Published in final edited form as:

Growth Horm IGF Res. 2014 February ; 24(1): 10–15. doi:10.1016/j.ghir.2013.11.001.

Discordance of IGF-1 and GH Stimulation Testing for Altered GH Secretion in Obesity

Takara L. Stanley, MD, Meghan N. Feldpausch, ANP, Caitlin A. Murphy, BS, Steven K. Grinspoon, MD, and Hideo Makimura, MD, PhD

Program in Nutritional Metabolism and Neuroendocrine Unit (T.L.S., M.N.F., C.A.M., S.K.G., H.M.), and Pediatric Endocrine Unit (T.L.S.), Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts 02114

Abstract

Objective—To investigate the concordance/discordance of IGF-1 and peak stimulated GH in identifying subjects with reduced GH secretion and to determine the physiological significance of any discordance in obese subjects.

Design, Patients and Methods—95 obese and 43 normal weight men and women underwent measurement of IGF-1 and GH stimulation testing with GH releasing hormone (GHRH)-arginine. Reduced IGF-1 and GH secretion were defined using pre-determined cut-points. Cardiovascular disease risk was determined by measuring carotid intima-media thickness (cIMT). In a second study, IGF-1 was measured in 52 obese men and women who underwent GH stimulation testing and overnight frequent blood sampling. The association of IGF-1 and peak stimulated GH with parameters of endogenous GH secretion was assessed.

Results—60% of obese subjects had normal IGF-1 and peak stimulated GH while 8.4% of obese subjects had reduced IGF-1 and GH secretion. Discordance rate for IGF-1 and peak GH was 31.6%. Subjects with both low IGF-1 and low peak GH had the highest cIMT, while subjects with both normal IGF-1 and peak GH had the lowest cIMT. Subjects with reduction in either IGF-1 or peak GH, had intermediate cIMT ($P=0.02$). IGF-1 and peak stimulated GH were associated with maximum and mean overnight serum GH and GH AUC as well as maximum peak mass and median pulse mass. Peak stimulated GH, but not IGF-1, was also associated with nadir overnight serum GH concentration and basal GH secretion.

Conclusion—Peak stimulated GH and IGF-1 demonstrate significant discordance in identification of subjects with reduced GH secretion in obesity. Subjects with reduction of either IGF-1 or peak GH had higher cIMT compared to subjects with both normal IGF-1 and peak GH. Subjects with reductions in both IGF-1 and peak GH had the highest cIMT. Peak GH, compared to

© 2013 Elsevier Ltd. All rights reserved.

Corresponding Author: Hideo Makimura, MD, PhD, Program in Nutritional Metabolism and Neuroendocrine Unit, Massachusetts General Hospital and Harvard Medical School, 55 Fruit St., LON 207, Boston, MA 02114, Tel: 617-726-8277, Fax: 617-724-8998, hmakimura@partners.org.

Clinical trials registration: The studies were registered at www.clinicaltrials.gov as NCT00562796 and NCT00675506.

Conflict of Interest Disclosures: Dr. Grinspoon has served as a consultant to Theratechnologies, Inc., Aileron Therapeutics, Inc., Alize Pharma SAS, F Hoffman-La Roche LTD, and EMD Serono Inc. all unrelated to this manuscript, and received investigator initiated research funds from Theratechnologies, Inc. Dr. Makimura has received investigator initiated research support from Pfizer, Inc. unrelated to the manuscript. Other authors have no conflicts to disclose.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

IGF-1, has broader associations with various parameters of endogenous GH secretion which support its utility in identifying those with reduced GH secretion.

Keywords

GH; IGF-1; GH stimulation test; GHRH-Arginine; deconvolution analyses; AutoDecon; frequent sampling; carotid intima-media thickness

Introduction

Endogenous [1-3] and stimulated GH secretion [4, 5] are reduced in obesity. Unlike the unequivocal effects of obesity to significantly lower GH, however, the effects of obesity on IGF-1 have been more controversial. While some studies have demonstrated reduced total IGF-1 levels in obesity [6, 7], other studies have demonstrated normal total IGF-1 in obesity [8, 9]. This discrepancy as to the effects of obesity on total IGF-1 may be secondary to the changes in IGF binding proteins, bioactive IGF-1, IGF-II and/or the hyperinsulinemia associated with obesity among other possibilities. Nonetheless, the reduced GH secretion in obesity is associated with pathophysiological sequelae including dyslipidemia, sub-clinical inflammation and increased carotid intima-media thickness (cIMT) [10-12]. Furthermore, we recently demonstrated normalization of GH/IGF-1 using a hypothalamic GH releasing hormone analog, tesamorelin, in obese subjects with reduced GH secretion results in improvement in these cardiovascular disease risk markers, including cIMT [13] suggesting a true physiologic role of the GH-IGF-1 axis in mediating some of the co-morbidities associated with obesity.

We hypothesize that reductions in IGF-1 and peak stimulated GH in obesity may represent different aspects of physiological GH secretion. To better understand the relationship between the potential reduction of IGF-1 in obesity and the reduced GH in obesity, we evaluated the concordance and discordance rate of IGF-1 and peak stimulated GH utilizing a standard stimulation testing paradigm with GH releasing hormone (GHRH) and arginine in the same patients. In addition, we assessed the physiological significance of the reductions in IGF-1 and peak stimulated GH by measuring cIMT as a measure of cardiovascular disease risk. Furthermore, we assessed the relationship of both IGF-1 and peak stimulated GH to parameters of endogenous GH secretion in obesity. While previous studies have assessed the relationship between IGF-1 and measures of endogenous GH secretion in otherwise healthy older men [14] and in adults with and without acromegaly [15], this is the first study, to our knowledge, to simultaneously assess in the same patients the relationships between both IGF-1 and peak stimulated GH to endogenous GH secretion parameters in obesity.

