Original Article

Extrauterine Growth Restriction Followed by Catch-Up Growth Reveals Adverse Effects on Aorta Microstructure: An Animal-Based Experimental Study

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ABSTRACT

Background: Preterm birth is a major global concern, often leading to extrauterine growth restriction (EUGR) and later need for catch-up growth. Excessive catch-up growth may predispose to long-term cardiovascular risk. The objective of this study was to investigate the histological alterations in the aortic microstructure of Wistar rats exposed to extrauterine growth restriction followed by varying degrees of catch-up growth.

Methods: This animal-based experimental study was conducted on 120 neonatal Wistar rats using a rodent litter-size manipulation model that mimics human extrauterine growth restriction (EUGR) and catch-up growth. Initially, 30 pups were fed normally (Group N), while 90 were undernourished (Group R) to simulate EUGR. From days 11 to 21, the undernourished rats were separated into two subgroups: normal growth (RN, n = 30) and catch-up growth (RC, n = 60). The RC subgroup was further subdivided into accelerated (RC_A) and slow (RC_S) catch-up growth based on growth velocity. On day 60, microscopic aortic parameters (lumen and wall areas, wall-to-lumen ratio, and collagen & elastin content) were assessed after euthanasia and compared using ANOVA with post hoc Tukey tests.

Results: Both RCA and RCS groups showed significantly reduced aortic luminal area and increased vessel wall area and wall-to-lumen ratio compared with RN and N groups ($p<0.001$). No significant difference was observed between RCA and RCS ($p>0.05$). Collagen and elastin contents of the tunica media did not differ significantly among groups ($p>0.05$).

Conclusion: Catch-up growth after extra-uterine growth restriction led to aortic wall changes, supporting the need for balanced rather than rapid catch-up growth in preterm infants.

Keywords: Fetal growth retardation; Catch-up growth; Aorta/ultrastructure; Aorta/pathology; Rats; Infant, Premature

INTRODUCTION

Preterm birth, defined by the World Health Organisation (WHO) as delivery before 37 completed weeks of gestation,[1] affects approximately 10% of all births worldwide, with South Asia contributing nearly one-third of the global burden.[2] Survivors of preterm birth often face lifelong health challenges, including impaired growth and metabolic complications. Following birth, preterm infants frequently experience extrauterine growth restriction (EUGR), a postnatal failure to achieve expected intrauterine growth rates, primarily due to nutritional deficits, increased metabolic demands, and immature organ systems.[3]

In neonatal care, fortified feeding regimens are used to promote catch-up growth, aiming to restore the growth trajectory toward that of term-born infants.[4] Catch-up growth is defined as "growth velocity above the statistical limits for that age or maturity, following a transient period of growth inhibition".[5] While early growth recovery supports survival and neurodevelopment, multiple studies link accelerated postnatal weight gain in preterm infants to later cardiovascular and metabolic risks, including hypertension, dyslipidaemia, and vascular remodelling.[6]

Experimental models in rodents have provided insight into these observations. Postnatal overfeeding in rats results in persistent adiposity, reduced lean mass, and an overweight phenotype that persists beyond weaning.[7] This altered body composition correlates with metabolic disturbances such as insulin resistance, abnormal lipid profiles, and increased expression of proinflammatory cytokines like leptin, all contributing to adverse cardiovascular outcomes.[7,8]

A systematic review of human preterm cohorts further associated rapid catch-up growth with increased blood pressure, higher cholesterol, endothelial dysfunction, and thickened arterial intima-media layers later in

