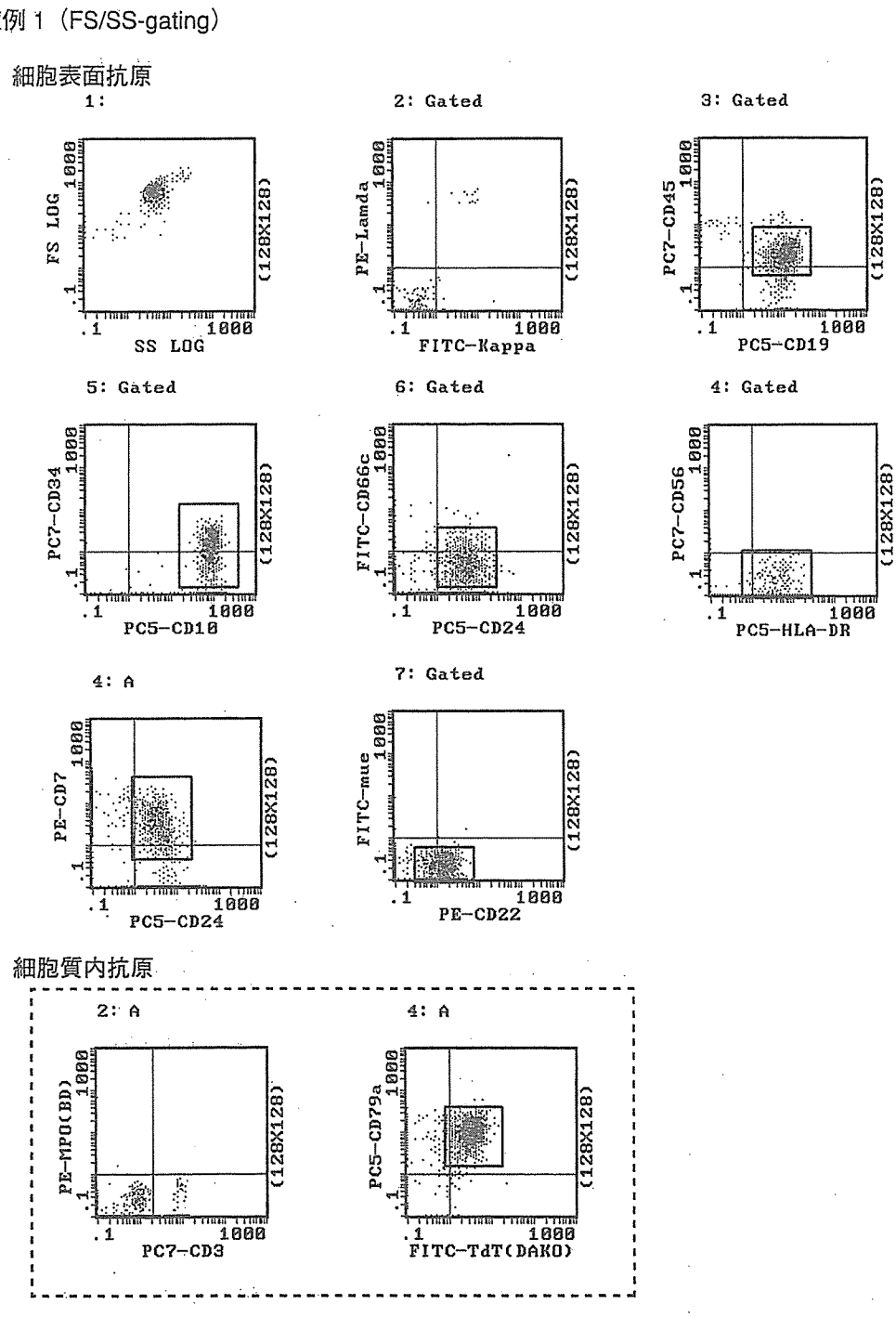

Figure 2　症例1
FS/SS-gatingで診断が容易であった，典型的なB-precursor ALL症例のマーカー結果のヒストグラムを示す。

は，標識する蛍光色素の違いによる反応性の差はほとんどの場合で認められず，標識色素の違いによる陽性/陰性の判定の不一致はなかった。

小児ALLでは，初診時検体中の芽球の割合が高い場合が比較的多いため，MoAbの組み合わせを工夫することによってCD45-gatingを行わなくても，診断に苦慮するケースは少ないと考えられた。実際にこのパネルを用いて行った解析の結果の一例をFigure 2に示す。9割以上の症例では症例1のように，芽球の割合が比較的高く，CD45-gatingを行わなくても，通常のFS/SS-gatingのみで診断可能であった（Figure 2）。しかし，一部の症例では芽球の占める割合が非常に低く，診断

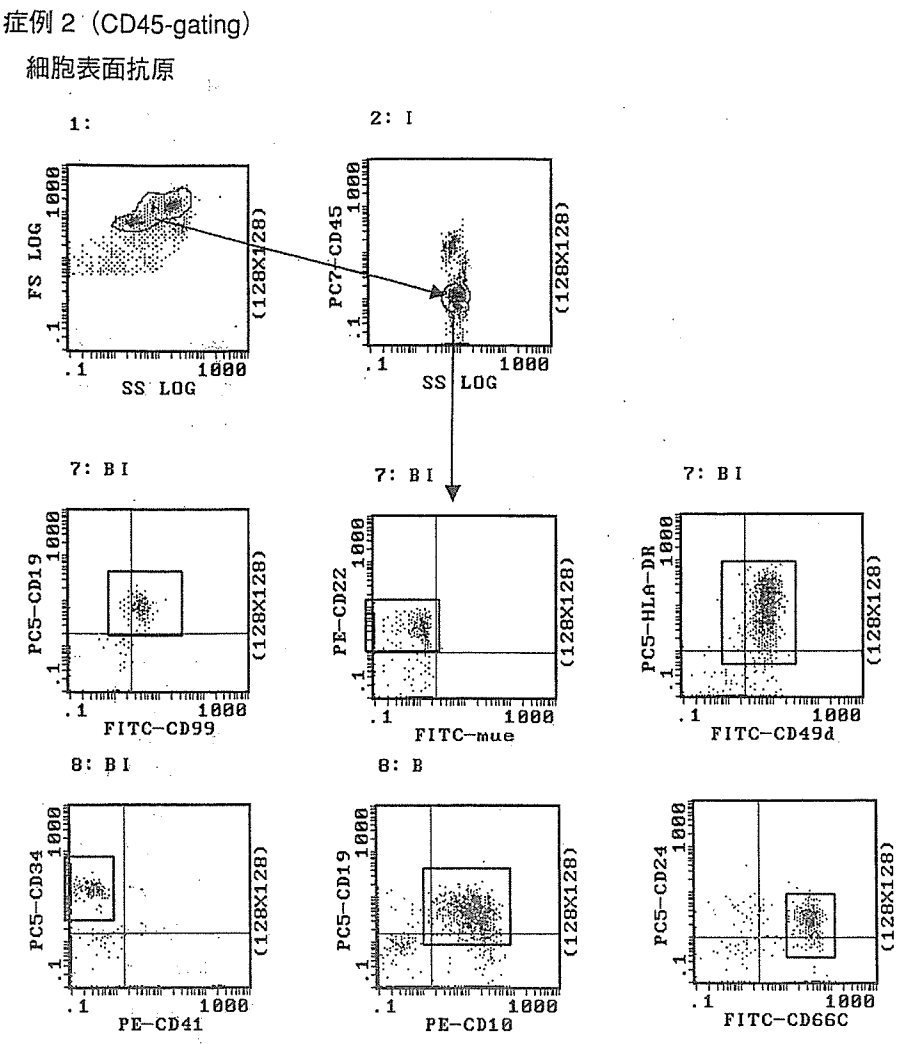

Figure 3 症例2
検体中の芽球の割合が低く，CD45-gatingが診断に有用であった，B-precursor ALL症例のマーカー結果のヒストグラムを示す。

にCD45-gatingが有用な場合も経験された（Figure 3）。

List mode data (LMD)コンペンセーションを用いた解析

FC500では，一度取得したLMDにコンピューター上でコンペンセーションをかけ直すことが可能である。検体量が少ないために再検査を行うことが困難な場合に非常に有用であった。実際に，データ取得時の蛍光補正が不充分であり，LMDコンペンセーションによって解析し直した例をFigure 4に示す。

考　　察

近年の化学療法を中心とした集学的治療の進歩により，小児ALL患者の予後は70-80%以上と飛躍的に改善してきた[1]。的確な治療を開始するためには，より正確で迅速な診断が求められるようになっており，FCMによるマーカー検査は，ALLの免疫学的診断において重要な役割を担っている[2]。しかし，一方で約15%の症例が不幸な転帰をたどると言われており[3]，治療成績の一層の向上のためには，治療開始時における的確なリスク分けが必要である。そのためには，従来用いられてきた細胞マーカーや特定の染色体異常の有無などによる病型診断に加えて，治療反応性を評価するためのMRDの検索が重要であるとの報告があいついでいる。また，今後あらたな予後マーカーを捜していく上で，臨床検体を用いたトランスレーショナルリサーチが不可欠であり，そのためには診断に用いた残余細胞を効率的に保存して，より有効に研究に活用して

