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REVIEW ARTICLE

Methodological Advances in Growth Hormone Stimulation Tests in Children with Short Stature

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ABSTRACT

Background: **The diagnosis of growth hormone deficiency in children with short stature relies on growth hormone stimulation testing. The conventional protocol, requiring two separate pharmacological tests on separate days, is invasive, time-consuming, costly, and carries safety risks, notably hypoglycemia from the insulin tolerance test.**
Methods: **This review is compiled after a relevant literature search from electronic databases including, but not limited to, Google Scholar, PMC, PubMed, Science Direct, and Scopus. The key words used for searching relevant literature include short stature in children, growth hormone stimulation test, peak growth hormone level, growth hormone stimulation test medications, etc.**
Results: **According to the published literature, endocrine factors play a significant role in the occurrence of short stature in children. Growth hormone deficiency is one of the more common endocrine causes, with an incidence ranging from 1/4,000 to 1/10,000. The growth hormone stimulation test is a classic laboratory method for diagnosing growth hormone deficiency. The transition from the traditional two-day growth hormone stimulation test to a simplified one-day protocol significantly shortens the diagnostic window, reduces the number of blood draws, and improves patient compliance and comfort, while maintaining diagnostic efficacy.**
Conclusions: **Our review highlights that the simplified one-day stimulation test can keep diagnostic efficiency while enhancing patient compliance and reducing both physical and psychological burdens. Optimizing the safety-diagnostic efficacy balance in growth hormone stimulation tests remains a research priority.**
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INTRODUCTION

The growth hormone (GH) stimulation test is a classical laboratory procedure for diagnosing short stature in children. The conventional GH stimulation tests protocol mandates two separate stimulation tests administered via different routes on separate days. However, this approach presents significant limitations for the pediatric population. The two-day testing process is not only more invasive and requires a higher frequency of blood sampling, but it also increases healthcare costs. Further-

more, the insulin-induced hypoglycemia test component carries a potential risk of hypoglycemia. In recent years, numerous clinicians and researchers have sought to optimize this testing protocol. Notably, the latest Chinese guidelines for the diagnosis and treatment of growth hormone deficiency (GHD) in children, released in 2024, recommend a single-day combined stimulation test-utilizing arginine in conjunction with either clonidine or levodopa-as a standard diagnostic method for children suspected of having GHD. This optimized protocol shortens the testing window from two days to one, reducing invasiveness [1]. This protocol significantly improves child-parent compliance, optimizes healthcare resource utilization, reduces family financial burdens, and demonstrates strong clinical value. This article reviews recent methodological improvements in growth hormone stimulation tests for children with short stature.

Etiology and classification of short stature in children

Pediatric short stature is clinically defined as a height below -2 SD (or the 3rd percentile) for age-, sex-, and ethnicity-matched peers. The causes of short stature are diverse, encompassing genetic, nutritional, endocrine, and disease-related factors.

Genetic factors significantly contribute to short stature, as many short children have similarly short parents, demonstrating a strong hereditary influence on height. In general, a child's final adult height is partly determined by parental height, as genetic factors modulate bone growth through the expression of growth hormone (GH) and other related genes [2].

Proper nutrition is essential for children's healthy growth and development. Protein, vitamins, minerals, and trace elements play crucial roles for bone growth and GH production [3]. Prolonged deficiency in balanced nutrition, particularly insufficient intake of key elements like calcium, zinc, and iron, can significantly impair height gain and may lead to nutritional short stature. Additionally, chronic diseases or long-term medication use may hinder growth in children. For instance, chronic kidney disease, congenital heart disease, and gastrointestinal disorders can impair nutrient absorption and metabolism, disrupting normal growth and development.

Endocrine dysfunction is a major contributor to short stature in children. GHD is one of the more common endocrine causes, with an estimated incidence ranging from 1/4,000 to 1/10,000 [4]. Since GH is secreted in a pulsatile manner under physiological conditions [5], its secretion is influenced by the integrity of the hypothalamus, pituitary gland, neurotransmitters, as well as brain structure and function. GH secretion displays significant interindividual variability, influenced by modifiable factors including physical activity, sleep quality, nutritional intake, and psychological stress. Consequently, random GH measurements fail to assess secretion. Therefore, the GH stimulation test serves as an essential tool for diagnosing GHD [6].

