Increases in bioactive IGF do not parallel increases in total IGF-I during growth hormone treatment of children born SGA

Mathilde Gersel Wegmann[1*], Rikke Beck Jensen[1*], Ajay Thankamony[2], Jan Frystyk[3,4], Edna Roche[5], Hilary Hoey[5] Jeremy Kirk[6], Guftar Shaikh[7], Sten-A. Ivarsson[8], Olle Söder[9], David B. Dunger[2,10], Anders Juul[1]

1. Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark
2. Department of Pediatrics, University of Cambridge, Cambridge Biomedical Campus, Cambridge CB2 0QQ, United Kingdom
3. Medical Research Laboratory, Department of Clinical Medicine, Faculty of Health, Aarhus University, Aarhus, Denmark
4. Endocrine Research Unit, Department of Endocrinology, Odense University Hospital & Department of Clinical Research, Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark.
5. Department of Pediatrics, The National Children's Hospital, Trinity College Dublin, The University of Dublin, Ireland
6. Department of Endocrinology, Birmingham Children's Hospital, Birmingham, United Kingdom
7. Department of Endocrinology, Royal Hospital for Children, Glasgow, United Kingdom
8. Department of Clinical Sciences, Endocrine and Diabetes Unit, University of Lund, Malmø, Sweden
9. Pediatric Endocrinology Unit, Department of Women's and Children's Health, Karolinska Institute, Stockholm, Sweden
10. Wellcome Trust-Medical Research Council Institute of Metabolic Science, University of Cambridge, Cambridge, UK

*Joint first authorship

Short title: Bioactive IGF in GH treated SGA children

Correspondence to:
Rikke Beck Jensen
Department of Growth and Reproduction
Rigshospitalet, section 5064
Blegdamsvej 9
DK-2100 Copenhagen, Denmark
Tel +45 3545 5064, Fax +45 3545 6054
Email: Rikke.beck.jensen@regionh.dk

Disclosure:

The NESGAS study was supported by an unrestricted research grant received from Novo Nordisk A/S (F-22717-20). The sponsor was not involved in the preparation of the study design, patient recruitment, collection of data, analysis and interpretation of data, writing of the report or in the decision to submit this paper.

Abstract

Background: Some children born small for gestational age (SGA) experience supra-physiological insulin-like growth factor-I (IGF-I) concentrations during GH treatment. However, measurements of total IGF-I concentrations may not reflect the bioactive fraction of IGF-I which reaches the IGF-I receptor at target organs. We examined endogenous IGF-bioactivity using an IGF-I kinase receptor activation (KIRA) assay that measures the ability of IGF-I to activate the IGF-IR in vitro.

Aim: To compare responses of bioactive IGF and total IGF-I concentrations in short GH treated SGA children in the North European Small for Gestational Age Study (NESGAS).

Material and method: In NESGAS, short SGA children (n=101, 61 males) received GH at 67µg/kg/day for 1 year. IGF-I concentrations were measured by Immulite immunoassay and bioactive IGF by in-house KIRA assay.

Results: Bioactive IGF increased with age in healthy pre-pubertal children (n=94). SGA children had low-normal bioactive IGF levels at baseline (-0.12 (1.8 SD), increasing significantly after one year of high-dose GH treatment to 1.1 (1.4) SD, $p<0.01$. Following high-dose GH, 68% (n=65) of SGA children had a total IGF-I concentration >2SD (mean IGF-I 2.8 SDS), whereas only 15% (n=15) had levels of bioactive IGF slightly above normal reference values. At baseline, bioactive IGF (SDS) was significantly correlated to height (SDS) ($r=0.29$, $p=0.005$), in contrast to IGF-I (SDS) ($r=0.17$, $p=0.10$). IGF-I (SDS) was inversely correlated to delta height (SDS) after one year of high-dose GH treatment ($r=-0.22$, $p=0.02$).

Conclusion: In contrast to total IGF-I concentrations, bioactive IGF stayed within the normal reference ranges for most SGA children during the first year of GH treatment.

Précis:

Despite supra-normal IGF-I concentrations, bioactive IGF stayed within the normal reference range for most SGA children during the first year of treatment with GH..

INTRODUCTION

Insufficient catch-up growth in some children born small for gestational age (SGA) may result in a low final height. Growth hormone (GH) treatment has a well-documented overall beneficial effect on final height in children born SGA and was approved for treatment of SGA children with persistent short stature in the EU in 2004 (1,2). Nevertheless, the growth response to GH treatment among SGA children is characterized by considerable variability (3), and some SGA children experience supra-physiological serum insulin-like growth factor-I (IGF-I) concentrations during treatment (4).

Long-term safety and mortality in patients treated with GH during childhood is an ongoing concern, but the debate has been characterized by conflicting data. Studies in large epidemiological cohorts of healthy adults have shown an association between elevated IGF-I levels and an increased risk of cancer and all-cause mortality (5–7). However, no studies have been able to establish a link between elevated IGF-I levels during GH treatment in childhood and increased morbidity or mortality later in life. Though, there is still a need for follow-up studies of long-term risk of disease after GH treatment in childhood. In the majority of clinical guidelines for the approved indications of GH treatment in childhood (e.g. SGA, Turner Syndrome and Prader-Willi Syndrome) it is recommended that serum IGF-I concentrations are kept within the normal reference range during GH treatment (2,8,9).

