Original article

The Wingate anaerobic test cannot be used for the evaluation of growth hormone secretion in children with short stature

Nitzan Dror, Liat Oren, Michal Pantanowitz, Alon Eliakim, Dan Nemet *

Child Health and Sports Center, Pediatric Department, Meir Medical Center, Sackler School of Medicine, Tel Aviv University, Kfar-Saba 44281, Israel

Abstract

Purpose: To assess the growth hormone (GH) response to the Wingate anaerobic test (WAnT) among children with short stature and suspected GH deficiency. We hypothesized that the GH response to the WAnT would be similar to the GH response to a commonly used pharmacologic provocation test.

Methods: Ten children (6 males and 4 females, age range 9.0–14.9 years) participated in the study. Each participant performed 2 tests: a standard all-out WAnT, cycling for 30 s against constant resistance, and a standardized pharmacologic test (clonidine or glucagon). Blood samples for GH were collected before and 10, 30, 45, and 60 min after the beginning of exercise. In addition, we collected pre- and post-exercise blood lactate levels.

Results: There was a significant increase in GH levels after the WAnT, yet in 9 of 10 participants, this increase was below the threshold for GH sufficiency. Peak GH after the WAnT was significantly lower compared to the pharmacologic GH provocation tests (with 9 of 10 demonstrating GH-sufficient response).

Conclusion: The traditional WAnT cannot be used as a GH provocation test. Further research is needed to develop anaerobic exercise protocols sufficient to promote GH secretion.

1. Introduction

The diagnosis of growth hormone (GH) deficiency in children with short stature is complex and challenging. GH is secreted from the pituitary gland in a pulsatile manner mainly during periods of deep sleep at night, whereas during most of the day GH levels are very low or even undetectable. Consequently, a single random blood sample for circulating GH levels cannot differentiate between a healthy and a GH-deficient child. To overcome this, several provocation tests aimed at stimulating pituitary GH release have been developed.[1] Most of these tests use pharmacologic agents[2] and present possible patient risk (e.g., hypoglycemia). Moreover, the interpretation of a normal GH response to pharmacologic stimuli may not necessarily reflect physiological GH secretion. These confounding factors emphasize the need for a more physiological stimulation test such as exercise or for the use of constant-level circulating substances,

such as insulin-like growth factor 1 and its binding proteins, for the diagnosis of childhood GH deficiency.[3]

Currently, GH deficiency is defined as failure to increase serum GH concentrations above a predetermined threshold level (e.g., 10 ng/mL, based on polyclonal hormonal assays) after a minimum of 2 GH stimulation tests. Two tests are generally required because false-negative responses (low GH levels in a GH-sufficient child) may occur. Moreover, the definition of GH deficiency in children may be even more challenging owing to the continuum between complete and partial GH deficiency based on the stimulated peak GH level (e.g., peak GH values of 7–10 ng/mL may be considered partial GH deficiency; however, peak GH levels below 5 ng/mL suggest more severe GH deficiency).[4] The artificial nature of pharmacologic provocation tests and the possibility that these tests might not always reflect GH under normal physiological conditions provided an impetus for a more physiological test. It was further suggested that the most important diagnostic role of "physiological" GH stimulation tests such as exercise in children with suspected partial GH deficiency. In these children, the response to pharmacologic provocation might be partial, but the response to physiological

stimulation will be blunted. Therefore, children with a partial GH response to the first provocation test should undergo an exercise test for GH secretion as the second preferred stimulation test.

Previous studies have shown that only relatively long (>10 min) and intense (above the lactic anaerobic threshold (LAT)) aerobic exercise induces GH secretion.[5] The fact that this type of exercise cannot truly be considered physiological because it does not reflect the type of exercise that children usually perform, combined with the complexity of such testing (several laboratory visits to determine peak aerobic power, LAT, and the relative testing intensity), led to an effort to use other types of exercise to provoke GH release. Recent studies have shown a significant increase in GH levels after the Wingate anaerobic test (WAnT) (30 s of supramaximal cycle exercise against resistance that is calculated relative to each individual's body mass) in young adults.[6,7] This is promising because the daily physical activity of children involves mainly spontaneous, short, anaerobic-type exercise, suggesting that the GH response to this kind of exercise will better represent the activity patterns of children. In addition, this type of exercise stimulation test for GH secretion requires only a single laboratory visit and as a consequence is less complicated and time-consuming and more cost-effective. Therefore, the aim of the present study was to assess the GH response to the WAnT among children with short stature and suspected GH deficiency. We hypothesized that the GH response to the WAnT would be similar to the GH response to a commonly used pharmacologic provocation test.