Growth hormone stimulation test

The GH stimulation test provokes the secretion of GH through various stimuli acting at the level of the hypothalamus or pituitary gland. The peak GH level achieved in response to these stimuli is compared to the expected peak levels in normal individuals to determine whether GHD is present [7]. A peak GH level < 5 ng/mL is widely accepted as the diagnostic threshold for GHD [8]. GH stimulation tests are categorized into physiological tests, which primarily include sleep and exercise, and pharmacological tests using specific drugs. Physiological stimulation tests, however, are often challenging to perform reliably in children. Sleep tests, for instance, require specific conditions and equipment; blood must be drawn during stage III or IV of sleep under electroencephalogram (EEG) monitoring to obtain an accurate GH measurement. Similarly, exercise tests necessitate achieving a defined intensity of physical activity to adequately stimulate GH secretion. These stringent requirements make it difficult to obtain consistent and reliable data from physiological stimulation tests in the pediatric population. Therefore, pharmacological stimulation tests, rather than exercise tests, are routinely employed in clinical practice for children suspected of having GHD. The Clinical Pathway for Short Stature (2019 Edition) specifies that two pharmacological tests acting via different mechanisms must be performed. One of these must be either the insulin-induced hypoglycemia test or the arginine test, with the other chosen from options such as the clonidine test or the L-DOPA test. Given that each individual pharmacological agent carries a false-positive rate of approximately 15%, combination tests utilizing a drug that suppresses somatostatin (e.g., arginine) along with one that promotes growth hormone-releasing hormone (e.g., clonidine or L-DOPA) are often used to improve diagnostic accuracy. These combined tests can be conducted over two days or within a single day. China's latest 2024 "Guidelines for the Diagnosis and Treatment of GHD in Children" recommend a single-day, combined stimulation test-specifically using arginine in conjunction with either clonidine or L-DOPA-as the standard diagnostic approach for children suspected of having GHD.

Conventional two-day GH stimulation test

The conventional two-day GH stimulation test serves as a widely used assessment method to determine whether children with short stature have GHD by stimulating GH secretion using two different pharmacological agents acting through distinct pathways. Agents commonly employed in this test include insulin, arginine, clonidine, neostigmine, and L-DOPA, each of which promotes GH release via a different mechanism.

The insulin-induced hypoglycemia test is regarded as a classical method within the conventional two-day GH stimulation test protocol. Due to its specific stimulatory effect on the pituitary GH secretory axis, it is consid-

ered the "gold standard" for diagnosing GHD. This test uses insulin to induce hypoglycemia, which stimulates central glucose receptors. This activation triggers monoaminergic neurons to promote the secretion of GHRH via α2-adrenergic receptors, while simultaneously inhibiting somatostatin release. This dual action activates the hypothalamic-pituitary axis, leading to the release of GH from the anterior pituitary [9]. In healthy children, GH secretion typically peaks 30 - 60 minutes after the induction of hypoglycemia, with reference levels usually ranging between 10 - 40 ng/mL. In contrast, children diagnosed with GHD consistently exhibit a peak GH response significantly below the diagnostic threshold, which is commonly defined as < 5 - 7 ng/mL [10]. Despite its high diagnostic sensitivity and physiological relevance, the insulin-induced hypoglycemia test requires strict adherence to safety protocols: blood glucose and vital signs must be monitored in real-time, and resuscitation measures must be available throughout the entire procedure to mitigate the risk of severe hypoglycemia.

The arginine stimulation test is a classical pharmacological method for evaluating pituitary GH secretory function. Its mechanism of action is primarily based on the regulation of amino acid metabolism. By increasing blood amino acid levels, arginine inhibits the secretion of somatostatin from the hypothalamus, thereby promoting the release of endogenous GH from the pituitary gland [11]. The peak GH response to arginine stimulation typically occurs 30 - 60 minutes after administration. Clinical data indicate that serum GH levels exhibit a stimulatory response following arginine challenge [12]. In children with GHD, this response is significantly blunted, typically characterized by a peak concentration below 3 ng/mL. In some severe cases of GHD, no significant GH peak may be detectable. Interestingly, compared to other stimulating agents, the GH secretion curve induced by arginine is characterized by a gradual rise and fall. Although the criteria for determining its peak level are relatively broad (typically assessed between 0.5 to 2 hours post-administration), it still provides a reliable basis for clinically evaluating pituitary GH secretory function [13]. This favorable pharmacokinetic profile makes arginine a widely used foundational component in combined stimulation tests.

