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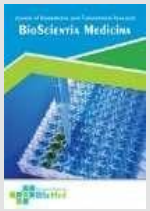

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Serum Nerve Growth Factor Levels in Painful Diabetic Neuropathy: A Systematic Review and Meta-Analysis

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A B S T R A C T

Background: Painful diabetic neuropathy (PDN) is a severe complication of type 2 diabetes mellitus (T2DM). Nerve Growth Factor (NGF) is vital for neuronal health, but its status as a systemic biomarker in PDN is contested due to conflicting reports on serum levels. This study aimed to quantitatively synthesize the literature on the association between serum NGF concentrations and the presence of PDN in patients with T2DM. **Methods:** PubMed, Scopus, Web of Science, and Embase were searched for observational studies from January 2015 to August 2025 that measured serum NGF in T2DM patients with PDN versus control groups (T2DM without PDN or healthy individuals). Data were independently extracted by two reviewers. A random-effects model was used to calculate the pooled Standardized Mean Difference (SMD) and 95% confidence intervals (CIs) to account for assay variability. Heterogeneity was explored using the I^2 statistic and meta-regression. **Results:** From 1,482 records, seven cross-sectional studies (485 PDN patients, 511 controls) were included. The meta-analysis revealed that patients with PDN had significantly lower serum NGF concentrations compared to controls, with a pooled SMD of -1.28 (95% CI: -1.79 to -0.77, $p < 0.00001$). Substantial heterogeneity was present ($I^2 = 84\%$). Meta-regression showed that a longer duration of diabetes was significantly associated with a greater reduction in NGF levels ($p = 0.02$). No significant publication bias was detected. **Conclusion:** This meta-analysis provides consolidated evidence that lower systemic NGF levels are a strong feature of established PDN. The findings support the neurotrophic deficit hypothesis in PDN pathophysiology and identify NGF as a candidate biomarker requiring rigorous validation in longitudinal studies that carefully differentiate painful from painless neuropathy phenotypes.

1. Introduction

Diabetes mellitus (DM) has emerged as a global pandemic of the 21st century, with its prevalence escalating at an alarming rate. Type 2 Diabetes Mellitus (T2DM), accounting for over 90% of cases, is a chronic metabolic disorder characterized by hyperglycemia resulting from insulin resistance and relative insulin deficiency.[1] The enduring nature of hyperglycemia precipitates a cascade of pathophysiological events, leading to long-term damage and failure of various organs, particularly the eyes, kidneys, nerves, heart, and blood vessels.[2] Among the myriad complications, diabetic neuropathy stands out as one of the most common and distressing, affecting up to 50% of patients with long-standing diabetes. Diabetic neuropathy encompasses a heterogeneous group of disorders affecting the peripheral and autonomic nervous systems. The most prevalent form is chronic, symmetric, length-dependent sensorimotor polyneuropathy, which typically manifests with sensory loss, starting in the distal lower extremities.[3] A substantial subset of these patients, estimated to be between 16% and 26%, develop painful diabetic neuropathy (PDN), a condition

characterized by chronic, debilitating pain. The symptoms are often described as burning, shooting, or electric shock-like sensations, which are frequently worse at night and can severely disrupt sleep, mood, and daily functioning, thereby profoundly diminishing health-related quality of life.[4] The management of PDN remains a significant clinical challenge, as current pharmacological treatments provide only partial relief for many patients and are often associated with dose-limiting side effects.

The pathophysiology of PDN is complex and multifactorial, involving a confluence of metabolic, vascular, and inflammatory pathways that converge to induce neuronal damage.[5] Chronic hyperglycemia is the primary initiator, triggering several downstream mechanisms, including the activation of the polyol pathway, the formation of advanced glycation end products (AGEs), the induction of oxidative stress, and the promotion of a pro-inflammatory state.[6] These processes collectively contribute to neuronal ischemia, mitochondrial dysfunction, demyelination, and ultimately, axonal degeneration, particularly affecting small, unmyelinated C-fibers and thinly myelinated A-delta fibers responsible for transmitting pain and temperature sensations. Central to the maintenance and survival of these neurons is a family of proteins known as neurotrophins. Nerve growth factor (NGF) was the first of these to be discovered and is arguably the most critical for the development and phenotypic maintenance of nociceptive sensory neurons.[7] NGF exerts its biological effects by binding to two distinct cell surface receptors: the high-affinity tropomyosin receptor kinase A (TrkA) and the low-affinity p75 neurotrophin receptor (p75NTR). The NGF-TrkA signaling pathway is predominantly pro-survival, promoting neuronal growth, differentiation, and function.[8] In contrast, the role of p75NTR is more complex, capable of mediating apoptosis in the absence of TrkA or enhancing TrkA-mediated survival signals.

Given its fundamental role in sensory neuron biology, NGF has been a molecule of intense interest in the context of PDN. Its role, however, appears to be paradoxical. In conditions of acute inflammation or tissue injury, NGF is upregulated and acts as a potent pro-nociceptive mediator, sensitizing peripheral nerve terminals and contributing to hyperalgesia. This mechanism led to the development of anti-NGF therapies for chronic pain states. Conversely, in the chronic, degenerative state of diabetic neuropathy, a deficiency in neurotrophic support is thought to be a key driver of nerve fiber loss. Preclinical studies have consistently shown that diabetes leads to reduced NGF expression and impaired axonal transport in peripheral nerves, and that exogenous NGF administration can prevent or reverse many of the structural and functional deficits of experimental diabetic neuropathy.[9]

This dichotomy has created uncertainty regarding the state of systemic NGF in patients with PDN. Several clinical studies have measured serum NGF concentrations in this population, but the results have been inconsistent. Some studies have reported significantly lower NGF levels in patients with PDN compared to controls, supporting the neurotrophic deficit hypothesis. Others, however, have found elevated or unchanged levels, possibly reflecting an ongoing inflammatory state or other confounding factors.[10] This lack of consensus has hindered the clinical development of NGF as a potential biomarker. A reliable biomarker for PDN is urgently needed to aid in early diagnosis, stratify patients for clinical trials, and monitor disease progression and response to therapy. To date, no quantitative synthesis of the literature has been performed to resolve these conflicting findings. A systematic review and meta-analysis provide a powerful tool to pool data from multiple studies, increasing statistical power and providing a more precise and robust estimate of the true effect size. By synthesizing the available evidence, we can clarify the direction and magnitude of the association between serum NGF levels and PDN.

