Effect of Gender on Metabolic and Neurodevelopmental Parameters of Postnatally Growth-Restricted Rats Undergoing Catch-Up Growth

Faiza Ikram[1], Muhammad Ali Rabani[2], Sidra Arshad[1], Barkat Ullah Khan[1] and Muhammad Usman Bashir[1]

[1]*Department of Physiology, CMH Multan Institute of Medical Sciences, Multan, Pakistan*
[2]*Department of Anatomy, CMH Multan Institute of Medical Sciences, Multan, Pakistan*

ABSTRACT

Objective: To determine the long-term effects of early postnatal malnutrition and various degrees of catch-up growth on metabolic (serum glucose, leptin, triacylglycerides) and neurodevelopmental parameters (learning and memory) among male and female rodent models, mimicking human preterm infants.

Study Design: Randomized controlled trial.

Place and Duration of the Study: CMH Multan Insitute of Medical Sciences, from September 2021 to December 2021.

Methdology: This study included 142 neonatal Wister rats, stratified into subgroups to mimic the human preterm infant model of postnatal malnutrition and catch-up growth. Metabolic consequences were assessed *via* serum analysis of glucose, leptin, and triacylglycerides. The neurocognitive comparison was made among subgroups *via* a passive avoidance test. Gender-specific comparison of all quantitative parameters was made among subgroups.

Results: Malnourished rats with accelerated catch-up growth achieved similar weight gain as normally fed rats when provided with *adlibitum* feeding in both males ($p = 0.92$) and females ($p > 0.99$). Rats undergoing accelerated catch-up growth exhibited higher fasting serum glucose levels compared to those undergoing no, or normal catch-up growth ($p < 0.001$). Malnourished female rats undergoing accelerated ($p = 0.007$), or no catchup growth ($p = 0.004$) exhibited significant deficits in learning and memory as compared to normally fed rats. Female malnourished rats with normal catchup growth exhibited no neurocognitive deficit as compared to normally fed rats ($p = 0.08$).

Conclusion: Accelerated catch-up growth effectively addresses somatic growth disparities, while normal catch-up growth offers more favourable metabolic and neurodevelopmental outcomes. Particularly, female malnourished rats exhibited poor neurodevelopment in response to both accelerated and no catch-up growth. Gender-specific variations in neurodevelopment underscore the need for personalised care approaches for preterm nutritional care.

Key Words: *Growth retardation, Leptin, Extrauterine growth restriction, Malnutrition, Neurodevelopment.*

How to cite this article: Ikram F, Rabani MA, Arshad S, Khan BU, Bashir MU. Effect of Gender on Metabolic and Neurodevelopmental Parameters of Postnatally Growth-Restricted Rats Undergoing Catch-Up Growth. *J Coll Physicians Surg Pak* 2024; **34(09)**:1029-1034.

INTRODUCTION

World Health Organization (WHO) defines preterm birth as birth before 37 completed weeks of gestation.[1] Worldwide, about 15 million children are born preterm every year.[1] In Pakistan, approximately 15.7% of births are preterm, contributing almost one million preterm births every year towards the global burden.[2]

Correspondence to: Dr. Faiza Ikram, Department of Physiology, CMH Multan Institute of Medical Sciences, Multan, Pakistan
E-mail: drfaizaikram@gmail.com
Received: March 28, 2024; Revised: July 19, 2024; Accepted: August 26, 2024
DOI: https://doi.org/10.29271/jcpsp.2024.09.1029

The nutritional management of preterm neonates is aimed at achieving growth patterns that approximate comparable intrauterine foetal growth velocities.[3] Although preterm infants can double or triple their weight during their first 2 to 3 months postnatally compared to the term infants, who take 4 to 5 months, still, a majority of preterm infants develop extrauterine growth restriction (EUGR) during their early postnatal life.[4] Malnutrition is the major cause of EUGR among preterm infants with comorbidities, who face altered nutritional uptake and utilisation along with increased energy requirements.[3]

Unfortunately, malnutrition during this "critical window" of development, can trigger "plasticity" *via* phenotypic adaptation of a genotype.[5] Developmental plasticity of genetic, nutritional, or endocrinal signalling as a consequence of malnutrition during the perinatal period, can persist in adult life, known as "nutritional programming."[6]