Clonidine, another commonly used provocative agent, is a selective α2-adrenergic agonist. It acts on α-adrenergic receptors in the central nervous system to stimulate the release of GHRH from the hypothalamus, thereby promoting GH secretion from the pituitary gland. In contrast to arginine, clonidine typically induces a more rapid GH response with a characteristically higher peak concentration. Blood sampling during the test is usually performed around 30 minutes after administration. Plasma GH levels rise sharply between 30 to 60 minutes post-administration, reaching a significant peak during this period [14]. In healthy children, the peak GH level following clonidine stimulation typically ranges from 10 to 30 ng/mL or even higher. In contrast, patients with GHD often exhibit a blunted response, with peak levels frequently remaining below 5 ng/mL. Similar to the arginine test, some individuals with severe GHD may show nearly undetectable GH peaks [15]. Due to its ability to rapidly stimulate GH release, clonidine is generally considered a sensitive diagnostic tool. However, its considerable inter-individual variability necessitates that clinicians interpret the test results within a comprehensive clinical context.

Neostigmine, on the other hand, acts by reversibly inhibiting acetylcholinesterase, leading to an increased concentration of acetylcholine. This, in turn, stimulates the secretion of GHRH, thereby promoting the release of GH from the anterior pituitary. During the test, administration of neostigmine rapidly provokes GH secretion, and blood samples are typically collected between 15 to 30 minutes after injection. Studies have shown that in normal children, the peak GH level following neostigmine stimulation generally ranges between 5 to 20 ng/mL, usually occurring within 15 to 30 minutes post-administration. Compared to other provocative agents, the peak response induced by neostigmine often exhibits a more gradual profile, which may contribute to its relatively higher reproducibility in certain clinical settings. However, in children with GHD, the peak GH level is typically below 5 ng/mL. While the results may demonstrate greater stability compared to some other agents, the sensitivity of the neostigmine test is generally considered lower.

In the L-DOPA GH stimulation test, L-DOPA - a precursor of dopamine - is converted in the body into dopamine or norepinephrine. These neurotransmitters stimulate the hypothalamic-pituitary axis, promoting the secretion of GHRH, which in turn induces GH release from the anterior pituitary gland. However, the stimulatory effect of L-DOPA is relatively mild, often resulting in lower peak GH levels, typically ranging between 3 to 12 ng/mL. The response is also characteristically gradual, requiring a longer duration to reach maximum concentration. In most clinical tests, the peak GH level following L-DOPA administration occurs between 30 minutes to 1 hour after ingestion, and this peak is generally lower than that observed with clonidine and arginine stimulation tests.

Currently, glucagon is recognized as a reliable provocative agent for the evaluation of GHD. It stimulates GH release by activating α-adrenergic receptors and can also induce rebound hypoglycemia following hyperglycemia to further promote GH secretion [16]. The test is typically performed via intramuscular injection of glucagon (dose: 0.03 mg/kg, maximum 1 mg), followed by blood sampling at 0, 30, 60, 90, 120, 150, and 180 minutes to measure GH levels. In normal children, the peak GH level usually occurs around 150 minutes and often exceeds 7 ng/mL, whereas children with GHD exhibit a blunted response. Advantages of this test include its safety profile, reproducibility, and independence from sex-based variations. However, limitations include the prolonged test duration, the requirement for intramus-

cular injection, and the influence of factors such as obesity, which may necessitate adjustment of diagnostic thresholds [17].

Although the two-day GH stimulation test holds diagnostic value for GHD, it is associated with several limitations. Firstly, the extended testing period-typically requiring two days to complete-imposes a significant burden on patients and contributes to higher medical costs. Secondly, the procedure is relatively complex, involving multiple blood draws, which markedly reduces compliance among both children and their parents. Thirdly, factors such as the type and dosage of pharmacological agents, intervals between blood sampling, and individual variability contribute to a relatively high rate of false-positive or false-negative results. Lastly, the insulin-induced hypoglycemia test carries a risk of hypoglycemia, which imposes constraints on its clinical applicability.

To address these limitations, the conventional two-day GH stimulation test should be optimized by reducing blood sampling points, refining drug combinations, and streamlining procedures. The following section discusses advances in simplified GH stimulation testing protocols.

Simplified one-day GH stimulation test

The simplified one-day GH stimulation test, as an optimized alternative to the conventional two-day protocol, has increasingly become an important option in clinical practice due to its reduced number of blood draws, shorter duration, and lower cost. Compared to the traditional two-day test, the one-day approach typically involves combinations of pharmacological agents designed to stimulate GH secretion more efficiently through a simplified procedure. Administration of stimuli and blood sampling are completed within a shorter timeframe to obtain GH response data. However, key factors such as the selection of drug combinations, sequence of administration, timing of blood sampling, and individual variability may affect the accuracy of the test.