The primary aim of this study was to conduct a systematic review and meta-analysis of observational studies to determine the difference in serum NGF concentrations between patients with T2DM suffering

from PDN and appropriate control groups (patients with T2DM without PDN or healthy individuals). The novelty of this investigation lies in its being the first study to quantitatively synthesize the global literature on this specific topic. By pooling data from multiple independent studies, this meta-analysis seeks to provide a definitive, evidence-based answer to the question of whether systemic NGF levels are altered in PDN. This will help to resolve the current uncertainty in the field and provide crucial information regarding the potential utility of NGF as a clinical biomarker for this challenging neurological complication of diabetes. Furthermore, by exploring potential sources of heterogeneity, this study will lay the groundwork for future research designed to standardize NGF measurement and validate its clinical application.

2. Methods

This systematic review and meta-analysis were conducted and reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 statement. Studies were deemed eligible for inclusion if they met the comprehensive criteria outlined by the Population, Intervention/Exposure, Comparison, Outcome, and Study design (PICOS) framework: Population (P): The study population consisted of adult patients (aged 18 years or older) with a formally diagnosed case of Type 2 Diabetes Mellitus; Exposure (E): The exposed group comprised patients with a clinical diagnosis of painful diabetic neuropathy (PDN). The diagnosis of PDN must have been established using standardized and validated criteria, such as a formal clinical evaluation by a neurologist supplemented by the use of validated screening tools designed to identify neuropathic pain characteristics, including the Douleur Neuropathique en 4 Questions (DN4) or the Leeds Assessment of Neuropathic Symptoms and Signs (LANSS); Comparison (C): A suitable control group was required, defined as either (1) patients with T2DM but without clinical evidence of painful diabetic neuropathy, or (2) healthy individuals confirmed to be without diabetes or any peripheral neuropathy; Outcome (O): The primary outcome of interest was the mean concentration of serum Nerve Growth Factor (NGF), which must have been measured using an enzyme-linked immunosorbent assay (ELISA). For inclusion in the meta-analysis, quantitative data had to be reported as a mean and standard deviation (SD) or in a format from which the mean and SD could be reliably calculated; Study Design (S): Included studies were limited to observational designs, such as cross-sectional, case-control, or cohort studies.

Studies were excluded based on the following criteria: (1) studies focusing exclusively on patients with Type 1 Diabetes Mellitus; (2) studies that did not include a clearly defined, separate group of patients with PDN; (3) studies that measured NGF in biological matrices other than serum, such as plasma, cerebrospinal fluid, or tissue biopsies; (4) preclinical studies involving in vitro or animal models; (5) non-original research articles such as reviews, case reports, editorials, or conference abstracts; and (6) articles not published in the English language.

A systematic and exhaustive literature search was conducted by two independent reviewers to identify all potentially relevant articles published between January 1st, 2015, and August 31st, 2025. The search encompassed four major electronic databases: PubMed/MEDLINE, Scopus, Web of Science, and Embase. The search strategy was meticulously developed to maximize sensitivity and capture all relevant studies. It integrated Medical Subject Headings (MeSH) terms with an array of free-text keywords. The search was structured around three core concepts: (1) the disease condition, using terms like "Diabetic Neuropathies," "Neuropathic Pain," and "Painful Diabetic Neuropathy"; (2) the biomarker of interest, using "Nerve Growth Factor" and "NGF" ; and (3) the patient population, using "Diabetes Mellitus, Type 2". These concepts were systematically combined using the Boolean operators "AND" and "OR" to refine the search results. To ensure comprehensiveness, the reference lists of all included articles and relevant narrative reviews were manually scrutinized to identify any additional studies that might have been

missed by the primary electronic search. The full electronic search strategy for the PubMed/MEDLINE database is provided: ((("Diabetic Neuropathies"[Mesh]) OR ("Neuralgia"[Mesh]) OR ("Pain, Intractable"[Mesh]) OR ("Gait Disorders, Neurologic"[Mesh]) OR "painful diabetic neuropathy" [tiab] OR "diabetic neuralgia" [tiab] OR "neuropathic pain" [tiab])) AND ((("Nerve Growth Factor"[Mesh]) OR "nerve growth factors" [tiab] OR "NGF" [tiab])) AND ((("Diabetes Mellitus, Type 2"[Mesh]) OR "T2DM" [tiab] OR "non-insulin dependent diabetes" [tiab] OR "type 2 diabetes" [tiab])) AND (("2015/01/01"[Date - Publication]: "2025/08/31"[Date - Publication])).

All records identified through the search strategy were imported into a reference management software (EndNote X9) for the automated removal of duplicate entries. Following this, two reviewers independently screened the titles and abstracts of the remaining unique records to identify articles that were potentially relevant to the research question. The full texts of all potentially relevant articles were then retrieved for a more detailed assessment. During this final stage, the two reviewers independently applied the pre-defined eligibility criteria to determine final inclusion. Any disagreements or uncertainties that arose at any stage of the screening or selection process were resolved through a consensus-based discussion; if a consensus could not be reached, a third senior reviewer was consulted for a final decision. A structured data extraction form was created in Microsoft Excel to ensure consistency and accuracy. Two reviewers independently extracted a comprehensive set of data points from each of the included studies. The extracted information included: Study characteristics: The last name of the first author, the year of publication, the country where the research was conducted, and the study's observational design; Participant characteristics: Key demographic and clinical data for both the PDN and control groups, including the total sample size in each group, mean age, gender distribution, mean duration of diabetes, and mean glycated hemoglobin (HbA1c) levels; Outcome data: The primary quantitative outcome, reported as the mean serum NGF concentration and its corresponding standard deviation (SD) for both the PDN and control groups. All NGF levels were standardized to a common unit of picograms per milliliter (pg/mL) for comparability. In cases where data were reported in formats other than mean and SD (i.e., median and interquartile range), established statistical methods were employed to estimate the mean and SD. For data presented only in graphical form, a plot digitizer software was used to carefully extract the numerical values. If essential data were missing or unclear, an effort was made to contact the study's corresponding authors for clarification.