Materials and Methods

Study subjects

New data and extended analyses from two previously completed clinical studies are reported in this manuscript. In the first study, a total of 138 normal weight and obese men and women age 18-55y were recruited at the Massachusetts General Hospital between November 2007 and March 2009. All 138 subjects underwent GHRH-arginine stimulation test and assessment of IGF-1. Data from a subset of these patients had previously been published demonstrating an association between peak stimulated GH and cardio-metabolic risk factors [11]. However, comparison of peak stimulated GH and IGF-1 as indicators of GH sufficiency had not previously been performed. Inclusion and exclusion criteria were previously reported in detail [11]. In brief, subjects without known pituitary dysfunction were included. Subjects on anti-diabetes medication, weight loss medications, or hormonal

medications including estrogen, testosterone, glucocorticoids, anabolic steroids, GHRH, GH, IGF-1 or any hormonal contraception including oral contraceptives, estrogen patch, depot provera, or IUD with hormones, were excluded. All female subjects had FSH <20 IU/liter.

In the second study, 60 obese men and women were recruited at the Massachusetts General Hospital between June 2008 and November 2010 for a 12 month, randomized, double-blind, interventional study with a GHRH analog, the results of which were recently published [13]. Fifty-two subjects completed both a GHRH-arginine stimulation test and overnight frequent blood sampling every 10 minutes for GH at baseline prior to any intervention. Data from this cohort regarding the association of IGF-1 and peak stimulated GH with parameters of endogenous GH secretion have not previously been reported. Inclusion and exclusion criteria were previously described in detail [13]. In brief, 18-55 year old men and women with BMI $\geq 30\text{ kg/m}^2$, waist circumference $\geq 102\text{ cm}$ (men) and 88 cm (women) and peak stimulated GH $\geq 9\text{ ug/L}$ after GHRH-arginine stimulation test were studied. Subjects on anti-diabetes medication, weight loss medications, or hormonal medications including estrogen, testosterone, or any hormonal contraception including oral contraceptives, estrogen patch, depot provera, or IUD with hormones, glucocorticoids, anabolic steroids, GHRH, GH, IGF-1 were excluded. All female subjects had FSH <20 IU/liter.

Both studies were approved by the Partners Institutional Review Board (IRB). All subjects provided written informed consent prior to participation in the respective study.

Assessment of GH secretion

Standardized GHRH-arginine stimulation testing was performed after an overnight fast as previously reported [5, 11, 13]. Subjects were given $0.1\text{ }\mu\text{g/kg}$ of GHRH1-29 followed by an infusion of arginine 0.5 g/kg (max dose 30 g) over 30 minutes. Blood sampling for GH concentration was performed at baseline and 30, 45, 60, 90 and 120 minutes.

Overnight frequent sampling was performed every 10 minutes as previously described [16]. Briefly, subjects had dinner at 1800 h and began fasting at 1900 h. Blood samples were drawn every 10 minutes from 2000 h until 0800 h the subsequent morning. Serum GH concentration parameters are presented as nadir, maximum, mean overnight and 12-hour area-under-the-curve (AUC). In addition, a previously well validated automated deconvolution algorithm AutoDecon [17] was used to assess pulsatile GH secretion characteristics including basal secretion, physiologic half life, number of secretion events over 12 hours (secretory bursts), maximum peak mass, median pulse mass, and median interpulse interval.

Biochemical assessments

GH was measured using the Beckman Access Ultrasensitive human GH assay, a paramagnetic particle, chemiluminescent immunoassay (Beckman Coulter, Chaska, MN). The assay has a >96% specificity with the 22kDa human GH molecule and has <4% cross reactivity with GH variants including the 20kDa fragment. This assay has an intra-assay variation of 1.90-2.78% and an inter-assay variation of 1.77-2.65% at concentrations of 2-10 $\mu\text{g/L}$. At concentrations of $0.005\text{ }\mu\text{g/L}$, the intra-assay coefficient of variation (CV) is 6.4% and inter-assay CV is 5.8%. The effective analytic sensitivity is $0.01\text{ }\mu\text{g/L}$. International Reference Preparation WHO international standard human GH 98/574 standards were used for calibration. The GH assays were run in batches throughout the course of both studies. Serum IGF-1 was measured by the Immulite 2000 assay (Siemens Healthcare Diagnostics, Deerfield, IL) at the MGH Clinical laboratories. The intra-assay CV for the serum IGF-1 ranges from 2.3 to 3.9% and the inter-assay CV ranges from 3.7-8.1%. The assay has an

analytical sensitivity of 20 µg/l. The IGF-1 samples from each study were batched and run in the same assay at the conclusion of the each study. Age-specific normal ranges for IGF-1 are as follows: age 18-24 years: 182-780 µg/l; age 25-39 years: 114-492 µg/l; age 40-54 years: 90-360 µg/l; age 55 years: 71-290 µg/l. Normal ranges for men and women are equivalent for these age groups using this assay.