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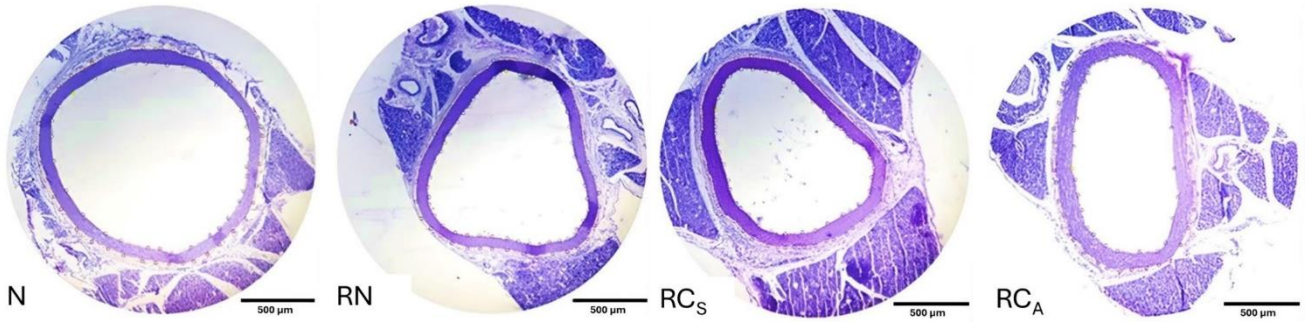

Figure 1: Photomicrograph showing the aorta lumen area enclosed within the inner selection line and the aorta wall area enclosed between the two selection lines for the representation images of subgroups N, RN, RCS, and RCA.

life.[9] Yet, despite growing evidence linking early growth patterns to long-term cardiovascular risk, the structural impact of extrauterine growth restriction and subsequent catch-up growth on the vascular wall remains insufficiently understood.

Wistar rats are well established as experimental models for cardiometabolic research.[10] Manipulating litter size effectively can simulate human EUGR and postnatal catch-up growth as previously explained.[11]

This study aimed to investigate the histological alterations in the aortic microstructure of Wistar rats exposed to extrauterine growth restriction followed by varying degrees of catch-up growth. Understanding these microscopic vascular changes may help explain how early nutritional programming contributes to later cardio-vascular disease risk and inform safer nutritional strategies for preterm infants.

MATERIALS AND METHODS

This animal-based experimental study was conducted in the Anatomy Department of CMH Multan Institute of Medical Sciences (CIMS), Pakistan, from October 2021 to June 2022, after approval from the Institutional Review Board and Ethical Committee (Case No. TW/25/CIMS).

Pregnant Wistar rats (n = 30), procured from University of Veterinary & Animal Sciences (UVAS), Lahore, were housed under controlled conditions (12-hour light/dark cycle, 26 ± 2°C temperature, 60 ± 5% humidity) with free access to pellet chow and water. After birth, pups were divided by simple random sampling into two groups: normally fed (Group N, n = 30; 8–10 pups/dam) and restricted (Group R, n = 90; 14–16 pups/dam) from postnatal day 2–10, using litter size manipulation to induce nutritional restriction. This period in rats corresponds to late gestation in humans,[11] comparable to the early ex-utero life of preterm infants.[12]

From postnatal day 11–21, undernourished pups (Group R) were reassigned either to normal growth (RN, n = 30; 8–10 pups/dam) or catch-up growth (RC, n = 60; 4–5 pups/dam) conditions.[10] This period in the rat is broadly representative of the first two years of life in humans, reflecting the period during which catch-up growth commonly occurs in human preterm infants.[11] Based on growth velocity, RC pups were further classified as accelerated (RCA) or slow catch-up (RCS) groups (n = 30 each).

On day 60, animals were sacrificed by chloroform inhalation followed by intracardiac potassium chloride injection (1–2 mmol/kg) to induce diastolic arrest. A 0.5 cm segment from the mid-thoracic aorta was collected, fixed in 10% NBF, dehydrated, embedded, and sectioned at 5–7 µm thickness.[13,14] Sections were stained with Haematoxylin and Eosin for general morphology and Orcein-Light Green for elastin and collagen visualisation.