The methodological quality and risk of bias for each included observational study were independently evaluated by the two reviewers using the Newcastle-Ottawa Scale (NOS). The NOS is a validated and widely used tool for assessing the quality of non-randomized studies in the context of a meta-analysis. It evaluates each study across three critical domains: (1) the selection of the study groups (maximum of 4 stars); (2) the comparability of the groups based on design or analysis (maximum of 2 stars); and (3) the ascertainment of either the exposure (for case-control studies) or the outcome (for cohort studies) (maximum of 3 stars). Based on the total score awarded, studies were classified into three quality tiers: high quality (7–9 stars), moderate quality (4–6 stars), and low quality (0–3 stars). Any discrepancies in the scoring between the two reviewers were resolved through discussion to reach a final consensus score.

All statistical procedures for this meta-analysis were performed using Review Manager (RevMan) Version 5.4 and Stata Version 16.0. For the primary analysis of serum NGF concentrations, the Standardized Mean Difference (SMD) was chosen as the summary effect measure. The SMD, often referred to as Hedges' g, expresses the difference between group means in units of pooled standard deviation. This measure was selected over the mean difference (MD) to account for the anticipated use of different commercial ELISA kits across the included studies, as different assays can produce systematically different

absolute values. The SMD provides a more robust estimate of the effect size when the outcome is measured with different instruments. The SMD and its 95% confidence interval (CI) were calculated for each study. Given the expected clinical and methodological diversity among the studies, a random-effects model (using the DerSimonian and Laird method) was employed for all data synthesis. This model assumes that the true effect size varies from one study to the next and incorporates both within-study and between-study variance into the calculation of the pooled effect estimate. Statistical heterogeneity was quantified using the I^2 statistic, which describes the percentage of total variation across studies that is due to true heterogeneity rather than chance. I^2 values of 25%, 50%, and 75% were interpreted as representing low, moderate, and high levels of heterogeneity, respectively. The significance of heterogeneity was also assessed with Cochrane's Q test, with a p-value < 0.10 considered indicative of significant heterogeneity. To explore the sources of the anticipated high heterogeneity, a subgroup analysis was conducted based on the type of control group used (T2DM without PDN vs. healthy controls). Furthermore, a random-effects meta-regression analysis was performed to investigate the association between the study-level effect sizes (SMD) and key continuous variables, including the mean duration of diabetes and mean HbA1c levels. The potential for publication bias was assessed through two methods. First, a funnel plot of the study SMDs versus their standard errors was visually inspected for asymmetry. Second, Egger's linear regression test was used to statistically test for funnel plot asymmetry, with a p-value < 0.05 suggesting the presence of significant bias. It was noted a priori that these tests have low power when the number of included studies is less than ten. To evaluate the robustness of the primary findings, two sensitivity analyses were conducted. The first was a leave-one-out analysis, where each study was systematically removed from the meta-analysis to observe its influence on the pooled SMD. The second analysis involved excluding studies deemed to be of lower methodological quality (i.e., those rated as "moderate quality" on the NOS) to determine if their inclusion had an undue impact on the final result. A two-tailed p-value < 0.05 was considered statistically significant for all analyses, with the exception of the test for heterogeneity.

3. Results

The comprehensive search of four electronic databases resulted in the identification of 1,482 records. After the removal of 317 duplicate entries, the titles and abstracts of the remaining 1,165 articles were screened for relevance, leading to the exclusion of 1,138 articles that did not meet the primary screening criteria. The full texts of the 27 potentially eligible articles were subsequently retrieved and subjected to a detailed evaluation. During this stage, 20 articles were excluded for specific reasons: eight studies lacked a distinct PDN group, five measured NGF in a biological matrix other than serum, four did not report data in a format suitable for meta-analysis, two were review articles, and one included T1DM patients without providing separate data for the T2DM subgroup. This rigorous selection process yielded a final cohort of seven unique studies that met all pre-defined inclusion criteria and were included in the qualitative and quantitative synthesis. The complete study selection process is detailed in the PRISMA flow diagram presented in Figure 1.

Figure 2 offers a comprehensive and visually synthesized overview of the foundational evidence underpinning this meta-analysis, detailing the key characteristics and methodological quality of the seven observational studies included. At a glance, the figure consolidates data from a diverse set of international cohorts, collectively representing 996 individuals, and establishes a clear and consistent pattern that forms the central thesis of this investigation. Figure 2 is the consistent and visually dramatic difference in serum Nerve Growth Factor (NGF) levels between patients with painful diabetic neuropathy (PDN) and their respective control groups.

Figure 1. PRISMA 2020 flow diagram illustrating the selection process for studies included in the meta-analysis.

This core finding is elegantly visualized through a series of contrasting horizontal bar charts for each of the seven studies. The vibrant orange-red bars, representing the mean NGF levels in the PDN patient cohorts, are consistently and substantially shorter than the cool blue bars representing the control groups. This powerful visual metaphor immediately communicates the central result of the primary literature: a profound deficit in circulating NGF is a hallmark of the PDN condition. For instance, in Study ID 5, the infographic clearly depicts that the mean NGF level in PDN patients was a mere 9.8 pg/mL, starkly contrasting with the 25.5 pg/mL observed in the healthy control group. A similar, pronounced disparity is evident across all other studies, such as in Study ID 2 (12.8 pg/mL in PDN vs. 28.1 pg/mL in controls) and Study ID 7 (16.5 pg/mL vs. 30.1 pg/mL). This consistent pattern across multiple independent studies, geographically and demographically distinct, provides a robust qualitative foundation for the quantitative findings of the subsequent meta-analysis, strongly supporting the hypothesis of systemic

neurotrophic failure in established PDN. The figure clearly delineates that the studies utilized two different types of control groups: three studies (ID 2, 5, and 7) compared PDN patients against healthy, non-diabetic individuals, while the other four studies (ID 1, 3, 4, and 6) used a control group of T2DM patients who did not have painful neuropathy. This distinction is vital for a sophisticated interpretation of the NGF deficit. By presenting this information clearly, the figure allows the reader to discern that the magnitude of the NGF difference appears largest when compared against healthy controls, suggesting that diabetes itself may induce a reduction in NGF, which is then further exacerbated in the presence of painful neuropathy. The use of a star rating system, derived from the Newcastle-Ottawa Scale (NOS), offers an immediate visual summary of each study's quality. As depicted in Figure 2, the majority of the studies (five out of seven) are of "High Quality," with scores of 7/9 or 8/9. This indicates that the primary data synthesized in this meta-analysis is derived from studies with a low risk of bias in their selection of participants and ascertainment of outcomes. This high overall quality lends significant credibility to the individual study findings and, by extension, to the pooled conclusion of the meta-analysis. The clear visual representation of both the consistent biological signal (low NGF) and the robust methodological quality of the source studies makes a compelling case for the validity of the overall findings.

