 2014) and fuvastatin (Anderssen et al., 2005) to reduce the progression of thickening. These therapies involve intensive lipid lowering and antihypertensive therapy with some also enhancing reduction of central fat (Waluś-Miarka et al., 2013). These therapeutic features of reducing carotid intima media thickness are also exhibited by beetroot due to its rich inorganic nitrate content. Dietary nitrates exert anti-oxidant properties which lower ROS and anti-inflammatory properties which maintain vascular integrity.

Therefore, carotid intima media thickness, which is a validated surrogate marker of preclinical atherosclerosis and is predictive of cardiovascular morbidity and mortality (McClintock et al., 2016; Stein et al., 2008), may be regulated by beetroot extract through its dietary ameliorative effects.

Smooth Muscle Density

There was a significant increase in the smooth muscle density in the CCA of rats fed on the HFHFr. Those administered beetroot in addition to the HFHFr presented with a lower smooth muscle density similar to that of controls.

The findings of this study are consistent with those of El Akoum et al., (2012) and Rodríguez-Lee et al., (2006) that have shown an increase in smooth muscle density following administration of a high fat diet, and Victorio et al., (2021) following a high fat high fructose diet. A proposed mechanism of this phenomenon is by a hormone induction and altered phenotypic expression by vascular smooth muscle cells (VSMC).

Physiologically, the high-fat diet has been suggested to increased blood pressure and down regulate expression of K(ATP) channels. This impairs channel-mediated relaxation responses and currents in VSMC, which underscores obesity-triggered increase in blood pressure (Fan et al., 2009). In addition, high fat diets correlate with an increase in systemic leptin and adiponectin that causes VSMC proliferation and hypertrophy in a concentration dependent manner (El Akoum et al., 2011). Further, increased adiponectin inhibits VSMC migration and promotes their differentiation (El Akoum et al., 2012).

Structurally, vascular smooth muscle cells (VSMC) undergo "phenotypic switching" from quiescent to synthetic phenotype showing less contractibility with increased proliferation and migration toward the intima (Mulvihill et al., 2004; Owens et al., 2004). In healthy conditions, the contractile phenotype is predominant and the vascular smooth muscle cells express a set of genes necessary for maintaining a high contractile ability (Beamish et al., 2010). Under a high fat high fructose diet, the expression of these genes are downregulated and differentiated smooth muscle cells switch to a synthetic phenotype, which is characterized by a high proliferation rate and synthesis of extracellular matrix, contributing to wall thickening and thrombosis (Beamish et al., 2010; Mulvihill et al., 2004).

With regards to the mechanisms of damage mentioned above, beetroot extract may pose as an ideal therapeutic dietary supplement attributed to its high nitrate content. The nitrates bind to an intracellular receptor in the smooth muscle cells mediating relaxation and vasodilation (Epstein et al., 1993). NO not only modulates contraction and oxygen uptake but is also involved in the breakdown of pathogens in macrophages and neutrophils, neurotransmission and more importantly, the inhibition of thrombocyte aggregation (Moncada et al., 1991).

Collagen and Elastic Fiber Density

The tunica media of the HFHFr experimental animals displayed a progressive increase in collagen fiber quantity over time. This increase in collagen fibers was also evident in the tunica adventitia. The beetroot fed experimental animals showed arterial collagen organization more similar to the controls. On the other hand, elastic fibers appeared further interspersed in the HFHFr fed experimental animals compared to controls, while those fed beetroot seemed to be protected from these structural changes.

The findings of the present study are consistent with those of Martínez-Martínez et al., (2021) and Santana et al., (2014) that have shown an increase in collagen fiber density and further interspersed elastic fibers following administration of a high fat-diet high-fructose diet.

The high fat high fructose diet is associated with hypertrophic vascular remodeling resulting in vascular fibrosis. Vascular fibrosis, being a dynamic process, results from the imbalance of ECM production and degradation. In the case of vascular collagen accumulation, it is a consequence of an increase in production and decrease in degradation(Gil-Ortega et al., 2016). The decrease in degradation has been suggested as being due to an increase in collagen fiber crosslinking, making it more difficult to degrade. Lysyl oxidase (LOX), the enzyme involved in the covalent cross-linking of collagen and elastin, has been held responsible for this vascular rigidity (Martínez-Martínez et al., 2016). The increased deposition and accumulation of collagen type I remains a consequence of long term adherence to a high-fat high-fructose diet (Leite et al., 2019; Martínez-Martínez et al., 2021).