In the bloodstream the majority of IGF-I circulates bound to IGF-binding proteins (IGFBPs) while approximately 1% circulates as unbound, free IGF-I (10). The IGFBPs, IGFBP-proteases (e.g. pregnancy associated plasma protein A and A2) as well as modifiers of IGFBP protease activity (e.g. stanniocalcin 1 and 2) affect the interaction between IGF-I and its IGFBPs, and thereby alter the bioactivity of IGF-I (11). Measurements of the concentration of total IGF-I by immunoassay, whereby IGF-I is stripped from the IGFBPs, do not take the modifying effects of IGFBPs and IGFBP-proteases into account. Therefore, we used the IGF-I kinase receptor activation (KIRA) assay, as this determines the ability of serum IGF-I to phosphorylate and thereby activate the IGF-I receptor (IGF-IR) (12,13). We believe this gives a biologically relevant estimate of the ability of serum IGF-I to activate the IGF-IR, and hence IGF-bioactivity. Indeed, a discrepancy between bioactive and total levels of IGF-I has been reported in both adults (14) and children (15) and it was reported that in adults, bioactive IGF concentrations correlated better with the diagnosis of GH deficiency (GHD) than total IGF-I levels (16).

In the current study we hypothesized that increased concentrations of IGF-I did not reflect the concentration of bioactive IGF. Accordingly, the aim of this study was to determine a normal reference range for bioactive IGF based on a cohort of healthy children and subsequently to evaluate responses of bioactive IGF and IGF-I

concentrations, respectively, with growth and metabolic responses in short GH treated SGA children in the North European Small for Gestational Age Study (NESGAS).

MATERIALS AND METHODS

Study population and design

NESGAS is a multicenter, randomized, parallel group study (EudraCT2005-001507-19) of GH treatment in short pre-pubertal children born SGA. The study population and design has been described in detail in previous publications (15, 26-29). In brief, all children received a fixed dose of 67 µg/kg/day of recombinant human GH (Norditropin®, Novo Nordisk, Bagsværd, Denmark) given as a daily subcutaneous injection during the first year of therapy to induce catch-up growth and identify non-responders. Data regarding weight, height and IGF-I using the Immulite assay have previously been published (26). One hundred and one (61 males) children from the NESGAS study were included in the current study. Only data from study entry and during the first year of GH therapy were included. The NESGAS study was performed according to the Helsinki II declaration and approved by the Ethical Committee or Institutional review board and national drug authorities in each study center. Written informed consent was obtained from parents or guardians of each child participating in the NESGAS study.

Laboratory measurements

Serum IGF-I and IGFBP-3 concentrations were determined using a solid-phase enzyme-labelled chemiluminescent immunometric assay (Immulite 2000, Diagnostic Products Corporation, LA, USA). Standards were calibrated against the WHO NIBSC IRR 87/518. The IGF-I detection limit was 20 ng/mL, inter-and intra-assay coefficients of variation (CVs) were 5.93% and 2.02%, respectively. The detection limit for IGFBP-3 was 500 ng/mL and inter-and intra-assay CVs were 5.23 % and 1.74 % respectively. IGF-I and IGFBP-3 SDS were calculated from our reference data based on serum samples from 1729 healthy children (911 girls) using the same assays (17,18).

IGFBP-1 and bioactive IGF were measured by in-house assays at Medical Research Laboratories, Aarhus University Hospital, Denmark. IGFBP-1 was measured by an in-house time-resolved immunofluorometric assay (TR-IFMA), with intra- and inter-assay CVs of 5 and 10%, respectively (19). Bioactive IGF was measured using the IGF-I KIRA assay, as described by Chen et. al. (21) with modifications (19). The detection limit was 0.1 ng/mL, and the intra-assay CV of samples 12%. The long-term inter-assay CV of a control sample was 20%. Samples were analyzed against a serial dilution of the WHO IGF-I reference preparation 02/254, and results expressed in ng/mL. Care was taken to analyze samples from the same individual in the same assay run. Insulin has a negligible cross-reactivity, whereas IGF-II cross-reacts with 12% (12). To acknowledge this, the output of the KIRA assay has been designated “bioactive IGF”.

Reference range for bioactive IGF in a cohort of healthy children

A subpopulation of 150 healthy children (75 males) aged 6 to 11 years from the COPENHAGEN Puberty Study (30, 31) were included. All children were healthy Caucasian, and prepubertal at evaluation. A single non-fasting blood sample was drawn from an antecubital vein between 8 and 12 o'clock. Blood was centrifuged and stored at -20 Celsius until analyses.

Other Assays

Plasma insulin and C-peptide levels were measured by a DELFIA assay using kits B080-101 and B081-101 respectively (Perkin Elmer Life Sciences, Turku, Finland) as described in detail previously (4). Plasma glucose and HbA1c were measured locally employing assays routinely used for clinical purposes.