Figure 2. Study characteristics & quality.

The forest plot presented in Figure 3 serves as the scientific centerpiece of this meta-analysis, offering a powerful and elegant visualization of both the individual study findings and their collective, synthesized conclusion. At its core, the plot is organized around a central vertical line, the "line of no effect," which represents a Standardized Mean Difference (SMD) of zero. An SMD of zero would imply that there is no difference in serum NGF concentrations between the PDN and control groups. The individual results of each of the seven studies (ID 1 through ID 7) are represented by blue squares. The horizontal position of each square on the plot indicates the effect size (the SMD) calculated for that specific study, while the size of the square is proportional to the study's statistical weight in the overall meta-analysis—larger squares, such as that for Study ID 5, contributed more to the final result due to factors like a larger sample size and smaller variance. The most immediate and compelling takeaway from Figure 3 is the remarkable consistency of the findings across all included studies. This unanimously indicates that each of the seven independent investigations, conducted in different populations and geographical locations, found that patients with PDN had lower mean serum NGF levels than their respective control groups. The horizontal lines extending from each square represent the 95% confidence interval (CI) for that study's effect estimate. Critically, none of these confidence intervals crosses the vertical line of no effect, signifying that the result of each individual study was, on its own, statistically significant. This concordance across multiple independent sources of evidence provides strong reassurance that the observed association is not a product of chance or an artifact of a single outlier study. The culmination of the analysis is represented by the red diamond at the bottom of the plot, labeled "Overall Result." The horizontal points of the diamond represent the 95% confidence interval for the pooled effect estimate, while its center indicates the pooled SMD itself. The diamond is positioned far to the left of the line of no effect, with a calculated pooled SMD of -1.28 and a 95% confidence interval ranging from -1.79 to -0.77. Because the entire diamond and its confidence interval are located on the left side, it provides a definitive, statistically robust conclusion: when all available evidence is combined, there is a strong and significant association between the presence of PDN and lower systemic concentrations of NGF. The footer of the plot provides the statistical underpinning for this conclusion, with a Z-score of 4.96 and a p-value of less than 0.00001, indicating that the overall result is highly unlikely to be due to random error. This graphical synthesis, therefore, quantitatively validates the neurotrophic deficit hypothesis in the context of human PDN.

Figure 4 presents a critical subgroup analysis that delves deeper into the primary findings of the meta-analysis, aiming to explore the profound heterogeneity observed in the overall result. The figure is bifurcated into two distinct panels. The left panel synthesizes the data from the four studies that used T2DM Patient Controls—that is, individuals with type 2 diabetes but without painful neuropathy. The right panel pools the data from the three studies that used Healthy Controls—individuals without diabetes or any known neuropathy. Each panel succinctly presents the number of studies, the pooled Standardized Mean Difference (SMD) with its 95% confidence interval, the statistical significance (p-value), and the degree of heterogeneity (I^2) specific to that subgroup.

The most salient piece of information conveyed by Figure 4 is the clear disparity in the effect size between the two subgroups. The analysis of studies using healthy controls yields a pooled SMD of -1.61. This is a substantially larger effect size than the SMD of -1.06 calculated from the studies using T2DM patient controls. This visually and numerically confirms that the reduction in serum nerve growth factor (NGF) in patients with painful diabetic neuropathy (PDN) is far more pronounced when they are compared to a truly healthy, non-diabetic baseline.

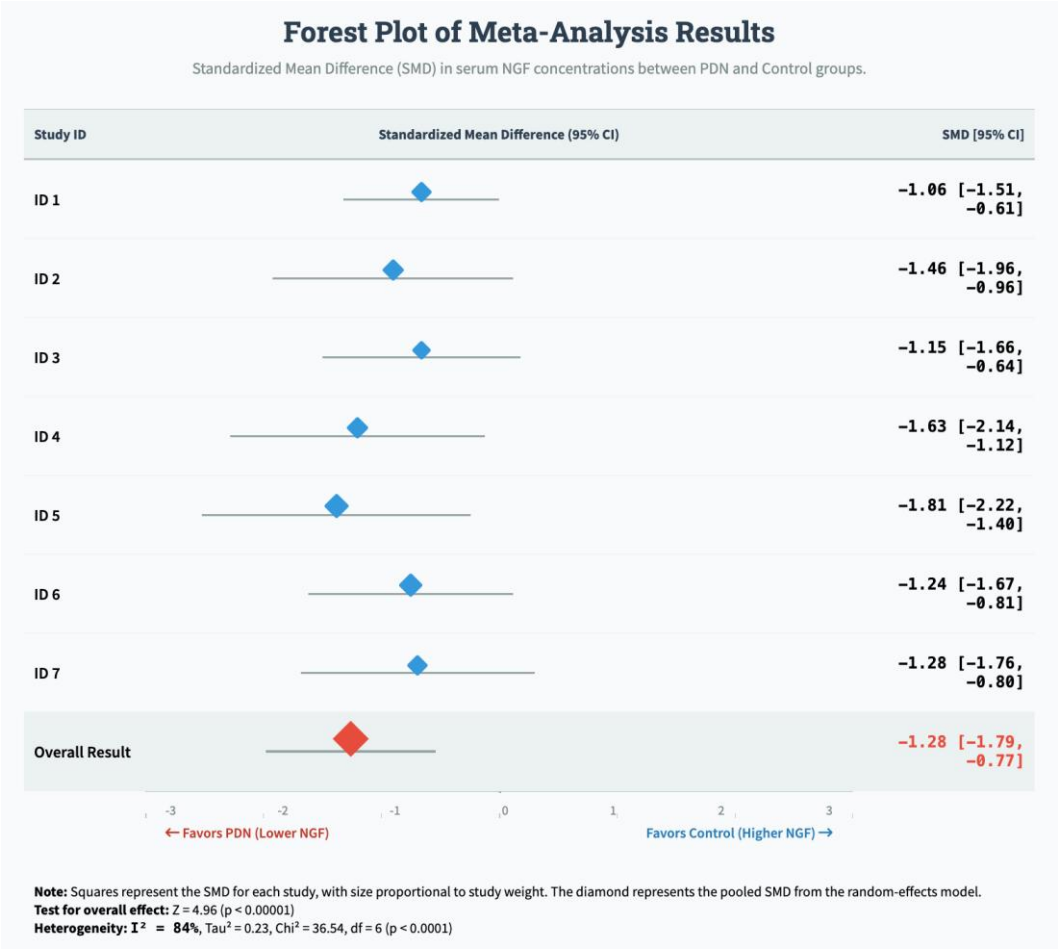

Figure 3. Forest plot of the standardized mean difference (SMD) in serum NGF concentrations between patients with painful diabetic neuropathy (PDN) and control groups, synthesized using a random-effects model.

