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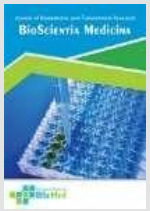

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# The Temporal Windows of Glycemic Injury: Association of Early and Late First-Week Hyperglycemia with Retinopathy of Prematurity in Low-Birth-Weight Infants

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**A B S T R A C T**

**Background:** Retinopathy of prematurity (ROP) is a leading cause of childhood blindness, driven by aberrant retinal vascular development in preterm infants. While hyperglycemia is a recognized risk factor, its impact may vary depending on its timing relative to the biphasic pathogenesis of ROP. This study aimed to generate a hypothesis regarding the differential association of hyperglycemia on postnatal day 1 versus day 7 with the incidence of ROP in a high-risk neonatal population. **Methods:** We conducted a retrospective, cross-sectional, hypothesis-generating study at Prof. Dr. I.G.N.G. Ngoerah General Hospital. Medical records of 68 preterm (<37 weeks gestation) and low-birth-weight (<2500 grams) infants who underwent ROP screening were reviewed. The exposures of interest were hyperglycemia (blood glucose >125 mg/dL) on postnatal day 1 (D1) and day 7 (D7). The primary outcome was the diagnosis of any stage of ROP. Statistical analysis involved Chi-square tests and exploratory multivariate logistic regression to adjust for select confounders. **Results:** Of the 68 infants included (mean gestational age 30.5 ± 2.2 weeks, mean birth weight 1447.5 ± 373.0 grams), 11 (16.2%) were diagnosed with ROP. Hyperglycemia was present in 29.4% of infants on D1 and 13.2% on D7. In logistic regression analysis, a strong statistical association was observed between hyperglycemia and ROP for both D1 (Adjusted Odds Ratio [AOR] = 55.7; 95% Confidence Interval [CI]: 5.1–611.0; p=0.001) and D7 (AOR = 74.5; 95% CI: 9.0–613.4; p<0.001). However, the profoundly wide confidence intervals indicate significant statistical instability and imprecision. **Conclusion:** This study found a strong, albeit statistically imprecise, association between hyperglycemia on both the first and seventh day of life and the incidence of ROP. These findings support the hypothesis that the timing of glycemic dysregulation may be critical, potentially impacting different phases of ROP pathogenesis. The results, while preliminary, underscore the need for larger, prospective studies to confirm these associations and elucidate the role of glycemic control in ROP prevention.

## 1. Introduction

Retinopathy of prematurity (ROP) represents a modern paradox in pediatric medicine: a disease born from the very success of neonatal care that saves the lives of increasingly premature infants.[1] This proliferative vascular disorder of the developing retina remains a principal cause of preventable childhood blindness worldwide. Annually, it is estimated that

ROP leads to irreversible visual impairment in over 32,000 children, with a tragic number progressing to complete blindness. Beyond vision loss, the sequelae of ROP extend to a lifetime of ophthalmological challenges, including a heightened risk of strabismus, high myopia, glaucoma, and deficits in visual field and contrast sensitivity. The global burden of ROP is substantial, though incidence rates vary widely, reflecting differences in neonatal intensive care standards and screening protocols.[2] The landmark early treatment for retinopathy of prematurity (ET-ROP) study in the United States reported that 68% of infants with a birth weight under 1,251 grams developed some stage of ROP. Data from the UK, US, South Korea, and Indonesia show incidences ranging from 3.8% to over 30%, underscoring the universal nature of this challenge.[3]

The pathogenesis of ROP is a complex, biphasic process intricately linked to the developmental arrest of the retina following premature birth.[4] Phase I, occurring roughly during the first few weeks of life, is characterized by the cessation of normal retinal vessel growth. This is precipitated by the abrupt loss of the placenta—the primary source of crucial growth factors, most notably Insulin-like Growth Factor 1 (IGF-1)—and exposure to a relatively hyperoxic extrauterine environment. This initial phase of vaso-cessation creates an avascular peripheral retina. As the metabolic demands of the maturing retina increase, this avascular zone becomes hypoxic, triggering Phase II. This second phase is a pathological response characterized by the profound upregulation of hypoxia-inducible factors (HIFs) and a subsequent surge in vascular endothelial growth factor (VEGF) production, leading to uncontrolled, abnormal neovascularization.[5] These fragile new vessels can bleed, form scar tissue, and ultimately cause tractional retinal detachment and blindness.

While prematurity and low birth weight are the sine qua non for ROP development, a constellation of other risk factors modulates its incidence and severity. These include supplemental oxygen therapy, sepsis, respiratory distress syndrome (RDS), and various nutritional deficiencies.[6] Among these, neonatal hyperglycemia has emerged as a particularly compelling and modifiable risk factor. Preterm infants are exquisitely vulnerable to glucose dysregulation due to a combination of immature pancreatic β-cell function, peripheral insulin resistance often exacerbated by stress and inflammation, and the high glucose load from essential parenteral nutrition.[7] Indeed, hyperglycemia is a common finding in this population, with studies reporting prevalence rates from 20% to as high as 88% in very low birth weight (VLBW) infants. The link between hyperglycemia and ROP is biologically plausible, yet the clinical evidence has been inconsistent, as highlighted by systematic reviews and meta-analyses.[8] Some studies demonstrate a strong association, while others find the link diminishes after adjusting for the severity of illness, suggesting hyperglycemia might merely be a marker of a sicker infant rather than an independent causal factor. This inconsistency may stem from methodological differences, but it also points to a more complex underlying relationship. Increasingly, research suggests that the pattern of glycemic dysregulation—specifically, high glucose variability—may be more injurious than sustained, stable hyperglycemia. Fluctuating glucose levels are thought to induce greater oxidative stress and endothelial dysfunction than stable high glucose.[9]