If adequate healthcare facilities and nutrition are ensured, preterm infants can subsequently catch up on their genetic growth potential.[3] The catch-up growth continues throughout the first year of life and is usually incomplete. Catch-up growth potentially benefits preterm infants in short-term survival and neurodevelopment, at the cost of metabolic disorders later in life.[3]

Epidemiologic studies have indicated that EUGR and the associated malnutrition in preterm infants can lead to impaired neurodevelopment.[7] Although controversial, recently protein-enriched formula feed recommendations have been made for catch-up growth in preterm infants.[8] Adult-onset obesity, insulin resistance (IR), cardiovascular disease, dyslipidaemia, and Type 2 diabetes have been reported in human preterm or small for gestational age (SGA) infants, who underwent rapid catch-up growth.[9,10] However, these results are inconclusive because of non-standardisation and paucity of the available data.

Despite the increasing burden of preterm births and thereby long-term adverse health consequences in the authors' community, very little data is available on the comparative effects of metabolic and neurodevelopmental consequences of various degrees of catch-up growth exclusively among postnatally growth-restricted preterm infants. This study intended to explore neuroendocrine markers in association with behavioural outcomes among a rodent model equivalent to human preterm infants, uniquely considering the influence of gender.

The objective of this study was to determine the long-term effects of early postnatal malnutrition and various degrees of catch-up growth on metabolic (serum glucose, leptin, triacylglycerides) and neurodevelopmental parameters (learning and memory) among male and female rodents, mimicking human preterm infants. The information gained from this research can inform the development of adequate nutritional intervention guidelines for preterm nutritional care.

METHODOLOGY

This randomised controlled trial was conducted at the CMH Multan Institute of Medical Sciences, Multan, Pakistan. Ethical approval was obtained from the Institutional Review Board and Ethical Committee vide letter No. TW/25/CIMS. The study duration was four months (September to December 2021) and involved 142 neonatal Wistar rats of both genders. The sample size was determined to maintain an average of 30 rats per group to adhere to the central limit theorem, whereas the rest (12) were excluded as mentioned below. Throughout the study, researchers were not blinded to the outcomes of the study.

Twenty-four pregnant Wistar rats were procured from a local veterinary research institute on the 14th day of gestation. These were placed in an institutional animal lab for acclimatisation before giving birth. The housing and care of the rats followed the guidelines set by the National Research Council (NRC) in 1996[11] and institutional protocols. Rats were provided with a standard diet and water *adlibitum*. Pups were left undisturbed with their dams on day one of postnatal life. On the second postnatal day, the first 142 pups delivered were randomly assigned to one of two groups using a lottery method:

a) Group N (Normal intake-n = 30): Comprising litters with 10 pups per dam, the normal average.

b) Group R (Restricted intake-n = 112): Comprising litters with 16 pups per dam to induce early postnatal malnutrition.[12]

Cross-fostered pups were closely monitored for adverse outcomes during this nutritional intervention, which continued from Day 2 to 11 of postnatal life. Based upon neural maturation, the early postnatal life of rats is considered equivalent to the early ex-utero life span of human preterm infants, during which malnutrition was introduced in this study.[13]

All 142 pups were weighed on day 11. Of the 112 pups in Group R, 108 were found to weigh below the 10th percentile compared to the 30 pups in Group N and were labelled as malnourished. The remaining four pups were excluded from the study. These malnourished pups were stratified by gender and redistributed by a lottery method into three subgroups:

Sub-group RC (Restricted then catch-up intake - n = 30): Comprising litters with 6 pups per dam to induce accelerated catch-up growth, Sub-group RN (Restricted then normal intake - n = 30): Comprising litters with 10 pups per dam to induce normal catch-up growth, and Sub-group RR (Restricted then restricted intake - n = 32): Comprising litters with 16 pups per dam to induce no catch-up growth.

The 30 pups in Group N continued to be fed normally in litters of 10 pups per dam. This nutritional intervention took place from Day 11 to 21 of their postnatal life, a period corresponding to the first two years of human life during which catch-up growth occurs in preterm human infants.[13] After Day 21, male and female pups were separated and weaned onto a standard rodent diet and fed *adlibitum*.