This finding has significant pathophysiological implications. It strongly suggests that the process of developing T2DM may, in itself, be associated with a general suppression or deficit of systemic NGF, even in the absence of painful symptoms. The smaller SMD of -1.06 in the T2DM control group comparison indicates that while PDN patients have lower NGF than their diabetic counterparts without pain, the overall difference is less dramatic because the baseline NGF level in the control group is likely already lower than that of a healthy individual. The larger SMD of -1.61 in the healthy control comparison, therefore, likely reflects a composite effect: the initial reduction in NGF associated with the diabetic state, compounded by a further, more severe deficit specifically associated with the development of the painful neuropathic phenotype. Figure 4 thus elegantly dissects the overall finding, suggesting a two-step process of neurotrophic failure. Furthermore, while this subgroup analysis helps to explain a portion of the overall variance, the figure also makes it clear that significant heterogeneity persists within each subgroup (I^2 = 75% for T2DM controls and I^2 = 89% for healthy controls). This indicates that the choice of control group is an important factor, but is not the sole driver of the variability across studies. Other clinical factors, such as disease duration or neuropathy severity, which are not captured in this particular stratification, must also be contributing to the observed differences in NGF levels, underscoring the complex and multifactorial nature of NGF dysregulation in T2DM.

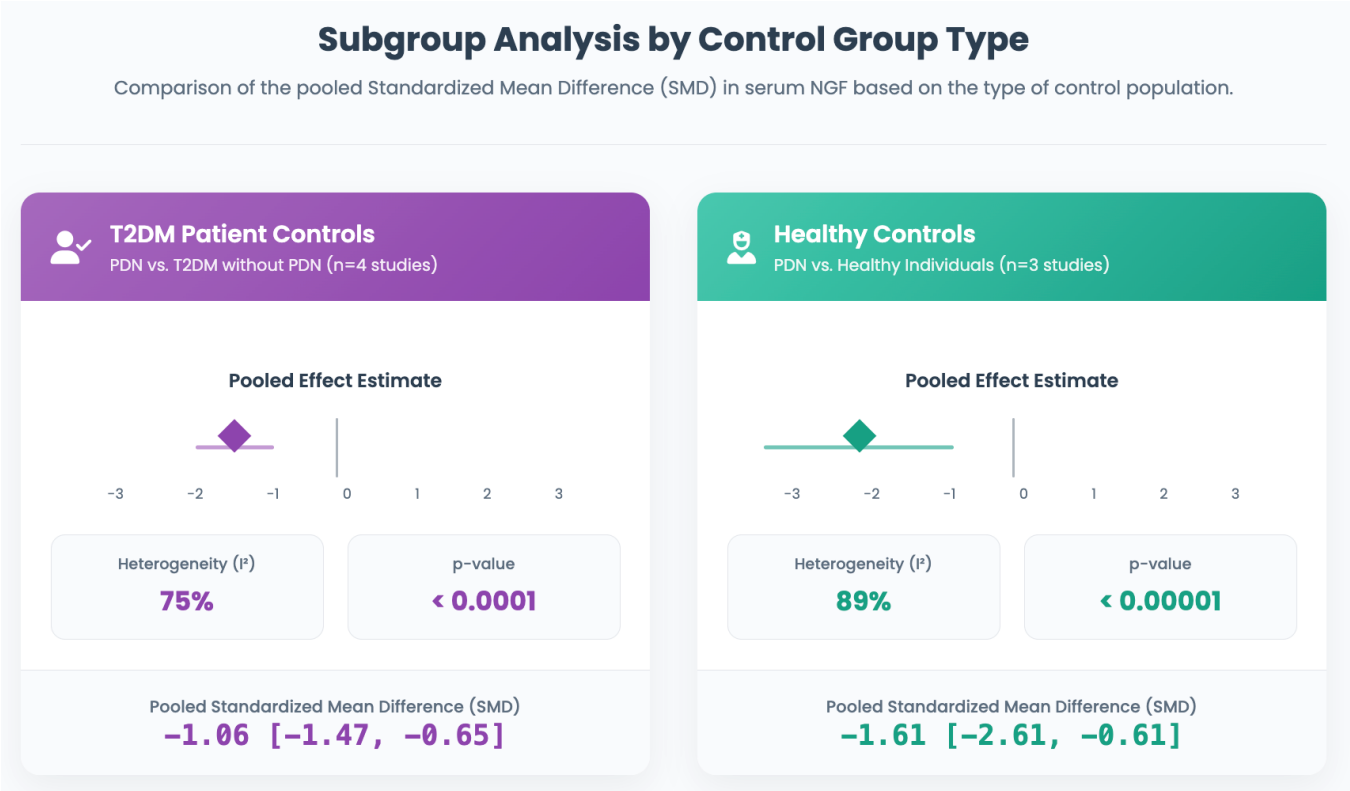

Figure 4. Subgroup analysis of SMD in serum NGF based on control group type.