As opposed to what was observed with collagen, the high-fat high-fructose diet is associated with a decrease in elastic fenestra. Although no changes in elastin levels is associated with high-fat high-fructose, there is a reduction in the fenestra number in the internal elastic lamina (Gil-Ortega et al., 2016). This reduction affects vascular mechanical

properties, thereby making the vessel stiffer (Briones et al., 2003).The rigidity is further increased due to an accumulation in fibronectin which is a major determinant of arterial stiffness and plays a pivotal role in cell matrix interactions (Sista et al., 2005). The relative level of collagen and elastin determine biomechanical properties of vessels, and the increase in collagen/elastin ratio observed in the high-fat high-fructose diet leads to increased stiffness. Correspondingly, there is an increase in the pulse wave velocity that results in lower vessel distensibility and compliance (Martínez-Martínez et al., 2021).

With regards to the mechanism of arterial stiffness as mentioned above, beetroot extract may pose as an ideal therapeutic dietary supplement (Ninfali et al., 2017). By mitigating the first steps involved in the vascular damage by ROS and the subsequent inflammatory process, beetroot through its anti-oxidant and inti-inflammatory contents, mainly nitrates and belatins, may be able to protect the common carotid artery from vascular remodeling and fibrosis.

CONCLUSION

The dietary administration of beetroot extract has been found to be ameliorative to structural changes to the common carotid artery following administration of a high-fat high-fructose diet. Therefore, dietary beetroot extract may be indicated to mitigate harmful changes following long term exposure to a high-fat high-fructose diet.

LIMITATIONS

The animals' absorption and their metabolism rates following the HFHFr and beetroot diet may have been a limitation in this study. This was, however, minimized by the use of in-bred rats which are genetically similar. In addition, tissue shrinkage during tissue processing may have altered the normal parameters. However, errors due to tissue processing were carried through all measurements.

STATEMENT OF CONFLICT OF INTEREST

The authors do not have any conflict of interest.

REFERENCES

1. Anderssen, S. A., Hjelstuen, A. K., Hjermann, I., Bjerkan, K., & Holme, I. (2005). Fluvastatin and lifestyle modification for reduction of carotid intima–media thickness and left ventricular mass progression in drug-treated hypertensives. *Atherosclerosis*, *178*(2), 387–397. https://doi.org/10.1016/j.atherosclerosis.2004.08.033

2. Arumbakti, A. D. (2019). Fructose Intake and Its Correlation with Carotid Intima-Media Thickness in Male Employees with Hypercholesterolemia in Harapan Kita National Cardiovascular Center Hospital, Jakarta, Indonesia. *World Nutrition Journal*, *3*(1), 8. https://doi.org/10.25220/WNJ.V03.i1.0003

3. Beamish, J. A., He, P., Kottke-Marchant, K., & Marchant, R. E. (2010). Molecular Regulation of Contractile Smooth Muscle Cell Phenotype: Implications for Vascular Tissue Engineering. *Tissue Engineering Part B: Reviews*, *16*(5), 467–491. https://doi.org/10.1089/ten.teb.2009.0630

4. Briones, A. M., González, J. M., Somoza, B., Giraldo, J., Daly, C. J., Vila, E., Carmen González, M., McGrath, J. C., & Arribas, S. M. (2003). Role of Elastin in Spontaneously Hypertensive Rat Small Mesenteric Artery Remodelling. *The Journal of Physiology*, *552*(1), 185–195. https://doi.org/10.1113/jphysiol.2003.046904

5. Bryda, E. C. (2013). The Mighty Mouse: The impact of rodents on advances in biomedical research. *Missouri Medicine*, *110*(3), 207–211.