The drug combinations used in the simplified one-day GH stimulation test typically include common provocative agents such as arginine, clonidine, insulin, and neostigmine. The selection and sequencing of these drugs, as well as the timing of their administration, are rationally designed based on their mechanisms of action, duration of effect, and the temporal characteristics of GH secretion they induce. The most common combination is the concurrent use of arginine and clonidine, which work synergistically through distinct mechanisms to promote GH release. The results from the arginine stimulation are often used for initial screening. If the peak GH level is low or near zero, the second phase of stimulation-using clonidine-is initiated. The specific procedure involves intravenous infusion of arginine over 30 minutes combined with oral administration of clonidine in the morning under fasting conditions. Blood samples are typically drawn at 30, 60, 90, 120, and 150 minutes after administration to measure GH levels [18]. However, numerous studies indicate that the GH peak generally occurs around 120 minutes, making sampling at 150 or 180 minutes potentially unnecessary [19]. In children with GHD, the peak GH level is typically below 5 ng/mL, indicating a significant impairment in GH secretion. Close monitoring is required post-test for potential adverse effects such as nausea, vomiting, and the occurrence of hematuria [20].

Another common drug combination is arginine paired with neostigmine. The implementation of this protocol is generally similar to the first phase following arginine injection. Approximately 30 to 60 minutes after arginine administration, neostigmine is injected, with the timing being relatively flexible. However, the stimulatory effect of this combination is usually milder, and the peak GH level tends to be lower than that observed with clonidine, typically ranging between 5 to 15 ng/mL. In children with GHD, the peak may sometimes fall below 3 ng/mL. As a result, this combination is more frequently used to further confirm the results of the arginine stimulation test.

Regarding the insulin-induced hypoglycemia test, its application in simplified one-day protocols is approached with caution due to the associated risk of hypoglycemia. It is typically reserved as a supplementary test when responses to prior pharmacological stimuli are insufficient or equivocal, and must be conducted under strict medical supervision [21].

The simplified one-day GH stimulation test enables efficient evaluation of GH secretion within a shortened timeframe through the rational arrangement of drug combinations, sequences, and sampling time points. The combination of arginine and clonidine is the most widely adopted regimen, while agents such as insulin are utilized supplementally. By strategically leveraging the stimulatory effects and peak profiles of each agent and integrating these findings with the patient's specific clinical presentation, this approach provides robust support for the early diagnosis and treatment of GHD in children.

Hormonal priming before GH stimulation test

Sex hormone priming prior to GH stimulation testing is a method designed to enhance the test's sensitivity and specificity. Multiple studies have demonstrated that sex hormone priming can significantly increase the peak GH response during the test, reduce false-positive diagnoses, and thereby avoid unnecessary GH treatment. However, priming protocols - such as dosage and treatment duration - have not yet been standardized, and some study results show heterogeneity, indicating the need for individualized priming strategies [22]. Overall, sex hormone priming holds important value in improving the diagnostic accuracy of GH stimulation test, but its clinical application should be comprehensively evaluated based on the patient's age, bone age, and pubertal status.

Influencing factors of GH stimulation tests

Factors influencing the results of GH stimulation tests include the timing of drug administration, the patient's age, body weight, health status, and other abnormalities of the endocrine system. Differences in the physiological effects of various pharmacological agents, individual patient responsiveness to these agents, and the precision of laboratory operations may all affect the accuracy of test data [23]. For instance, regarding body weight, there is significant overlap in the mean peak GH levels between obese children without GHD and those with genuine GHD. Moreover, a negative correlation has been observed between body mass index standard deviation scores and peak GH levels. This inverse relationship may lead to overdiagnosis of GHD in overweight or obese children [24]. In terms of age, pre-pubertal children at older ages tend to exhibit lower peak GH responses, indicating that age is an important confounding variable in GH stimulation test results [25]. In summary, in clinical practice, it is essential to account for these influencing factors and interpret test results comprehensively to ensure diagnostic accuracy and reliability.