Somatic growth analysis involved a serial weighing of the pups every 10th day throughout this study i.e., till day 60 of postnatal life, using a digital scale precise up to 0.1g. Neurodevelopmental outcomes were assessed using the Passive Avoidance Test (PAT).[14] This test was conducted on all rats on Day 45 (training day) and Day 46 (testing day). The test apparatus consisted of two Perspex boxes, one dark and lightproof, the other white and transparent to light. On training day, rats were trained to avoid the dark chamber due to the aversive stimulus of the brief electric shock they received there on Day 45. On Day 46, they were given an extended time to enter the dark chamber, and no electric shock was administered. The correct learned behaviour was taken as an increased latency to enter the dark chamber or a greater chance of getting "timed out" on testing day. At postnatal Day 60, blood samples were collected from each rat after an overnight fast *via* terminal cardiac puncture. Rats were anaesthetised using isoflurane, and blood samples were collected and sera were separated. Separated

sera were used for glucose measurement by the glucose oxidase-mediated peroxidase method. The rest of the sera were used for the analysis of serum leptin and Triacylglycerides (TAG) using Glory Science Co, Ltd. Rat ELISA kits for leptin (Ref. No. 31070-1, Lot No. 201904) and triglycerides (Ref. No. 35613-4, Lot No. 201914).

Data analysis was conducted using Statistical Package for Social Sciences (SPSS) version 26. Data were expressed as mean ± standard error of the mean (SEM) unless otherwise specified. Three-way mixed ANOVA was used to compare the effect of independent variables (gender, nutritional group, and day of life) on body weight. Serum parameters (leptin, TAG, and fasting glucose) were analysed by two-way ANOVA, with group and gender as independent variables. For ANOVA, values of Fisher's statistic (F), probability value (p) and effect size partial eta-squared (η^2) were calculated. The impact of dietary interventions on passive avoidance was assessed *via* Kaplan-Meier analysis among male and female rats. All statistical analysis was carried out after looking for the necessary assumptions, considering a significance level of $p < 0.05$.

RESULTS

On Day 11, pups in groups N and R weighed 15.97 ± 0.29 grams and 12.36 ± 0.22 grams, respectively. Figure 1 A and B show the serial increments in the weights, of male and female pups, respectively.

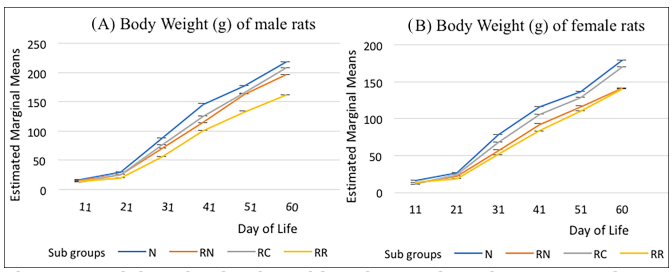

Figure 1: Weight gain of male and female pups from day 11 to 60 of postnatal life.
N: Normally fed, RN: Restricted then normal intake, RC: Restricted then catch-up intake, RR: Restricted then restricted intake. Error bars at ± 1SEM. Error bars too small are not visible.

Three-way interaction between the day of life, gender, and body weight was statistically significant $F(6.17, 234.43) = 2.56$, $p = .02$, partial $\eta^2 = 0.06$). Table I indicates that both male and female malnourished pups in subgroups RN and RC had gained weight up to normally fed rats by Day 21. When shifted to *ad-libitum*, all malnourished rats again weighed significantly less as compared to the normally fed rats at Day 31 ($p < 0.05$). However, by the end of the study at day 60 of postnatal life, male ($p = 0.92$) and female ($p > 0.99$) pups undergoing accelerated catch-up growth had gained weight comparable to the normally fed rats.

The interaction effect between gender and subgroups on the fasting serum glucose level of rats was statistically significant ($p = 0.03$). Figure 2A illustrates that the mean serum glucose levels were higher in subgroup RC, as compared to subgroup RN ($p < 0.001$) and subgroup RR ($p < 0.001$).

The interaction effect between gender and subgroup on fasting serum leptin levels ($p = 0.97$) and TAG levels ($p = 0.48$) was not statistically significant. Subsequently, the main effect for subgroups was insignificant for serum leptin ($p = 0.31$) but significant for serum TAG ($p = 0.001$). Figures 2B and 2C show the pairwise comparison of serum leptin and TAG concentrations among subgroups, respectively.