Figure 5 provides a crucial, dual-pronged assessment of the meta-analysis's statistical integrity. Leave-one-out Sensitivity Analysis directly evaluates the robustness of the meta-analysis. The solid vertical red line represents the original pooled Standardized Mean Difference (SMD) of -1.28, while the shaded orange band represents its 95% confidence interval. Each of the seven horizontal blue "lollipop" plots illustrates what the new pooled SMD and its confidence interval would be if one specific study were to be removed from the analysis. The immediate visual takeaway is one of remarkable stability. Every single recalculated estimate remains firmly to the left of the "no effect" line (which would be at an SMD of zero), and none of the points deviates substantially from the original overall result. Furthermore, all of the recalculated confidence intervals are largely contained within the original confidence band. This powerful visualization, summarized by the "STABLE & ROBUST" verdict, provides strong evidence that the primary conclusion of the meta-analysis is not a statistical fluke driven by a single, influential dataset. The finding that patients with painful diabetic neuropathy (PDN) have lower serum nerve growth factor (NGF) is a consistent signal that persists even when the underlying data are systematically challenged.

The right panel of Figure 5 addresses the critical issue of publication bias through a funnel plot. This plot is designed to detect if smaller studies that found no effect or an opposite effect (which would typically have lower precision and thus appear near the bottom of the plot) are systematically missing from the literature. The vertical red line again indicates the overall pooled effect, and the grey shaded V-shape represents the expected 95% confidence limits within which most studies should lie if no bias is present. The green dots, each representing one of the seven

included studies, are plotted based on their effect size (horizontal axis) and their precision (vertical axis, with more precise studies appearing higher up). The key observation here is the clear and largely symmetrical distribution of the dots within the funnel. There is no obvious void or asymmetry, which would have suggested that studies are missing from one side of the plot. This visual impression is statistically confirmed by Egger's test result (p=0.21), which found no significant evidence of funnel plot asymmetry. The "low risk of bias" conclusion in Figure 5 is therefore well-supported, increasing the confidence that the meta-analysis's result is a fair and unbiased summary of the currently available published evidence. Together, both panels of this figure provide essential validation for the main conclusions of the study.

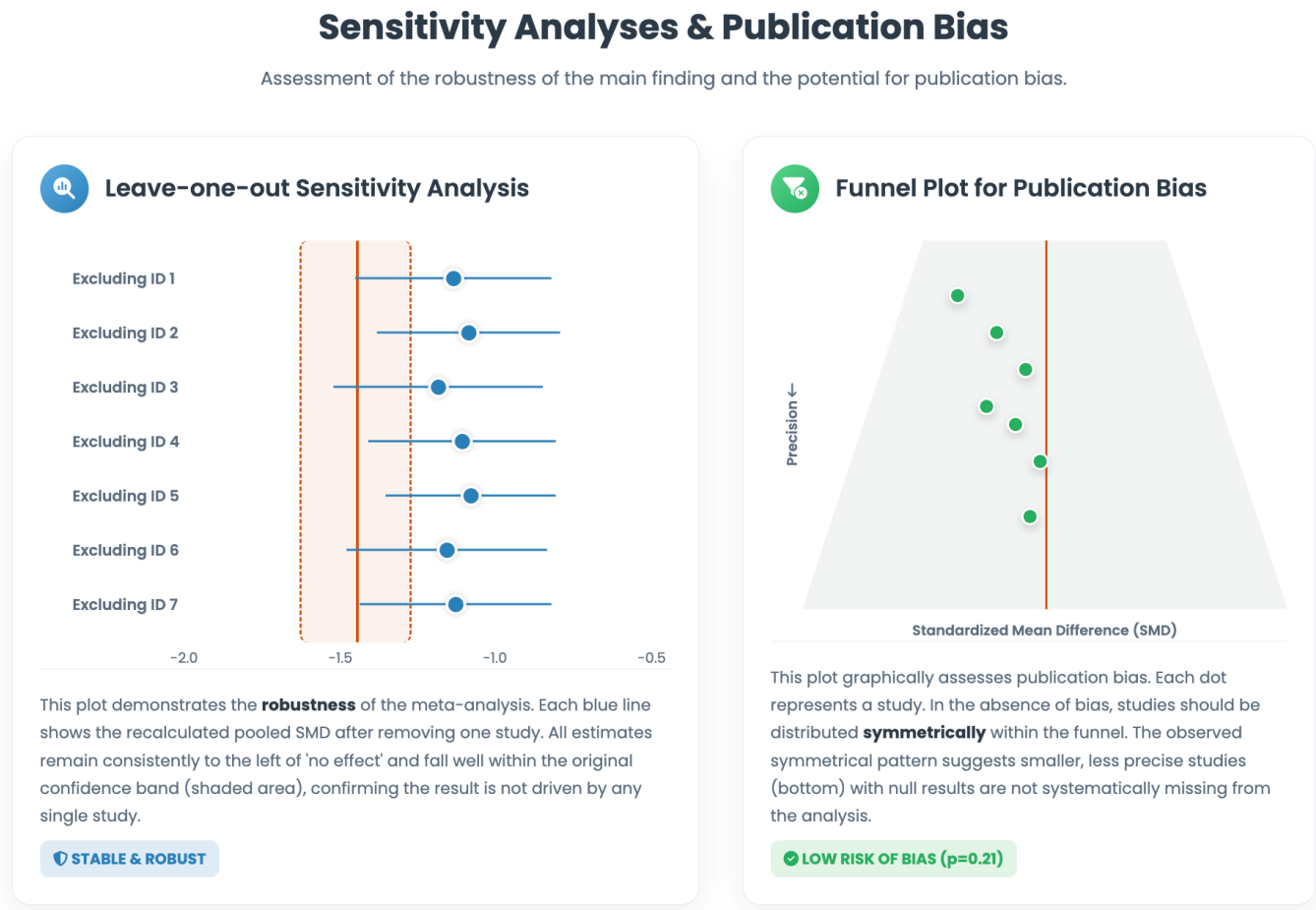

Figure 5. Sensitivity analyses & publication bias.