Safety of GH stimulation tests

The GH stimulation test is clinically valuable for diagnosing short stature in children, but safety concerns remain a critical factor influencing its selection and application. Particularly in the conventional two-day testing protocol, the use of different provocative agents and their associated adverse effects introduces potential risks during the procedure. Although the insulin-induced hypoglycemia test is regarded as the "gold standard" for diagnosing GHD, its safety profile is particularly concerning. Since insulin stimulates the hypothalamic-pituitary axis to release GH by inducing hypoglycemia, the procedure may lead to adverse reactions such as dizziness, palpitations, weakness, or even loss of consciousness. In severe cases, it may trigger seizures, profound hypoglycemia, or other life-threatening conditions [26].

In addition to insulin, other provocative agents also carry certain safety concerns. For example, clonidine may cause drowsiness, fatigue, dizziness, and gastrointestinal discomfort during the test, particularly requiring cautious use in children with cardiovascular conditions [27]. Although arginine is relatively safe, some children may experience side effects such as nausea, vomiting, and hematuria. The L-DOPA stimulation test is often accompanied by mild hypoglycemia and gastrointestinal discomfort, which may further increase the physiological and psychological burden for some children. Furthermore, neostigmine, as an acetylcholinesterase inhibitor, may induce cholinergic side effects [28], especially at high doses or in drug-sensitive individuals.

The safety concerns associated with conventional stimulation tests are not only related to the mechanisms of the pharmacological agents themselves but are also closely tied to the frequency of blood sampling, the duration of the test, and individual patient differences [14]. Repeated venipuncture causes physical trauma to the child, while the prolonged testing process may induce psychological stress such as anxiety and fear, adversely affecting both the child and their family. Particularly in younger children or those with comorbid conditions, the complexity and discomfort of the procedure may reduce compliance, thereby indirectly compromising the accuracy of diagnostic results.

In recent years, simplified stimulation tests have significantly enhanced procedural safety. The one-day stimulation test reduces the risk of hypoglycemia by optimizing drug combinations and decreasing the use of insulin, while also shortening test duration and minimizing the number of blood draws, thereby alleviating both physical and psychological burdens on children. These improvements have not only enhanced the safety profile of the test but have also increased compliance among pediatric patients and their parents. However, although simplified protocols demonstrate favorable safety and feasibility, their applicability across different pediatric populations requires further validation. Future research should focus on the development of individualized testing strategies and the advancement of non-invasive or minimally invasive diagnostic technologies. These efforts aim to maintain diagnostic accuracy while minimizing potential risks associated with the testing process.

In summary, the GH stimulation test plays a crucial role in the diagnosis of short stature in children, and continuous methodological advancements have expanded its clinical diagnostic and therapeutic potential. The conventional two-day stimulation test, utilizing multiple pharmacological agents with distinct mechanisms, provides essential information for assessing GH secretory function. However, its complexity, time-consuming nature, and invasiveness have limited its widespread use in routine clinical practice. The introduction of the simplified one-day stimulation test has significantly improved diagnostic efficiency while enhancing patient compliance and reducing both physical and psychological burdens. This evolution marks a transition from complexity toward greater practicality and effectiveness in GH stimulation testing.

Nevertheless, current stimulation tests still exhibit certain limitations. The false-positive and false-negative rates associated with conventional protocols affect diagnostic accuracy, while the simplified tests still need further optimization in the aspects of drug excitation mechanism, peak time point, and applicability of different individuals [29]. Additionally, safety concerns related to methods such as the insulin-induced hypoglycemia test demand ongoing attention. Achieving a balance between ensuring safety and improving diagnostic sensitivity and specificity remains a key focus for future research.

Future research may focus on the following directions. First, conducting multicenter, large-sample clinical studies to further validate the applicability of various stimulation tests, particularly their efficacy in complex

cases or special populations. Second, exploring non-invasive or minimally-invasive alternative methods, such as utilizing imaging techniques or biomarkers to assist in evaluating GH secretory function, thereby reducing patient discomfort and risks [30]. Third, developing more precise and efficient stimulation protocols based on existing pharmacological agents to shorten test duration and minimize disruption to patients. Ongoing research and technological advancements will enhance GH stimulation tests' accuracy and improve patient experience. As a critical tool for diagnosing short stature in children, its future development will benefit more patients and elevate related research standards.

CONCLUSION

The transition from the traditional two-day growth hormone stimulation test to a simplified one-day protocol significantly shortens the diagnostic window, reduces the number of blood draws, and improves patient compliance and comfort, while maintaining diagnostic efficacy. Balancing safety with diagnostic accuracy remains a key research focus.

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