2. Materials and methods

2.1. Participants

Ten children (6 males and 4 females, age range 9.0–14.9 years, body weight 34.5 ± 9.4 kg, body height 139.7 ± 10.4 cm, body mass index 17.2 ± 2.9 kg/m^2, body mass index percentile $30.7\% \pm 30.8\%$; mean $\pm$ SEM) participated in the study. Only 1 participant was overweight. Five participants were prepubertal, and 5 were at Tanner stages 2–3 for pubic hair. Participants were children who were evaluated for short stature and impaired growth rate in the endocrine clinic at the Meir Medical Center, Sackler School of Medicine, Tel Aviv University, and were requested to perform a provocation test for GH secretion. The study was approved by the Meir Medical Center Institutional Review Board (Trial registration number: NCT01934270), and appropriate informed consent was obtained from all the participants and their parents.

2.2. Anaerobic test for GH secretion

The WAnT was performed using the Lode Corival cycle ergometer (Lode B.V., Groningen, The Netherlands). Seat height was adjusted to each participant's satisfaction, and clips with straps were used to prevent the feet from slipping off the pedals. Each participant cycled 30 s against constant resistance. For female participants resistance was set to 0.53 N·m per kilogram body weight (<14 years of age) or 0.67 N·m per kilogram body weight (≥14 years of age). In male participants, resistance was set at 0.55 N·m per kilogram body weight (<14 years of age) or 0.70 N·m per kilogram body weight (≥14 years of age).[8] Partici-

pants were instructed to pedal as fast as possible throughout the test period and were verbally encouraged throughout the test.

In each test maximal power output, mean power output, minimal power output, and fatigue index were measured. All power output measurements are based on 5 s averages that were calculated by the WAnT computer software and were reported in watts per kilogram (W/kg). Maximal power output (peak power) was calculated from the highest 5 s work output. Mean power output, which reflects the anaerobic capacity, was calculated as the mean power output throughout the 30 s of the test. Minimal power output was calculated as the lowest 5 s work output. Fatigue index was calculated as the percentage of power output drop from the maximal power output throughout the test.[8]

In a separate visit, each participant performed an additional commonly used GH provocation test (i.e., clonidine test or glucagon test) using standard protocols.

2.3. Blood sampling and analysis

Tests were performed in the morning after an overnight fast. However, water was given *ad libitum* before testing to avoid dehydration. An indwelling venous catheter was inserted 30 min before the first blood draw, after allowing subjects to rest and sit quietly. In the WAnT, blood samples were collected before and 10, 30, 45, and 60 min after the beginning of the exercise test. Lactate levels were collected before, immediately after, and 10 min after the WAnT. In the clonidine test, blood samples were collected before and 30, 60, 90, and 120 min after the beginning of the exercise test. In the glucagon test, blood samples were collected before and 60, 90, 120, 150, and 180 min after the beginning of the exercise test. Blood samples were immediately spun at 3000 rpm and at 4°C for 20 min. All serum specimens from each individual for each test were analyzed in the same batch by an experienced technician, who was blinded to the type of provocation test and to the order of the samples.

2.3.1. GH

GH serum concentrations were determined by means of solid phase, 2-site, chemiluminescent immunometric assay with the Siemens IMMULITE 2000 immunoassay system (Siemens Healthcare, Erlangen, Germany) using murine monoclonal anti-GH antibody. Intra-assay coefficient of variability (CV) was 2.9%–4.6%, interassay CV was 4.2%–6.6%, and analytical sensitivity was 0.01 ng/mL. Normal values in our laboratory are 0.1–7.5 ng/mL.

2.3.2. Lactate

Plasma lactate levels were measured by the COBAS INTEGRA 400 system (Roche Diagnostics Ltd., Rotkreuz, Switzerland) using the enzymatic colorimetric method. Intra-assay CV was 0.7%–0.8%, interassay CV was 1.1%, and analytical sensitivity was 2 mg/dL. Normal values in our laboratory are 4.5–19.8 mg/dL.