Statistics

Normal distributed data were presented as mean (SD), while non-normal distributed data were presented as median (interquartile range). Age and gender corrected SD-scores for IGF-I measured by Immulite were calculated from our reference data based on samples from 1,729 healthy children, as previously published (17,18). Age and gender corrected SD-scores for bioactive IGF were calculated using a normal reference population of 150 healthy children. Differences between the sexes were compared by independent sample t-test or Mann-Whitney test and ANOVA test or Kruskal-Wallis test when appropriate. A correlation matrix was completed using Spearman non-parametric correlations. P-values <0.05 were considered significant. The statistical analyses were performed using statistical package PASW (version 22; SPSS Inc., Chicago, IL).

RESULTS

Baseline concentrations of bioactive IGF in short SGA children were within the normal range of healthy children (Table 1, Figure 1), although in the lower part of the reference ranges. Moreover, bioactive IGF concentrations at baseline were significantly lower in boys (-1.4 SDS (-2.7 to -0.2)) (median (25-75 percentile)) compared to girls (-0.2 SDS (-1.4 – 0.4)) (p=0.002) (Table 1, Figure 1). In contrast, there were no significant differences in total IGF-I concentrations (SDS), weight (SDS) or height (SDS) between boys and girls at baseline (Table 1).

Bioactive IGF (SDS), weight (SDS) and height (SDS) did not differ between genders after one year of GH treatment (Table 1, Figure 1) and thereby a significantly greater change was found in bioactive IGF among boys (+2.7 SDS (1.2 – 4.6)) than girls (+1.2 SDS (0.5 – 1.6)) (p=0.004) after one year of GH treatment (Table 1, Figure 2a). Changes in total IGF-I concentrations (SDS) (Figure 2b) and height (SDS) (Figure 2c) were similar in girls and boys.

After one year of GH treatment only 15% (n=15) of the children in the NESGAS cohort had levels of bioactive IGF above 2 SD (figure 2a) whereas 68% (N=65) of the children had concentrations of total IGF-I (SDS) above the normal range (> 2SD) (Figure 2b).

Bioactive IGF (SDS) correlated significantly with IGF-I (SDS) (r=0.35, p=0.001) and IGFBP-3 (SDS) (r=0.36, p=0.001) at baseline (table 2). Bioactive IGF (SDS) was significantly correlated with height (SDS) and weight (SDS) at baseline (table 2) but did not correlate to changes in height (SDS) after one year of GH treatment. In contrast, concentrations of IGF-I (SDS) and IGFBP-3 (SDS) were not associated with height (SDS) or weight (SDS) at baseline but correlated inversely with changes in height (SDS) after one year of treatment. Insulin sensitivity determined by HOMA-S were negatively correlated with bioactive IGF (r=-0.29, p=0.007), IGF-I (r=-0.27, p=0.01), IGFBP-3 (r=-0.33, p=0.005) and insulin secretion (r=-0.47, p<0.001). Furthermore, we observed a significant positive association between HOMA-S and IGFBP-1 as well as with change in height from baseline to 1 year (Table 2). IGFBP-1 was negatively correlated to delta height (SDS) after one year of high-dose GH treatment (Table 2).

The change in bioactive IGF (SDS) from baseline to 1 year was not associated with either height (SDS) at baseline (r=-0.16, p=0.14) or change in height (SDS) during the first year of treatment (r=0.12, p=0.29). In contrast the change in IGF-I (SDS) was correlated with change in height (SDS) during the first year of treatment (r=0.46, p<0.0001).

The molar ratio of IGF-I to IGFBP-3 has been suggested to reflect IGF-I bioavailability, and therefore we also determined IGFBP-3. However, we failed to observe correlations between changes in the IGF-I/IGFBP-3 ratio and changes in bioactive IGF (-0.03, p=0.8). Furthermore, the ratio correlated neither to baseline height nor height changes (data not shown).

DISCUSSION

In this cohort of SGA children treated for one year with GH, we show that the concentration of bioactive IGF was within the normal range in the majority of children, despite elevated total IGF-I concentrations. On the other hand, total IGF-I concentrations correlated better with the growth response during the first year of GH treatment than bioactive IGF, whereas only bioactive IGF correlated to height and weight at baseline. Insulin sensitivity was related to both bioactive IGF and total IGF-I concentrations as well as the binding proteins and growth response during the first year of treatment. To our knowledge this is the first study to explore bioactive IGF in a cohort of short GH treated SGA children, and we find it of interest that bioactive IGF stays within the normal range during the first year of treatment with GH.

The IGF-I response in vivo is controlled by the IGFBPs that can inhibit as well as stimulate IGF-I mediated effects at the cellular level. The ability of IGF-I to stimulate the IGF-IR is believed to be partly dependent on

IGFBP proteolysis, as cleavage of IGFBPs lower their ligand affinity, causing IGF-I to become liberated and hence IGF-IR accessible (20). Many proteases have been identified (MID:12466191), but the most thoroughly investigated enzymes as regards liberation of IGF-I and stimulation of growth include PAPP-A, which cleaves IGFBP-3 and IGFBP-5, and PAPPA, which cleaves IGFBP-4. The KIRA assay is a well-recognized assay for direct measurements of the biological active amount of IGF-I (12,13). Nevertheless, it is still controversial whether activation of IGF-IR in transfected cells in an artificially environment is representative of the endogenous activation of the IGF-IR and whether it can be translated into a biological response in cells *in vivo* (21). However, our findings of a stronger correlation between bioactive IGF and height and weight before start of GH treatment suggest that bioactive IGF reflects the biological active IGF-I and the endogenous secretion of GH. On the other hand, the IGF-I concentration was associated with change in height during the first year of treatment with supra-physiological GH doses which may mirror the relation between IGF-I and insulin sensitivity.