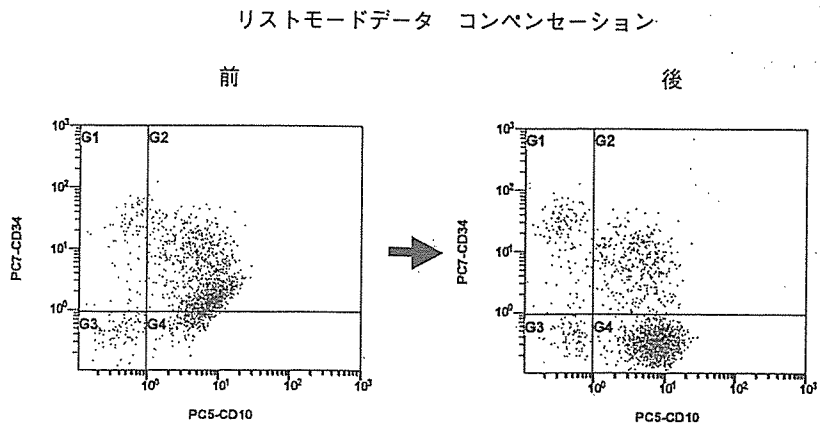

Figure 4　リストモードコンペンセーション
一度取得したリストモードデータにコンピューター上でコンペンセーションをかけ直した一例を示す。

行くことが必要である。

今回われわれが行った検討では，DG-FCMを用いた4カラー解析は，小児ALLの細胞表面マーカー検査において，より少ない細胞数で正確かつ迅速な診断が可能であり，貴重な検体を節約して用いる上で有用であると考えられた。例えば，我々が用いている抗体のパネルでは，細胞質内抗原として必須の検査項目と考えられるCD3，CD79a，TdT，MPOを1本のチューブで測定することが可能である(Table 1)。また，FC500はLMDをワークステーション上で蛍光補正し直すことが可能であり，再検が困難な臨床検体の解析には有用性が高いと考えられた。Luiderら[4]も，FC500を用いた5カラー解析により，白血病の診断パネルにおいて，4カラー解析で17チューブ必要であったものを13チューブに，リンパ腫においては，13チューブから7チューブに減らすことができ，MoAbにかかるコスト（約20%の削減），解析にかかる時間・労力を削減できたと報告している。

FCMは，白血病などの造血器腫瘍における初期の免疫学的な病型診断とともにMRDの検出においても広く用いられるようになってきている[5-7]。われわれのXLおよびFC500を用いた4カラー解析による検討でも，これまでの報告と同様に-10^4程度までのMRDを検出することが可能であり（data not shown），今後DG-FCMを用いたマルチカラー解析がMRD検索においても大きな力を発揮することが期待される。

1チューブについて染色する抗体の項目数を増やすことは，検体量節約のみではなく，複数の抗体で同時に染色することにより各抗原の発現様式を多角的に解析することができる，という利点がある。例えば，抗体の組み合わせを工夫することにより，B-precursor ALL細胞上に発現するCD33，CD65，CD66cといったabberantな抗原の発現をより正確に解析することも可能である。しかし，一回に染色する抗体数を増やすと，蛍光補正がより複雑になることや，抗体同士の競合によってそれぞれの抗体の反応性が修飾されること，等の問題点が生じる。今回われわれが行った4カラー解析では，MoAbの組み合わせを工夫することによって，蛍光補正は比較的容易に行うことができた。また，抗体同士の競合に関する問題も，十分な事前検討を行うことにより，回避することが可能と考えられる。

一方，小児ALLの場合，その多くの症例ではCD45-gatingは必ずしも必要ではないと考えられる。しかし，一部には芽球の割合が低いために，やはりCD45-gatingが必要な症例が確実に存在することも事実である。検体量を節約しつつ，CD45-gatingが必要な症例にも対応するために，現在5カラー/ CD45-gatingを用いた解析のシステムについて検討を行っている。

本論文の要旨は，第15回日本サイトメトリー学会（2005年7月，名古屋）で発表した。

参考文献

1）Pui C-H, Evans WE. Treatment of acute lymphoblastic leukemia. N Engl J Med. 2006;354:166-178.
2）Howard SC, Campana D, Coustan-Smith E, et al. Development of a regional flow cytometry center for diagnosis of childhood leukemia in central America.

Leukemia. 2005;19:323-325.
3) Gaynon PS. Childhood acute lymphoblastic leukemia and relapse. Br J Haematol. 2005;131:579-587.
4) Luider J, Cyfra M, Johnson P, Auer I. Impact of the new Beckman coulter cytomics FC500 5-color flow cytometer on a regional flow cytometry clinical laboratory service. Lab Hematol. 2004;10:102-108.
5) Campana D, Coustan-Smith E. Detection of minimal residual disease in acute leukemia by flow cytometry. Cytometry. 1999;38:139-152.
6) Dworzak MN, Froschl G, Printz D, et al. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. Blood. 2002;99:1952-1958.
7) Borowitz MJ, Pullen DJ, Shuster JJ, et al. Minimal residual disease detection in childhood precursor-B-cell acute lymphoblastic leukemia: relation to other risk factors. A children's oncology group study. Leukemia. 2003;17: 1566-1572.

別冊請求先：〒157-0074　東京都世田谷区大蔵2-10-1
国立成育医療センター研究所　発生・分化研究部　塩沢裕介
TEL: 03-5494-7120, 内線4620　FAX: 03-3416-0181　E-mail: y-shio@mte.biglobe.ne.jp

Clin Pediatr Endocrinol 2006; **15**(1), 15-21

Original Article

No Improvement of Adult Height in Non-growth Hormone (GH) Deficient Short Children with GH Treatment

Toshiaki Tanaka[1, 2], Kenji Fujieda[1], Susumu Yokoya[1], Akira Shimatsu[1], Katsuhiko Tachibana[1], Hiroyuki Tanaka[1], Takakuni Tanizawa[1], Akira Teramoto[1], Toshiro Nagai[1], Yoshikazu Nishi[1], Yukihiro Hasegawa[1], Kunihiko Hanew[1], Keinosuke Fujita[1], Reiko Horikawa[1], Goro Takada[3], Masahiro Miyashita[4], Tadashi Ohno[5] and Kazuo Komatsu[6]

[1]*Study Group of Growth Hormone Treatment, the Foundation for Growth Science, Japan*

[2]*Department of Clinical Laboratory Medicine, National Center for Child Health and Development, Tokyo, Japan*

[3]*Akita University, Akita, Japan,* [4]*Akita Red Cross Hospital, Akita, Japan,* [5]*Ohno Clinic, Akita, Japan*

[6]*Akita Kumiai General Hospital, Akita, Japan*

Abstract. It is still in doubt whether the standard-dose growth hormone (GH) used in Japan (0.5 IU/kg/week, 0.167 mg/kg/week) for growth hormone deficiency is effective for achieving significant adult height improvement in non-growth hormone deficient (non-GHD) short children. We compared the growth of GH-treated non-GHD short children with that of untreated short children to examine the effect of standard-dose GH treatment on non-GHD short children. GH treatment with recombinant human growth hormone (rhGH) was started before the age of 11 yr in 64 boys and 76 girls with non-GHD short stature registered at the Foundation for Growth Science who have now reached their adult height. In 119 untreated boys and 127 untreated girls whose height standard deviation score (SDS) was below −2 SD at the age of 6 yr, height growth was followed until 17 yr. Height SDS was significantly lower before GH treatment in the GH-treated group than at the age of 6 yr in the untreated group, in both sexes. Adult height and adult height SDS were significantly greater in the untreated group than in the GH-treated group, in both sexes, although the change in height SDS did not differ significantly. Height SDS was significantly lower before GH treatment in the GH-treated group than at the age of 6 yr in the untreated group, so 57 boys and 57 girls whose height SDS at the age of 6 yr in the untreated group closely matched the height SDS before GH treatment in the GH-treated group were chosen for comparison. Height SDS did not differ significantly between the GH-treated group before GH treatment and the untreated group at the age of 6 yr, nor were there differences between these subgroups in adult height, adult height SDS, or height SDS change, in either sex. The effect of GH treatment is reported to be dose-dependent and doses over 0.23 mg/kg/week are reported to be necessary to improve adult height in non-GHD short children. Currently, the GH dose is fixed at 0.175 mg/kg/week in Japan, and we expected to find, and indeed concluded, that ordinary GH treatment in Japanese, non-GHD short children does not improve adult height.

Key words: non-GHD short children, GH treatment, GH dosage

Received: May 18, 2005
Accepted: July 15, 2005
Correspondence: Dr. Toshiaki Tanaka, Department of Clinical Laboratory Medicine, National Center for Child Health and Development, 2-10-1, Ohkura, Setagaya-ku, Tokyo 157-8535, Japan
E-mail: tanaka-t@ncch.go.jp

Introduction

The effectiveness of growth hormone (GH) treatment on adult height in non-growth hormone deficient (non-GHD) children is controversial. A recent double-blind, placebo-controlled study clearly demonstrated that children with idiopathic short stature treated with high-dose GH are significantly taller than placebo-treated short children when they reach adult height (1). It is still unclear whether the standard dose used in Japan has any significant efficacy on increasing the adult height of non-GHD short children.