Aortic lumen area, vessel wall area, wall-to-lumen ratio, and collagen and elastic content per unit area were calculated. Digital micrographs (100× for wall and lumen area, 400× for collagen and elastin) were captured through an Olympus binocular microscope with a 20 MP camera. Image analysis was performed in Fiji ImageJ (v1.53t, NIH, USA) using scale calibration and colour thresholding. Aortic wall area (mm^2) was calculated as:

$$\text{Aorta Vessel Wall} = \text{Area}_{OW} - \text{Area}_{IW}$$

Where Area_{OW} represented the total Aorta cross-sectional area, including the wall and lumen, and Area_{IW} represented the area of the lumen (Figure 1). The Wall to Lumen Area Ratio was calculated as:

$$\text{Aorta Wall to Lumen Area Ratio} = \frac{\text{Aorta Vessel Wall Area } (mm^2)}{\text{Aorta Lumen Area } (mm^2)}$$

Elastin and collagen quantification followed the colour-based morphometric method based on the unique brown and green colours, respectively, by ImageJ, as explained by Hong et al.[15] (Figure 2). All analyses were performed

using SPSS version 26. Group comparisons were made using one-way ANOVA with Tukey post-hoc testing. A p-value ≤0.05 was considered statistically significant.

RESULTS

The mean ± SD values for the histological parameters of all the groups and overall ANOVA p-values are presented in Table 1. The aortic luminal area (mm²) was significantly lower among restricted pups with accelerated (RCA) or slow (RCS) catch-up growth compared with those showing subsequent normal growth (RN) (p<0.001 for both). Subgroup RCS also had a smaller lumen than the normally fed controls (N) (p=0.01), while no difference was found between RCA and RCS (p=0.68).

The aortic vessel wall area (mm²) was significantly greater in both RCA and RCS groups than in RN and N (p<0.001 for all pair-wise comparisons), with no significant difference between RCA and RCS (p=0.73). Similarly, the wall-to-lumen area ratio was higher in both catch-up growth subgroups (RCA, RCS) compared with RN and N (p<0.001 for all comparisons). Figure 1 shows the representative histological images for morphometric measurements for all four groups. In contrast, the collagen and elastin contents of the tunica media did not differ significantly among subgroups (p=0.21 and p=0.50, respectively, for overall ANOVA). Representative histological images for all groups are shown in Figure 2.

Table 1: Pairwise comparison for the effect of nutritional intervention subgroups on aortic microscopic parameters of rats

Parameter	N	RN	RCS	RCA	p-value
Aortic luminal area (mm²)	0.83 ± 0.27	0.91 ± 0.17	0.66 ± 0.19	0.72 ± 0.18	<0.001*
Aortic vessel wall area (mm²)	0.35 ± 0.1	0.33 ± 0.05	0.43 ± 0.10	0.45 ± 0.09	<0.001*
Aortic wall to lumen ratio	0.44 ± 0.09	0.37 ± 0.06	0.67 ± 0.14	0.64 ± 0.14	<0.001*
Collagen content per unit area in tunica media of aorta (%)	42.11 ± 14.44	42.34 ± 20.79	38.3 ± 15.52	48.38 ± 21.17	0.21
Elastin content per unit area in tunica media of aorta (%)	51.93 ± 18.22	53.5 ± 22.3	65.1 ± 17.42	58.75 ± 21.04	0.5

*p-value: significant at <0.05, computed for overall ANOVA

Abbreviations: N: Normally Fed, **RN:** Restricted then normal Growth, **RCA:** Restricted then Accelerated catch-up growth, **RCS:** Restricted then slow catch-up growth