In the current cohort the increase in bioactive IGF stayed within the normal range for most of the children whereas the IGF-I concentration was above the normal range in 68% of the children treated with GH for a year. In a Dutch of GH treated children with Prader-Willi Syndrome, almost all the children had IGF-I SDS levels > 2 SD, but only one child had a bioactive IGF concentration above the normal reference (22). That study also revealed that serum bioactive IGF concentrations correlated with neither duration of GH treatment nor GH dose. These findings align nicely with ours, even though the two bioassays are not strictly identical (15,23). Bioactive IGF has been proposed to be a better screening tool in diagnosing GHD in adulthood than IGF-I concentrations, showing a sensitivity of 82% for bioactive IGF vs. 62% for IGF-I concentration (16). Based on the same cohort of adults with GHD, another study reported that the majority of GHD patients had subnormal bioactive IGF levels despite normalization of IGF-I concentrations during GH treatment and those with normalized bioactive IGF had significantly higher concentrations of IGF-I (14). Furthermore, the authors concluded that bioactive IGF in large part was independent of total IGF-I, as 70-75% of the variation in bioactivity was unexplained by total IGF-I (24). In our study, IGF-I concentrations explained 12% only and in conjunction the two studies indicate that the two measurement represent different entities of the IGF-system. Hence, these results suggest that GH dosing by titration of IGF-I concentrations is effective during physiological GH replacement of GHD children, but less so during pharmacological intervention with GH in non-GHD patients like short SGA children.

Among the present SGA children girls were found to have significantly higher baseline levels of bioactive IGF (SDS) as compared to boys, whereas boys had a significantly greater change in bioactive IGF-I SDS during GH therapy, leading to equal levels after one year. The same pattern was not reflected in the IGF-I concentrations or height. These findings are in accordance with previous findings in children with PWS (15). The gender difference in bioactive IGF during childhood could reflect differences in sensitivity to IGF-I and

insulin between boys and girls born SGA and it may be speculated that these differences could influence timing of puberty in GH treated SGA children. However, opposed to our data, a former study reported that adult females with GHD appeared to have significantly lower levels of bioactive and total IGF-I compared to men (16), which is in agreement with the generally recognized fact that adult females with GHD are less sensitive to GH and therefore need larger doses of GH in order to normalize IGF-I levels.

IGF-I mediates the growth promoting actions of GH by stimulating cell proliferation and survival. Since IGF-I has mitogenic and anti-apoptotic effects *in vitro*, the role of IGF-I (and IGF-II) in cancer growth and development has been extensively investigated in both cellular and animal models, but the evidence of a cancerogenic effect in humans is weak (25). However, large epidemiological cohort studies of healthy adults have shown that IGF-I concentrations within the upper reference range is linked to an increased risk of cancer (5,6). Therefore, the long-term safety and mortality in patients treated with GH during childhood is an ongoing concern and this was reinforced by the first results of a large cross-Europe cohort, the Safety and Appropriateness of Growth Hormone treatments in Europe (SAGhE) study, published in 2012. The SAGhE study was established to examine mortality risk and cancer incidence in a large register study including almost 24,000 people across Europe. The first results from the French register showed an increase all-cause mortality and increased mortality from bone tumors and cardiovascular disease (26). However, the following studies from other countries did not confirm this and the overall conclusion was that the results did not generally support a carcinogenic effect of GH (26–28). These findings have subsequently been supported by other studies (29,30) as well as in a meta-analysis (31). Nevertheless, the uncertainty regarding IGF-I and risk of neoplasia has created a concern among treating physicians and generally guidelines for GH treatment of children recommend to keep serum IGF-I levels within the normal reference range (below 2SD) to increase safety of the treatment (2,8,9). However, we previously demonstrated in the NESGAS cohort that titration of the GH dose to keep IGF-I levels below 2SD proved less effective in terms of height gain than current dosing regimens for short SGA children (15). Thus, it has been speculated that some of these SGA children are less sensitive to IGF-I and that they may depend on continuously supra-physiological levels of IGF-I to maintain sufficient growth. In this context, we find it of interest that our study showed that the serum concentrations of bioactive IGF stayed within the normal range during high-dose GH treatment despite of elevated concentrations of IGF-I.

In conclusion, our results show for the first time that bioactive IGF levels are mainly kept within normal ranges despite elevated total IGF-I concentrations during one year of GH treatment of short SGA children. Titration of GH dose in SGA patients according to their total IGF-I concentration resulted in very low doses of GH and a low growth response in a previous study Further studies are needed to investigate the potential clinical role of bioactive IGF-I in the monitoring of GH treated children.