Body Composition

Anthropometric measurements including height and weight were obtained after an overnight fast. Waist and hip circumference were measured in a standardized fashion in triplicate on each patient by a trained research dietitian.

Carotid Intima Media Thickness

Measurement of cIMT was performed as previous described [11]. Briefly, ultrasound images were obtained using a high resolution 5-12 MHz linear array transducer (HDI 5000 SONOS CT; ATL Ultrasound, Bothell, Washington). Digital Images were captured using a high-quality video frame capture card. Edge detection and mean cIMT calculation were accomplished with an in-house computer program. The published reproducibility of the technique is excellent with a SD of 0.007 mm [18]. The average cIMT over the length of the measured segments on the left is reported.

Statistical Methods

The primary endpoint of the new data analyzed from the first study was to define the concordance and discordance rate of IGF-1 and peak stimulated GH after GHRH-arginine stimulation testing to identify subjects with reduced GH secretion. A secondary and exploratory end point included an assessment of cardiovascular risk, as determined by cIMT, in relation to IGF-1 and peak GH. The primary aim of the second study was to relate, for the first time, IGF-1 and peak stimulated GH to specific parameters of endogenous GH secretion determined from frequent sampling in obese subjects.

Demographic and baseline variables that are continuous were presented as mean±SEM for normally distributed variables and median (interquartile range) for variables that are not normally distributed. To describe the concordance/discordance rates of reduced IGF-1 and peak stimulated GH in obesity, we used age specific normal ranges of our clinically utilized IGF-1 assay, and we used a cut-off value of 4.2 µg/l after GHRH-arginine stimulation test for peak stimulated GH, based on Corneli et al [19]. The cut-off of 4.2 µg/l is also similar to the cut-off of 4.1 µg/l identified by Biller et al. as the value least likely to result in misclassification of GH deficiency with the GHRH-arginine stimulation in comparison with the gold standard, insulin tolerance test among a primarily obese subject cohort [20]. As a sensitivity analysis, we re-evaluated the concordance and discordance rates using the lowest quartile of IGF-1 and peak GH to identify subjects with reduced IGF-1 and peak stimulated GH secretion, rather than the strict cut-off points. A sensitivity analyses was also performed to assess for concordance/discordance rates of IGF-1 and peak stimulated GH after stratifying the sample population by median age. Overall ANOVA was utilized to assess the significance of cIMT across obese subjects stratified by IGF-1 and peak stimulated GH in study 1. For the primary end point in the second study, univariate regression analysis with the non-parametric Spearman's rank correlation coefficient ρ was performed to assess the association of IGF-1 and peak stimulated GH to parameters of endogenous GH secretion as IGF-1 and the majority of parameters describing endogenous GH secretion were not normally distributed. Statistical analyses were performed using JMP 10.0.0 (SAS Institute). All reported P values are two-sided.

Results

Characteristics of Normal Weight and Obese Subjects in Study 1

Forty-three normal weight and 95 obese men and women were studied in study 1. Normal weight subjects were 43 (29; 48) years old and 60.5% male. The obese subjects were also 43 (35; 48) years old and were 54.7% male. The two groups had similar age, gender, race, ethnicity and tobacco use (all $P > 0.10$). The two groups differed significantly for weight, BMI, waist and hip circumference, and waist:hip ratio by design (all comparisons $P < 0.0001$). Normal weight subjects had higher IGF-1 (146 (116; 207) vs. 133 (103; 178) $\mu\text{g/l}$; $P = 0.04$), fasting GH (0.36 (0.19; 0.87) vs. 0.05 (0.02; 0.16) $\mu\text{g/l}$; $P < 0.0001$) and peak stimulated GH (43.28 (19.91; 59.76) vs. 6.25 (4.34; 9.96) $\mu\text{g/l}$; $P < 0.0001$) (all comparisons normal weight versus obese) (Table 1).

Concordance of IGF-1 and peak stimulated GH in Study 1

Of the 43 normal weight subjects, all subjects (100%) had peak GH $> 4.2 \mu\text{g/l}$, and 39 subjects (90.7%) had IGF-1 above the age-specific lower limit resulting in a concordance of 90.7% (39 out of 43 subjects) and discordance of 9.3 % (4 out of 43 subjects) among normal weight subjects.

Of the 95 obese subjects, 72 subjects (75.8%) had peak GH $> 4.2 \mu\text{g/l}$ and similarly, 72 subjects (75.8%) had IGF-1 above the age-specific lower limit. However, these were not the same 72 subjects. Amongst the obese subjects, 57 subjects (60%) were characterized as having normal GH secretion by both peak stimulated GH $> 4.2 \mu\text{g/l}$ and IGF-1 above the age-specific lower limit, while 8 subjects (8.4%) were characterized as having reduced GH secretion by both peak stimulated GH $< 4.2 \mu\text{g/l}$ and IGF-1 below the age-specific lower limit. Fifteen subjects (15.8%) had peak GH $> 4.2 \mu\text{g/l}$ but IGF-1 below the age-specific lower limit, while another 15 subjects (15.8%) had peak GH $< 4.2 \mu\text{g/l}$ but IGF-1 above the age-specific lower limit, for an overall discordance rate of 31.6% (Table 2).

In both the normal weight and obese groups, neither frequency of low IGF-1 levels nor frequency of peak GH $< 4.2 \mu\text{g/L}$ was different by gender.

