

Table 1: Effect of nutritional intervention on growth velocity of rats, Day 11 - 60 of postnatal life

Subgroup Pair (a-b)	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	Mean ± SEM	P-value	
a	b	Day 11- 21		Day 21- 31		Day 31- 41		Day 41- 51		Day 51- 60	
N	RN	28.29± 42.53	1.00	71.52± 20.79	0.01*	-45.79± 28.15	0.64	-109.89± 24.88	<.001*	46.36± 18.36	0.08
N	RC	-183.06± 42.53	<.001*	1.36± 20.79	1.00	-26.6± 28.15	1.00	-59.02± 24.88	0.12	-19.78± 18.36	1.00
N	RR	152.65± 41.86	0.002*	43.87± 20.46	0.20	-88± 27.71	0.01*	-102.09± 24.49	<.001*	16.54± 18.07	1.00
RN	RC	-211.35± 42.53	<.001*	-70.17± 20.79	0.01*	19.19± 28.15	1.00	50.87± 24.88	0.26	-66.14± 18.36	0.01*
RN	RR	124.36±41.86	0.02*	-27.66± 20.46	1.00	-42.22± 27.71	0.78	7.81± 24.49	1.00	-29.82± 18.07	0.61
RC	RR	335.71± 41.86	<.001*	42.51± 20.46	0.24	-61.41± 27.71	0.17	-43.07± 24.49	0.49	36.31± 18.07	0.28

*Significant at Bonferroni adjusted alpha level of $p < 0.05$, SEM: Standard error of mean

N; Normally Fed, RN; Restricted then normal Growth, RC; Restricted then Accelerated catch-up growth, RR; Restricted then restricted growth, (N/RN/RC: $n=30$ each, RR: $n=32$).

Univariate ANCOVA for the serum glucose of rats in subgroups was statistically significant ($p < 0.001$), while adjusting for body weight. Figure 2 shows pairwise comparisons indicating that mean serum glucose concentrations of N and RC were significantly higher than RN and RR, $p < 0.01$.

RR; Restricted then restricted growth. (N/RN/RC: $n=30$ each, RR: $n=32$)

Table 2: Effect of nutritional intervention on “Timed-out” event of Passive avoidance Test

Sub-groups	Training Day		Testing Day	
	Entered dark chamber. n (%)	Timed-out at 60 sec n (%)	Entered dark chamber. n (%)	Timed-out at 120 sec n (%)
N	25 (83.3%)	5 (16.7%)	25 (83.3%)	5 (16.7%)
RN	24 (80%)	6 (20%)	27 (90%)	3 (10%)
RC	29 (96.7%)	1 (3.3%)	29 (96.7%)	1 (3.3%)
RR	25 (78.1%)	7 (21.9%)	32 (100%)	0 (0%)

N; Normally Fed, RN; Restricted then normal Growth, RC; Restricted then Accelerated catch-up growth, RR; Restricted then restricted growth. (N/RN/RC: $n=30$ each, RR: $n=32$)

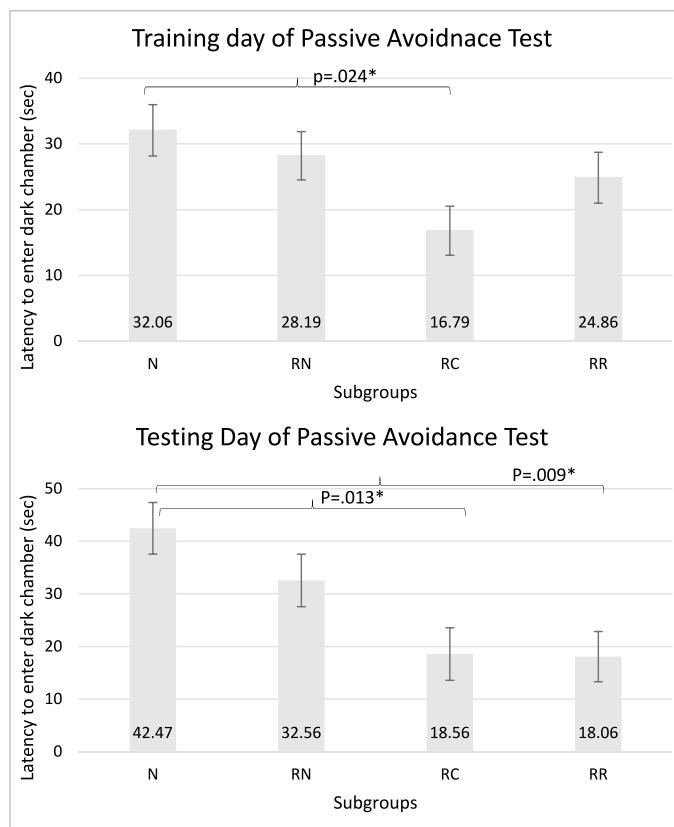


Figure 3: Effect of nutritional intervention subgroups on passive avoidance test performance

*Significant at Bonferroni adjusted alpha level of $p < 0.05$, Error bars: $\pm 1SEM$,

N; Normally Fed, RN; Restricted then normal Growth, RC; Restricted then Accelerated catch-up growth,

Univariate ANCOVA for insulin ($p=0.15$) and leptin ($p=0.97$), both adjusted for final body weight, showed no significant subgroup effect. Therefore, post hoc testing was not performed. Figure 2 presents the adjusted means for insulin and leptin levels.

Univariate ANCOVA for serum triglyceride (TAG) levels showed a significant subgroup effect ($p=0.001$), adjusting for body weight. Figure 2 shows that subgroup RN had significantly higher TAG levels than RC ($p=0.014$) and RR ($p=0.001$).