It is known that approximately 60% of non-GHD short children show a tendency toward delayed puberty and reach an adult height within the normal range (> –2 SD) without any treatment (2). Therefore, the normalization of adult height following GH treatment does not prove the efficacy of GH treatment in these patients, unless we establish this through comparison of the resulting adult heights with the adult heights of untreated non-GHD short children. In Japan, since it is now practically impossible to study the efficacy of GH treatment on adult height in non-GHD short children in a controlled study, we compared data on file at the Foundation for Growth Science and epidemiological data in Akita Prefecture (2).

The Foundation for Growth Science was founded in 1977 and has been monitoring the use of GH by its registration system, which includes judgment of eligibility to start and to continue GH treatment, and a system for reporting adverse events. The data on the application sheets were utilized as they are the largest database of GH treatment in Japan. The epidemiological data in Akita Prefecture was collected through the collaborative activity of pediatric endocrinologists and the local committee of education.

Subjects and Methods

The following two groups were compared:

1) GH-treated group: 64 boys and 76 girls for whom data were on file at the Foundation for Growth Science and had started growth hormone (GH) treatment with recombinant growth hormone (rhGH) at a dose of 0.5 IU (0.167 mg)/kg/week before the age of 11 yr and thereafter reached their adult height (as defined below). They were diagnosed as having non-GHD short stature because their peak GH values were below 10 ng/ml in two GH provocation tests but over 10 ng/ml in at least one test. Their tallest height after their height velocity became less than 2 cm/yr was defined as their adult height. The age at adult height estimation was 16.89 ± 1.01 yr and 15.34 ± 0.93 yr in boys and girls, respectively.

2) Untreated group: 119 boys and 127 girls whose height SDS was below –2 SD at 6 years of age were followed until 17 yr old. Height at the age of 17 yr was defined as adult height in these patients.

In the second comparison, height SDS at start of GH treatment in the GH-treated group and at the age of 6 yr in the untreated group were matched and 57 boys and 57 girls were selected from the both groups.

The height standard of the national survey of 1990 was used for the calculation of height SDS. Student's t-test was used for comparisons of the two groups. The significance level was set at 0.05.

Results

In the GH treated group, the age at the start of GH was 10.7 ± 0.9 yr and 9.6 ± 0.9 yr, and the duration of GH treatment was 6.2 ± 1.0 yr and 5.7 ± 1.0 yr for boys and girls, respectively. Table 1 shows the height SDS before GH treatment in the GH-treated group and the height SDS at the age of 6 yr in the untreated group, and adult height and adult height SDS in both sexes, as

Table 1 Comparison of clinical characteristics between GH-treated and untreated groups of non-GHD short children

	boys		girls	
	GH-treated	untreated	GH-treated	untreated
n	64	119	76	127
height SDS at start of GH or at 6 yr	−2.68 ± 0.36*	−2.36 ± 0.36	−2.87 ± 0.63*	−2.39 ± 0.43
adult height	159.2 ± 4.8*	161.1 ± 4.3	146.0 ± 6.0*	148.8 ± 4.4
adult height SDS	−2.01 ± 0.86*	−1.66 ± 0.76	−2.38 ± 1.20*	−1.83 ± 0.88
change in height SDS	0.67 ± 0.77	0.71 ± 0.80	0.49 ± 0.85	0.56 ± 0.68

*$p<0.05$ vs untreated.

Table 2 Comparison of clinical characteristics between the GH-treated and untreated groups of non-GHD short children, when GH-treated short children were matched with untreated short children whose height SDS at 6 yr of age was similar to the height SDS of GH-treated short children before treatment

	boys		girls	
	GH-treated	untreated	GH-treated	untreated
n	57	57	57	57
height SDS at start of GH or at 6 yr	−2.61 ± 0.39	−2.60 ± 0.39	−2.68 ± 0.47	−2.68 ± 0.49
adult height	159.8 ± 4.8	161.1 ± 5.1	147.4 ± 5.1	146.8 ± 4.4
adult height SDS	−1.90 ± 0.81	−1.66 ± 0.91	−2.11 ± 1.02	−2.21 ± 0.95
change in height SDS	0.71 ± 0.77	0.94 ± 0.93	0.57 ± 0.85	0.47 ± 0.68

well as the change in height SDS in the GH-treated group between before GH treatment and after attaining adult height and in the untreated group between the ages of 6 yr and 17 yr. In both sexes, the height SDS was significantly smaller in the GH-treated group before GH treatment than in the untreated group at the age of 6 yr, and adult height and adult height SDS were significantly smaller in the GH-treated group than in the untreated group. Change in height SDS, however, did not differ significantly: gains in height SDS were observed not only in the GH-treated group but also in the untreated group.

Because there was a significant difference between height SDS in the GH-treated group before GH treatment and height SDS in the untreated group at the age of 6 yr, 57 boys and 57 girls whose height SDS at the age of 6 yr in the untreated group closely matched the height SDS of patients in the GH-treated group before GH treatment were chosen and compared with the GH-treated group. Table 2 shows height SDS in the GH-treated group before GH treatment and in the untreated group at the age of 6 yr, adult height, and adult height SDS in both sexes. The changes in height SDS in the GH-treated group between before GH treatment and adult height and in the untreated group between the ages of 6 yr and 17 yr are also shown. No significant differences were found in height SDS between the GH-treated group before GH treatment and the untreated group at the age of 6 yr, or in adult height or adult height SDS, nor did the change in height SDS differ significantly between the GH-treated group and the untreated group.

Table 3 Adult height after GH treatement in non-GHD short children in Europe and the United States

	dose (mg/kg/week)	n	Height SDS			AH-PAH		AH-start (ΔSDS)
			At start	PAH	AH	Δcm	ΔSDS	
Wit[3]	0.19	53 (FSS)	−2.6		−1.9			0.8
	0.2	36 (NFSS)	−2.8		−1.3			1.4
Bernascovi[4]	0.21	71	−2.8	−1.7	−1.7	0	0	1.1
Leschek[1]	0.23	22	−2.7	−2.1	−1.77		0.32	0.93
	placebo	11	−2.8	−2.3	−2.34		−0.14	0.42
Hintz[6]	0.3	57 (m)	−2.9	−2.5	−1.7	5	0.8	1.2
		23 (f)	−2.7	−2.6	−1.6	5.9	1	1.1
MaCaughey[5]	0.33	8 (f)	−2.5	−1.8	−1.1	3.5	0.7	1.3
Wit[7]	0.24	17	−3.26	−2.5	−1.69	5.4		1.55
	0.24→0.37	16	−3.08	−2.6	−1.48			1.52
	0.37	17	−2.88	−2.3	−1.12	7.2		1.85

( ): Reference No. PAH: Predicted adult height, AH: Adult height, FSS: Familial short stature, NFSS: Non-familial short stature. m: male, f: female.

Discussion

Table 3 shows data on GH treatment outcomes in non-GHD short children from six important studies conducted in Europe and the United States (1, 3–7), which have used recombinant hGH. Evaluation reveals some problems with these reports: 1) ages at the start of treatment and durations of therapy differ; 2) therapeutic doses differ; and 3) therapeutic effects cannot be evaluated, since most reports did not include untreated controls. Many reports, like the studies cited in Table 3, evaluate the effect of GH treatment by comparing the measured outcome with predicted adult height (PAH) instead of by comparison with controls. Despite these problems, it is clear that in all the studies higher doses than the Japanese standard dose were used and the change in height SDS from the start of GH treatment to adult height was greater in these studies than that of this study. The change in height SDS reported by the studies varied from 0.8 to 1.85 SD (Table 3). The studies employing higher doses (5–7) of GH achieved more improvement.

Leschek *et al.* (1) conducted a randomized, double-blind, placebo-controlled trial. Sixty-eight non-GHD short children received either GH (0.23 mg/kg/week) or placebo. Adult height was finally evaluated in 22 GH-treated children and 11 placebo-treated children and was significantly greater in the GH-treated group than in the placebo-treated group by 0.51 SDS. However the injection frequency was three times per week in both groups. When these resuits are compared with those of Wit *et al.* (7) who used daily injections of a similar dose of GH, the effect on adult height was greater in daily injection.

McCaughey (5) compared GH effects with untreated controls, though few in number. At a dose of 30 IU/m^2/week (about 0.33 mg/kg/week), adult height was 7.5 cm taller on average in GH-treated children after a mean treatment period of 6.2 yr than in the untreated control group. Hintz *et al.* (6) estimated the effect of GH treatment on the adult height of non-GHD short children at a dose of 0.3 mg/kg/week for 5.5–6.0 yr, the difference between predicted adult height before treatment and achieved adult height was 9.2 cm greater in boys and 5.7 cm in girls than the corresponding difference in the untreated historical controls.

Wit *et al.* (7) compared the adult height of 50 GH-treated patients with idiopathic short stature. Patients were treated with GH at 0.24 mg/kg/week, 0.24 mg/kg/week for the first year and at 0.37 mg/kg/week thereafter, or 0.37 mg/kg/week, and their mean height SDS increased by 1.55, 1.52, and 1.85 SD, respectively. They concluded that the effect of GH treatment on adult height was dose-dependent and that regimens increasing dosage from the second year were less efficacious.