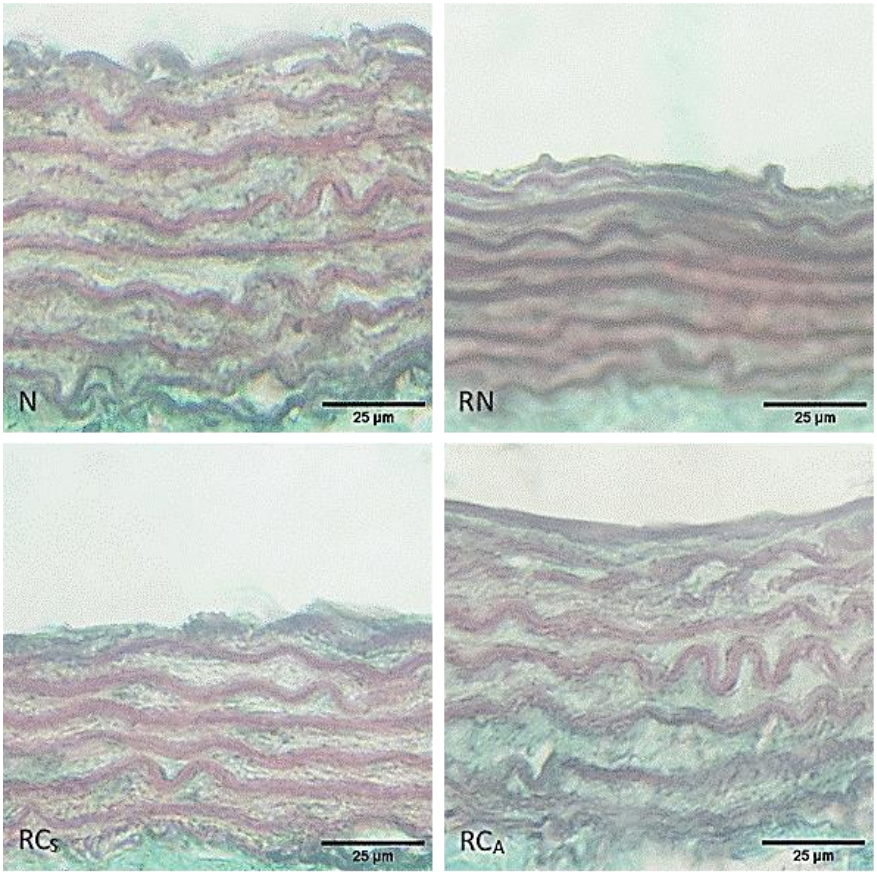

Figure 2: Photomicrographs showing the aorta wall stained with Orcein and counterstained with light for the representative images of subgroups N, RN, RC_S, and RC_A

DISCUSSION

This study investigated aortic microstructure in rats following extrauterine growth restriction (EUGR) and subsequent catch-up growth. Our primary finding was significant adverse remodelling: rats in both the slow (RCS) and accelerated (RCA) catch-up groups exhibited a larger aortic vessel wall area and a decreased luminal area. This resulted in a markedly higher wall-to-lumen ratio compared to normally fed controls (N) and the restricted-then-normal-growth group (RN).

These structural changes are classic histological markers associated with an increase in peripheral vascular resistance. This inward hypertrophic remodelling, a thicker wall and narrower lumen, corresponds to prehypertensive vascular changes[16] and indicates cardiovascular work overload.[17] These results align with previous rodent models where postnatal overfeeding was linked to adult cardiovascular disorders, including aortic narrowing and pro-atherogenic changes.[18] Postnatal overfeeding is known to cause adverse vascular adaptations, including increased aortic thickness,[8,19] and our findings strongly corroborate this link between early catch-up growth and detrimental vascular remodelling.

While our two catch-up subgroups both exhibited adverse aortic remodelling with no statistically significant difference between them, Ye et al.[20] found that moderate catch-up growth did not lead to adverse vascular remodelling in IUGR-rats. This suggests that magnitude/velocity may moderate the effect and that the absolute level of catch-up (and not just any catch-up) matters.

A second key finding was that, in contrast to some prior literature, there was no significant difference in the mean content of collagen or elastin per unit area of the tunica media across any subgroup. This unaltered matrix density differs from reports that observed increased collagen and decreased elastin in postnatally overfed rats.[8,18] The discrepancy may be due to the timing of the sacrifice. Our assessment at day 60 (corresponding to early adulthood) may represent an earlier stage of vascular pathology. At this point, the increased wall area may be driven primarily by smooth muscle cell hypertrophy or hyperplasia, rather than significant matrix rearrangement. Vascular remodelling likely begins with smooth muscle hypertrophy and phenotypic switching, while matrix changes such as fibrosis or elastin degradation appear later, as suggested by recent studies.[21]

These experimental findings resonate with human studies. Rapid postnatal growth acceleration in infants is linked to increased adiposity.[22] Systematic reviews also associate rapid catch-up growth in preterm cohorts with adverse long-term outcomes, including higher blood pressure,[23] hypercholesterolemia,[22] and altered intima-media thickness.[22,24] Our results provide histomorphological evidence that this early nutritional insult may program fixed vascular changes, predisposing individuals to hypertension and atherosclerosis.