Univariate analysis for passive avoidance test latency showed significant subgroup differences on training ($p=0.03$) and testing ($p=0.004$) days. Figure 3 shows significantly longer latency in N compared with RC on training ($p=0.024$) and testing ($p=0.013$) days, and longer latency in N compared with RR on testing day ($p=0.009$).

Chi-square analysis of the proportion of rats who timed out showed no significant subgroup difference on train-

ing day ($p=0.15$), but was significant on testing day ($p=0.04$). Table 2 shows subgroup-wise percentages of timed-out rats.

Discussion

This study explores the interplay between early postnatal malnutrition, catch-up growth, and their impacts on glucose homeostasis and neurodevelopment in a rat model. During the catch-up growth phase, malnourished rats with accelerated growth exhibited significantly higher growth velocity than normally fed rats, aligning with previous research indicating short-term growth advantages.²² Despite initial growth retardation, preterm infants typically catch up to their genetic growth potential with adequate nutrition.²³

However, the subsequent normalization of growth velocity between malnourished and normally fed rats by the study's end emphasizes the transient nature of these effects, contrary to studies suggesting persistent weight gain and obesity after rapid catch-up growth.²⁴ Many observational studies and review articles among human preterm infants have reported a high risk of later obesity among protein supplemented formula-fed infants.^{24,25} Previous rodent research has shown similar results relating early postnatal overfeeding to overweight and adiposity later in life.²⁶

Notably, serum glucose concentrations were significantly higher in malnourished rats with rapid catch-up growth compared to those undergoing normal or no catch-up growth. This suggests that the metabolic consequences of catch-up growth extend beyond the immediate growth phase, emphasizing the need for careful consideration of nutritional interventions in the postnatal period. These results support the concept of nutritional programming, indicating that extremely low birth weight (EUGR) infants not only fail to thrive but also experience long-term adverse consequences, including an increased risk of adult-onset type 2 Diabetes Mellitus and cardiovascular diseases among overweight preterm infants.^{23,27} However, current study showed no difference in serum glucose of normally fed rats and rats with rapid catch-up growth. Previously, rodent, and human studies have variably associated preterm birth and rapid catch-up growth with deranged glucose homeostasis; still, the results are inconclusive because of heterogeneity of data available.^{3,14,28}

Surprisingly, serum insulin and leptin levels did not show significant differences among subgroups when adjusting for body weight, raising concerns about the intricate relationship between catch-up growth, metabolic factors, and the potential role of compensatory

mechanisms. Insulin and leptin are both critical regulators in growth and metabolism.²⁹ Previous studies have linked accelerated catch-up growth to increased insulin resistance and deranged leptin levels.^{3,30} Inadequate nutrition during the perinatal period often results in altered leptin levels, which can adversely impact hypothalamic development and energy balance regulation.²⁹ The variation observed in this study may be due to the timing of the assessment. Measurements on day 60 may not capture the significant effects of insulin and leptin, which could be more evident during catch-up growth or later metabolic adaptation. A strength of this study is the use of ANCOVA to adjust for weight at assessment, a factor often not fully addressed in other studies. Although ANCOVA controlled key confounders, it may not have captured hormonal or stage-specific metabolic influences on insulin and leptin.

In contrast, serum triglyceride levels demonstrated significant differences, with malnourished rats undergoing normal catch-up growth showing higher mean levels compared to those with restricted catch-up growth. Previously, catch-up growth was also associated with an increased risk of dyslipidemia and cardiovascular disorders among low birth weight and preterm infants.³ These findings underscore the need for a comprehensive understanding of the metabolic consequences associated with catch-up growth in the context of early malnutrition.

In this study, malnourished rats with normal catch-up growth demonstrated significantly better learned behavior than rats with rapid or no catch-up growth. Conversely, previous studies largely associate accelerated catch-up growth with improved neurodevelopment of preterm infants at the cost of metabolic derangements.^{22,24} Consistent with this study, 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.³¹ Conversely, previous studies largely associate accelerated catch-up growth with improved neurodevelopment of preterm infants at a cost of metabolic derangements.^{22,24} However, the direction and magnitude of the relation between catch-up growth velocity and neurocognitive/ metabolic consequences are still controversial.

In conclusion, this study provides valuable insights into the intricate relationship between early postnatal malnutrition, catch-up growth, and subsequent metabolic and neurodevelopmental outcomes. These findings offer a foundation for future research and potential interventions in the realm of developmental biology and pediatric medicine.

Limitations and Recommendations:

This study is limited to using a rat model, which may not perfectly mirror the complexities of human development. The results from this rat model may not directly apply to human infants due to differences in metabolism and neurodevelopment. Also, the Passive Avoidance Test measures only one aspect of neurodevelopment. Future research could explore additional markers of metabolic health and extend the observation period to elucidate the long-term consequences further. More sophisticated measures of neurocognitive outcome could be used, such as the Morris Water Maze, T-Maze, or Barnes Maze.

Conclusion

Catch-up growth at a normal velocity after early post-natal malnutrition preserves metabolic health while limiting neurodevelopmental deficit. Accelerated catch-up growth, though transiently beneficial for growth, can increase vulnerability to neurodevelopmental deficits. These findings urge a nuanced approach in developmental biology and pediatric medicine for effective interventions and improved outcomes among preterm infants.

Ethical Approval: The Institutional Review Board/Ethical Committee, CMH Multan Institute of Medical Sciences (CIMS), Multan approved this study vide Case No. TW/25/ CIMS.

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Authors' Contribution

FI: Conception & design, acquisition of data, analysis & interpretation, drafting of article, critical revisions for important intellectual content, final approval of the version to be published

AR: acquisition of data, analysis & interpretation, drafting of article

SA: Acquisition of data, analysis & interpretation

UB: Critical revisions for important intellectual content

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