1. **Lee PA, Chernausek SD, Hokken-Koelega AC, Czernichow P.** International Small for Gestational Age Advisory Board consensus development conference statement: management of short children born small for gestational age, April 24-October 1, 2001. *Pediatrics* 2003;111(6 Pt 1):1253–1261.
2. **Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A.** Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society. *J Clin Endocrinol Metab* 2007;92(3):804–810.
3. **Bang P, Bjerknes R, Dahlgren J, Dunkel L, Gustafsson J, Juul A, Kristrom B, Tapanainen P, Aberg V.** A comparison of different definitions of growth response in short prepubertal children treated with growth hormone. *Horm Res Paediatr* 2011;75(5):335–345.
4. **Jensen RB, Thankamony A, O'Connell SM, Salgin B, Kirk J, Donaldson M, Ivarsson SA, Soder O, Roche E, Hoey H, Dunger DB, Juul A.** Baseline IGF-I levels determine insulin secretion and insulin sensitivity during the first year on growth hormone therapy in children born small for gestational age. Results from a North European Multicentre Study (NESGAS). *Horm Res Paediatr* 2013;80(1):38–46.
5. **Major JM, Laughlin GA, Kritz-Silverstein D, Wingard DL, Barrett-Connor E.** Insulin-like growth factor-I and cancer mortality in older men. *J Clin Endocrinol Metab* 2010;95(3):1054–1059.
6. **Carlzon D, Svensson J, Petzold M, Karlsson MK, Ljunggren Ö, Haghsheno MA, Damber JE, Mellström D, Ohlsson C.** Insulin-like growth factor i and risk of incident cancer in elderly men - Results from MrOS (Osteoporotic Fractures in Men) in Sweden. *Clin. Endocrinol. (Oxf).* 2016;84(5):764–770.
7. **Burgers AM, Biermasz NR, Schoones JW, Pereira AM, Renehan AG, Zwahlen M, Egger M, Dekkers OM.** Meta-analysis and dose-response metaregression: circulating insulin-like growth factor I (IGF-I) and mortality. *J Clin Endocrinol Metab* 2011;96(9):2912–2920.
8. **Gravholt CH, Andersen NH, Conway GS, Dekkers OM, Geffner ME, Klein KO, Lin AE, Mauras N, Quigley CA, Rubin K, Sandberg DE, Sas TCJ, Silberbach M, Söderström-Anttila V, Stochholm K, Van Alfen-Van DerVelden JA, Woelfle J, Backeljauw PF, Bamba V, Bonfig NB, Braverman AC, Breech LL, Brickman WJ, Brown NM, Bryant N, Cernich J, Chernausek S, Christin-Maitre S, Corathers SD, Crawford A, Crenshaw ML, Davenport ML, De Backer J, Eagle K, Gawlik A, Gutmark-Little I, Hay D, Hiratzka L, Hong DS, Hovatta O, Hultcrantz M, Johnson WH, Kanaka-Gantenbein C, Karnis MF, Knickmeyer RC, Kristrøm B, Lajiness-O'Neill RR, Landin-Wilhelmsen K, Law JR, Lippe B, Lopez L, Mawson L, Mazzanti L, Mortensen KH, Popovic J, Prakash S, Ranallo KC, Rappold GA, Roos-Hesselink J, Rosenfield R, Ross J, Roulot-Marullo D, Saidi A, Santen RJ, Scurlock CC, Sheanon NM, Smyth A, Van Hagen IM, Verlinde F, Wasniewska M, Young LT.** Clinical practice guidelines for the care of girls

and women with Turner syndrome: Proceedings from the 2016 Cincinnati International Turner Syndrome Meeting. *Eur. J. Endocrinol.* 2017;177(3):G1–G70.