2.4. Statistical analysis

Two-way repeated-measure analysis of variance with Bonferroni corrections was used to assess the effect of the WAnT on GH levels with time serving as the within-group factor and

type of provocative test as the between-group factor. Data are presented as mean ± SEM. Significance was set at $p < 0.05$.

3. Results

All participants completed the WAnT. Peak power was 7.9 ± 2.3 W/kg, mean power was 5.5 ± 1.1 W/kg, and fatigue index was $60.8\% \pm 17.6\%$ (mean ± SEM). There was a significant increase in GH levels after the WAnT ($p < 0.05$, Fig. 1). In all participants, GH_{peak} was seen in the 10- or 30-min sample, except in 1 participant who experienced GH_{peak} before the WAnT. However, GH_{peak} was significantly lower after the WAnT compared to the other GH provocation test ($p < 0.01$, Fig. 2). Only 1 participant had GH increase above 7.5 ng/mL after the WAnT (i.e., 15.3 ng/mL), and, in this participant, GH_{peak} occurred before the exercise test. In

Fig. 1. Changes in growth hormone (GH) level after the Wingate anaerobic test. * $p < 0.05$, compared with pre-test.

Fig. 2. Pre-peak growth hormone (GH) levels after the Wingate anaerobic test (WAnT) and commonly used pharmacologic GH provocation tests. *$p < 0.01$, compared with GH_{peak} after WAnT.

contrast, GH_{peak} was greater than 7.5 ng/mL in all participants except one (i.e., 3.6 ng/mL) after the pharmacologic provocation tests. There was no correlation between the GH response to the WAnT and the GH response to the pharmacologic provocation test. There was no difference in GH increase after the WAnT between prepubertal and pubertal participants. There were no correlations between any of the WAnT indices (peak power, mean power, and fatigue index) and the GH response to the WAnT. There was a significant increase in lactate levels after the WAnT (11.5 ± 2.0 mg/dL, 42.0 ± 7.9 mg/dL, and 53.3 ± 21.9 mg/dL before, immediately after, and 10 min after, respectively; $p < 0.005$).

4. Discussion

Short stature is among the most common causes for referral to the pediatric endocrinology clinic. The most common causes of short stature are familial (genetic) short stature and delayed (constitutional) growth, which are considered normal variants of growth. A major goal in the evaluation of children with short stature is to identify the fraction of children with pathologic, genetic, systemic, and endocrine causes.[9] GH deficiency is an important treatable endocrine cause for short stature. Because of the pulsatile nature of GH secretion, the diagnosis of GH deficiency relies on the GH response to provocation tests and additional information from auxological data and measurements of insulin-like growth factor 1. However, provocative GH testing has several limitations because it relies on GH assays of variable accuracy, and the reproducibility of the tests has not been adequately documented. Moreover, most pediatric endocrinologists define a "normal" GH response as a serum GH concentration of >10 ng/mL, although the ideal threshold may vary slightly with the laboratory and the assay used.[9] To overcome some of these limitations, a similar single monoclonal GH assay has been used since 2010 for GH measurement nationwide in Israel. As a result, the cutoff levels were changed; a normal response is now defined as serum GH concentration of >7.5 ng/mL and severe GH deficiency as GH levels <5 ng/mL. With these cutoff levels, none of the participants in the present study had a normal GH response to the WAnT (7 showed GH response of <5 ng/mL and could be categorized as having severe GH deficiency). In fact, only 1 subject demonstrated normal GH levels; this level was measured before the exercise stimulation, reflecting probably a spontaneous GH pulse or a stress response to the testing procedure. Although one can speculate that the blunted GH response to exercise reflects "true" GH deficiency, the fact that all participants showed a normal response to the pharmacologic provocation test suggests that the WAnT cannot be used as a sufficient stimulus for GH secretion. Moreover, a sufficient GH response to the WAnT was also not seen in any of the 5 normal-height children (GH_{peak} 2.6 ± 2.3 ng/mL (mean ± SEM), unpublished data), suggesting that the WAnT is not a suitable GH stimulus even in normal-height children.