Figure 3 shows the survival distributions of latency time to enter the dark chamber for male and female rats in subgroups. The log-rank comparison showed significantly different survival distribution of subgroups on the testing day of the PAT, only for the female rats ($X^2 = 10.91$, $p = 0.01$), but no significantly different survival distributions for male rats ($X^2 = 6.65$, $p = 0.08$).

Malnourished female rats with subsequent accelerated ($\chi^2 = 7.4$, $p = 0.007$) or no catchup ($\chi^2 = 8.43$, $p = 0.004$) growth had a significantly lower latency time to enter the dark chamber on the testing day as compared to normally-fed rats. Whereas the female malnourished rats with subsequent normal growth showed no significant difference ($\chi^2 = 3.15$, $p = 0.08$) from normally-fed rats.

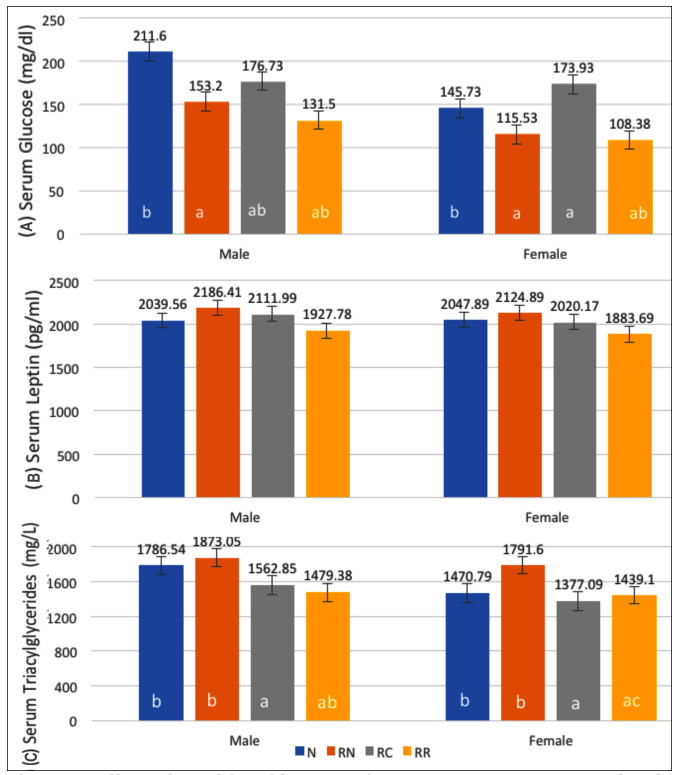

Figure 2: Effect of nutritional intervention on serum parameters of male and female rats in groups; 2A (glucose), 2B (leptin), and 2C (Triacylglycerides).
N: Normally fed, RN: Restricted then normal intake, RC: Restricted then catch-up intake, RR: Restricted then restricted intake.
Error bars at ± 1SEM. Bars carrying different alphabets differ significantly from each other within each set of comparisons, at Bonferroni adjusted p-value of <0.05.

Table I: Effect of nutritional intervention on weight of male and female pups.

Subgroup Pair (a-b)		Mean Diff. (gm)	p-value	Mean Diff. (g)	p-value	Mean Diff. (g)	p-value	Mean Diff. (g)	p-value	Mean Diff. (g)	p-value	Mean Diff. (g)	p-value
a	b	Male Day 11		Female		Male Day 21		Female		Male Day 31		Female	
N	RN	2.4	0.001*	2.6	<0.001*	4.13	0.12	4.73	0.05	18.33	<.001*	21.2	<0.001*
N	RC	5.	<0.001*	4.93	<0.001*	4	0.15	2.67	0.78	12.73	0.009*	10.67	0.04*
N	RR	3.85	<0.001*	2.9	<0.001*	9.75	<0.001*	8.45	<0.001*	32.18	<.001*	27.51	<0.001*
RN	RC	2.6	<0.001*	2.33	0.002*	-0.13	01	-2.07	1	-5.6	0.92	-10.53	0.05
RN	RR	1.45	0.13	0.3	1.000	5.62	0.01*	3.72	0.2	13.84	0.003*	6.31	0.62
RC	RR	-1.15	0.39	-2.03	0.01*	5.75	0.01*	5.79	0.01*	19.44	<.001*	16.84	<0.001*
		Day 41				Day 51				Day 60			
N	RN	32.6	<0.001*	23.6	<0.001*	14.47	0.24	19.67	0.03*	22.73	0.02*	37.87	<0.001*
N	RC	21.8	0.001*	10.47	0.34	12.73	0.42	8.33	01	11.07	0.92	9.73	1
N	RR	45.85	<0.001*	33.01	<0.001*	44.41	<0.001*	26.66	0.001*	56.67	<0.001*	40.10	<0.001*
RN	RC	-10.8	0.29	-13.1	0.11	-1.73	1	-11.33	0.64	-11.7	0.79	-28.1	0.002*
RN	RR	13.25	0.09	9.41	0.49	29.95	<0.001*	6.99	1.000	33.93	<0.001*	2.24	1
RC	RR	24.05	<0.001*	22.54	<0.001*	31.68	<0.001*	18.33	0.05	45.6	<0.001*	30.37	0.001*