While the association between hyperglycemia and ROP has been investigated, the existing literature has not sufficiently explored the differential impact of the timing of the hyperglycemic insult.[10] The biphasic nature of ROP pathophysiology suggests that the developmental stage of the retina could dictate its vulnerability to metabolic derangement. The novelty of this study lies in its specific focus on comparing two distinct time points within the first postnatal week. By analyzing hyperglycemia on day 1 separately from day 7, we aim to uncover preliminary evidence for "temporal windows of vulnerability," a concept that has not been thoroughly examined in clinical ROP research. This approach moves beyond simply asking if hyperglycemia is a risk factor to asking when it

might be most detrimental, thereby providing a more nuanced perspective on the interplay between metabolic state and retinal development. Given the existing gaps in the literature, the primary aim of this study was to conduct an exploratory analysis to generate a specific, testable hypothesis: that the association between hyperglycemia and ROP differs depending on its timing within the first week of postnatal life. By comparing the impact of hyperglycemia on day 1 with that on day 7, we sought to provide preliminary evidence on whether these represent distinct windows of vulnerability, potentially corresponding to the different pathophysiological phases of ROP. This work is intended to serve as a foundational step, providing the rationale and direction for future, more definitive prospective investigations into this critical area of neonatal care.

## 2. Methods

This study was conducted using an analytical observational design with a retrospective, cross-sectional approach. This design was explicitly chosen for its suitability for hypothesis generation, utilizing existing clinical data to explore preliminary associations that can inform the design of future, more definitive prospective cohort studies. The research was carried out by reviewing medical records from the Department of Ophthalmology, the Neonatal Intensive Care Unit (NICU), and the Medical Records Unit of Prof. Dr. I.G.N.G. Ngoerah General Hospital in Denpasar, Indonesia, a tertiary referral center. The data collection period spanned from March 2024 to February 2025, covering records of infants admitted in the preceding years. The study population consisted of preterm infants with low birth weight who were admitted to the NICU and underwent ROP screening as part of standard clinical care. We employed a consecutive sampling strategy to identify all eligible patients from the hospital's medical records database, aiming to minimize selection bias. The inclusion criteria were precisely defined as: (1) infants born at a gestational age of <37 weeks with a birth weight of <2500 grams who underwent ROP screening according to institutional protocol; (2) availability of a complete medical record, including demographic data (gestational age, birth weight, gender), ophthalmological examination results for ROP, key laboratory values, and a comprehensive neonatal history; and (3) specifically, documented blood glucose level measurements for both postnatal day 1 and day 7 of life. The exclusion criterion was any medical record with incomplete or missing data for the key variables under investigation, which would preclude their inclusion in the analysis. After applying these criteria, a final sample of 68 infants was included in the study.

Data were retrospectively extracted from inpatient medical records by trained research staff. A standardized data extraction form was used to ensure consistency. The primary outcome was the presence of ROP. The diagnosis was recorded as a binary variable (Yes/No) based on the findings of indirect ophthalmoscopy performed by a pediatric ophthalmologist as documented in the medical record. While the stage of ROP was often recorded, for the purpose of this analysis with a small number of cases, all stages of ROP were grouped together to create the binary outcome. The primary exposure variable was hyperglycemia, measured at two distinct time points: postnatal day 1 (D1) and day 7 (D7). Consistent with common clinical definitions in neonatology, hyperglycemia was defined as any recorded blood glucose concentration >125 mg/dL. For the analysis, this was treated as a binary variable (Yes/No) for each time point. Several potential confounding variables were extracted from the medical records, including: gestational age (in weeks), birth weight (in grams), gender, respiratory distress syndrome (RDS), patent ductus arteriosus (PDA), documented sepsis (based on clinical signs and/or positive blood cultures), requirement for supplemental oxygen therapy, and multiple births (gemelli).

All data were entered into a database and analyzed using the Statistical Package for the Social Sciences (SPSS) version 29. The characteristics of the study sample were summarized using descriptive statistics.

Continuous variables with a normal distribution (such as gestational age, birth weight, glucose levels) were presented as means and standard deviations (SD). Categorical variables (such as gender, presence of ROP, hyperglycemia, comorbidities) were presented as frequencies and percentages. To assess the initial association between the primary exposures (hyperglycemia on D1 and D7) and the outcome (ROP), we used the Chi-square test or Fisher's exact test where appropriate. For this cross-sectional data, we calculated the Prevalence Ratio (PR) with its 95% Confidence Interval (CI) to estimate the magnitude of the association. A p-value of <0.05 was considered statistically significant. To explore the independent association of hyperglycemia with ROP while accounting for other factors, we performed an exploratory multivariate logistic regression analysis. Two separate models were built: one for D1 hyperglycemia and one for D7 hyperglycemia. Potential confounders considered for inclusion were RDS, PDA, and gemelli status. Sepsis and supplemental oxygen use, despite their known biological importance, were excluded from the final regression models. This decision was necessitated by a lack of variability in the data (94.1% of infants had sepsis and 80.9% received oxygen), which can lead to statistical issues such as quasi-complete separation and model instability. The results of the logistic regression are presented as Adjusted Odds Ratios (AOR) with their corresponding 95% CIs and p-values. This study was conducted in accordance with the ethical principles of the Declaration of Helsinki. As a retrospective study using anonymized data from medical records, direct patient contact was not required. Approval was sought from the relevant ethics committee (Faculty of Medicine, Universitas Udayana, Denpasar, Indonesia) prior to the commencement of data collection.