One study in Japan found that after 4.2 yr of GH treatment at a dose of 0.5 IU/kg/week (0.167 mg/kg/week), the average adult height of 9 GH-treated non-GHD short boys, 154.2 cm, was significantly shorter than the adult height of 18 untreated short boys, 162.0 cm (8). Bone age at the onset of puberty did not differ significantly between the groups, but pubertal height gain was significantly greater in the untreated boys. Yet the approximately 1-yr bone age difference at the start of treatment, though it was not statistically significant, might cause to early epiphyseal closure and hence lower the adult height in the GH-treated group rather than GH treatment itself. Another study by the same group (Kawai *et al.* (9)) compared adult height in 11 GH-treated non-GHD short girls treated at the same dose, 0.5 IU/kg/week for 4.2 yr on average, with 11 untreated short girls, and found no significant difference.

The long-term effect of GH treatment is usually evaluated in terms of adult height. Since adult height, however, depends heavily on height SDS at the start of GH treatment (10), change in height SDS from the start of GH treatment to adult height is a more precise evaluation. In our study, the tallest height after the timing of less than 2 cm/yr of height velocity was defined as adult height, and adult height was taller in untreated short children than in GH-treated non-GHD short children. But this is because the pre-treatment height SDS of the GH-treated non-GHD short children was significantly lower than the baseline height SDS of the untreated short children. Adjusted for pre-treatment height SDS, adult height SDS was not significantly different between the GH-treated patients and the untreated controls. In no situation did the change in height SDS in the two groups differ significantly. It can only be concluded that, in this study, GH treatment in non-GHD short children was ineffective in improving adult height.

In GHD, there is a significant positive correlation between age at onset of puberty and age at the start of GH treatment (11–13). Increasing evidence points to an important role for GH in gonadal function through induction of local production of IGF-I in the ovary or in the testis and by increase of gonadotropin-dependent gonadal functions such as sex steroid production and ovulation (13–16). These facts demonstrate that GH accelerates pubertal onset and that the resulting relatively early pubertal development adversely effects decompensates the catch-up growth observed for the first few years following initiation of GH treatment. The lack of improvement in adult height in GH-treated non-GHD short children is attributable mainly to the early induction of puberty by GH treatment and the relatively poor response to GH in non-GHD short children, even though GH treatment improves growth velocity during the first two or three years (10). It is well known that adult height shows a strong positive correlation with height at the onset of puberty in GH-treated short children (11). Therefore, to achieve normal adult height in GH-treated short children, height must be normalized at onset of puberty.

In this study, puberty was not analyzed since the onset of the puberty is often not correctly evaluated and in our experience tends to be judged later than its actual occurrence. Therefore, the prepubertal height SDS and adult height SDS were analyzed. The GH dosage used in Japan now (0.175 mg/kg/week) induces catch-up growth for only a few years, but in non-GHD short children it seems insufficient to normalize

height by the onset of puberty and to increase height SDS to an extent exceeding natural improvement in untreated short children.

Natural improvement of height SDS in short children has been reported to range between 0.4 and 0.7 SD (1, 2, 17), as it did in our study. To prove that GH treatment is effective in non-GHD short children, it is necessary to achieve a gain in height SDS greater than 1 SD through GH treatment. Leschek *et al.* (1) report 0.93 SD improvement on average by GH at a dose of 0.23 mg/kg/week injected thrice a week, significantly greater than the natural improvement in placebo-treated short children (0.42 SD on average). Other studies employing higher doses reported greater than 1 SD improvement (5–7).

There may be a small possibility that the real adult height is different from the adult height defined in this study. It is possible that both GH treated and untreated groups will grow after the adult height of this definition. GH-treated non-GHD short children will grow after the growth velocity drops below 2 cm/yr, but such growth is around 5 mm in our experience. Untreated non-GHD short children have a tendency of delayed puberty. Boys with delayed puberty will grow after 17 yr, but the growth is less than 1 cm with a few exceptions (18). Therefore, these minimal differences in adult height do not essentially change our conclusion.

The above findings and considerations demonstrate that the effect of GH treatment is dose-dependent and suggest that doses over 0.23 mg/kg/week are necessary to achieve meaningful improvements in adult height in non-GHD short children. We conclude that GH treatment in non-GHD short children in Japan does not improve the adult height of treated children because the GH dose currently being administered, 0.175 mg/kg/week, is insufficient.

Acknowledgement

This study was supported by the Grants from the Foundation for Growth Science and from the Ministry of Health, Labour and Welfare.

References

1. Leschek EW, Rose SR, Yanovski JA, Troendle JF, Quigley CA, Chipman JJ, *et al.* Effect of growth hormone treatment on adult height in peripubertal children with idiopathic short stature: a randomized, double-blind, placebo-controlled trial. J Clin Endocrinol Metab 2004;89:3140–8.
2. Komatsu K, Okamura T, Takada G, Miyashita M, Ohno T, Tanaka T. Analysis of natural growth of children with short stature at prepuberty or at final height. I. Follow-up of healthy children at Akita Prefecture. J Jpn Pediatr Soc 1997;101:610–6.
3. Wit JM. Growth hormone treatment of idioapthic short stature in KIGS. In: Ranake MB, Wilton P, editors. Growth hormone therapy in KIGS-10 years' experience. Heidelberg, 1999, p.225–43.
4. Bernasconi S, Street ME, Volta C, Mazzardo G. Final height in non-growth hormone deficient children treated with growth hormone. Clin Endocrinol (Oxf) 1997;47:261–6.
5. McCaughey ES, Mulligan J, Voss LD, Betts PR. Randomized trial of growth hormone in short normal girls. Lancet 1998;351:940–4.
6. Hintz R, Attie KM, Baptista J, Roche A. Effect of growth hormone treatment on adult height of children with idiopathic short stature. N Engl J Med 1999;340:502–7.
7. Wit JM, Rekers-Mombarg LT, Cutler GB, Crowe B, Beck TJ, Roberts K, *et al.* Growth hormone (GH) treatment to final height in children with idiopathic short stature: evidence for a dose effect. J Pediatr 2005;146:45–53.
8. Kawai M, Momoi T, Yorifuji T, Yamanaka C, Sakai H, Furusho K. Unfavorable effects of growth hormone therapy on the final height of boys with short stature not caused by growth hormone deficiency. J Pediatr 1997;130:205–9.
9. Kawai M, Momoi T, Yorifuji T, Muroi J, Uematsu A, Yamanaka C, *et al.* Growth hormone treatment does not improve the final height of girls with short stature not caused by growth hormone deficiency. Clin Pediatr Endocrinol 1998;7:93–8.

10. Tanaka T, Cohen P, Clayton P, Laron Z, Hintz RL, Sizonenko PC. Diagnosis and management of growth hormone deficiency in childhood and adolescence. Part 2: growth hormone treatment in growth hormone deficient children. GH & IGF Research 2002;12:323–41.
11. Tanaka T, Yoshizawa A, Tanae A, Hibi I, Shizume K. Relationships between puberty and growth at adolescence in growth-hormone-deficient males: effect of growth hormone and of associated gonadal suppression therapy. Horm Res 1990;33(suppl 4):102–5.
12. Price DA, Shalet SM, Clayton PE. Management of idiopathic growth hormone deficient patients during puberty. Acta Paediatr Scand 1998;347(Suppl):44–51.
13. Tanaka T. Pubertal aspects of idiopathic growth hormone deficiency. In: Tanake MB, Gunnarsson R, editors. Progress in growth hormone therapy-5 years of KIGS. Mannheim: J&J Verlag; 1994: p.112–28.
14. Tres LL, Smith EP, Van Wyk JJ, Kierszenbaum AL. Immunoreactive sites and accumulation of somatomedin-C in rat Sertoli-spermatogenic cell cocultures. Exp Cell Res 1986;16:33–50.
15. Davoren JB, Hsueh AJW. Growth hormone increases ovarian levels of immunoreactive somatomedin C/insulin-like growth factor I in vivo. Endocrinology 1986;118:888–90.
16. Adashi EY, Resnick CE, D'Ercole AJ, Svoboda NE, Van Wyke JJ. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr Rev 1985;6:400–20.
17. Wit JM, Rekers-Mombarg LT. Final height gain by GH therapy in children with idiopathic short stature is dose dependent. J Clin Endocrinol Metab 2002;87:604–11.
18. Okamura T, Takada G, Miyashita M, Ohno T, Watanabe T, Tanaka T. Growth of school children in Akita Prefecture. Clin Pediatr Endocrinol 1994;3(suppl 5):155–7.