*Follow-up pairwise comparisons for Three-way mixed ANOVA by post-hoc Tukey test, *p-value adjusted for multiple comparisons: Significant at <0.05, Diff.: Difference. N: Normally fed, RN: Restricted then normal intake, RC: Restricted then catch-up intake, RR: Restricted then restricted intake.*

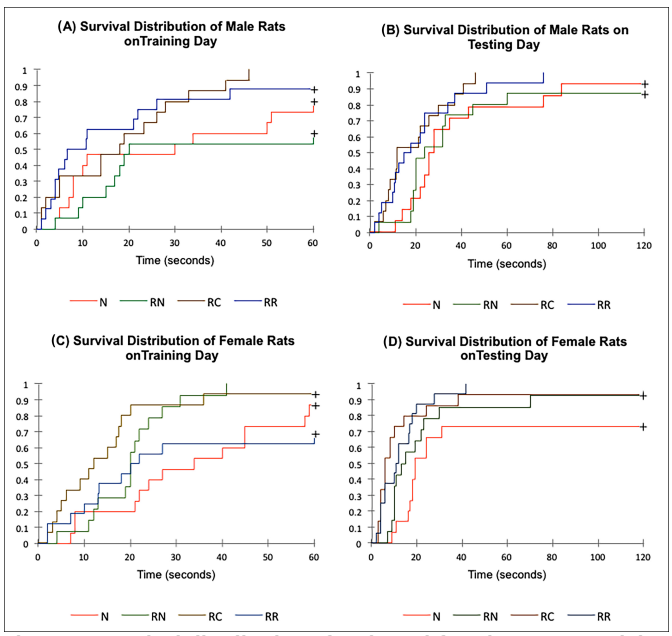

Figure 3: Survival distribution of male and female rats on training and testing days of passive avoidance test.
N: Normally fed, RN: Restricted then normal intake, RC: Restricted then catch-up intake, RR: Restricted then restricted intake.

DISCUSSION

The study explored gender-specific effects of early postnatal malnutrition and varying catch-up growth in a rodent model mimicking human preterm infants. The results highlighted the importance of gender-specific nutritional intervention to limit the long-term metabolic and neurocognitive consequences.

In the current study, pups in large litters, experiencing early postnatal malnutrition, grew significantly smaller than normally-fed rats. Previous literature suggests this effect is likely influenced by altered milk intake, nutrient utilisation, and social interactions.[15] Despite potential confounders of dam-pup and pup-pup interactions, this rodent model is widely accepted as equivalent to the early postnatal life of human preterm infants.[16]

In contrast to previous studies associating early postnatal overfeeding with later overweight and adiposity,[9,13] this study revealed that accelerated catch-up growth merely compensated for somatic growth deficiencies following early-life malnutrition. A meta-analysis of 19 studies, published in 2020, reported that early childhood weight gain velocity is a significant contributor towards obesity in adult life.[17] This discrepancy in results may be due to variability in the degree and duration of catch-up growth in the existing literature.

The current study suggested that rats undergoing accelerated and normal catch-up growth exhibited higher fasting serum glucose and TAG levels, respectively, suggesting a potential link to IR and dyslipidaemia. This aligns with previous studies reporting an association between human preterm birth and an increased risk of metabolic derangements and cardiovascular disorders, particularly among females.[17,18] Striking a balance is crucial, as both early postnatal malnutrition and accelerated catch-up growth among preterm infants have been associated with adult-onset metabolic implications.