**3. Results**

Table 1 showed a detailed clinical portrait of the 68 preterm infants who constituted the study cohort. This population was characterized by a significant degree of immaturity and clinical fragility, providing a critical context for the study's primary investigation into retinopathy of prematurity (ROP). The data revealed that the infants were extremely premature, with a mean gestational age of 30.5 weeks (± 2.2 weeks). This was coupled with a mean birth weight of 1447.5 grams (± 373.0 grams), placing the cohort, on average, within the very low birth weight category. The demographic composition was nearly balanced, consisting of 36 males (52.9%) and 32 females (47.1%). The primary outcome of interest, retinopathy of prematurity, was diagnosed in 11 infants, corresponding to a notable incidence of 16.2% within this high-risk group. The metabolic profile of the cohort, a central focus of the study, was detailed through blood glucose measurements. On the first day of life, the mean blood glucose level was 120.9 mg/dL (± 39.8 mg/dL), a value approaching the clinical threshold for hyperglycemia. This level subsequently decreased to a mean of 107.7 mg/dL (± 28.9 mg/dL) by day seven, illustrating a dynamic glycemic course during the critical first week of postnatal life. Furthermore, the table 1 powerfully illustrates the immense clinical burden carried by these infants. An overwhelming majority suffered from conditions known to be associated with prematurity and adverse outcomes. Sepsis was nearly universal, affecting 64 infants (94.1%), while respiratory distress syndrome (RDS) was also highly prevalent, diagnosed in 57 infants (83.8%). Consequently, the need for clinical intervention was substantial, with 55 infants (80.9%) requiring supplemental oxygen therapy. Other significant clinical factors included the presence of a patent ductus arteriosus (PDA) in 9 infants (13.2%) and the occurrence of multiple births (gemelli) in 11 infants (16.2%). In summary, Table 1 effectively establishes the study's setting within a population of clinically vulnerable, very low birth weight infants. It highlights not only their profound immaturity but also a high incidence of ROP set against a backdrop of near-universal comorbidity from sepsis and respiratory distress.

## Table 1. Baseline Characteristics of the Study

A detailed summary of the 68 preterm infants included in the Retinopathy of Prematurity (ROP) study.

| VARIABLE | VALUE |
|---|---|
| Gestational Age (weeks), Mean ± SD | 30.5 ± 2.2 |
| Birth Weight (grams), Mean ± SD | 1447.5 ± 373.0 |
| Gender, n (%) | |
| Male | 36 (52.9) |
| Female | 32 (47.1) |
| Retinopathy of Prematurity, n (%) | 11 (16.2) |
| Blood Glucose (mg/dL), Mean ± SD | |
| Day 1 | 120.9 ± 39.8 |
| Day 7 | 107.7 ± 28.9 |
| **Clinical Conditions & Interventions** | |
| Supplemental Oxygen Use, n (%) | 55 (80.9) |
| Sepsis, n (%) | 64 (94.1) |
| Patent Ductus Arteriosus (PDA), n (%) | 9 (13.2) |
| Respiratory Distress Syndrome (RDS), n (%) | 57 (83.8) |
| Multiple Births (Gemelli), n (%) | 11 (16.2) |

Table 2 showed a striking and statistically significant relationship between the presence of hyperglycemia and the incidence of retinopathy of prematurity (ROP) at two distinct time points during the critical first week of postnatal life. The data, presented as a bivariate analysis, offers a clear initial look at the powerful association between this metabolic disturbance and retinal pathology. The analysis for the first day of life revealed a dramatic disparity in ROP outcomes based on glycemic status. Among the 20 infants who experienced hyperglycemia, exactly half (50.0%) were subsequently diagnosed with

ROP. In stark contrast, ROP was a rare event in the 48 infants with normal blood glucose levels, occurring in only one infant (2.1%). This profound difference is quantified by the Prevalence Ratio (PR), which was a staggering 24.0 (95% CI: 3.3 – 175.3). This value indicates that the prevalence of ROP was 24 times higher in infants who were hyperglycemic on their first day of life compared to their normoglycemic peers. The association was highly statistically significant ($p < 0.001$), underscoring that this finding is extremely unlikely to be a result of random chance. The analysis was repeated for day seven, and the potent association between hyperglycemia and ROP persisted. In the group of 9 infants with hyperglycemia on day seven, the incidence of ROP was even more pronounced, affecting a substantial majority of 77.8%. This stood in sharp contrast to the 59 infants without hyperglycemia, where the incidence of ROP was only 6.8%. While the Prevalence Ratio for the day seven analysis was lower than for day one, it remained exceptionally high at 11.5 (95% CI: 4.2 – 31.5). This signifies that the prevalence of ROP was still more than 11 times greater for infants with elevated blood sugar at the one-week mark. This relationship was also confirmed to be highly statistically significant ($p < 0.001$). Table 2 powerfully illustrates that hyperglycemia, whether occurring immediately after birth or later in the first week, is a profound risk factor for the development of ROP. The magnitude of the prevalence ratios at both time points highlights the clinical importance of this metabolic derangement as a potential predictor for this sight-threatening condition.