Sensitivity Analyses using Quartiles in Study 1

Sensitivity analysis utilizing quartiles rather than discrete cut-off points was performed. Among the entire cohort of obese subjects investigated in Study 1, the lowest quartile was defined for IGF-1 by a value of $< 107 \mu\text{g/l}$ and for peak stimulated GH by a value of $< 5.015 \mu\text{g/l}$. Of the 43 normal weight subjects, all subjects (100%) had peak stimulated GH above the lowest quartile, and 37 (86.1%) had IGF-1 above the lowest quartile. Within the obese subjects, 61 of 95 subjects (64.2%) had peak stimulated GH above the lowest quartile while 68 of 95 subjects (71.6%) had IGF-1 above the lowest quartile. Again, these represented different subjects. Forty-seven subjects (49.5%) had both peak stimulated GH and IGF-1 above the lowest quartile, and 13 subjects (13.7%) had both peak stimulated GH and IGF-1 below the lowest quartile. Fourteen subjects (14.7%) had peak stimulated GH above the lowest quartile but IGF-1 below the lowest quartile, and 21 subjects (22.1%) had peak stimulated GH below the lowest quartile but IGF-1 above the lowest quartile for an overall discordance rate of 36.8%.

Sensitivity Analyses using Age in Study 1

Additional sensitivity analyses with the subjects stratified by their median age of 43 years were also performed. Amongst subjects younger than age 43 years, the overall discordance rate was 5% in normal weight subjects and 23.4% in obese subjects. In normal weight subjects younger than the median age of 43 years only one subject had an IGF-1 below the

age specific cut-off point and no subjects had a peak stimulated GH $>4.2 \mu\text{g/l}$, for a discordance rate of 5% (1 out of 20). Amongst obese subjects younger than age 43 years, 35 out of 40 subjects had a normal IGF-1 and peak stimulated GH $>4.2 \mu\text{g/l}$ and one subject had an IGF-1 below the age specific normal range and a peak stimulated GH $>4.2 \mu\text{g/l}$ for an overall concordance rate of 76.6%. Five subjects (10.6%) had IGF-1 below the age specific normal range but peak stimulated GH $>4.2 \mu\text{g/l}$, and six subjects (12.8%) had normal IGF-1 but peak stimulated GH $>4.2 \mu\text{g/l}$ (overall discordance rate of 23.4%)

Amongst subjects older than 43 years, the overall discordance rate was 13% in normal weight subjects and 39.6% in obese subjects. In normal weight subjects older than the median age of 43 years, three subjects had an IGF-1 below the age specific normal range, and no subject had a peak stimulated GH $>4.2 \mu\text{g/l}$ for a discordance rate of 13% (3 out of 23). Amongst obese subjects older than age 43 years, 22 out of 48 subjects had had a normal IGF-1 and a peak stimulated GH $>4.2 \mu\text{g/l}$, and 7 had an IGF-1 below the age-specific normal range and a peak stimulated GH $>4.2 \mu\text{g/l}$ for an overall concordance rate of 60.4%. Ten subjects (20.8%) had an IGF-1 below the age-specific normal range but peak stimulated GH $>4.2 \mu\text{g/l}$, and nine subjects (18.8%) had a normal IGF-1 but peak stimulated GH $>4.2 \mu\text{g/l}$ (overall discordance rate of 39.6%).

Implications for low GH and low IGF-1 in obesity

Ninety-five obese subjects were stratified into sub-groups based on age-associated normal ranges for IGF-1 and peak stimulated GH $>4.2 \mu\text{g/l}$. Subjects with both low IGF-1 and peak stimulated GH $>4.2 \mu\text{g/l}$ had the highest cIMT among the four groups by overall ANOVA. Furthermore, a dosage effect was seen with subjects having normal IGF-1 and peak stimulated GH $>4.2 \mu\text{g/l}$ having the lowest cIMT and progressively higher cIMT in the two discordant groups [cIMT: 0.680 ± 0.019 mm for subjects with peak GH $>4.2 \mu\text{g/l}$ and IGF-1 above age-associated lower limit; versus 0.723 ± 0.040 mm for subjects with peak GH $>4.2 \mu\text{g/l}$ but IGF-1 below the age-associated lower limit; versus 0.768 ± 0.053 mm for subjects with peak GH $>4.2 \mu\text{g/l}$ and IGF-1 below age-associated lower limit; versus 0.873 ± 0.097 mm for subjects with peak GH $>4.2 \mu\text{g/l}$ and IGF-1 below the age-associated lower limit; overall $P=0.02$ by ANOVA] (Figure 1).

Characteristics of Obese Subjects in Study 2

Fifty-two obese subjects completed both the GHRH-arginine stimulation test and the overnight frequent sampling procedures. Subjects' demographics are presented in Table 3. Subjects had a mean age of 41.4 ± 1.1 years and BMI of $38.3 \pm 0.6 \text{ kg/m}^2$ with a waist circumference of 123 ± 2 cm.