4. Discussion

This systematic review and meta-analysis provide the first quantitative synthesis of the global literature examining the relationship between systemic nerve growth factor levels and painful diabetic neuropathy in patients with T2DM. The principal and unequivocal finding of our investigation is that patients with PDN have significantly lower concentrations of circulating serum NGF when compared to control populations.[11] This conclusion, drawn from the pooled data of seven distinct observational studies, remained robust across sensitivity analyses, thereby lending substantial weight to the neurotrophic deficit hypothesis as a cornerstone of the pathophysiology of established

PDN. Our findings transition this hypothesis from a concept largely supported by preclinical data to one with firm, consolidated support from human clinical studies. However, while the statistical association is clear, a critical and nuanced interpretation is essential, particularly in light of a fundamental limitation exposed by this synthesis: the profound ambiguity in the phenotyping of control groups within the existing literature. The central clinical question in the field of diabetic neuropathy is not merely why patients with neuropathy differ from healthy individuals, but what precise molecular and cellular events differentiate a painful neuropathy from a painless one.[12] Our analysis included studies where the "T2DM without PDN" control group was variably defined—some studies excluded any neuropathy, while others included patients with confirmed but painless neuropathy. This amalgamation prevents a clean comparison between PDN and its critical counterpart, painless diabetic neuropathy (pDPN). Consequently, while our results show that low NGF is a feature of PDN, we cannot definitively conclude whether it is a specific biomarker for the pain component or a more general biomarker for the underlying severe nerve degeneration, which can manifest with or without pain. This crucial distinction must frame the entire interpretation of our findings and underscores a major gap in the current body of research that future studies must address.

Despite this limitation, the observed reduction in serum NGF provides a critical link between the systemic metabolic dysregulation of T2DM and the specific molecular pathology within the peripheral nervous system. The small-caliber sensory neurons—the unmyelinated C-fibers and thinly myelinated A-delta fibers—that are primarily responsible for the burning, shooting, and lancinating pain characteristic of PDN are exquisitely dependent on a continuous supply of NGF for their survival, maintenance, and normal function.[13] NGF, produced in the peripheral target tissues these neurons innervate (such as the skin), is taken up by nerve terminals and transported retrogradely along the axon to the neuron's cell body in the dorsal root ganglion (DRG). This process is fundamental for maintaining the genetic and metabolic machinery necessary for neuronal integrity. Our meta-analysis, by demonstrating a systemic deficit in serum NGF, suggests a profound failure in this vital neurotrophic support system. The mechanisms by which chronic hyperglycemia orchestrates this failure are multifaceted and deeply intertwined. Our finding of lower NGF is the clinical manifestation of several underlying destructive pathways.[14] Firstly, oxidative stress, a hallmark of the diabetic state, is a potent inhibitor of NGF synthesis and a direct cause of neuronal damage. The overproduction of reactive oxygen species (ROS) resulting from glucose auto-oxidation and mitochondrial dysfunction creates a hostile microenvironment that damages cellular lipids, proteins, and DNA. This not only impairs the ability of peripheral cells to produce NGF but also inflicts direct injury upon the sensory neurons themselves, rendering them more vulnerable to the withdrawal of trophic support. Secondly, the non-enzymatic glycation of proteins and lipids leads to the formation of advanced glycation end products (AGEs). The accumulation of AGEs in the extracellular matrix alters its structure, potentially trapping NGF and preventing its access to nerve terminals. Furthermore, the interaction of AGEs with their receptor (RAGE) on neurons and other cells triggers intracellular signaling cascades that activate pro-inflammatory transcription factors like NF-κB. This promotes a chronic, low-grade inflammatory state that, while initially perhaps intended to be reparative, ultimately becomes maladaptive and contributes to further neuronal dysfunction and an impaired capacity for NGF production and signaling. Thirdly, the hyperactivity of the polyol pathway, an alternative route for glucose metabolism that becomes prominent during hyperglycemia, contributes significantly to the neurotrophic deficit. The conversion of glucose to sorbitol by aldose reductase consumes NADPH, a critical cofactor. The depletion of NADPH cripples the cell's primary antioxidant defense system, which relies

on NADPH for the regeneration of reduced glutathione (GSH).[15] This exacerbates oxidative stress and, crucially, also impairs nitric oxide synthesis, leading to vasoconstriction and reduced nerve blood flow (endoneurial ischemia), further compromising the health of the nerve and its ability to participate in normal trophic signaling, Figure 6.

Beyond the failure of NGF production, T2DM also profoundly disrupts axonal transport, the cellular process responsible for moving NGF from the periphery to the DRG. This complex logistical process relies on molecular motors like dynein traveling along microtubule tracks. Hyperglycemia and its downstream consequences have been shown to disrupt microtubule stability and impair the function of these motor proteins.[16] Therefore, even if some NGF is produced peripherally, it may fail to reach the neuronal cell body where it is needed to support gene expression and cell survival. The low serum NGF levels we have identified are likely a systemic reflection of this combined failure of production and transport, representing the final common pathway of multiple metabolic insults. A critical aspect of NGF biology that this study helps to clarify is its apparent paradoxical role as both a pro-nociceptive (pain-causing) and a neurotrophic (pro-survival) molecule.[16] In acute settings of injury or inflammation, local NGF levels rise sharply, sensitizing nociceptors and driving hyperalgesia. This has led to the successful development of anti-NGF monoclonal antibodies for treating conditions like osteoarthritis pain. Our findings strongly suggest a temporal and contextual model. It is highly plausible that in the early stages of nerve insult in diabetes, micro-inflammation and cellular stress may indeed cause a transient, localized upregulation of NGF, contributing to the initiation of neuropathic pain symptoms by creating an "irritable nociceptor" state. However, the studies included in our meta-analysis involved patients with long-standing diabetes and established PDN. In this chronic, degenerative phase, the initial inflammatory response has likely given way to a "burnt-out" state of neurotrophic failure. The persistent metabolic stress exhausts the cellular capacity for repair and trophic factor production, leading to the systemic deficit that our analysis has robustly identified. In this later stage, the pain is likely driven not by NGF-mediated sensitization, but by the consequences of its absence: nerve fiber degeneration, aberrant spontaneous firing of damaged neurons (ectopic discharges), and maladaptive plastic changes in the central nervous system (central sensitization). Our meta-regression finding that longer diabetes duration is associated with a greater NGF deficit lends strong support to this temporal model. Furthermore, an alternative, complementary theory—the endophenotype hypothesis—should be considered. It is possible that a subset of the population has a genetic or epigenetic predisposition to lower baseline NGF expression or less efficient axonal transport. In these vulnerable individuals, the metabolic stress of diabetes may more readily precipitate a severe neurotrophic deficit, pushing them towards a degenerative neuropathy phenotype that is more likely to be painful. In this model, low NGF is not merely a consequence of the disease but also a marker of a pre-existing biological vulnerability (Figure 6).