Notably, the current study sheds light on the gender-specific impact of catch-up growth on neurodevelopmental outcomes. Malnourished female rats undergoing accelerated catch-up growth in the current study exhibited significant deficits in learning and memory, contrary to previous literature. Previous studies have demonstrated improved neurodevelopmental outcomes of preterm infants by accelerated catch-up growth achieved *via* protein and fatty acids-supplemented formula feed.[19,20] Conversely, rats undergoing normal catch-up growth exhibited neurodevelopmental advantage in the current study, emphasising the importance of optimal catch-up growth for preterm infants' neurocognition. Similarly, Beyerlein *et al.* proposed a linear relationship between cognition and weight gain velocity from −1 to +2 standard deviation, and no further advantage at >+2 standard deviation.[21]

Based on previous studies, rapid weight gain, leptin, or insulin dysregulation can be the cause of poor neurocogni-

tion among malnourished rats with rapid or no catch-up growth. In addition to a satiety signal, leptin also contributes to perinatal brain development.[22] In the current study, serum leptin concentration was not different among various subgroups at day 60 of postnatal life. However, this study is limited in not accessing the serum leptin levels of rats in early neonatal life when malnutrition and catch-up growth interventions were carried out. Another limitation of this rodent model is its dependency on nutritional intervention, but the specifics of the milk quality and quantity could not be detailed. Also, the neurodevelopmental findings need conformation with more sophisticated tests for better generalisation.

Despite the limitations, the study's focus on preterm infant models and gender disparities is highly relevant to public health. These results have the potential to inform nutritional guidelines and interventions for this vulnerable population, owing to its multidisciplinary perspectives.

CONCLUSION

Accelerated catch-up growth effectively addresses somatic growth disparities, while normal catch-up growth offers more favourable metabolic and neurodevelopmental outcomes. In particular, female malnourished rats exhibited poor neurodevelopment in response to both accelerated and no catch-up growth. Gender-specific variations in neurodevelopment underscore the need for personalised care approaches for preterm care.

ETHICAL APPROVAL:
An approval was obtained from the Institutional Review Board and Ethical Committee vide letter No. TW/25/CIMS.

PATIENTS' CONSENT:
Not applicable.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
FI: Conceived idea, analysis and interpretation of data and manuscript writing.
MAR: Collection and analysis of data, manuscript writing, and literature search.
SA: Collection of data and critical revision of the manuscript.
BUK: Collection of data and critical revision of the manuscript.
MUB: Supervision, literature search, and critical revision of the manuscript.