6. Carrillo, C., Rey, R., Hendrickx, M., del Mar Cavia, M., & Alonso-Torre, S. (2017). Antioxidant Capacity of Beetroot: Traditional vs Novel Approaches. *Plant Foods for Human Nutrition*, *72*(3), 266–273. https://doi.org/10.1007/s11130-017-0617-2

7. Carrizzo, A., Izzo, C., Forte, M., Sommella, E., Di Pietro, P., Venturini, E., Ciccarelli, M., Galasso, G., Rubattu, S., Campiglia, P., Sciarretta, S., Frati, G., & Vecchione, C. (2020). A Novel Promising Frontier for Human Health: The Beneficial Effects of Nutraceuticals in Cardiovascular Diseases. *International Journal of Molecular Sciences*, *21*(22), 8706. https://doi.org/10.3390/ijms21228706

8. Chhikara, N., Kushwaha, K., Sharma, P., Gat, Y., & Panghal, A. (2019). Bioactive compounds of beetroot and utilization in food processing industry: A critical review. *Food Chemistry*, *272*, 192–200. https://doi.org/10.1016/j.foodchem.2018.08.022

9. El Akoum, S., Cloutier, I., & Tanguay, J.-F. (2012). Vascular Smooth Muscle Cell Alterations Triggered by Mice Adipocytes: Role of High-Fat Diet. *Journal of Atherosclerosis and Thrombosis*, *19*(12), 1128–1141. https://doi.org/10.5551/jat.13482

10. El Akoum, S., Lamontagne, V., Cloutier, I., & Tanguay, J.-F. (2011). Nature of fatty acids in high fat diets differentially delineates obesity-linked metabolic syndrome components in male and female C57BL/6J mice. *Diabetology & Metabolic Syndrome*, *3*(1), 34. https://doi.org/10.1186/1758-5996-3-34

11. Epstein, F. H., Moncada, S., & Higgs, A. (1993). The L-Arginine-Nitric Oxide Pathway. *New England Journal of Medicine*, *329*(27), 2002–2012. https://doi.org/10.1056/NEJM199312303292706

12. Fan, L.-H., Tian, H.-Y., Yang, M.-L., Ma, A.-Q., Hu, Z., Bai, X.-J., & Cao, Y.-X. (2009). High-fat diet may impair KATP channels in vascular smooth muscle cells. *Biomedicine & Pharmacotherapy*, *63*(2), 165–170. https://doi.org/10.1016/j.biopha.2008.01.005

13. Fang, N., Han, W., Gong, D., Zou Chen, & Fan, Y. (2015). Atorvastatin Treatment for Carotid Intima-Media Thickness in Chinese Patients With Type 2 Diabetes: A Meta-Analysis. *Medicine*, *94*(44), e1920. https://doi.org/10.1097/MD.0000000000001920

14. Gil-Ortega, M., Martín-Ramos, M., Arribas, S. M., González, M. C., Aránguez, I., Ruiz-Gayo, M., Somoza, B., & Fernández-Alfonso, M. S. (2016). Arterial stiffness is associated with adipokine dysregulation in non-hypertensive obese mice. *Vascular Pharmacology*, *77*, 38–47. https://doi.org/10.1016/j.vph.2015.05.012

15. Grand View Research. (2020). *Nutraceutical Market Size, Share & Trends Analysis Report By Product (Dietary Supplements, Functional Foods, Functional Beverages), By Region, And Segment Forecasts, 2020—2027*. https://www.grandviewresearch.com/industry-analysis/nutraceuticals-market

16. Gundersen, H. J., Bagger, P., Bendtsen, T. F., Evans, S. M., Korbo, L., Marcussen, N., Møller, A., Nielsen, K., Nyengaard, J. R., & Pakkenberg, B. (1988). The new stereological tools: Disector, fractionator, nucleator and point sampled intercepts and their use in pathological research and diagnosis. *APMIS: Acta Pathologica, Microbiologica, et Immunologica Scandinavica*, *96*(10), 857–881. https://doi.org/10.1111/j.1699-0463.1988.tb00954.x

17. Hirsiger, S. C. (2010). *Effect of High-Fat Diet on the Regulation of Vascular Tone in Arteries of Young Mice*. https://doi.org/10.5167/UZH-68245

18. Kim, J.-Y., & Shim, S. H. (2019). Medicinal Herbs Effective Against Atherosclerosis: Classification According to Mechanism of Action. *Biomolecules & Therapeutics*, *27*(3), 254–264. https://doi.org/10.4062/biomolther.2018.231

19. Leite, S., Cerqueira, R. J., Ibarrola, J., Fontoura, D., Fernández-Celis, A., Zannad, F., Falcão-Pires, I., Paulus, W. J., Leite-Moreira, A. F., Rossignol, P., López-Andrés, N., & Lourenço, A. P. (2019). Arterial Remodeling and Dysfunction in the ZSF1 Rat Model of Heart Failure With Preserved Ejection Fraction. *Circulation: Heart Failure*, *12*(7). https://doi.org/10.1161/CIRCHEARTFAILURE.118.005596