## Table 2. Bivariate Association: Hyperglycemia and ROP

Analysis of the relationship between hyperglycemia on Day 1 and Day 7 and the incidence of Retinopathy of Prematurity (ROP).

| HYPERGLYCEMIA STATUS | ROP (YES) n (%) | ROP (NO) n (%) | TOTAL | PREVALENCE RATIO (95% CI) | P-VALUE |
|---|---|---|---|---|---|
| **Day 1 Analysis** | | | | | |
| Yes (>125 mg/dL) | 10 (50.0) | 10 (50.0) | 20 | **24.0** (3.3 – 175.3) | <0.001 |
| No (≤125 mg/dL) | 1 (2.1) | 47 (97.9) | 48 | Reference | — |
| **Day 7 Analysis** | | | | | |
| Yes (>125 mg/dL) | 7 (77.8) | 2 (22.2) | 9 | **11.5** (4.2 – 31.5) | <0.001 |
| No (≤125 mg/dL) | 4 (6.8) | 55 (93.2) | 59 | Reference | — |

Table 3 showed the results of the multivariate logistic regression analysis, a sophisticated statistical method designed to isolate the independent impact of each risk factor on the development of retinopathy of prematurity (ROP) after mathematically adjusting for the influence of other variables in the model. This

analysis provides a more nuanced view than the initial bivariate comparison, revealing which factors remain significant predictors when considered simultaneously. The analysis for the first day of life produced a powerful and unequivocal primary finding. After controlling for the effects of respiratory distress syndrome (RDS), Patent Ductus Arteriosus (PDA), and multiple births, hyperglycemia on day 1 emerged as an exceptionally strong and statistically significant independent predictor of ROP. The Adjusted Odds Ratio (AOR) for hyperglycemia was a staggering 55.7. This value can be interpreted to mean that the odds of an infant developing ROP were nearly 56 times higher if they experienced hyperglycemia on their first day of life, compared to infants who did not, even when holding the other clinical factors constant. This dramatic odds ratio, however, must be viewed in the context of its 95% Confidence Interval (CI), which ranged from 5.1 to 611.0. The vastness of this interval indicates significant statistical imprecision, a common result in studies with smaller sample sizes. While the true effect size is uncertain, the fact that the lower bound of the interval (5.1) is substantially greater than 1.0 provides strong confidence that a true and powerful association exists. The statistical significance was further confirmed by the p-value of 0.001, indicating that this result is highly unlikely to be a product of random chance. In this same model, the other clinical factors did not demonstrate a statistically significant independent association with ROP. RDS (AOR 0.5), PDA (AOR 1.3), and multiple births (AOR 4.2) all had confidence intervals that crossed 1.0 and non-significant p-values (0.6, 0.8, and 0.4, respectively). This suggests that within this model, hyperglycemia was the dominant predictive factor. The second model, focusing on risk factors present at day seven, reinforced and amplified the findings from the day one analysis. Again, hyperglycemia (Day 7) was the most significant predictor. The AOR for hyperglycemia at this later time point was even more pronounced, at 74.5. This suggests that after adjusting for other factors, the odds of developing ROP were nearly 75 times higher for infants with hyperglycemia on day seven. Similar to the first model, this powerful association was accompanied by a very wide 95% CI of 9.0 to 613.4, again highlighting statistical imprecision in the magnitude of the effect. Nevertheless, with a lower bound of 9.0, the evidence for a strong association is robust. The finding was of the highest statistical significance, with a p-value of <0.001. In this model, neither PDA (AOR 0.13) nor multiple births (AOR 1.39) reached statistical significance, with p-values of 0.078 and 0.76, respectively. Table 3 provides compelling evidence that hyperglycemia, both in the immediate postnatal period and later in the first week, stands out as the most powerful independent predictor of ROP in this cohort. The other clinical conditions, while known risk factors, did not show a significant independent effect after accounting for the overwhelming influence of hyperglycemia in these models.

**4. Discussion**

This study embarked on an exploratory investigation into the temporal relationship between early-life hyperglycemia and the development of ROP in a cohort of preterm, low-birth-weight infants.[9] Our findings reveal a powerful statistical association between hyperglycemia on both postnatal day 1 and day 7 and the subsequent diagnosis of ROP. The magnitude of the observed associations is striking and suggests that metabolic dysregulation in the first week of life could be a formidable determinant of this sight-threatening disease.[10] To truly appreciate the significance of these findings, one must delve into the intricate dance of molecular and cellular events that govern retinal development and how a seemingly simple metabolic substrate like glucose can profoundly disrupt this delicate process. The retina of a full-term infant is a marvel of developmental precision, with a vascular network that has meticulously grown from the optic nerve to the far peripheral ora serrata, perfectly matching its metabolic needs. This process, known as vasculogenesis, is orchestrated by a complex interplay of growth factors, transcription factors, and metabolic

signals within the stable, low-oxygen environment of the womb. A central conductor of this orchestra is Insulin-like growth factor 1 (IGF-1), a hormone supplied in abundance by the placenta and amniotic fluid. IGF-1 does not directly cause vessels to grow; rather, it acts as a crucial permissive factor, creating an environment in which other pro-angiogenic signals, primarily vascular endothelial growth factor (VEGF), can function effectively to guide endothelial cell proliferation, migration, and tube formation.[11]

