HOSTED BY
ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Journal of Sport and Health Science xx (2016) 1–4

www.jshs.org.cn

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

Received 26 August 2015; revised 21 February 2016; accepted 28 March 2016
Available online

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.

Keywords: Anaerobic; Exercise; Growth hormone; Lactate; Provocation test; Short stature

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

Peer review under responsibility of Shanghai University of Sport.
* Corresponding author.
E-mail address: dan.nemet@clalit.org.il (D. Nemet).

http://dx.doi.org/10.1016/j.jshs.2016.06.002

Please cite this article in press as: Nitzan Dror, Liat Oren, Michal Pantanowitz, Alon Eliakim, Dan Nemet, The Wingate anaerobic test cannot be used for the evaluation of GH secretion in children with short stature, Journal of Sport and Health Science (2016), doi: 10.1016/j.jshs.2016.06.002

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% ± 30.8%; mean ± 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/kg body weight (≤14 years of age) or 0.67/kg body weight (≥14 years of age). In male participants, resistance was set at 0.55/kg body weight (≤14 years of age) or 0.70/kg body weight (≥14 years of age).[8] Participants 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 5s averages that were calculated by the WAnT computer software and were reported in watts/kg. Maximal power output (peak power) was calculated from the highest 5s 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 5s 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 $p < 0.05$.

Please cite this article in press as: Nitzan Dror, Liat Oren, Michal Pantanowitz, Alon Eliakim, Dan Nemet, The Wingate anaerobic test cannot be used for the evaluation of GH secretion in children with short stature, Journal of Sport and Health Science (2016), doi: 10.1016/j.jshs.2016.06.002