9. **Deal CL, Tony M, Höybye C, Allen DB, Tauber M, Christiansen JS, Ambler GR, Battista R, Beauloye V, Berall G, Biller BMK, Butler MG, Cassidy SB, Chihara K, Cohen P, Craig M, Farholt S, Goetghebeur M, Goldstone AP, Greggi T, Grugni G, Hokken-Koelega AC, Johannsson G, Johnson K, Kemper A, Kopchick JJ, Malozowski S, Miller J, Mogul HR, Muscatelli F, Nergårdh R, Nicholls RD, Radovick S, Rosenthal MS, Sipilä I, Tarride J-E, Vogels A, Waters MJ.** Growth Hormone Research Society Workshop Summary: Consensus Guidelines for Recombinant Human Growth Hormone Therapy in Prader-Willi Syndrome. *J. Clin. Endocrinol. Metab.* 2013;98(6):E1072–E1087.
10. **Frystyk J.** Free insulin-like growth factors - Measurements and relationships to growth hormone secretion and glucose homeostasis. *Growth Horm. IGF Res.* 2004;14(5):337–375.
11. **Jones JI.** Insulin-like growth factors and their binding proteins: biological actions. *Endocr. Rev.* 2004;16(1):3–34.
12. **Chen J-W, Ledet T, Orskov H, Jessen N, Lund S, Whittaker J, De Meyts P, Larsen MB, Christiansen JS, Frystyk J.** A highly sensitive and specific assay for determination of IGF-I bioactivity in human serum. *Am. J. Physiol. Endocrinol. Metab.* 2003;284(6):E1149-55.
13. **Ramadhin C, Pillay B, Olaniran AO.** Cell-based assays for IGF-I bioactivity measurement: Overview, limitations and current trends. *Growth Factors* 2014;32(3–4):130–138.
14. **Varewijck AJ, Lamberts SWJ, Van Der Lely AJ, Neggers SJCMM, Hofland LJ, Janssen JAMJL.** Changes in circulating IGF1 receptor stimulating activity do not parallel changes in total IGF1 during GH treatment of GH-deficient adults. *Eur. J. Endocrinol.* 2015;173(2):119–127.
15. **Bakker NE, Van Doorn J, Renes JS, Donker GH, Hokken-Koelega ACS.** IGF-1 levels, complex formation, and IGF bioactivity in growth hormone-treated children with Prader-Willi syndrome. *J. Clin. Endocrinol. Metab.* 2015;100(8):3041–3049.
16. **Varewijck AJ, Lamberts SWJ, Uitterlinden P, Hofland LJ, Janssen JAMJL.** IGF-I bioactivity better reflects growth hormone deficiency than total IGF-I. *J. Clin. Endocrinol. Metab.* 2011;96(7):2248–2254.
17. **Sorensen K, Aksglaede L, Petersen JH, Andersson AM, Juul A.** Serum IGF1 and insulin levels in girls with normal and precocious puberty. *Eur.J.Endocrinol.* 2012;166(5):903–910.
18. **Sørensen K, Aksglaede L, Munch-Andersen T, Aachmann-Andersen NJ, Leffers H, Helge JW, Hilsted L, Juul A.** Impact of the growth hormone receptor exon 3 deletion gene polymorphism on glucose metabolism, lipids, and insulin-like growth factor-I levels during puberty. *J. Clin. Endocrinol. Metab.* 2009;94(8):2966–2969.
19. **Reinhard M, Frystyk J, Jespersen B, Bjerre M, Christiansen JS, Flyvbjerg A, Ivarsen P.** Effect

of hyperinsulinemia during hemodialysis on the insulin-like growth factor system and inflammatory biomarkers: a randomized open-label crossover study. *BMC Nephrol.* 2013;14(1):80.

20. **Baxter RC.** Insulin-like growth factor (IGF)-binding proteins: interactions with IGFs and intrinsic bioactivities. *Am. J. Physiol. Endocrinol. Metab.* 2000;278(6):E967-76.
21. **Varewijck AJ, Lamberts SWJ, Van Der Lely AJ, Neggers SJCMM, Hofland LJ, Janssen JAMJL.** The introduction of the IDSiSYS total IGF1 assay may have farreaching consequences for diagnosis and treatment of GH deficiency. *J. Clin. Endocrinol. Metab.* 2015;100(1):309–316.
22. **Bakker NE, Van Doorn J, Renes JS, Donker GH, Hokken-Koelega ACS.** IGF-1 levels, complex formation, and IGF bioactivity in growth hormone-treated children with Prader-Willi syndrome. *J. Clin. Endocrinol. Metab.* 2015;100(8):3041–3049.
23. **Chen J-W, Ledet T, Ørskov H, Jessen N, Lund S, Whittaker J, De Meyts P, Larsen MB, Christiansen JS, Frystyk J.** A highly sensitive and specific assay for determination of IGF-I bioactivity in human serum. *Am. J. Physiol. Metab.* 2003;284(6):E1149–E1155.
24. **Brugts MP, Ranke MB, Hofland LJ, Van Der Wansem K, Weber K, Frystyk J, Lamberts SWJ, Janssen JAMJL.** Normal values of circulating insulin-like growth factor-I bioactivity in the healthy population: Comparison with five widely used IGF-I immunoassays. *J. Clin. Endocrinol. Metab.* 2008;93(7):2539–2545.
25. **Clayton PE, Banerjee I, Murray PG, Renehan AG.** Growth hormone, the insulin-like growth factor axis, insulin and cancer risk. *Nat. Rev. Endocrinol.* 2011;7(1):11–24.
26. **Carel JC, Ecosse E, Landier F, Meguellati-Hakkas D, Kaguelidou F, Rey G, Coste J.** Long-term mortality after recombinant growth hormone treatment for isolated growth hormone deficiency or childhood short stature: preliminary report of the French SAGhE study. *J.Clin.Endocrinol.Metab* 2012;97(2):416–425.
27. **Savendahl L, Maes M, bertsson-Wikland K, Borgstrom B, Carel JC, Henrard S, Speybroeck N, Thomas M, Zandwijken G, Hokken-Koelega A.** Long-term mortality and causes of death in isolated GHD, ISS, and SGA patients treated with recombinant growth hormone during childhood in Belgium, The Netherlands, and Sweden: preliminary report of 3 countries participating in the EU SAGhE study. *J.Clin.Endocrinol.Metab* 2012;97(2):E213–E217.
28. **Swerdlow AJ, Cooke R, Beckers D, Borgström B, Butler G, Carel JC, Cianfarani S, Clayton P, Coste JL, Deodati A, Ecosse E, Gausche R, Giacomozzi C, Hokken-Koelega ACS, Khan AJ, Kiess W, Kuehni CE, Mullis PE, Pfaffle R, Sävendahl L, Sommer G, Thomas M, Tidblad A, Tollerfield S, Van Eycken L, Zandwijken GRJ.** Cancer risks in patients treated with growth hormone in childhood: The SAGhE European cohort study. *J. Clin. Endocrinol. Metab.* 2017;102(5):1661–1672.
29. **Mo D, Hardin DS, Erfurth EM, Melmed S.** Adult mortality or morbidity is not increased in

childhood-onset growth hormone deficient patients who received pediatric GH treatment: an analysis of the Hypopituitary Control and Complications Study (HypoCCS). *Pituitary* 2014;17(5):477–485.