Relationship of IGF-1 and Peak Stimulated GH with GH Pulse Parameters in Study 2

GH concentration and secretion parameters for this obese cohort are shown in Table 3. Regression analyses to investigate associations between IGF-1 and peak stimulated GH with parameters of overnight GH concentration demonstrated that both IGF-1 and peak stimulated GH were significantly associated with maximum and mean overnight serum GH concentrations, as well as GH AUC and the deconvolution parameters of maximum peak mass and median pulse mass (Table 4). However, peak stimulated GH but not IGF-1 was associated with nadir overnight serum GH concentration ($\rho=0.31$; $P=0.03$ vs. $\rho=0.11$; $P=0.44$) and with basal GH secretion ($\rho=0.37$; $P=0.007$ vs. $\rho=0.13$; $P=0.37$) upon deconvolution (comparisons for peak stimulated GH versus IGF-1). Neither of the parameters was associated with the deconvolution parameters of physiologic half life of GH, the number of discrete secretion events, or median interpulse interval (Table 4).

Peak stimulated GH and IGF-1 demonstrated a positive correlation with each other ($\rho=0.28$, $P=0.04$).

Discussion

In this study we demonstrate a reduction of both IGF-1 and peak GH after GHRH-arginine stimulation in subjects with abdominal obesity. However, there is significant discordance between IGF-1 and peak stimulated GH in identifying obese subjects with reduced GH secretion. This discordance may have patho-physiological significance in relation to the obese subjects' cardiovascular disease risk. Furthermore, while there is significant overlap in the association of IGF-1 and peak stimulated GH to parameters of endogenous GH secretion, we have identified subtle differences in these associations which may contribute to the discordance.

Analyses from the current study on the discordance rates of IGF-1 and peak GH, and relationship of each to GH pulse parameters, advance our understanding of the physiology and optimal testing strategies for reduced GH in obesity. While IGF-1 is a commonly used initial screening test to identify reduced GH secretion in patients with pathologic GH deficiency caused by central nervous system pathology or trauma, provocative GH stimulation testing remains the gold standard to confirm the diagnosis. In otherwise healthy obese individuals, the role of IGF-1 and GH stimulation testing are less clear. Our data suggest that there is discordance between IGF-1 and peak stimulated GH values in a significant percentage of the population. Among obese subjects, 15.8% of subjects demonstrated a normal IGF-I but reduced peak stimulated GH, and vice versa, 15.8% demonstrated a low IGF-I, but normal peak stimulated GH. Stated differently, concordance between IGF-I and peak GH was seen in only 68% of obese subjects. This discordance between peak GH and IGF-1 suggests the two tests may be identifying different populations of obese subjects. In addition, we performed a sensitivity analysis evaluating the effects of aging on the concordance/discordance rate in this study. We demonstrate an overall discordance rate of 5% in normal weight subjects and 23.4% in obese subjects younger than age 43 years, and an overall discordance rate of 15% in normal weight subjects and 39.6% in obese subjects older than age 43 years. This suggests a greater likelihood of discordance between IGF-1 and peak stimulated GH as age advances, despite the use of age specific cut-off values for IGF-1. The discordance between the IGF-1 and stimulated GH testing may be significant as we demonstrate in an exploratory study that reduction in either peak GH or IGF-1 is associated with higher cIMT compared to subjects with normal peak GH and IGF-1 and furthermore that reductions in both peak GH or IGF-1 result in even greater cIMT.

We previously demonstrated obese subjects with reduced GH secretion have increased cardiovascular disease risk above and beyond traditional risk markers [11] and further supported this association in a double-blind, randomized, placebo-controlled study demonstrating improvement in cIMT, serum triglycerides and high sensitivity C-reactive protein after treatment with a GHRH analog in obese subjects with reduced GH secretion [13]. However, more careful phenotyping to better distinguish the pathological significance of obese subjects with discordant IGF-1 and peak stimulated GH may be necessary. Of note all the healthy controls had a normal peak GH response to GHRH-arginine $>4.2 \mu\text{g/l}$ (or greater than the lowest quartile), but 9% had a reduced IGF-I. The clinical significance of this is unclear at the current time but this may identify a subset of subjects with sub-clinical cardiovascular disease risk. The significance of this finding requires further study.

Using a cohort of obese patients, in whom overnight frequent sampling of GH was performed, we demonstrate a significant association between IGF-1 and maximum and mean overnight serum GH concentrations and GH AUC and the deconvolution parameters

of maximum peak mass and median pulse mass. Peak stimulated GH was similarly associated with these parameters and in addition was also associated with nadir overnight serum GH concentration and basal GH secretion upon deconvolution analyses. Overall, this suggests that peak stimulated GH may be more broadly associated with parameters of endogenous pulsatile and non-pulsatile (or basal) GH secretion compared to IGF-1. Although it is well known that obese subjects demonstrate reductions in endogenous and stimulated GH secretion, this is the first study that we are aware of comparing IGF-1, provocative stimulation testing and parameters of endogenous GH secretion in otherwise healthy obese adults.

In a prior study evaluating older adults (age 59.4-69.9), Hindmarsh et al. also demonstrated IGF-1 was associated with mean 24 hour serum GH concentration from q20 minute frequent sampling over 24 hours [14]. Using Fourier transformation, the investigators also demonstrated an association between IGF-1 and peak but not trough GH secretion, similar to our findings [14]. In a study evaluating subjects with and without Acromegaly, Faje and Barkan demonstrated a strong association between IGF-1 and basal but not pulsatile GH secretion [15]. The investigators studied 18 acromegalic and 19 normal adults and assessed GH secretion using CLUSTER program from frequent blood sampling obtained every 10-20 minutes over 24 hours. IGF-1 was significantly associated with 24 hour mean GH concentration as well as mean valley and nadir GH secretion but not with mean GH pulse mass [15]. The discrepancies between our studies and this prior study may be secondary to the interval of frequent sampling employed and to phenotypic characteristics of the subjects studied, e.g., obese, older, acromegalic as well as the specific algorithms used for analyses.