**Table 3. Multivariate Logistic Regression Analysis**

Adjusted odds ratios for risk factors associated with Retinopathy of Prematurity (ROP).

| RISK FACTOR | ADJUSTED ODDS RATIO (AOR) | 95% CONFIDENCE INTERVAL | P-VALUE |
|---|---|---|---|
| **Day 1 Model** | | | |
| Hyperglycemia (Day 1) | **55.7** | 5.1 – 611.0 | 0.001 |
| Respiratory Distress Syndrome (RDS) | 0.5 | 0.02 – 8.7 | 0.6 |
| Patent Ductus Arteriosus (PDA) | 1.3 | 0.2 – 10.1 | 0.8 |
| Multiple Births (Gemelli) | 4.2 | 0.05 – 3.5 | 0.4 |
| **Day 7 Model** | | | |
| Hyperglycemia (Day 7) | **74.5** | 9.0 – 613.4 | <0.001 |
| Patent Ductus Arteriosus (PDA) | 0.13 | 0.14 – 1.25 | 0.078 |
| Multiple Births (Gemelli) | 1.39 | 0.17 – 11.63 | 0.76 |

The premature birth of an infant is a cataclysmic event for this developing system. The infant is violently disconnected from its placental lifeline, leading to an immediate and precipitous drop in serum IGF-1 levels.[12] Simultaneously, the retina is exposed to the relatively hyperoxic environment of extrauterine life, even if the infant is only breathing room air. This combination of low IGF-1 and high oxygen acts as a powerful brake on normal vascular development. The process of vasculogenesis stalls, a phenomenon known as vaso-cessation. This is the hallmark of Phase I ROP. Our finding of a strong association between hyperglycemia on day 1 and the development of ROP suggests that this early metabolic derangement acts as a potent "second hit" upon this already compromised system. An acute hyperglycemic event on the very first day of life is not a benign metabolic fluctuation; it is a profound systemic insult that can derail the fragile, remaining processes of normal vessel growth.[13] The mechanisms are multifactorial. Firstly, the preterm infant's immature pancreas cannot mount an adequate insulin response to a high glucose load. The resulting state of relative hypoinsulinemia has direct consequences for IGF-1, as insulin is a key stimulus for hepatic IGF-1 production. Thus, hyperglycemia can further deepen the already critical IGF-1 deficiency, reinforcing the blockade on normal vascular development.[13]

Secondly, and perhaps more importantly, hyperglycemia is a potent generator of oxidative stress. The metabolism of excess glucose through alternative pathways, such as the polyol pathway, and the non-enzymatic glycation of proteins lead to a massive surge in the production of reactive oxygen species (ROS).[14] The developing retina, with its high metabolic rate and rich concentration of polyunsaturated fatty acids, is exquisitely vulnerable to oxidative damage. ROS directly attack the endothelial cells that form the growing blood vessels, inducing apoptosis and arresting their migration. They also deplete the cell's natural antioxidant defenses, such as glutathione, leaving the vasculature defenseless against further injury.[15] Therefore, a hyperglycemic insult on day 1 can be conceptualized as a metabolic firestorm that scorches the leading edge of the developing retinal vasculature, halting its progress and expanding the dangerous avascular zone that will later become the crucible for Phase II pathology. This direct cellular toxicity, combined with the systemic suppression of the IGF-1 axis, provides a compelling biological explanation for why hyperglycemia in this first window of vulnerability is so strongly associated with ROP.[16]

If day 1 hyperglycemia is about arresting normal development, day 7 hyperglycemia is about promoting abnormal, pathological development. By the end of the first week of life, the consequences of the initial vaso-cessation begin to manifest. The now-expanded avascular peripheral retina, populated by rapidly maturing photoreceptors and neurons, becomes increasingly starved of oxygen. This growing hypoxia is the trigger for the switch to Phase II ROP. The cellular response to hypoxia is governed by a master transcription factor, hypoxia-inducible factor 1-alpha (HIF-1α). In normal oxygen conditions, HIF-1α is rapidly degraded. In a hypoxic environment, however, it becomes stabilized, translocates to the nucleus, and activates a cascade of genes designed to restore oxygen homeostasis.[17] Chief among these is the gene for VEGF. The result is a massive, uncontrolled surge in VEGF production by the hypoxic retinal tissue. This VEGF is the primary driver of the pathological neovascularization that defines severe ROP. It signals the rapid growth of new blood vessels, but these vessels are fundamentally abnormal. They are leaky, disorganized, and grow not within the plane of the retina where they are needed, but aberrantly forward into the vitreous humor.[17]