30. **Wilton P, Mattsson AF, Darendeliler F.** Growth hormone treatment in children is not associated with an increase in the incidence of cancer: Experience from KIGS (Pfizer international growth database). *J. Pediatr.* 2010;157(2):265–270.

31. **Deodati A, Ferroli BB, Cianfarani S.** Association between growth hormone therapy and mortality, cancer and cardiovascular risk: Systematic review and meta-analysis. *Growth Horm. IGF Res.* 2014;24(4):105–111.

Legend to figures:

Figure 1: Bioactive IGF concentrations (µg/L), top row represents a normal reference population (grey dots), middle row represents baseline concentration and bottom row represents concentrations at 1 yr. Solid lines reflect mean +/- 2 SD, dotted lines reflect -1 SD and +1SD.

Figure 2: Blue lines are boys and red lines are girls. Figure 2a: Changes in bioactive IGF during first year of growth hormone treatment, Figure 2b: Changes in IGF-I SDS during first year of growth hormone treatment, Figure 2c: Changes in Height SDS during first year of growth hormone treatment.

Table 1: Baseline and 1year characteristics

		All children (N =101)	**Female** (N = 40)	**Male** (N = 61)	**P value**	
					Mann-Whitney	**T-test**
	N					
Baseline						
Age (years)	101	6.2 (1.7)	5.8 (1.3)	6.5 (1.8)		0.06
Weight (SDS)	101	-3.2 (1.0)	-3.2 (1.0)	-3.1 (1.1)		0.30
Height (SDS)	101	-3.4 (0.8)	-3.5 (0.9)	-3.4 (0.7)		0.27
Bioactive IGF-I (µg/L)	94	1.6 (0.7)	1.8 (0.7)	1.5 (0.6)		0.03
Bioactive IGF-I (SDS)	94	-1.2 (1.8)	-0.5 (1.6)	-1.6 (1.8)		0.005
IGF-I (ng/mL)	95	81.9 (62.1 – 111.0)	91.5 (65.0 – 118.8)	79.0 (57.2 – 110.0)	0.25	
IGF-I (SDS)	95	-1.2 (1.2)	-1.2 (1.1)	-1.1 (1.3)		0.87
IGFBP-3 (ng/mL)	95	2870 (2380-3475)	2870 (2580-3560)	2780 (2235-3467)	0.23	
IGFBP-3 (SDS)	95	-0.92 (-1.5 to -0.01)	-0.92 (-1.37-0.12)	-0.93 (-1.73 to -0.04)	0.53	
IGFBP-1 (ng/mL)	95	239 (174-320)	258 (179-350)	233 (171-294)	0.27	
1 year of GH therapy						
Age (years)	99	7.3 (1.6)	6.9 (1.4)	7.5 (1.7)		0.10
Weight (SDS)	96	-2.2 (1.0)	-2.2 (0.9)	-2.1 (1.2)		0.70
Height (SDS)	99	-2.4 (0.8)	-2.5 (0.9)	-2.4 (0.8)		0.58
Bioactive IGF-I (µg/L) 1 year	94	2.9 (0.9)	3.0 (0.9)	2.9 (0.9)		0.60
Bioactive IGF-I (SDS) 1 year	94	1.1 (1.4)	1.1 (1.0)	1.1 (1.6)		0.99
IGF-I (ng/mL)	95	312.0 (225.0 – 394.0)	338.0 (282.8 – 453.5)	308.0 (217.0 – 359.5)	0.05	
IGF-I (SDS)	95	2.8 (1.5)	2.9 (1.5)	2.8 (1.5)		0.67
IGFBP-3 (ng/mL)	95	4555 (4055-5082)	4600 (4207-5275)	4475 (3975-5000)	0.13	
IGFBP-3 (SDS)	95	1.25 (0.65-1.86)	1.17 (0.79-1.94)	1.30 (0.62-1.86)	0.77	
IGFBP-1 (ng/mL)	95	165 (134-220)	173 (138-216)	160 (132-234)	0.83	

Data are presented as Mean (SD) or median (interquartile range). Comparison between the sexes was analysed by Independent T-test or Mann-Whitney test when appropriate.