The mechanism for the lack of GH response to the WAnT is not clearly understood. Previous studies have indicated that the aerobic exercise—induced GH_{peak} occurs about 25–30 min after the start of the exercise, irrespective of the exercise duration,[10] and occurs a few minutes earlier in females.[11] In addition, it was
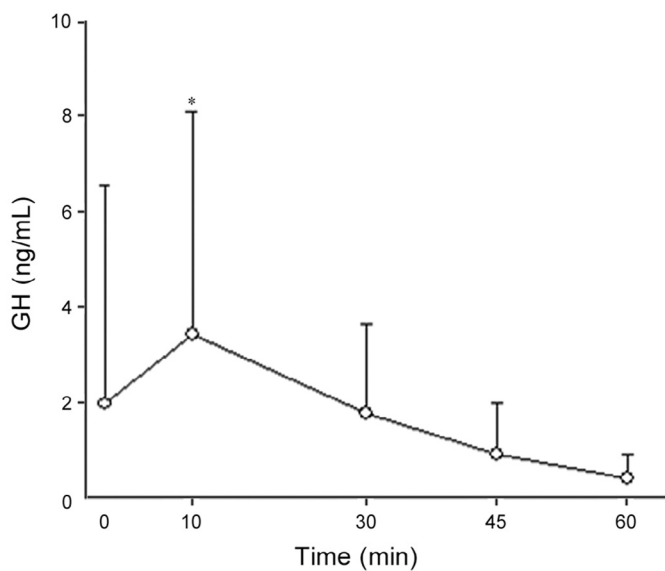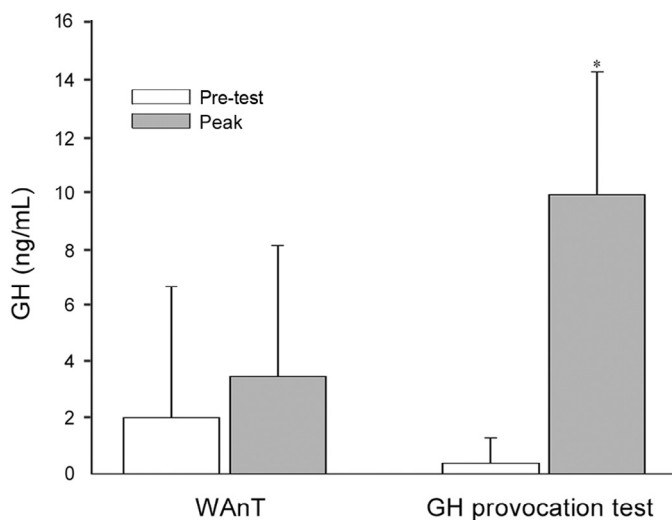
demonstrated that GH$_{peak}$ after anaerobic exercise (interval training) occurs earlier (10–15 min from the beginning of exercise).[12] This was the rationale for our GH sampling times of 10, 30, and 45 min after the start of exercise. Therefore, it is possible, yet very speculative, that a different sampling timing could detect a greater GH response.

In addition, anticipation anxiety from the GH provocation test may lead to an anticipation-related GH$_{peak}$ before the GH provocation test. Sometimes such a peak can prevent a sufficient GH response to any stimulation test. In fact, this is one of the reasons that 2 failed GH provocation tests are required before a diagnosis of GH deficiency is made. Whether an anticipatory effect was greater before the WAnT (compared to the pharmacologic test) as a result of the stress from exercise itself is not known.

Peak and mean anaerobic power and fatigue index of the present study participants were within normal values. Therefore, it is unlikely that the cause of the reduced GH response was the fact that the participants did not reach their maximal effort. Moreover, there were no correlations between the WAnT indices (peak power, mean power, and fatigue index) and the GH response to the WAnT. Peak plasma lactate level after the WAnT was relatively low (53.3 ± 21.9 mg/dL, which equals 5.9 ± 2.4 mmol/L). This is probably associated with the known reduced glycolitic enzymatic activity and anaerobic capacity of children.[13] This is important because it was previously thought that circulating GH levels increase only in response to exercise intensity above, but not below, LAT,[5] and that exercise loads of 75%–90% of maximal aerobic power yielded a greater GH increase than milder loads.[14] However, other studies have shown that during constant exercise, increases in exercise intensity (25%, 75%, 100%, 125%, and 175% of LAT) resulted in increased GH secretion in a linear dose-dependent manner.[15] Therefore, it is possible that the reduced GH response to the WAnT in our study was related to the relatively lower anaerobic capacity and lactate response of children.