The current study has some limitations. Although we were able to procure GHRH¹⁻²⁹ for the study, to perform a standardized GH-arginine test for all subjects reported here, we did not simultaneously assess patients with other stimulation testing algorithms, including the gold standard, the insulin-tolerance test or glucagon tests and we relied on a single measurement of IGF-1. The second study was limited to an evaluation of obese men and women with a relative reduction in GH, defined by a peak stimulated GH of $9 \mu\text{g/l}$, a cutoff capturing 75% of obese subjects in our prior study. Utilizing this cutoff in Study 2 limits the generalizability of the study to relate peak GH to frequent sampling parameters among all obese patients, but nonetheless, the findings remain applicable to our population of interest, the majority of obese subjects with reduced GH secretion. In addition, the study was limited to an overnight assessment of endogenous GH secretion, rather than a 24 hour assessment. We also did not measure bioavailable or “free” IGF-1, which is generally reported as normal to increased in obesity due to the suppressive effects of hyperinsulinemia on IGFBP-1 [6, 8]. However, as measurement of bioavailable IGF-1 is not yet standard in clinical settings, this is not a significant limitation to the clinical relevance of the current study. In this regard, further studies are necessary to assess whether IGF binding-protein dynamics may play a role in the discrepancy between reduced GH and IGF-1 in obesity. We also did not account for the effects of physical activity or nutritional intake in the two studies which may independently affect GH or IGF-1. Finally, new data and analyses from both studies are cross sectional in nature, thereby limiting our conclusions on causality.

In summary, we demonstrate a significant 31.6% discordance between IGF-1 and peak stimulated GH in identifying obese subjects with reduced GH secretion. This discordance may have pathophysiological significance as we demonstrate increased cIMT with reductions in either IGF-1 or peak stimulated GH and a further increase in cIMT with reductions in both IGF-1 and peak stimulated GH. We further demonstrate similarity in the association between IGF-1 and peak stimulated GH to various parameters of endogenous overnight GH secretion, including mean and maximum overnight serum GH concentration as well as GH AUC and maximum GH pulse mass and median pulse mass in obese adults.

However, we also demonstrate subtle differences in this relationship, with peak stimulated GH, but not IGF-1 demonstrating a significant association between peak stimulated GH and nadir overnight GH serum concentrations and basal GH secretion. Further studies assessing the mechanisms of reduced GH in obese subjects are needed. These studies may support the need to develop strategies to augment GH in obese patients with reduced GH secretion identified by either IGF-1 or stimulation testing.

Acknowledgments

We gratefully acknowledge the nurses and staff at the MGH Clinical Research Center as well as the subjects who participated in the study.

Funding/Support: National Institutes of Health grant K23DK089910 to Stanley, R01HL085268, P30DK040561 and K24DK064545 to Grinspoon, K23DK087857 to Makimura, M01RR01066 and UL1RR025758, Harvard Clinical and Translational Science Center, from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Center For Research Resources or the National Institutes of Health.

References

- Iranmanesh A, Lizarralde G, Veldhuis JD. Age and relative adiposity are specific negative determinants of the frequency and amplitude of growth hormone (GH) secretory bursts and the half-life of endogenous GH in healthy men. *J Clin Endocrinol Metab.* 1991; 73:1081–1088. [PubMed: 1939523]
- Vahl N, Jorgensen JO, Skjaerbaek C, Veldhuis JD, Orskov H, Christiansen JS. Abdominal adiposity rather than age and sex predicts mass and regularity of GH secretion in healthy adults. *Am J Physiol.* 1997; 272:E1108–1116. [PubMed: 9227458]
- Pijl H, Langendonk JG, Burggraaf J, Frolich M, Cohen AF, Veldhuis JD, Meinders AE. Altered neuroregulation of GH secretion in viscerally obese premenopausal women. *J Clin Endocrinol Metab.* 2001; 86:5509–5515. [PubMed: 11701729]
- Bonert VS, Elashoff JD, Barnett P, Melmed S. Body mass index determines evoked growth hormone (GH) responsiveness in normal healthy male subjects: diagnostic caveat for adult GH deficiency. *J Clin Endocrinol Metab.* 2004; 89:3397–3401. [PubMed: 15240621]
- Makimura H, Stanley T, Mun D, You SM, Grinspoon S. The effects of central adiposity on growth hormone (GH) response to GH-releasing hormone-arginine stimulation testing in men. *J Clin Endocrinol Metab.* 2008; 93:4254–4260. [PubMed: 18765508]
- Frystyk J, Brick DJ, Gerweck AV, Utz AL, Miller KK. Bioactive insulin-like growth factor-I in obesity. *J Clin Endocrinol Metab.* 2009; 94:3093–3097. [PubMed: 19470623]
- Attia N, Tamborlane WV, Heptulla R, Maggs D, Grozman A, Sherwin RS, Caprio S. The metabolic syndrome and insulin-like growth factor I regulation in adolescent obesity. *J Clin Endocrinol Metab.* 1998; 83:1467–1471. [PubMed: 9589640]
- Nam SY, Lee EJ, Kim KR, Cha BS, Song YD, Lim SK, Lee HC, Huh KB. Effect of obesity on total and free insulin-like growth factor (IGF)-1, and their relationship to IGF-binding protein (BP)-1, IGFBP-2, IGFBP-3, insulin, and growth hormone. *Int J Obes Relat Metab Disord.* 1997; 21:355–359. [PubMed: 9152736]
- Saitoh H, Kamoda T, Nakahara S, Hirano T, Nakamura N. Serum concentrations of insulin, insulin-like growth factor(IGF)-I, IGF binding protein (IGFBP)-1 and -3 and growth hormone binding protein in obese children: fasting IGFBP-1 is suppressed in normoinsulinaemic obese children. *Clin Endocrinol (Oxf).* 1998; 48:487–492. [PubMed: 9640416]
- Utz AL, Yamamoto A, Hemphill L, Miller KK. Growth hormone deficiency by growth hormone releasing hormone-arginine testing criteria predicts increased cardiovascular risk markers in normal young overweight and obese women. *J Clin Endocrinol Metab.* 2008; 93:2507–2514. [PubMed: 18445664]
- Makimura H, Stanley T, Mun D, Chen C, Wei J, Connelly JM, Hemphill LC, Grinspoon SK. Reduced growth hormone secretion is associated with increased carotid intima-media thickness in obesity. *J Clin Endocrinol Metab.* 2009; 94:5131–5138. [PubMed: 19837914]