20. Mandarim-de-Lacerda, C. A. (2003). Stereological tools in biomedical research. *Anais Da Academia Brasileira De Ciencias*, *75*(4), 469–486. https://doi.org/10.1590/s0001-37652003000400006

21. Martínez-Martínez, E., Rodríguez, C., Galán, M., Miana, M., Jurado-López, R., Bartolomé, M. V., Luaces, M., Islas, F., Martínez-González, J., López-Andrés, N., & Cachofeiro, V. (2016). The lysyl oxidase inhibitor (β-aminopropionitrile) reduces leptin profibrotic effects and ameliorates cardiovascular remodeling in diet-induced obesity in rats. *Journal of Molecular and Cellular Cardiology*, *92*, 96–104. https://doi.org/10.1016/j.yjmcc.2016.01.012

22. Martínez-Martínez, E., Souza-Neto, F., Jiménez-González, S., & Cachofeiro, V. (2021). Oxidative Stress and Vascular Damage in the Context of Obesity: The Hidden Guest. *Antioxidants*, *10*(3), 406. https://doi.org/10.3390/antiox10030406

23. McClintock, T. R., Parvez, F., Wu, F., Islam, T., Ahmed, A., Rani Paul, R., Shaheen, I., Sarwar, G., Rundek, T., Demmer, R. T., Desvarieux, M., Ahsan, H., & Chen, Y. (2016). Major dietary patterns and carotid intima-media thickness in Bangladesh. *Public Health Nutrition*, *19*(2), 218–229. https://doi.org/10.1017/S136898001500124X

24. Mirmiran, P., Houshialsadat, Z., Gaeini, Z., Bahadoran, Z., & Azizi, F. (2020). Functional properties of beetroot (Beta vulgaris) in management of cardio-metabolic diseases. *Nutrition & Metabolism*, *17*(1), 3. https://doi.org/10.1186/s12986-019-0421-0

25. Moncada, S., Palmer, R. M., & Higgs, E. A. (1991). Nitric oxide: Physiology, pathophysiology, and pharmacology. *Pharmacological Reviews*, *43*(2), 109–142.

26. Mulvihill, E. R., Jaeger, J., Sengupta, R., Ruzzo, W. L., Reimer, C., Lukito, S., & Schwartz, S. M. (2004). Atherosclerotic Plaque Smooth Muscle Cells Have a Distinct Phenotype. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *24*(7), 1283–1289. https://doi.org/10.1161/01.ATV.0000132401.12275.0c

27. Ninfali, P., Antonini, E., Frati, A., & Scarpa, E.-S. (2017). C-Glycosyl Flavonoids from *Beta vulgaris Cicla* and Betalains from *Beta vulgaris rubra*: Antioxidant, Anticancer and Antiinflammatory Activities-A Review: Pharmacological Activities of Green and Red Beets. *Phytotherapy Research*, *31*(6), 871–884. https://doi.org/10.1002/ptr.5819

28. Oren, A., Vos, L. E., Uiterwaal, C. S. P. M., Grobbee, D. E., & Bots, M. L. (2003). Cardiovascular Risk Factors and Increased Carotid Intima-Media Thickness in Healthy Young Adults: The Atherosclerosis Risk in Young Adults (ARYA) Study. *Archives of Internal Medicine*, *163*(15), 1787. https://doi.org/10.1001/archinte.163.15.1787

29. Owens, G. K., Kumar, M. S., & Wamhoff, B. R. (2004). Molecular Regulation of Vascular Smooth Muscle Cell Differentiation in Development and Disease. *Physiological Reviews*, *84*(3), 767–801. https://doi.org/10.1152/physrev.00041.2003

30. Qu, B., & Qu, T. (2015). Causes of changes in carotid intima-media thickness: A literature review. *Cardiovascular Ultrasound*, *13*(1), 46. https://doi.org/10.1186/s12947-015-0041-4

31. Ratchford, E. V., & Evans, N. S. (2014). Carotid artery disease. *Vascular Medicine*, *19*(6), 512–515. https://doi.org/10.1177/1358863X14557722

32. Rodríguez-Lee, M., Östergren-Lundén, G., Wallin, B., Moses, J., Bondjers, G., & Camejo, G. (2006). Fatty Acids Cause Alterations of Human Arterial Smooth Muscle Cell Proteoglycans That Increase the Affinity for Low-Density Lipoprotein. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *26*(1), 130–135. https://doi.org/10.1161/01.ATV.0000191659.94059.62