Our finding that hyperglycemia on day 7 has an even stronger association with ROP than day 1 hyperglycemia points to its role as a powerful amplifier of this pathological Phase II response. Persistent or recurrent hyperglycemia at this later time point acts as a potent fuel for the fire of neovascularization. The mechanisms are again multifaceted and synergistic. Hyperglycemia itself, independent of hypoxia, has been shown to upregulate VEGF expression in retinal cells.[18] This creates a "dual-stimulus" environment where both hypoxia and high glucose are pushing the VEGF accelerator. Furthermore, hyperglycemia profoundly promotes a pro-inflammatory state. It activates key inflammatory pathways, such as the NF-κB pathway, leading to the production of inflammatory cytokines that further stimulate angiogenesis. It also leads to the formation of advanced glycation end-products (AGEs), which are proteins or lipids that have been non-enzymatically glycated. These AGEs can bind to their receptor (RAGE) on endothelial cells and macrophages, triggering a chronic inflammatory and pro-angiogenic response.[19] This inflammatory milieu not only promotes the growth of new vessels but also contributes to the breakdown of the blood-retinal barrier, making the new vessels even more leaky and prone to hemorrhage. Finally, the vessels that grow in this hyperglycemic, inflammatory soup are structurally inferior. Hyperglycemia impairs the function of pericytes, the mural cells that wrap around capillaries and provide them with structural support and stability. The loss of pericyte coverage is a classic hallmark of diabetic retinopathy and likely plays a role in ROP as well, contributing to the fragility and tortuosity of the neovascular complexes.[20] Thus, the stronger association of ROP with day 7 hyperglycemia can be understood as its contribution to a "perfect

storm" of pathological signals. It synergizes with hypoxia to drive VEGF to extreme levels, it creates a pro-inflammatory environment that supports abnormal vessel growth, and it contributes to the structural instability of the very vessels it helps to create. While day 1 hyperglycemia sets the stage by creating a large avascular zone, day 7 hyperglycemia provides the critical fuel and inflammatory context for that avascular zone to erupt into sight-threatening proliferative disease. This distinction between disrupting normal growth and promoting abnormal growth is central to our temporal hypothesis and provides a clear, pathophysiologically-grounded explanation for our findings.

While our analysis highlights the unique role of hyperglycemia, it is crucial to understand that ROP is a multifactorial disease, and these risk factors do not act in isolation. Hyperglycemia engages in a dangerous synergy with other common insults in the NICU environment. The relationship with supplemental oxygen is particularly important. Both hyperoxia (in Phase I) and hyperglycemia are potent generators of oxidative stress. When they occur together, their capacity to damage the retinal endothelium is likely multiplicative, not merely additive.[20] Similarly, sepsis, which was nearly universal in our cohort, is a state of profound systemic inflammation. The inflammatory cytokines released during sepsis can cause insulin resistance, leading to or worsening hyperglycemia. In turn, hyperglycemia can impair immune function, making the infant more susceptible to infection. In the context of the retina, the combination of sepsis-induced inflammation and hyperglycemia-induced inflammation creates an overwhelmingly hostile environment for the developing vasculature, further driving the pathological processes of ROP. Understanding these interactions is key to appreciating the full impact of glycemic dysregulation in the complex clinical picture of the preterm infant.