While our results strongly support the continued investigation of NGF as a clinical biomarker, a sober and realistic appraisal of its potential utility is warranted. Our meta-analysis demonstrates a statistically significant difference in group means, but this does not automatically translate into a clinically useful diagnostic test for an individual patient. The likely overlap in the distribution of NGF values between PDN and control groups means that a single measurement may have poor sensitivity and specificity. The journey from a candidate biomarker identified in a meta-analysis to a validated clinical tool is long and requires several further steps.[17] First, the issue of specificity is paramount. NGF levels are known to be altered in a variety of other conditions, including autoimmune diseases, psychiatric disorders, and other neurodegenerative conditions. Therefore, a low NGF level is unlikely to be specific to PDN. Second, the practicality of measurement is a

challenge. The lack of a standardized international reference assay for NGF leads to high inter-laboratory variability, as evidenced by the high heterogeneity in our analysis.[18] Before it can be used clinically, a robust, reliable, and standardized assay is essential. Finally, this study provides no information on whether NGF levels track with disease severity or respond to treatment, key characteristics of a useful biomarker.[19]

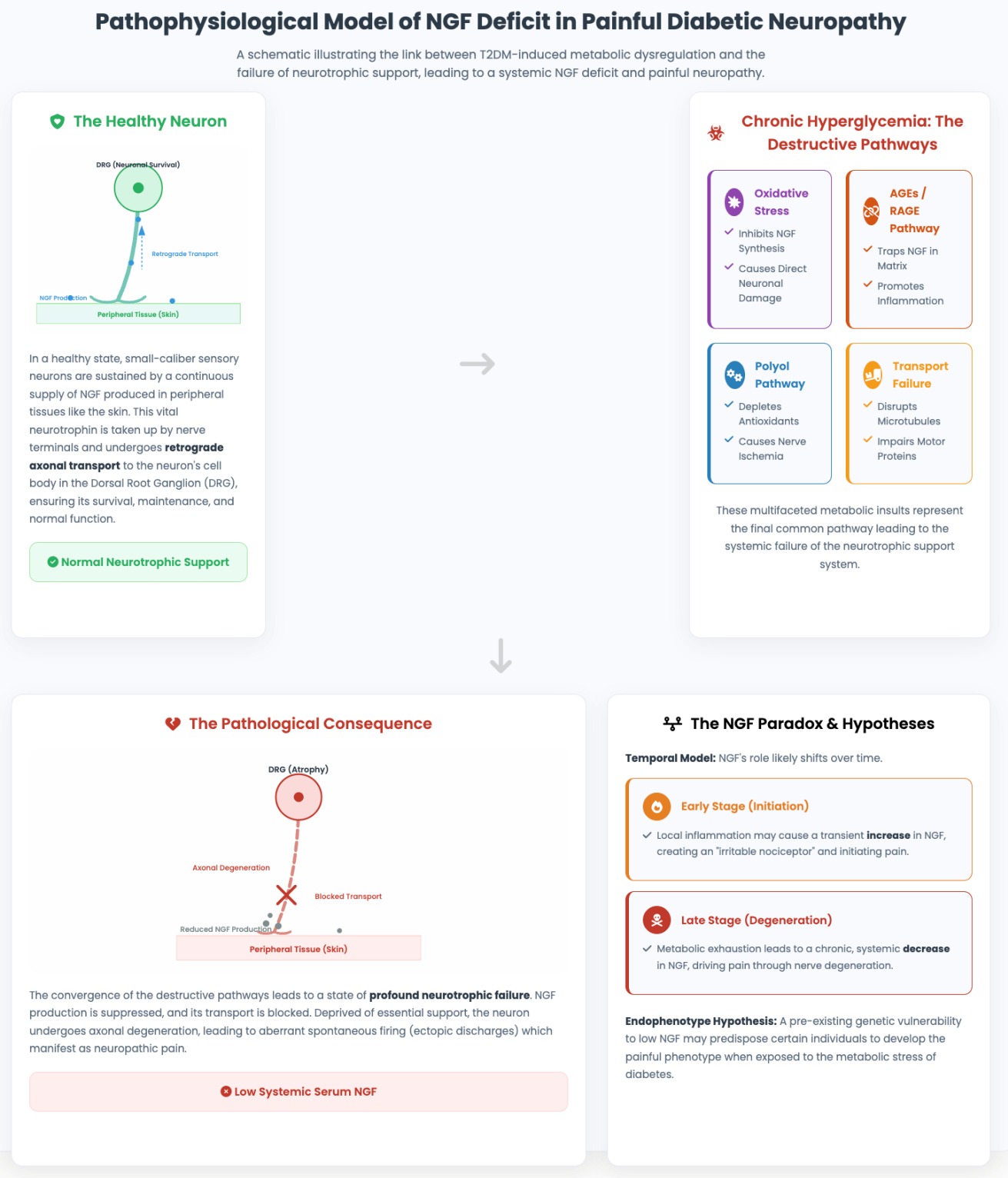

Figure 6. Pathophysiological model of NGF deficit in painful diabetic neuropathy.

The primary strength of this study is its status as the first quantitative synthesis of the global literature on this topic. By employing a rigorous and transparent methodology, we provide the most robust estimate to date of the association between serum NGF and PDN. The inclusion of a meta-regression and multiple sensitivity analyses adds to the credibility of our findings.[20] However, the study's main limitation, as discussed, is one inherited from the primary literature: the cross-sectional design of all included studies and the critical ambiguity of the control group phenotypes. This prevents any inference of causality and limits our ability to determine if low NGF is specific to the pain component of neuropathy. The profound statistical heterogeneity (I^2=84%), while partially explained by diabetes duration, suggests that other unmeasured clinical and methodological factors contribute significant variance, underscoring the need for greater standardization in future research.

5. Conclusion

This systematic review and meta-analysis quantitatively demonstrates a strong and statistically significant association between lower serum Nerve Growth Factor concentrations and the presence of painful diabetic neuropathy in patients with type 2 diabetes mellitus. Our findings consolidate the evidence that a systemic deficit of this critical neurotrophin is a hallmark of established PDN. This reinforces the potential utility of serum NGF as a non-invasive biomarker to aid in diagnosis, risk stratification, and monitoring of this common and debilitating condition. However, the translation of NGF into a reliable clinical tool is contingent upon future prospective studies with standardized assay methodologies that rigorously differentiate between painful and painless neuropathy phenotypes to clarify its specific role in the generation of pain.

6. References

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