Table 2: Correlation matrix

	Bioactive IGF (SDS)	IGF-I (SDS)	IGFBP-3 (SDS)	IGFBP-1 (ng/mL)	Height (SDS)	Delta height (SDS)	Weight (SDS)	BMI (SDS)	Insulin sensitivity (HOMA-S)	Insulin secretion
Bioactive IGF (SDS)	1									
IGF-I (SDS)	0.35***	1								
IGFBP-3 (SDS)	0.62***	0.78***	1							
IGFBP-1 (ng/mL)	-0.18	-0.15	-0.19	1						
Height (SDS)	0.29**	0.17	0.15	-0.02	1					
Delta height (SDS)	-0.06	-0.22*	-0.35**	0.22*	0.004	1				
Weight (SDS)	0.37***	0.20	0.20	-0.03	0.59***	0.04	1			
BMI (SDS)	0.18	0.10	0.11	0.10	0.16	0.24*	0.80***	1		
Insulin sensitivity (HOMA-S)	-0.29**	-0.27*	-0.33**	0.42***	-0.08	0.28**	-0.22*	-0.09	1	
Insulin secretion	0.18	0.13	0.23	0.25*	0.17	0.06	0.07	-0.12	-0.47***	1

Spearman non-parametric correlations

*correlation is significant at the 0.05 level (two-tailed); **correlation is significant at the 0.01 level (two-tailed); ***correlation is significant at the 0.001 level (two-tailed)

All variables are baseline values except Delta Height (SDS) (height (SDS) at 1 yr – height (SDS) at baseline)

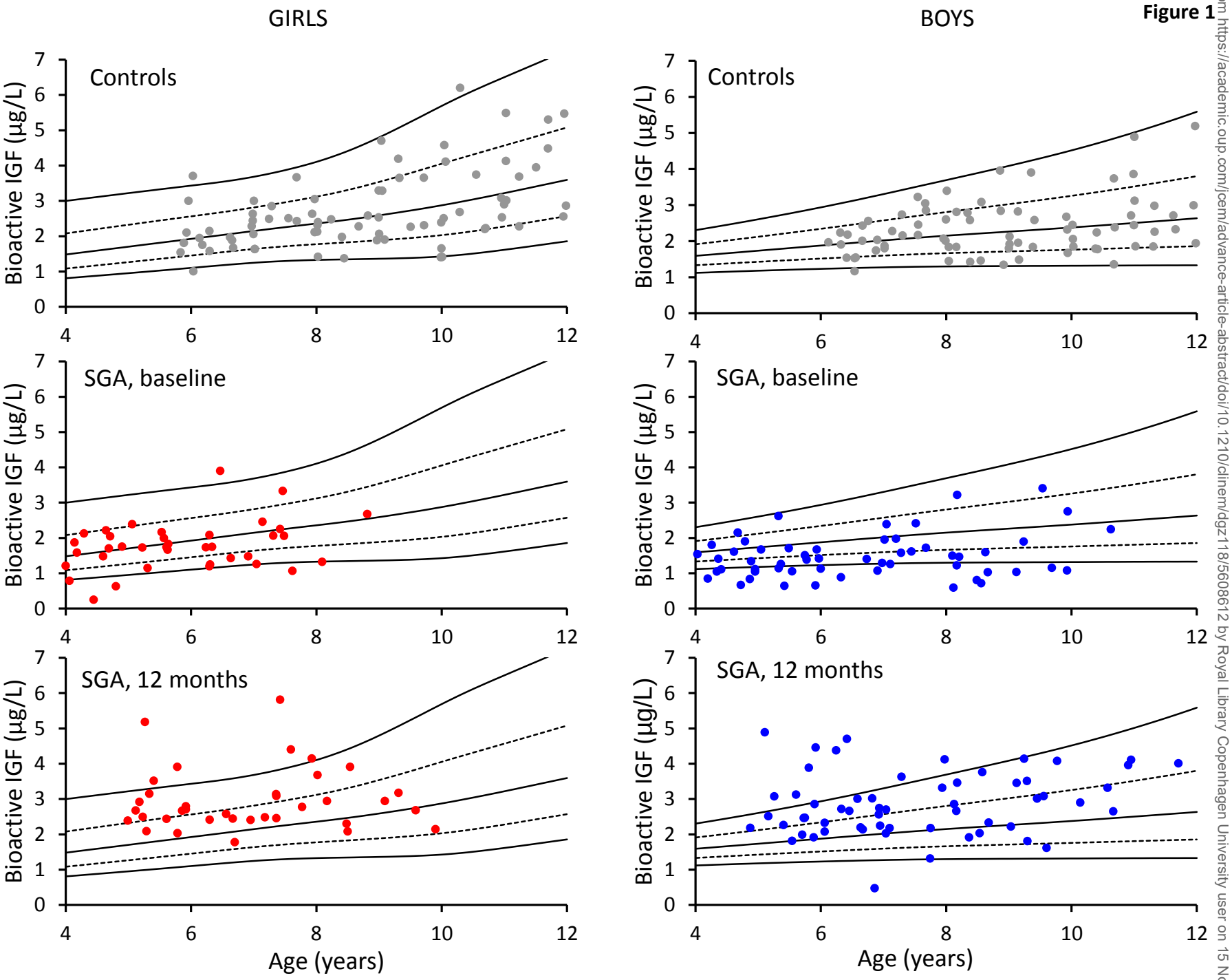

Figure 1

Figure 2