12. Makimura H, Feldpausch MN, Stanley TL, Sun N, Grinspoon SK. Reduced growth hormone secretion in obesity is associated with smaller LDL and HDL particle size. *Clin Endocrinol (Oxf)*. 2012; 76:220–227. [PubMed: 21819438]
13. Makimura H, Feldpausch MN, Rope AM, Hemphill LC, Torriani M, Lee H, Grinspoon SK. Metabolic effects of a growth hormone-releasing factor in obese subjects with reduced growth hormone secretion: a randomized controlled trial. *J Clin Endocrinol Metab*. 2012; 97:4769–4779. [PubMed: 23015655]
14. Hindmarsh PC, Fall CH, Pringle PJ, Osmond C, Brook CG. Peak and trough growth hormone concentrations have different associations with the insulin-like growth factor axis, body composition, and metabolic parameters. *J Clin Endocrinol Metab*. 1997; 82:2172–2176. [PubMed: 9215290]
15. Faje AT, Barkan AL. Basal, but not pulsatile, growth hormone secretion determines the ambient circulating levels of insulin-like growth factor-I. *J Clin Endocrinol Metab*. 2010; 95:2486–2491. [PubMed: 20190159]
16. Stanley TL, Chen CY, Branch KL, Makimura H, Grinspoon SK. Effects of a Growth Hormone-Releasing Hormone Analog on Endogenous GH Pulsatility and Insulin Sensitivity in Healthy Men. *J Clin Endocrinol Metab*. 2011; 96:150–158. [PubMed: 20943777]
17. Johnson ML, Pipes L, Veldhuis PP, Farhy LS, Nass R, Thorner MO, Evans WS. AutoDecon: a robust numerical method for the quantification of pulsatile events. *Methods Enzymol*. 2009; 454:367–404. [PubMed: 19216935]
18. Chan R, Kaufhold J, Hemphill LC, Lees RS, Karl WC. Anisotropic Edge-Preserving Smoothing in Carotid B-mod Ultrasound for Improved Segmentation and Intima-Media Thickness (IMT) Measurement. *Computers in Cardiology*. 2000; 27:37–40.
19. Corneli G, Di Somma C, Baldelli R, Rovere S, Gasco V, Croce CG, Grottoli S, Maccario M, Colao A, Lombardi G, Ghigo E, Camanni F, Aimaretti G. The cut-off limits of the GH response to GH-releasing hormone-arginine test related to body mass index. *Eur J Endocrinol*. 2005; 153:257–264. [PubMed: 16061832]
20. Biller BM, Samuels MH, Zagar A, Cook DM, Arafah BM, Bonert V, Stavrou S, Kleinberg DL, Chipman JJ, Hartman ML. Sensitivity and specificity of six tests for the diagnosis of adult GH deficiency. *J Clin Endocrinol Metab*. 2002; 87:2067–2079. [PubMed: 11994342]

Figure 1.

Carotid intima-media thickness across obese subjects in study 1 (n=95) stratified by IGF-1 and peak stimulated GH. P value determined by overall ANOVA.

Table 1

Distribution of sample population in study 1 stratified by BMI. Study 1 assesses the rate of concordance and discordance between IGF-1 and peak stimulated GH in identifying subjects with reduced GH secretion. Continuous variables are presented as mean $\pm$ SEM for normally distributed variables and median (interquartile range) for variables that are not normally distributed.