REFERENCES

1. Chawanpaiboon S, Vogel JP, Moller AB, Lumbiganon P, Petzold M, Hogan D*, et al.* Global, regional, and national estimates of levels of preterm birth in 2014: a systematic review and modelling analysis. *Lancet Glob Health* 2019; **7(1)**:e37-e46. doi: 10.1016/S2214-109X(18)30451-0.
2. AMANHI GASG. Population-based rates, risk factors and consequences of preterm births in South-Asia and sub-Saharan Africa: A multi-country prospective cohort study. *J Glob Health* 2022; **12**:004011. doi: 10.7189/jogh.12.04011.
3. Greer FR, Norlin S, Olsen IE. How Should the Preterm Infant Grow? *Curr Pediatr Rep* 2020; **8**:202-8. doi: 10.1007/s401 24-013-0029-1.
4. Silveira RC, Procianoy RS. Preterm newborn's postnatal growth patterns: How to evaluate them. *J Pediatr* 2019; **95**: S42-8. doi: 10.1016/j.jped.2018.10.006.
5. Buchanan KL, Meillere A, Jessop TS. Early life nutrition and the programming of the phenotype. In: Costantini D, Marasco V, Eds. Development Strategies and Biodiversity: Darwinian Fitness and Evolution in the Anthropocene: ed. 1st, Switzerland; Springer; 2022. p. 161-214.
6. de Rooij SR. Are brain and cognitive reserve shaped by early life circumstances? *Front Neurosci* 2022; **16**:825811. doi: 10.3389/fnins.2022.825811.
7. Castanys-Munoz E, Kennedy K, Castaneda-Gutierrez E, Forsyth S, Godfrey KM, Koletzko B*, et al.* The 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. doi: 10.1111/apa.13868.
8. Rizzo V, Capozza M, Panza R, Laforgia N, Baldassarre ME. Macronutrients and Micronutrients in Parenteral Nutrition for Preterm Newborns: A Narrative Review. *Nutrients* 2022; **14(7)**:1530. doi: 10.3390/nu14071530.
9. Casirati A, Somaschini A, Perrone M, Vandoni G, Sebastiani F, Montagna E*, et al.* Preterm birth and metabolic implications on later life: A narrative review focused on body composition. *Front Nutr* 2022; **9**:978271. doi: 10.3389/fnut. 2022.978271.
10. Yoshida-Montezuma Y, Stone E, Iftikhar S, De Rubeis V, Andreacchi AT, Keown-Stoneman C*, et al.* The association between late preterm birth and cardiometabolic conditions across the life course: A systematic review and meta-analysis. *Paediatr Perinat Epidemiol* 2022; **36(2)**:264-75. doi: 10.1111/ppe.12831.
11. Kumar S, Rajput MK, Tickoo SB. Laws, regulations, policies and guidelines governing the care and use of laboratory animals. In: Nagarajan P, Gudde R, Srinivasan R., Eds. Essentials of Laboratory Animal Science: Principles and Practices. Singapore: Springer; 2021. p. 23-38.
12. Widdowson EM, Mc CR. Some effects of accelerating growth. I. General somatic development. *Proc R Soc Lond B Biol Sci* 1960; **152(947)**:188-206. doi: 10.1098/rspb.1960.0032.
13. Alexeev EE, Lonnerdal 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. doi: 10.1186/s12899-015-0017-5.
14. Jou MY, Lonnerdal B, Griffin IJ. Effects of early postnatal growth restriction and subsequent catch-up growth on body composition, insulin sensitivity, and behaviour in neonatal rats. *Pediatr Res* 2013; **73(5)**:596-601. doi: 10.1038/pr. 2013.27.
15. Fiorotto ML, Burrin DG, Perez M, Reeds PJ. Intake and use of milk nutrients by rat pups suckled in small, medium, or large litters. *Am J Physiol* 1991; **260(6 Pt 2)**:R1104-13. doi: 10.1152/ajpregu.1991.260.6.R1104.

16. Bouret SG. Developmental programming of hypothalamic melanocortin circuits. *Exp Mol Med* 2022; **54(4)**:403-13. doi: 10.1038/s12276-021-00625-8.

17. Ou-Yang MC, Sun Y, Liebowitz M, Chen CC, Fang ML, Dai W, *et al.* Accelerated weight gain, prematurity, and the risk of childhood obesity: A meta-analysis and systematic review. *PLoS One* 2020; **15(5)**:e0232238. doi: 10.1371/journal.pone.0232238.

18. Wells JC, Sawaya AL, Wibaek R, Mwangome M, Poullas MS, Yajnik CS*, et al.* The double burden of malnutrition: Aetiological pathways and consequences for health. *Lancet* 2020; **395(10217)**:75-88. doi: 10.1016/S0140-6736(19)32472-9.

19. Silveira RC, Corso AL, Procianoy RS. The influence of early nutrition on neurodevelopmental outcomes in preterm infants. *Nutrients* 2023; **15(21)**:4644. doi: 10.3390/nu1521 4644.

20. Fang PC, Kuo HK, Huang CB, Ko TY, Chen CC, Chung MY. The effect of supplementation of docosahexaenoic acid and arachidonic acid on visual acuity and neurodevelopment in larger preterm infants. *Chang Gung Med J* 2005; **28 (10)**:708-15. Available from: http://www.ncbi.nlm. nih.gov/pubmed/16382755

21. Beyerlein A, Ness AR, Streuling I, Hadders-Algra M, von Kries R. Early rapid growth: no association with later cognitive functions in children born not small for gestational age. *Am J Clin Nutr* 2010; **92(3)**:585-93. doi: 10.3945/ajcn.2009.29116.

22. Maffei M, Giordano A. Leptin, the brain and energy homeostasis: From an apparently simple to a highly complex neuronal system. *Rev Endocr Metab Disord* 2022; **23(1)**: 87-101. doi: 10.1007/s11154-021-09636-2.