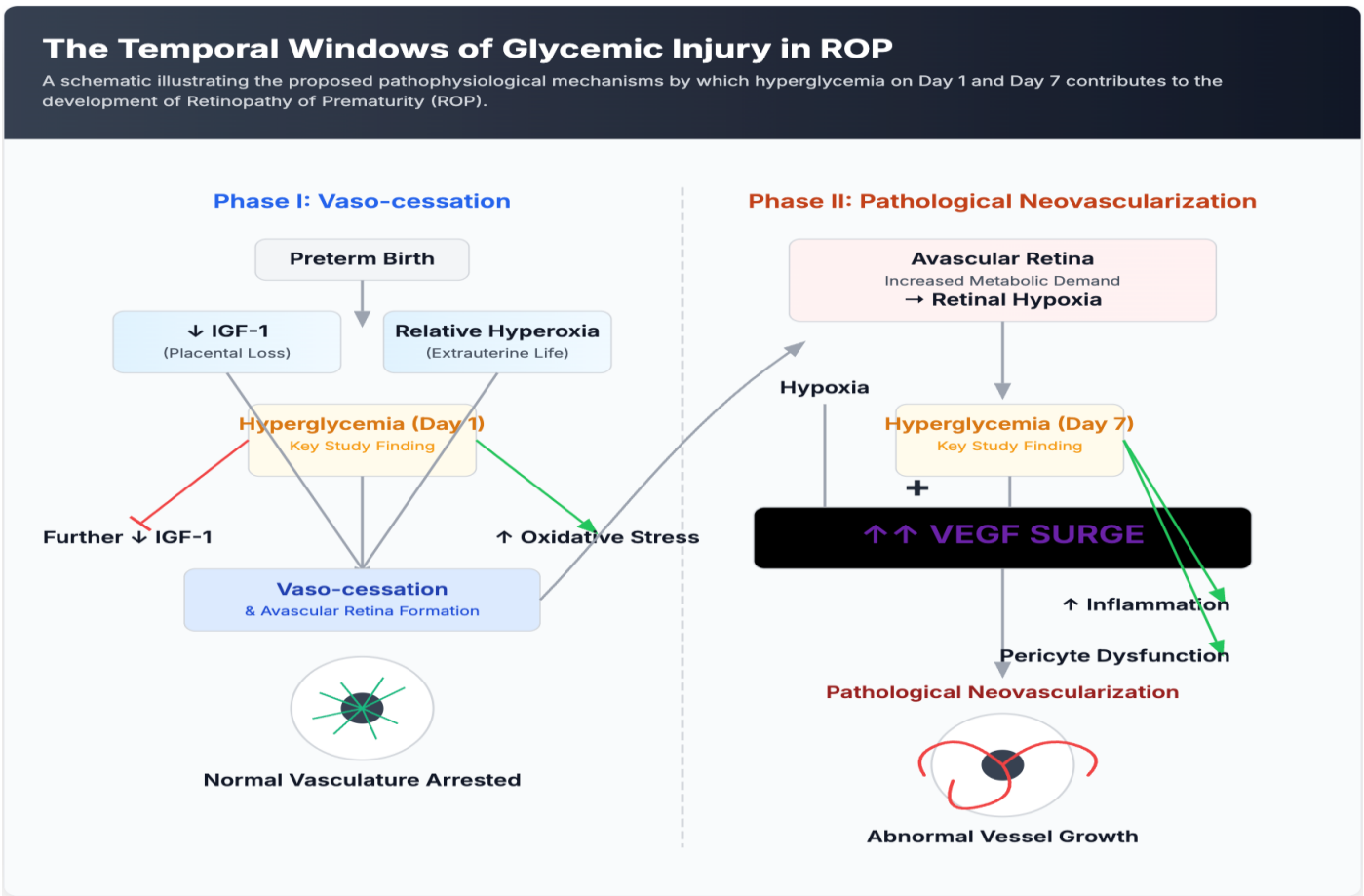


Figure 1. The temporal windows of glycemic injury in ROP.

Figure 1 showed a comprehensive and elegant schematic that visually articulates the proposed pathophysiological mechanisms linking early-life hyperglycemia to the development of retinopathy of prematurity (ROP). The figure thoughtfully deconstructs this complex disease into two distinct but interconnected phases, each representing a temporal window of vulnerability. It powerfully illustrates how the study's key findings—the detrimental impact of hyperglycemia on both day one and day seven—can be mapped onto these separate pathological stages, providing a robust biological narrative for the observed clinical associations. The left panel of the diagram meticulously details the events of Phase I, a period defined by the cessation of normal retinal vascular development. This initial phase lays the critical groundwork for all subsequent pathology, and the figure explains it as a cascade of insults beginning with the foundational event of a Preterm Birth. For a developing fetus, the womb is a precisely calibrated environment, characterized by low oxygen tension and a constant, life-sustaining infusion of growth factors from the placenta. A premature birth represents a violent expulsion from this stable world into an environment for which the infant's immature systems are profoundly unprepared. The diagram illustrates that this event immediately precipitates two primary insults. The first is a precipitous decrease in Insulin-like Growth Factor 1, or IGF-1. The figure notes this is a direct consequence of placental loss. In utero, the placenta is the primary source of this vital hormone, which acts as an essential permissive factor for the healthy, outward growth of retinal blood vessels from the optic nerve toward the periphery. It does not directly command vessel growth, but rather creates the necessary biological environment in which other growth signals can function properly. The sudden severance from the placenta causes systemic IGF-1 levels to plummet, effectively removing a key support structure for the developing retinal vasculature.

The second primary insult is the exposure to Relative Hyperoxia. The diagram correctly notes this is a consequence of extrauterine life. While neonatologists may carefully titrate supplemental oxygen, even the oxygen concentration in room air is significantly higher than the hypoxic conditions of the womb. For the developing retina, this relative hyperoxia is a paradoxical and powerful shock. Instead of promoting growth, it signals the already-present blood vessels that oxygen is plentiful, which in turn suppresses the production of Vascular Endothelial Growth Factor (VEGF), the very protein that drives the extension of the vascular network. The combination of low IGF-1 and hyperoxia-induced low VEGF creates a powerful braking mechanism, grinding the normal, orderly process of vasculogenesis to a halt. It is upon this already compromised system that the figure introduces the study's primary finding for this period: Hyperglycemia on Day 1. The diagram positions this not as a minor metabolic fluctuation, but as a significant, independent pathological event that drastically worsens the existing situation. The figure illustrates two distinct pathways by which early-life hyperglycemia inflicts its damage. Firstly, it leads to a Further decrease in IGF-1. The biological link here is the relationship between glucose, insulin, and IGF-1 production. In a preterm infant with an immature pancreas, hyperglycemia often results in a state of relative hypoinsulinemia. Since insulin is a key stimulus for the liver's production of IGF-1, the hyperglycemic state serves to deepen the already-critical IGF-1 deficit, further ensuring that the conditions for normal vessel growth are not met. This is a classic example of adding insult to injury, as the metabolic derangement reinforces the primary developmental blockade.