CURRENT MEDICAL RESEARCH AND OPINION®
VOL. 22, NO. 10, 2006, 1973–1979

0300-7995
doi:10.1185/030079906X132460

ORIGINAL ARTICLE

Effect of growth hormone treatment on trunk fat accumulation in adult GH-deficient Japanese patients: a randomised, placebo-controlled trial

Kazuo Chihara[a], Yuzuru Kato[b], Kazue Takano[c], Akira Shimatsu[d], Hitoshi Kohno[e], Toshiaki Tanaka[f] and Minoru Irie[g]

[a] *Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan*

[b] *Division of Endocrinology, Metabolism, Hematology and Oncology, Shimane University, Izumo, Japan*

[c] *Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women's Medical University, Tokyo, Japan*

[d] *Clinical Research Center for Endocrine and Metabolic Disease, Kyoto Medical Center, Kyoto, Japan*

[e] *Department of Endocrinology and Metabolism, Fukuoka Children's Hospital, Fukuoka, Japan*

[f] *Division of Endocrinology and Metabolism, National Center for Child Health and Development, Tokyo, Japan*

[g] *Toho University, Tokyo, Japan*

Address for correspondence: Kazuo Chihara, Division of Endocrinology/Metabolism, Neurology and Hematology/Oncology, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan. Fax: +81 78 382 5898; email: chiharak@med.kobe-u.ac.jp

Key words: Abdominal obesity – GH deficiency – GH treatment – Trunk fat

ABSTRACT

Objective: Patients with growth hormone deficiency (GHD), both Japanese and Caucasian, have an abnormal body composition with pronounced abdominal obesity. This study aimed to evaluate changes in trunk fat with GH treatment.

Design: Double-blind, placebo-controlled study.

Patients and measurements: Sixty-one Japanese adult GH deficient patients (mean age 37 years) were randomised to either GH, titrated to 0.012 mg/kg/day, ($n = 30$) or placebo ($n = 31$) for 24 weeks. Body composition, by dual-energy X-ray absorptiometry (DXA), was evaluated at a central laboratory for trunk fat, total body fat and lean body mass. Serum lipid levels were also determined centrally.

Results: At baseline, 26 (42.6%) patients had a body mass index (BMI) ≥ 25 kg/m^2, the threshold for obesity-related complications for Japanese subjects. Median trunk fat mass (FM) was ≥ 9.0 kg for each treatment and gender group, higher than the cut-off for increased age-adjusted risk for cardiovascular complications reported in the

normal Japanese population. After 24 weeks of GH treatment, the change in percentage trunk FM was –3.4 ± 0.6%, versus 0.4 ± 0.6% with placebo ($p < 0.001$). Change in total body FM was –2.8 ± 0.5% with GH and 0.0 ± 0.5% with placebo, indicating that the decrease in trunk fat was more pronounced than for total body fat. Total and low density lipoprotein (LDL)-cholesterol were both significantly ($p < 0.001$) decreased compared with placebo. One patient discontinued due to a subdural haematoma and one had GH dose reduced due to hyperglycaemia.

Conclusions: Japanese patients with GHD have abnormal central fat accumulation, which is reduced by GH treatment over 24 weeks. This may reduce cardiovascular risk but the GH dose should be individualised to maintain IGF-I in the normal range.

Introduction

One of the most prominent features of the adult growth hormone deficiency (GHD) syndrome is the abnormal body composition with increased body fast mass (FM) and decreased lean body mass (LBM)[1,2]. Specifically, the increase in FM in these subjects is characterised by pronounced abdominal obesity[3], a well known risk factor for the development of metabolic abnormalities leading to cardiovascular complications[4,5]. In fact, epidemiological studies in Caucasian subjects have shown that the risk of cardio- and cerebrovascular morbidity and mortality in adult hypopituitary patients with GHD is increased compared to the normal population[6–8]. The increased vascular risk, with high prevalence of angina pectoris, myocardial infarction, hypertension and hyperlipidaemia, has been confirmed in adult Japanese GH deficient subjects by epidemiological surveys and cohort studies[9–11].

There is, therefore, evidence demonstrating that, in spite of differences in racial intrinsic factors and in nutritional habits, Japanese adult GH deficient subjects carry the same causes and risks for cardiovascular complications as Caucasian patients. GH replacement in adult patients with GHD decreases overall fat mass and improves lipid status, and in Caucasian patients, studies suggested that the FM reduction involves predominantly abdominal fat, thus acting on one of the primary causes of increased risk[3]. Studies in the normal Japanese population have examined the relationship of direct measurements of body fat distribution to cardiovascular complications and provided age-adjusted cut-off values for dual-energy X-ray absorptiometry (DXA) trunk FM for increased cardiovascular risk[12]. Based on this background, we designed a study to assess upper body obesity by DXA measurement of truncal fat in adult GH deficient patients and to examine the hypothesis that GH replacement has a significant effect on this measure.

Patients and methods

This was a 24-week, randomised, placebo-controlled, double-blind study performed in 20 Japanese study centres. All patients gave informed consent and the study was performed with appropriate ethical approval and according to the Declaration of Helsinki. Japanese patients aged 20–64 years, with organic or idiopathic, isolated or multiple, childhood onset (CO) as well as adult onset (AO) GHD were recruited. AO patients were defined as having onset of GHD at 18 years or above and CO patients as having had onset before 18 years of age. For diagnosis of GHD, the criteria set in the GRS guidelines were used[13]; patients had to have a serum GH peak < 3.0 µg/L in the insulin tolerance test (ITT) or, in the presence of a contraindication to the ITT, in an arginine or glucagon test. For patients with isolated GHD confirmation was required by a peak GH value < 3.0 µg/L in an additional test to the ITT, either arginine or glucagon test. Replacement therapy for other missing hormones had to be stable and adequate during the 3 months prior to entering the study and throughout the study. Malignancy, diabetes, severe organ dysfunction, severe hypertension and pharmacologic treatment with glucocorticoids were major exclusion criteria.

A total of 61 patients, 32 with CO and 29 with AO GHD were enrolled and randomly assigned to treatment with either recombinant human GH (Nutropin AQ, Genentech Inc., San Francisco, USA) or placebo, with stratification by gender and onset. GH was started at a dose of 0.003 mg/kg/day for the first 4 weeks, then increased to 0.006 mg/kg/day until Week 12 and thereafter given at the final dose of 0.012 mg/kg/day until the end of the study at 24 weeks; maximum total daily dose had to not exceed 1.0 mg/day. In the event of side effects that were thought to be GH-related at the final dose of 0.012 mg/kg/day, a 50% dose reduction was permitted at the physician's discretion.

At the baseline, 12- and 24-week visits, LBM, total FM and trunk FM were measured by DXA. All DXA measurements performed in each investigative centre were evaluated centrally; a hard copy of the DXA scan data was sent to the DXA Central Evaluation Committee which was responsible for the analysis of the individual patient scans in a blinded manner. Serum IGF-I concentrations, as well as triglycerides and total, high density lipoprotein (HDL-) and

LDL-cholesterol levels, were measured centrally (Hachioji Laboratories, SRL Inc., Japan). Standard deviation (SD) scores were calculated from serum IGF-I concentrations by comparison to age- and gender-matched subjects[14].

Safety was assessed by the recording of treatment-emergent adverse events, laboratory test values and blood pressure. Laboratory determinations were performed centrally and included measurements of liver and kidney function, thyroid hormones and glycosylated haemoglobin (HbA_{1c})concentrations.

All results were analysed on the full dataset in an intent-to-treat basis. Change in the percentage trunk fat for GH-treatment versus placebo was the primary efficacy measure of the study. The baseline to 12-week and baseline to 24-week differences in actual and percentage changes in body composition between GH- and placebo-treated patients were compared by analysis of variance (ANOVA) after adjustment for gender and onset. Group comparisons were made using Fisher's exact test, chi-square test and Mann–Whitney test as appropriate. Significance levels were set at a two-sided level of 5%.

Results

The baseline demographic, anthropometric and diagnostic criteria of the patients by assigned treatment group are presented in Table 1. The patients were well randomised and no significant differences were seen in baseline presentation between GH and placebo-treated patients. Mean age of GH-treated and placebo-treated patients were 40 ± 14 years and 35 ± 11 years ($p = 0.155$), respectively. More than two thirds of the patients had an organic cause of hypopituitarism and GHD, and all except one placebo-treated patient had multiple pituitary deficiencies. Peak GH values in the stimulation tests, as well as baseline IGF-I status demonstrated severe GHD for all patients. In total for the two treatment groups, 42.6% of the patients had a BMI ≥ 25.0 kg/m^2.

Baseline DXA body composition values are presented in Table 2 for male and female subjects by treatment group. The median trunk fat mass values in males were 9.4 kg in the GH-treated and 9.0 kg in the placebo group and in females were 9.1 kg in the GH-treated group and 11.6 kg in the placebo group.