5. Conclusion

In summary, although 30 s supramaximal exercise better represents the daily pattern of physical activity in children (spontaneous, short, anaerobic-type exercise bursts), the GH response to the WAnT cannot be used as a GH provocation test. It is possible that a better anaerobic protocol for GH secretion and one that would better mimic children's activity patterns would include several shorter 10–20 s maximal sprints that last altogether about 10 min. Further research is needed to develop other anaerobic exercise protocols that would be sufficient to promote GH secretion.

Acknowledgment

Authors' contributions

ND was involved in the study design, patient testing and drafting the initial manuscript; LO and MP carried out the exercise testing and were involved in data analysis; AE and DN conceived of and designed the study, interpreted the results, and drafted the initial manuscript and following revision. All authors have read and approved the final version of the manuscript, and agree with the order of presentation of the authors.

Competing interests

The authors declare that they have no competing interests.

References

1. Frasier SD. A preview of growth hormone stimulation tests in children. *Pediatrics* 1974;**53**:929–37.
2. Cowell CT, Dietsch S. Adverse events during growth hormone therapy. *J Pediatr Endocrinol Metab* 1995;**8**:243–52.
3. Rosenfeld RG, Albertsson-Wikland K, Cassorla F, Frasier SD, Hasegawa Y, Hintz RL, et al. Diagnostic controversy: the diagnosis of childhood growth hormone deficiency revisited. *J Clin Endocrinol Metab* 1995;**80**:1532–40.
4. Pescowitz OH, Eugster EA, editors. *Pediatric endocrinology: mechanisms, manifestations, and management*. Philadelphia, PA: Lippincott Williams & Wilkins; 2004.p.172–90.
5. Felsing NE, Brasel JA, Cooper DM. Effect of low and high intensity exercise on circulating growth hormone in men. *J Clin Endocrinol Metab* 1992;**75**:157–62.
6. Stokes K, Nevill M, Frystyk J, Lakomy H, Hall G. Human growth hormone responses to repeated bouts of sprint exercise with different recovery periods between bouts. *J Appl Physiol* 2005;**99**:1254–61.
7. Eliakim A, Nemet D, Most G, Rakover N, Pantanowitz M, Meckel Y. Effect of gender on the GH-IGF-I response to anaerobic exercise in young adults. *J Strength Cond Res* 2014;**28**:3411–5.
8. Bar-Or O. The Wingate anaerobic test. An update on methodology, reliability and validity. *Sports Med* 1987;**6**:381–94.
9. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, et al. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: a summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. *J Clin Endocrinol Metab* 2008;**93**:4210–7.
10. Eliakim A, Nemet D. Exercise provocation test for growth hormone secretion: methodologic considerations. *Pediatr Exerc Sci* 2008;**20**:370–8.
11. Wideman L, Weltman JY, Shah N, Story S, Veldhuis JD, Weltman A. Effects of gender on exercise-induced growth hormone release. *J Appl Physiol* 1999;**87**:1154–62.
12. Meckel Y, Eliakim A, Seraev M, Zaldivar F, Cooper DM, Sagiv M, et al. The effect of a brief sprint interval exercise on growth factors and inflammatory mediators. *J Strength Cond Res* 2009;**23**:225–30.
13. Kaczor JJ, Ziolkowski W, Popinigis J, Tarnopolsky MA. Anaerobic and aerobic enzyme activities in human skeletal muscle from children and adults. *Pediatr Res* 2005;**57**:331–5.
14. Sutton J, Lazarus L. Growth hormone in exercise: comparison of physiological and pharmacological stimuli. *J Appl Physiol* 1976;**41**:523–7.
15. Pritzlaff-Roy CJ, Wideman L, Weltman JY, Abbott R, Gutgesell M, Hartman ML, et al. Gender governs the relationship between exercise intensity and growth hormone release in young adults. *J Appl Physiol* 2002;**92**:2053–60.