One of the key limitations of the study is that we did not record functional parameters like blood pressure. While the histological markers correlate with prehypertensive states, these findings require confirmation with direct functional measurements. Furthermore, the generalizability of rodent findings to the complexity of human preterm infants remains a key limitation.

Despite these constraints, this study uniquely compares different catch-up growth velocities after EUGR. A critical finding is that significant vascular hypertrophy was present in both slow (RCS) and accelerated (RCA) catch-up groups, with no statistical difference between them for aortic parameters. This suggests that catch-up growth itself, regardless of velocity, may initiate adverse remodelling. In contrast, the RN (restricted-then-normal-growth) group showed largely preserved vascular morphology, supporting the notion that adequate, not maximum, growth velocity is associated with better cardiovascular outcomes.[25]

CONCLUSION

Catch-up growth following early postnatal restriction produced structural changes in the aortic wall, suggesting potential early vascular remodelling. These findings support the concept that optimal, rather than maximal, catch-up growth may be preferable in preterm or growth-restricted infants.

Conflict of Interest: None to mention.

Author Contributions

HBSS: Conception and design, analysis and interpretation of data, drafting the article, critical revision for important intellectual content, and final approval.

MAR: Conception and design, analysis and interpretation of data.

AI: Analysis and interpretation of data, drafting the article.

AH: Acquisition of data, conception and design, analysis and interpretation.

FI: Conception and design, analysis and interpretation of data.

REFERENCES

1. De Costa A, Moller AB, Blencowe H, Johansson EW, Hussain-Alkhateeb L, Ohuma EO, et al. Study protocol for WHO and UNICEF estimates of global, regional, and national preterm birth rates for 2010 to 2019. PLoS One. 2021;16(10):e0258751.