	Normal weight (n=43)	Obese (n=95)	P
Demographics			
Age (years)	43 (29; 48)	43 (35; 48)	0.76
Gender (n, male [%])	26 (60.5%)	52 (54.7%)	0.53
Race (n, Caucasian [%])	28 (65.1%)	49 (51.6%)	0.14
Ethnicity (n, Hispanic [%])	3 (7.0%)	11 (11.6%)	0.41
Tobacco use (pack years)	0 (0; 5)	0.2 (0; 7.5)	0.29
Body composition by anthropometrics:			
Weight (kg)	67.6 (61.7; 73.9)	106.8 (97.3; 122.8)	<0.0001
Body mass index (kg/m ²)	22.6 (21.3; 23.6)	36.9 (33.1; 41.3)	<0.0001
Waist circumference (cm)	80 $\pm$ 1	119 $\pm$ 1	<0.0001
Hip circumference (cm)	95 $\pm$ 1	122 $\pm$ 1	<0.0001
Waist:Hip ratio	0.84 $\pm$ 0.01	0.97 $\pm$ 0.01	<0.0001
IGF-1 (μ g/l)	146 (116; 207)	133 (103; 178)	0.04
Fasting GH (μ g/l)	0.36 (0.19; 0.87)	0.05 (0.02; 0.16)	<0.0001
Peak GH (μ g/l)	43.28 (19.91; 59.76)	6.25 (4.34; 9.96)	<0.0001

Table 2

Rate of concordance and discordance between IGF-1 and peak stimulated GH in identifying subjects with reduced GH secretion among (A) normal weight and (B) obese subjects. Relative GH deficiency was defined as peak stimulated GH $< 4.2 \mu\text{g/l}$ and age-specific lower limit of IGF-1.

A. Normal weight subjects		
	Peak GH	
	4.2 $\mu\text{g/l}$ (n [%])	>4.2 $\mu\text{g/l}$ (n [%])
Low IGF-1 (n [%])	0 (0%)	4 (9.3%)
Normal IGF-1 (n [%])	0 (0%)	39 (90.7%)
B. Obese subject		
	Peak GH	
	4.2 $\mu\text{g/l}$ (n [%])	>4.2 $\mu\text{g/l}$ (n [%])
Low IGF-1 (n [%])	8 (8.4%)	15 (15.8%)
Normal IGF-1 (n [%])	15 (15.8%)	57 (60%)

Table 3

Distribution of subjects in study 2 assessing the relationship between IGF-1 and peak stimulated GH to parameters of endogenous GH secretion defined from overnight frequent blood sampling. Continuous variables are presented as mean $\pm$ SEM for normally distributed variables and median (interquartile range) for variables that are not normally distributed.

Demographics:	N=52
Age (years)	41.4 $\pm$ 1.1
Gender (n, male [%])	35 (67.3%)
Race (n, Caucasian [%])	32 (61.5%)
Ethnicity (n, Hispanic [%])	8 (15.4%)
Current or past tobacco use (n [%])	29 (55.8%)
Body composition by anthropometrics:	
Weight (kg)	116.1 $\pm$ 2.8
Body mass index (kg/m ²)	38.3 $\pm$ 0.6
Waist circumference (cm)	123 $\pm$ 2
Hip circumference (cm)	125 $\pm$ 1
Waist:Hip ratio	0.98 $\pm$ 0.01
GHRH-Arginine Stimulation Test:	
Fasting GH (μ g/l)	0.04 (0.02; 0.11)
Peak GH (μ g/l)	4.82 $\pm$ 0.35
IGF-1: (μ g/l)	115 (86; 148)
Serum GH from overnight frequent sampling:	
Nadir GH overnight (μ g/l)	0.01 (0.01; 0.02)
Maximum GH overnight (μ g/l)	0.91 (0.44; 1.62)
Mean overnight GH (μ g/l)	0.17 (0.07; 0.29)
GH AUC (ng/ml)	102.1 (49.5; 199.4)
Deconvolution Analyses of overnight frequent sampling:	
Basal secretion (ng/ml/min)	0.0010 (0.0005; 0.0015)
GH Physiologic half life (min-1)	12.77 $\pm$ 0.63
Number of secretion events	11.1 $\pm$ 0.5
Maximum peak mass (ng/ml)	1.46 (0.68; 2.52)
Median pulse mass (ng/ml)	0.33 (0.17; 0.50)
Median interpulse interval (min-1)	35.59 (28.77; 54.83)

Table 4

Relationship between IGF-1 and peak stimulated GH on GHRH-arginine stimulation test to parameters of endogenous GH secretion defined from overnight frequent blood sampling. Deconvolution analyses were performed using an automated algorithm Auto-Decon. Univariate regression analyses were performed using the non-parametric Spearman's ρ .

Parameter	Peak stimulated GH		IGF-1	
	ρ	P	ρ	P
Peak GH ($\mu\text{g/l}$)	N/A	N/A	0.28	0.04
IGF-1 ($\mu\text{g/l}$)	0.28	0.04	N/A	N/A
Serum GH from overnight frequent sampling:				
Nadir GH overnight ($\mu\text{g/l}$)	0.31	0.03	0.11	0.44
Maximum GH overnight ($\mu\text{g/l}$)	0.60	<0.0001	0.45	0.0009
Mean overnight GH ($\mu\text{g/l}$)	0.54	<0.0001	0.32	0.02
GH AUC (ng/ml)	0.50	0.0002	0.31	0.03
Deconvolution Analyses of overnight frequent sampling:				
Basal secretion (ng/ml/min)	0.37	0.007	0.13	0.37
GH Physiologic half life (min-1)	0.07	0.62	0.02	0.88
Number of secretion events	-0.01	0.94	-0.04	0.79
Maximum peak mass (ng/ml)	0.52	<0.0001	0.42	0.002
Median pulse mass (ng/ml)	0.42	0.002	0.36	0.01
Median interpulse interval (min-1)	0.19	0.18	0.004	0.98