Secondly, and perhaps more directly damaging at a cellular level, hyperglycemia leads to a profound increase in Oxidative Stress. High concentrations of glucose overwhelm the normal metabolic pathways, shunting excess sugar into alternative routes that generate a flood of reactive oxygen species. These highly unstable molecules wreak havoc on the delicate endothelial cells that form the leading edge of the developing retinal vessels. They damage cellular

membranes, proteins, and DNA, triggering apoptosis, or programmed cell death. This oxidative storm effectively scorches the cellular machinery required for vessel migration and proliferation. The diagram elegantly shows these three parallel insults—the foundational lack of IGF-1 and exposure to hyperoxia, compounded by the hyperglycemic toxicities—all converging on the central outcome of Phase I: Vaso-cessation and Avascular Retina Formation. This is the critical pathological consequence. The outward march of blood vessels stops prematurely, leaving a vast, barren periphery of the retina without a blood supply. The figure concludes this panel with a simple yet effective diagram of an eye, labeled Normal Vasculature Arrested, visually summarizing this state of arrested development. This incomplete vasculature is not a stable endpoint; it is a ticking time bomb, setting a dangerous stage for the subsequent phase of the disease.

The right panel of the diagram illustrates the tragic and often destructive response to the injury established in Phase I. This second phase is driven by the consequences of having a large, Avascular Retina. As the neurons and photoreceptors in this unperfused peripheral tissue continue to mature, their metabolic demand for oxygen steadily increases. Eventually, this demand outstrips the passive oxygen supply, leading to a state of profound and worsening Retinal Hypoxia. This hypoxia is the critical trigger that flips the switch from a state of arrested development to a state of pathological, uncontrolled growth. The diagram shows that this retinal hypoxia acts as a powerful distress signal. In response to low oxygen, retinal cells stabilize a key transcription factor called HIF-1-alpha, which in turn activates a host of genes designed to restore oxygenation. The most important of these is the gene for VEGF. This results in a massive, uncontrolled upregulation of VEGF production from the hypoxic retina—a desperate cry for new blood vessels. It is at this critical juncture that the figure introduces the study's second key finding: Hyperglycemia on Day 7. This later metabolic insult does not occur in a vacuum; it acts upon a system already primed for a pathological response by hypoxia. The figure powerfully illustrates the synergistic effect of these two factors. Hypoxia alone drives VEGF production, but the combination of hypoxia and hyperglycemia results in a much more extreme and pathological outcome, which the diagram labels as an increase in VEGF SURGE. This is because hyperglycemia itself can independently stimulate VEGF production in retinal cells. When this glucose-driven stimulation is layered on top of the powerful hypoxia-driven stimulation, the result is an explosive overproduction of VEGF that far exceeds any physiological need.

This VEGF surge is the central engine of Phase II pathology, but the diagram shows that day seven hyperglycemia contributes to the disaster through other mechanisms as well. It causes a significant increase in Inflammation.[18] High glucose fuels inflammatory pathways within the retina, leading to the recruitment of immune cells and the production of inflammatory cytokines. Furthermore, it promotes the formation of advanced glycation end-products, which are proteins that become damaged by sugar molecules. These end-products further stoke the flames of inflammation, creating a toxic, pro-angiogenic environment. Hyperglycemia also causes pericyte dysfunction. Pericytes are crucial support cells that wrap around capillaries, providing them with structural stability and regulating blood flow.[19] Hyperglycemia is toxic to pericytes, causing them to die off.[20] The loss of pericyte coverage leaves the newly forming blood vessels weak, unstable, and prone to leaking. Figure 1 demonstrates that the combination of these deleterious events—the massive VEGF surge, the intense inflammatory environment, and the structural instability from pericyte loss—inevitably leads to the final, sight-threatening outcome: Pathological Neovascularization. The diagram concludes with a compelling visual of Abnormal Vessel Growth, depicting disorganized, tortuous blood vessels that, instead of growing within the plane of the retina to supply the hypoxic tissue, grow chaotically forward into the vitreous humor. These abnormal vessels are fragile, leak fluid, and bleed easily. They

form fibrous scar tissue that contracts and pulls on the retina, which can ultimately lead to tractional retinal detachment and irreversible blindness. In its totality, Figure 1 provides a masterful narrative of a two-act tragedy. It shows how hyperglycemia first acts as an accomplice in arresting normal development in Phase I, and then returns as a primary accelerant, fanning the flames of the pathological response in Phase II. The schematic thus provides a clear, compelling, and biologically plausible explanation for the study's findings, highlighting that the timing of a hyperglycemic insult is critically important in determining the nature of its devastating impact on the vulnerable preterm retina.[20]

**5. Conclusion**

This study identified a strong and statistically significant association between hyperglycemia on both the first and seventh postnatal days and the incidence of retinopathy of prematurity in preterm, low-birth-weight infants. Our analysis, grounded in the biphasic pathophysiology of the disease, suggests a compelling narrative of two distinct temporal windows of glycemic injury. Early hyperglycemia, occurring in the first days of life, appears to disrupt the foundational process of normal vasculogenesis by exacerbating the post-natal fall in IGF-1 and inflicting direct oxidative damage on developing vessels. In contrast, later hyperglycemia, occurring around day seven, appears to act as a powerful accelerant for the pathological neovascularization of Phase II, synergizing with hypoxia and inflammation to fuel the growth of the abnormal vessels that threaten sight. The primary value of this study lies not in providing definitive answers, but in constructing a detailed, biologically plausible hypothesis that can and should be tested in future, more rigorous prospective research. Confirming this temporal association and further elucidating its underlying mechanisms could have profound implications for clinical practice, potentially leading to time-sensitive strategies for glycemic management aimed at protecting the vulnerable preterm retina during its most critical periods of development.

**6. References**


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