Table 1. *Baseline characteristics of Japanese GH deficient patients, by assigned treatment group*

	GH ($n = 30$)	Placebo ($n = 31$)	p-value
Age (years), mean ± SD	40 ± 14	35 ± 11	0.155
Onset, *n* (%)			1.000
childhood, *n* (%)	16 (53.3%)	16 (51.6%)	
adult, *n* (%)	14 (46.7%)	15 (48.4%)	
Male/female, *n/n*	15/15	17/14	0.800
Aetiology of GH deficiency			0.749
idiopathic, *n* (%)	5 (16.7%)	7 (22.6%)	
organic, *n* (%)	25 (83.3%)	24 (77.4%)	
Duration of GHD (years), mean ± SD	15.8 ± 9.1	13.5 ± 8.2	0.413
Height (cm), mean ± SD	159.7 ± 8.3	159.2 ± 7.4	0.634
BMI (kg/m^2), mean ± SD	24.8 ± 3.2	24.2 ± 4.2	0.609
≥ 25.0 kg/m^2, *n* (%)	14 (46.7)	12 (38.7)	0.445
< 25.0 kg/m^2, *n* (%)	16 (53.3)	19 (61.3)	0.585
Peak GH (μg/L), mean ± SD	0.25 ± 0.56	0.17 ± 0.27	0.171

Table 2. *Body composition measurements, from dual-energy X-ray absorptiometry, at baseline, by gender and assigned treatment (mean ± SD [median])*

	Males		Females	
	GH	Placebo	GH	Placebo
Fat mass (kg)	17.6 ± 5.1 (19.4)	17.8 ± 5.8 (18.9)	20.4 ± 5.4 (18.6)	22.1 ± 8.0 (20.7)
Fat mass (%)	27.1 ± 5.6 (27.1)	32.0 ± 8.8 (30.4)	39.5 ± 6.3 (38.8)	38.6 ± 7.3 (41.2)
Trunk fat mass (kg)	9.6 ± 3.1 (9.4)	8.8 ± 3.3 (9.0)	10.0 ± 2.8 (9.1)	11.3 ± 3.9 (11.6)
Trunk fat mass (%)	27.7 ± 5.9 (28.0)	30.3 ± 9.4 (27.8)	37.5 ± 7.5 (37.8)	37.9 ± 7.8 (40.6)
Lean body mass (kg)	44.9 ± 7.4 (4.2)	36.6± 10.1 (3.3)	29.7 ± 5.8 (3.1)	32.0 ± 5.1 (3.0)
Lean body mass (%)	70.0 ± 5.6 (70.3)	65.2 ± 8.7 (67.1)	57.8 ± 6.1 (58.1)	58.7 ± 6.9 (56.4)

There were three GH-treated and four placebo-treated patients who did not complete the 24-week study; the reason for discontinuation was an adverse event for one placebo patient and withdrawal of patient consent for the remainder. With GH treatment, serum IGF-I levels increased significantly in the GH-treated group (Table 3) overall, as well as for both CO and AO patients. Values at Week 12 were not as high as at Week 24, reflecting the stepwise increase in dosage. Overall, AO patients had higher mean IGF-I concentrations and SD scores than CO patients at baseline as well as under GH replacement. At the 24-week endpoint, AO patients had a mean SD score of 2.74 ± 1.84 and CO a mean SD score of –0.07 ± 1.60.

The primary efficacy measure was the change in percentage of fat mass in the trunk with GH treatment versus placebo. Mean changes from baseline to Week 12 and to the endpoint at Week 24 are presented in Table 4, together with the changes from baseline for total body FM and LBM. At Week 24, GH-treated patients had lost 3.4 ± 0.6% of trunk fat and the placebo-treated group had gained 0.4 ± 0.6%; the difference, 3.8 ± 0.8%, was statistically significant ($p < 0.001$). Over the same time period, GH-treated patients lost 2.8 ± 0.5% of total FM compared with no change (0.0 ± 0.5%) in the placebo group ($p < 0.001$), which indicated that the trunk fat reduction was more pronounced that the overall body fat reduction. The ratio of % trunk FM/% total FM decreased from baseline to Week 24 by –1.2 ± 0.5 ($p = 0.023$ for within-group change) in the GH-treated patients.

In parallel with the changes in the DXA FM measures, a significant increase in DXA LBM for GH versus placebo was observed. The net increase in LBM after 24 weeks of GH treatment was 1.17 ± 0.28 kg (Table 4).

Table 3. *IGF-I concentrations and SD scores after 12 and 24 weeks of GH or placebo treatment in Japanese CO and AO GHD patients (mean ± SD [median])*

	Childhood onset		Adult onset	
	GH ($n = 16$)	Placebo ($n = 15$)	GH ($n = 13$)	Placebo ($n = 15$)
IGF-I, μg/L				
baseline	58 ± 41 (43)	63 ± 27 (59)	82 ± 42 (77)	71 ± 36 (71)
Week 12	156 ± 86 (140)	69 ± 32 (65)	255 ± 137 (250)	71 ± 35 (71)
Week 24	194 ± 101 (205)	63 ± 29 (57)	337 ± 152 (320)	74 ± 38 (82)
IGF-I SD score				
baseline	–2.71 ± 1.19 (–2.83)	–2.75 ± 0.85 (–2.68)	–1.18 ± 1.05 (–1.32)	–1.62 ± 0.83 (–1.40)
Week 12	–0.70 ± 1.64 (–0.74)	–2.63 ± 0.96 (–2.56)	1.67 ± 1.89 (1.96)	–1.62 ± 0.87 (–1.33)
Week 24	–0.07 ± 1.60 (0.00)	–2.76 ± 0.92 (–2.64)	2.74 ± 1.84 (2.47)	–1.52 ± 0.90 (–1.50)

Table 4. *Change from baseline in body composition measured by DXA after 12 and 24 weeks of GH or placebo treatment of adult Japanese GHD patients*

	GH	Placebo	*p*-value*
Total fat mass (kg)			
Week 12	–0.72 ± 0.19	–0.21 ± 0.19	0.069
Week 24	–1.64 ± 3.20	0.18 ± 0.31	< 0.001
Total fat mass (%)			
Week 12	–1.3 ± 0.3	–0.4 ± 0.3	0.032
Week 24	–2.8 ± 0.5	0.0 ± 0.5	< 0.001
Trunk fat mass (kg)			
Week 12	–0.46 ± 0.13	–0.16 ± 0.12	0.112
Week 24	–1.05 ± 0.19	0.12 ± 0.19	< 0.001
Trunk fat mass (%)			
Week 12	–1.6 ± 0.4	–0.3 ± 0.4	0.009
Week 24	–3.4 ± 0.6	0.4 ± 0.6	< 0.001
Lean body mass (kg)			
Week 12	0.78 ± 0.21	0.36 ± 0.20	0.155
Week 24	1.17 ± 0.28	0.37 ± 0.27	0.046
Lean body mass (%)			
Week 12	1.3 ± 0.3	0.4 ± 0.3	0.033
Week 24	2.8 ± 0.5	–0.0 ± 0.5	< 0.001

*p-value for between group difference in change from baseline, by ANOVA adjusted for gender and GHD onset

Baseline values for serum total cholesterol and LDL-cholesterol concentrations were comparable in GH and placebo patients (Table 5). Serum total cholesterol was significantly ($p < 0.001$) decreased, from 217 ± 36 mg/dL to 192 ± 35 mg/dL, in the GH-treated patients and remained virtually unchanged in the placebo group. The between-group difference for the change from baseline to Week 24 was highly significant ($p < 0.001$). The same pattern of change was also seen for LDL-cholesterol values. There were no significant within-group changes or between-group differences for HDL-cholesterol (data not shown).

Treatment emergent adverse events were reported by 28 of 30 patients (93.3%) in the GH group and 27 of 31 subjects (87.1%) in the placebo group. There were no significant differences between the two groups in the incidence of adverse events or the incidence of events considered to have a possible causal relationship to the investigational product. In the GH group all adverse events were mild or moderate, and none was severe. Discontinuation due to adverse events was necessary in only one patient who developed a subdural haematoma during placebo treatment. One patient had increased HbA_{1C} level with hyperglycaemia that required a GH dose decrease, and one patient experienced a decrease in the T_4 level requiring an increase in the dose of thyroid hormone.

Discussion

The main purpose of the present study was to assess the short term effect of GH replacement on obesity status in a cohort of adult GHD Japanese patients using a double-blind, placebo-controlled design. In the Japanese population, obesity-associated risk increases at lower levels of BMI, waist or waist/hip ratio than in Caucasians. Obesity-related complications, such as hyperglycaemia, hyperlipidaemia and hypertension, significantly increase in the Japanese population at values higher than 25 kg/m^2 for BMI[15,16]. Using this threshold, it has recently been reported that up to 30% of adult GHD patients were obese[17]. In the present study, an even higher proportion of patients (46.7% males and 38.7% females) had a BMI higher than 25 kg/m^2. Thus, overall obesity is, as in Caucasians, a presenting symptom of adult GHD in Japanese subjects.

In population studies, variables indicating fat distribution, such as waist or waist/hip ratio, are used as predictive factors for cardiovascular risk[18]. These measures, however, are less suitable when precise measurement is required to quantify treatment effects in smaller cohorts and, for this reason, direct quantification of regional fat mass is preferable. Bengtsson *et al.*[3] used CT scanning and showed decreases in abdominal fat accumulation with GH treatment in Caucasian adult GHD patients. We have used DXA trunk fat mass to assess upper body obesity, because this measure has been validated as a good surrogate of abdominal fat mass in healthy Japanese subjects[19]. In addition, cut-off points for trunk fat have been developed for cardiovascular risk in the Japanese population; a cut-off of 7.8 kg trunk fat for males and 8.9 kg for females was reported to increase the age-adjusted risk for cardiovascular complications in Japanese subjects[12]. Median values for trunk fat in the present study indicated that more than 50% of the patients had abnormal upper body fat accumulation and resulting increased cardiovascular risk. Therefore, our data confirm and extend previous descriptions of body composition abnormalities in Japanese patients with the adult GHD syndrome.