2. Cao G, Liu J, Liu M. Global, regional, and national incidence and mortality of neonatal preterm birth, 1990-2019. JAMA Pediatr. 2022;176(8):787–96.
3. Peila C, Spada E, Giuliani F, Maiocco G, Raia M, Cresi F, et al. Extrauterine growth restriction: Definitions and predictability of outcomes in a cohort of very low birth weight infants or preterm neonates. Nutrients. 2020;12(5):1224.
4. Arslanoglu S, Boquien CY, King C, Lamireau D, Tonetto P, Barnett D, et al. Fortification of human milk for preterm infants: update and recommendations of the European Milk Bank Association (EMBA) Working Group on Human Milk Fortification. Front Pediatr. 2019;7:76.
5. Tian A, Meng F, Li S, Wu Y, Zhang C, Luo X. Inadequate linear catch-up growth in children born small for gestational age: Influencing factors and underlying mechanisms. Rev Endocr Metab Disord. 2024;25(4):805–16.
6. Sixtus RP, Dyson RM, Gray CL. Impact of prematurity on lifelong cardiovascular health: Structural and functional considerations. NPJ Cardiovasc Health. 2024;1(1):2.
7. Su X, Peng D. Emerging functions of adipokines in linking the development of obesity and cardiovascular diseases. Mol Biol Rep. 2020;47(10):7991–8006.
8. Josse M, Rigal E, Rosenblatt-Velin N, Rochette L, Zeller M, Guenancia C, et al. Programming of cardiovascular dysfunction by postnatal overfeeding in rodents. Int J Mol Sci. 2020;21(24):9427.
9. Ong KK, Kennedy K, Castañeda-Gutiérrez E, Forsyth S, Godfrey KM, Koletzko B, et al. Postnatal growth in preterm infants and later health outcomes: a systematic review. Acta Paediatr. 2015;104(10):974–86.
10. Whishaw IQ, Kolb B. Analysis of behavior in laboratory rats. In: Mark A. Suckow RPW, editor. The laboratory rat. 3rd ed. Elsevier; 2020. p. 215–42.
11. Alexeev EE, Lönnerdal B, Griffin IJ. Effects of postnatal growth restriction and subsequent catch-up growth on neurodevelopment and glucose homeostasis in rats. BMC Physiol. 2015;15(1):3.
12. Zeiss CJ. Comparative milestones in rodent and human postnatal central nervous system development. Toxicol Pathol. 2021;49(8): 1368–73.
13. Layton C, Bancroft JD, Suvarna SK. Fixation of tissues. In: Suvarna SK, Layton C, Bancroft JD, editors. Bancroft's Theory and Practice of Histological Techniques. 8th ed. Berkeley, CA: Elsevier; 2018. p. 40–63.
14. Robbins N, Thompson A, Mann A, Blomkalns AL. Isolation and excision of murine aorta; a versatile technique in the study of cardiovascular disease. J Vis Exp T. 2014;(93):e52172.
15. Hong JH, Kim DH, Rhyu IJ, Kye YC, Ahn HH. A simple morphometric analysis method for dermal microstructure using color thresholding and moments. Skin Res Technol. 2020;26(1):132–6.
16. Schiffrin EL. How structure, mechanics, and function of the vasculature contribute to blood pressure elevation in hypertension. Can J Cardiol. 2020;36(5):648–58.
17. Bornstein AB, Rao SS, Marwaha K. Left Ventricular Hypertrophy. Treasure Island, FL: StatPearls Publishing; 2022.
18. Sánchez-García G, Del Bosque-Plata L, Hong E. Postnatal overnutrition affects metabolic and vascular function reflected by physiological and histological changes in the aorta of adult Wistar rats. Clin Exp Hypertens. 2018;40(5):452–60.
19. Junior MDF, Cavalcante KVN, Ferreira LA, Lopes PR, Pontes CNR, de Bessa A de SM, et al. Postnatal early overfeeding induces cardiovascular dysfunction by oxidative stress in adult male Wistar rats. Life Sci. 2019;226(April):173–84.
20. Ye L, Huang Y, Chen K, Hang C, Ying Y, Zu L, et al. Early postnatal moderate catch-up growth in rats with nutritional intrauterine growth restriction preserves pulmonary vascular and cognitive function in adulthood. Exp Ther Med. 2024 Mar 5;27(5):183.
21. Yu Y, Cai Y, Yang F, Yang Y, Cui Z, Shi D, et al. Vascular smooth muscle cell phenotypic switching in atherosclerosis. Heliyon. 2024 Sep 30;10(18):e37727.
22. Félix NDDC, Nóbrega MMLD. Síndrome metabólica: análise conceitual no contexto da enfermagem. Rev Lat Am Enfermagem. 2019;27:e3154.
23. Castanys-Muñoz E, Kennedy K, Castañeda-Gutiérrez E, Forsyth S, Godfrey KM, Koletzko B, et al. Systematic review indicates postnatal growth in term infants born small-for-gestational-age being associated with later neurocognitive and metabolic outcomes. Acta Paediatr. 2017;106(8):1230–8.
24. Sebastiani G, García-Beltran C, Pie S, Guerra A, López-Bermejo A, de Toledo JS, et al. The sequence of prenatal growth restraint and postnatal catch-up growth: normal heart but thicker intima-media and more pre-peritoneal fat in late infancy. Pediatr Obes. 2019;14(3):e12476.
25. Kim YJ, Shin SH, Lee ES, Jung YH, Lee YA, Shin CH, et al. Impact of size at birth and postnatal growth on metabolic and neurocognitive outcomes in prematurely born school-age children. Sci Rep. 2021;11(1):1–8.