33. Santana, A. B. C., Souza Oliveira, T. C. de, Bianconi, B. L., Barauna, V. G., Santos, E. W. C. O., Alves, T. P., Silva, J. C. S., Fiorino, P., Borelli, P., Irigoyen, M. C. C., Krieger, J. E., & Lacchini, S. (2014). Effect of High-Fat Diet upon Inflammatory Markers and Aortic Stiffening in Mice. *BioMed Research International*, *2014*, 1–12. https://doi.org/10.1155/2014/914102

34. Sigala, F., Oikonomou, E., Antonopoulos, A. S., Galyfos, G., & Tousoulis, D. (2018). Coronary versus carotid artery plaques. Similarities and differences regarding biomarkers morphology and prognosis. *Current Opinion in Pharmacology*, *39*, 9–18. https://doi.org/10.1016/j.coph.2017.11.010

35. Sista, A. K., O'Connell, M. K., Hinohara, T., Oommen, S. S., Fenster, B. E., Glassford, A. J., Schwartz, E. A., Taylor, C. A., Reaven, G. M., & Tsao, P. S. (2005). Increased aortic stiffness in the insulin-resistant Zucker *fa/fa* rat. *American Journal of Physiology-Heart and Circulatory Physiology*, *289*(2), H845–H851. https://doi.org/10.1152/ajpheart.00134.2005

36. Statista. (2021). Market value of nutraceuticals in the United States from 2014 to 2025. *Market Value of Nutraceuticals in the United States from 2014 to 2025*. https://www.statista.com/statistics/910097/us-market-size-nutraceuticals/

37. Stein, J. H., Korcarz, C. E., Hurst, R. T., Lonn, E., Kendall, C. B., Mohler, E. R., Najjar, S. S., Rembold, C. M., & Post, W. S. (2008). Use of Carotid Ultrasound to Identify Subclinical Vascular Disease and Evaluate Cardiovascular Disease Risk: A Consensus Statement from the American Society of Echocardiography Carotid Intima-Media Thickness Task Force Endorsed by the Society for Vascular Medicine. *Journal of the American Society of Echocardiography*, *21*(2), 93–111. https://doi.org/10.1016/j.echo.2007.11.011

38. Toklu, H. Z., Muller-Delp, J., Sakarya, Y., Oktay, S., Kirichenko, N., Matheny, M., Carter, C. S., Morgan, D., Strehler, K. Y. E., Tumer, N., & Scarpace, P. J. (2017). *HIGH DIETARY FRUCTOSE DOES NOT EXACERBATE THE DETRIMENTAL CONSEQUENCES OF HIGH FAT DIET ON BASILAR ARTERY FUNCTION*. 28.

39. Victorio, J. A., Guizoni, D. M., Freitas, I. N., Araujo, T. R., & Davel, A. P. (2021). Effects of High-Fat and High-Fat/High-Sucrose Diet-Induced Obesity on PVAT Modulation of Vascular Function in Male and Female Mice. *Frontiers in Pharmacology*, *12*, 720224. https://doi.org/10.3389/fphar.2021.720224

40. Waluś-Miarka, M., Wojciechowska, W., Miarka, P., Kloch-Badełek, M., Woźniakiewicz, E., Czarnecka, D., Sanak, M., Małecki, M., & Idzior-Waluś, B. (2013). Intima-media thickness correlates with features of metabolic syndrome in young people with a clinical diagnosis of familial hypercholesterolaemia. *Kardiologia Polska*, *71*(6), 566–572. https://doi.org/10.5603/KP.2013.0119

41. WHO. (2021). WHO: Obesity and overweight. *Obesity and Overweight*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

42. Yokoi, H., Nohara, R., Daida, H., Hata, M., Kaku, K., Kawamori, R., Kishimoto, J., Kurabayashi, M., Masuda, I., Sakuma, I., Yamazaki, T., Yoshida, M., & for the Justification for Atherosclerosis Regression Treatment (JART) Investigators. (2014). Change in Carotid Intima-Media Thickness in a High-Risk Group of Patients by Intensive Lipid-Lowering Therapy With Rosuvastatin: Subanalysis of the JART Study. *International Heart Journal*, *55*(2), 146–152. https://doi.org/10.1536/ihj.13-216