Significant changes in body composition occurred with GH replacement, versus placebo, and these changes were in line with other published studies in both Caucasians and Japanese[1,3,17,20–22]. Specifically, the magnitude of the changes at 6 months for LBM and total FM were comparable with those seen in recent studies in Japanese patients, which also used DXA and a similar GH dose regimen but with different GH preparations[17,21]. On average in the present study, total

Table 5. Serum total and LDL-cholesterol concentrations at baseline and after 24 weeks of GH or placebo treatment in Japanese GHD patients

	GH	Placebo	*p*-value
Total cholesterol (mg/dL)			
baseline (mean ± SD)	217 ± 36	222 ± 47	
24-weeks (mean ± SD)	192 ± 35 (196)	224 ± 47	
change (mean ± SE)	−26 ± 5	1 ± 5	< 0.001
LDL-cholesterol (mg/dL)			
baseline (mean ± SD)	126 ± 31	134 ± 42	
24-weeks (mean ± SD)	108 ± 31	139 ± 42	
change (mean ± SE)	−18 ± 4	5 ± 4	< 0.001

Normal ranges: total cholesterol 150–219 mg/dL; LDL-cholesterol 70–139 mg/dL

FM at Week 24 had decreased by 1.64 kg, and trunk fat by 1.05 kg. Since the total FM change included the trunk fat change, it follows that almost 65% of total fat loss took place in the truncal region and, in fact, the change in the ratio of % fat in the trunk/% fat in the body was statistically significant. Thus, a 6 month GH treatment had a statistically and clinically significant effect on body fat distribution in Japanese adult GHD subjects. Baseline total cholesterol and LDL cholesterol concentrations, as well as the magnitude of their change under GH, were comparable with other results and confirm the short-term lipid-lowering effect of GH in Japanese adult GHD patients[17,21].

With the fixed dosage regimen used in the study, mean IGF-I levels were normalised for most patients. Baseline as well as GH-stimulated levels were higher in AO compared with CO patients, consistent with results in Caucasians but also in Japanese[16,19,20–23]. In addition, the median IGF-I SD score at 24 weeks in GH-treated AO patients indicated that a proportion of these patients had an IGF-I level exceeding the upper normal range. However, higher than normal IGF-I levels have previously been reported in studies with fixed dose regimens and, while this cannot be avoided in the context of a double-blind, placebo-controlled trial, an individualised dose regimen as recommended by the GRS guidelines[13] would avoid excessive IGF-I stimulation.

At the time of study, GH replacement in adult GH deficient patients was not an approved indication in Japan. There were 20 study centres involved in recruiting the 61 patients enrolled in the study, which may be a limitation; however, all of the investigators involved in this careful study were highly experienced in the treatment of hypopituitarism. The investigators provided replacement therapies for other missing pituitary hormones, which had to be stable and adequate for at least 3 months before enrolment. While GH is believed to affect the risk of cardiovascular disease directly, it is recognised that this will also be affected by the other elements of the hypopituitarism. GH replacement for adult GH deficient patients has been an approved indication in other countries for some years and the GH doses in the present study were based on previously published results in Caucasian patients. These doses may be higher than optimal but in order to maintain the blind they could not be individualised according to IGF-I and had to be fixed doses.

The safety profile in the present 6-month study does not differ from that reported in other studies with similar design, GH dosage and duration, either in Caucasians or in Japanese. In spite of the chosen dosing algorithm, by which GH dose was progressively increased, reporting rate of adverse events was high (93.3% in the GH-treated and 87.1% in the placebo-treated patients). However, reported adverse events did not disclose any uncommon pattern and the safety profile did not differ from that reported in other similar studies, either in Caucasians or in Japanese. Thus, we assume that the high reporting rate primarily reflected the careful attitude of investigators previously inexperienced with adult GH replacement because this was not an approved indication in Japan at the time of the study.

Conclusion

This study confirms and extends previous studies on the short-term effects of GH replacement in adult Japanese GHD patients. Although overall less obese than Caucasians, Japanese patients with the adult GHD syndrome have abnormal central fat accumulation, which is highly responsive to GH replacement. Follow-up studies will be required to confirm this beneficial effect over the longer term and to show if, as postulated, it will translate into a clinically measurable reduction in cardiovascular risk.

Acknowledgements

Declaration of interest: This study was funded by Sumitomo Pharmaceutical Co., Tokyo, Japan. The authors would like to thank Dr Peter Bates, Cambridge Medical Writing Services, UK, for help in preparation of the manuscript.

The authors are grateful to the following investigators and study sites in Japan, who participated in the study:

Dr K. Fujieda, Asahikawa Medical College, Hokkaido; Dr T. Tajima, Hokkaido University, Hokkaido; Dr T. Watanabe, Fukushima Medical University, Fukushima; Dr Y. Hirata, Tokyo Medical and Dental University, Tokyo; Dr T. Nishikawa, Yokohama Rosai Hospital, Kanagawa; Dr H. Sekihara, Yokohama City University Hospital, Kanagawa; Dr K. Hashizume, Shinshu University, Nagano; Dr Y. Oki and Dr Y. Nakagawa, Hamamatsu University School of Medicine, Shizuoka; Dr M. Mayumi, Fukui Medical University, Fukui; Dr T. Akamizu, Kyoto University, Kyoto; Dr K. Fujita, Osaka City General Hospital, Osaka; Dr S. Ida, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka; Dr K. Iida, Kobe University, Hyogo; Dr H. Sawada, Miyazaki Medical College, Miyazaki; Dr S. Nishiyama and Dr H. Mabe, Kumamoto University, Kumamoto; Dr M. Fukunaga and Dr T. Sone, Kawasaki Medical School, Okayama.

References

1. Carroll PV, Christ ER, the Growth Hormone Research Society Scientific Committee. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. J Clin Endocrinol Metab 1998;83:382-95
2. Shalet SM, Toogood A, Rahim A, Brennan BM. The diagnosis of growth hormone deficiency in children and adults. Endocrine Rev 1998;19:203-23
3. Bengtsson B-A, Eden S, Lonn L, Kvist H, et al. Treatment of adults with growth hormone (GH) deficiency with recombinant human GH. J Clin Endocrinol Metab 1993;76:309-17
4. Larsson B, Svardsudd K, Welin L, Wilhemsen L, et al. Abdominal adipose tissue distribution, obesity, and risk of cardiovascular disease and death: follow-up of participants in the study of men born in 1913. Br Med J 1984;288:1401-4
5. Empana JP, Ducimetiere P, Charles MA, Jouveon X. Sagittal abdominal diameter and risk of sudden death in asymptomatic middle-aged men: the Paris Prospective Study. Circulation 2004;110:2581-5
6. Tomlinson JW, Holden N, Hills RK, Wheatley K, et al. Association between premature mortality and hypopituitarism. Lancet 2001;357:425-31
7. Beshyah SA, Johnston DG. Cardiovascular disease and risk factors in adults with hypopituitarism. Clin Endocrinol 1999;50:1-15
8. Bülow B, Hagmar L, Mikoczy Z, Nordstöm CH, et al. Increased cerebrovascular mortality in patients with hypopituitarism. Clin Endocrinol 1997;46:75-81
9. Murakami Y, Yokoyama T, Oiso Y, Kato Y. Clinical features of adult hypopituitary patients in a national survey. In: Kato Y, editor. Annual report of the Japan Study Group on Hypothalamo-Pituitary Disorders. 2002. p. 170-6 [in Japanese]
10. Murakami Y, Kato Y. Hypercholesterolemia and obesity in adult patients with hypopituitarism: a report of a nation-wide survey in Japan. Endocr J 2003;50:759-65
11. Irie M, Itoh Y, Miyashita Y, Tsushima T, et al. Complications in adults with growth hormone deficiency – a survey study in Japan. Endocr J 2004;51:479-85
12. Ito H, Nakasuga K, Ohshima A, Maruyama T, et al. Detection of cardiovascular risk factors by indices of obesity obtained from anthropometry and dual-energy x-ray absorptiometry in Japanese individuals. Int J Obesity 2003;27:232-7
13. Growth Hormone Research Society. Consensus guidelines for the diagnosis and treatment of adults with growth hormone deficiency: summary statement of the Growth Hormone Research Society workshop on adult growth hormone deficiency. J Clin Endocrinol Metab 1998;83:379-81
14. Shimatsu A, Fujieda K, Haniu K, Tanaka T, et al. Clinical study of measurements of IGF-I, IGF-II and IGFBP-3 using an IRMA kit. Report No 1. Study in adults. Horumon to Rinsho 1996;44:1129-38 [in Japanese]
15. Yoshiike N, Matsumura Y, Zaman MM, Yamaguchi M. Descriptive epidemiology of body mass index in Japanese adults in a representative sample from the National Nutrition Survey 1990–1994. Int J Obesity 1998;22:684-7
16. The Examination Committee of Criteria for 'Obesity Disease' in Japan, Japan Society for the Study of Obesity. New criteria for 'obesity disease' in Japan. Circulation J 2002;66:987-92
17. Chihara K, Koledova E, Shimatsu A, Kato Y, et al. Adult GH deficiency in Japanese patients: effects of GH treatment in a randomised, placebo-controlled trial. Eur J Endocrinol 2004;151:343-50
18. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO Consultation on Obesity. WHO Geneva: WHO Technical Report Series No 894; 1998
19. Ito H, Ohshima A, Ohto N, Ogasawara M, et al. Relation between body composition and age in healthy Japanese subjects. Eur J Clin Nutr 2001;55:462-70
20. Irie M, Shizume K, Takano K, Kato Y, et al. Growth hormone replacement therapy in adults with growth hormone deficiency: a double-blind, placebo-controlled crossover trial in Japan. Endocrinol Metab 1995;2(Suppl B):17-23
21. Chihara K, Kato Y, Kohno H, Takano K, et al. Efficacy and safety of growth hormone (GH) in the treatment of adult Japanese patients with GH deficiency: a randomised, placebo-controlled study. Growth Horm IGF Res 2006;16:132-42
22. Attanasio AF, Lamberts SWJ, Matranga AMC, Birkett MA, et al. Adult growth hormone (GH)-deficient patients demonstrate heterogeneity between childhood onset and adult onset before and during human GH treatment. J Clin Endocrinol Metab 1997;82:82-8
23. Kehely A, Bates PC, Frewer P, Birkett M, et al. Short-term safety and efficacy of human GH replacement in 595 adults with GH deficiency: a comparison of two dosage algorithms. J Clin Endocrinol Metab 2002;87:1974-9

CrossRef links are available in the online published version of this paper:
http://www.cmrojournal.com
Paper CMRO-3533_3, *Accepted for publication*: 15 August 2006
Published Online: 05 September 2006
doi:10.1185/030079906X132460

Endocrine Journal 2006, **53** (6), 853–858

Growth Hormone (GH) Effects on Central Fat Accumulation in Adult Japanese GH Deficient Patients: 6-month Fixed-dose Effects Persist during Second 6-month Individualized-dose Phase

KAZUO CHIHARA, AKIRA SHIMATSU*, YUZURU KATO**, HITOSHI KOHNO***, TOSHIAKI TANAKA#, KAZUE TAKANO## AND MINORU IRIE###

Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan
**Clinical Research Center for Endocrine and Metabolic Disease, Kyoto Medical Center, Kyoto 612-8555, Japan*
***Division of Endocrinology, Metabolism, Hematology and Oncology, Shimane University, Izumo 693-8501, Japan*
****Department of Endocrinology and Metabolism, Fukuoka Children's Hospital, Fukuoka 810-0063, Japan*
#Division of Endocrinology and Metabolism, National Center for Child Health and Development, Tokyo 157-8535, Japan
##Department of Medicine, Institute of Clinical Endocrinology, Tokyo Women's Medical University, Tokyo 162-8666, Japan
###Toho University, Tokyo 143-8540, Japan

Abstract. Both Japanese and Caucasian adults with GH deficiency (GHD) have pronounced abdominal obesity, which is associated with increased risk of cardiovascular complications. We investigated the effects of GH treatment in 27 adult Japanese GHD patients, 15 with adult onset (AO) and 12 with childhood onset (CO) GHD. Patients initially received GH titrated to 0.012 mg/kg/day for 24 weeks in a double-blind design and the dose was then individualized for each patient according to IGF-I for a further 24 weeks. Dual-energy x-ray absorptiometry (DXA) data were evaluated for percentages of trunk fat, total body fat and lean body mass. Serum IGF-I and lipid concentrations were determined at a central laboratory. There were 25 patients who completed 48 weeks of treatment, with 7, 6 and 12 patients then receiving GH at 0.003, 0.006 and 0.012 mg/kg/day, respectively. With the reductions in dose when individualized between weeks 24 and 48, mean serum IGF-I level was reduced and excessively high values, observed in AO patients on the fixed GH dose, were no longer seen. The decrease from baseline in trunk fat was similar at week 24 (–3.8 ± 3.3%, $p<0.001$) and week 48 (–3.1 ± 3.7%, $p<0.001$), and the difference between changes was not significant. Total cholesterol was decreased from baseline by –24 ± 28 mg/dl ($p<0.001$) at week 24 and –17 ± 28 mg/dl ($p = 0.007$) at week 48. Two patients had elevated HbA1c levels: one continued GH treatment after a dose reduction and the other discontinued due to persistent impaired glucose tolerance. Therefore, excessively high IGF-I levels can be avoided by individualized dosing during long-term GH treatment. Individualized dosing maintains the decrease in abdominal fat in adult Japanese GHD patients and should reduce the cardiovascular risk.

Key words: Adult GH deficiency, Trunk fat, GH treatment, Abdominal obesity, Japan

(*Endocrine Journal* **53**: 853–858, 2006)

ABNORMAL body composition with increased body fat mass (FM) and decreased lean body mass (LBM) is one of the characteristic features of the adult GH deficiency (GHD) syndrome [1, 2]. The increase in fat mass in these patients is particularly in the abdominal area [3]. Epidemiological studies have shown that this abdominal obesity is a risk factor for development of metabolic abnormalities leading to cardiovascular complications [4, 5]. In studies in Japanese adult patients with GHD it has been shown that there is an increased risk of cardio- and cerebro-vascular morbidity and

Received: January 18, 2006
Accepted: September 7, 2006
Correspondence to: Dr. Kazuo CHIHARA, Division of Endocrinology/Metabolism, Neurology and Hematology/Oncology, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

mortality compared to the normal population [6–8], similar to that previously shown in Caucasian patients [5, 9–11].

GH replacement decreases overall fat mass and improves lipid status in adult GHD patients, and in Caucasian patients it has been shown that the fat reduction involves predominantly abdominal fat, thus acting on a primary cause of increased cardiovascular risk [3]. In a previous double-blind, placebo-controlled trial [12] we have shown that short-term GH replacement significantly reduces trunk (*i.e.* abdominal) FM as well as the ratio of trunk FM/total FM in adult GHD patients. In a study by Ito *et al.* [13], these measures have been shown to be better predictors of cardiovascular risk in the Japanese population than overall obesity measures such as body mass index (BMI) and total body FM. In the present study, we report the long-term effects of GH replacement on these measures and show that the beneficial effects on central obesity persist over time and with GH dose adjustment.

Patients and Methods

In this study, 27 Japanese adult GHD patients were treated with GH (Nutropin AQ®, Genentech Inc., San Francisco, USA) for a total of 48 weeks. During the first 24 weeks, patients participated in the GH-treated arm of a randomized, parallel-group, placebo-controlled, double-blind phase [12]; the GH-treated group then entered a 24-week open phase. Twenty Japanese clinical centers participated in the study; all patients gave informed consent and the study was performed with appropriate ethical approval according to the Declaration of Helsinki.

All patients had multiple pituitary hormone deficiency with GHD. Baseline demographic characteristics have been presented previously; there were no significant differences between treatment groups at baseline [12]. Mean age was 39 ± 14 years, the male/female ratio was 15/12, and 15 patients had childhood onset (CO) while 12 had adult onset (AO) GHD. The primary cause of hypopituitarism was tumor for 66.7% of the patients; the remaining patients were either idiopathic (18.5%) or had other causes of hypopituitarism (14.8%). GH treatment was started at a dose of 0.003 mg/kg/day for the first 4 weeks and then increased to 0.006 mg/kg/day until week 12; thereafter GH was given at a dose of 0.012 mg/kg/day until the end of the 24-week placebo-controlled phase. At the end of the double-blind phase, the dose was reduced again to 0.003 mg/kg/day and increases were then performed between weeks 28–48, adjusting the dose for each patient according to their IGF-I levels. This dose adjustment, tailored to the features of individual patients, was based on the GRS Consensus Guidelines [14] where the serum IGF-I level was kept between –2 standard deviations (SD) and +2 SD (normal range of IGF-I level by gender and age).

At the baseline, and the 12, 24, 36 and 48 week visits, LBM, total FM and trunk FM were measured by dual-energy X-ray absorptiometry (DXA). All DXA measurements performed in each investigative center were evaluated centrally; a hard copy of the DXA scan data was submitted to the DXA Central Evaluation Committee, and this Committee was responsible for the analysis of each individual patients' scans in a blinded manner. Serum IGF-I concentrations, as well as triglyceride and total, HDL- and LDL-cholesterol levels, were measured centrally (Hachioji Laboratories, SRL Inc., Japan).

Safety was assessed by recording of treatment-emergent adverse events, laboratory test values and blood pressure. Laboratory determinations were performed centrally and included measurements of liver and kidney function, thyroid hormones and glycosylated haemoglobin (HbA1c) concentrations.

Standard deviation (SD) scores were calculated from serum IGF-I concentrations by comparison to age- and gender-matched subjects [15]. Changes from baseline and differences between time points were analyzed from Student t-tests. All results were analysed on the full dataset on an intent-to-treat basis and assessed at a 2-sided significant level of 5%.

Results

Fixed vs. individualized GH dose regimen and IGF-I patterns

The patients were on a fixed GH dose regimen during the first 24 weeks, with a final dose of 0.012 mg/kg/day; this dose was then adjusted to maintain a normal IGF-I level from the beginning of the open-label phase. As a result, at the end of the 48-week study period only 12 patients (48.0%) were still on the full dose of 0.012 mg/kg/day. For the remaining patients, 6 (23.0%) had moved to 0.006 mg/kg/day and 7 (28.0%)