Childhood malignant disease and consequences for growth hormone secretion, intellectual function and cardiovascular risk

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2005

Citation for published version (APA):
Link, K. (2005). Childhood malignant disease and consequences for growth hormone secretion, intellectual function and cardiovascular risk Lund University, Faculty of Medicine Doctoral Dissertation Series
Survivors of childhood acute lymphoblastic leukaemia, with radiation-induced GH deficiency, exhibit hyperleptinaemia and impaired insulin sensitivity, unaffected by 12 months of GH treatment

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(Received 18 May 2004; returned for revision 28 June 2004; finally revised 13 August 2004; accepted 1 September 2004)

Summary

OBJECTIVE Adult survivors of childhood acute lymphoblastic leukaemia (ALL) often exhibit GH deficiency (GHD), due to prophylactic cranial radiotherapy (CRT). It is not known whether the observed risk for adiposity in these patients is associated with impaired insulin sensitivity and whether the insulin sensitivity is affected by GH replacement therapy.

SUBJECTS AND DESIGN Eleven patients with GHD (median age 29 years), previously given prophylactic CRT for ALL, and 11 sex-, age- and body mass index (BMI)-matched controls were investigated with bioimpedance analysis (BIA) and analysis of serum leptin, serum free fatty acids (FFA) and serum insulin. Insulin sensitivity was measured by a euglycaemic–hyperinsulinaemic clamp technique (IS-clamp). Moreover, the effects of 12 months of individually titrated GH treatment (median dose 0.5 mg/day) on these parameters were investigated.

RESULTS At baseline, the patients had lower fat free mass (FFM) (P = 0.003), higher percentage fat mass (FM) (P = 0.05), serum insulin (P = 0.02) and serum leptin/kg FM (P = 0.01) than controls. The patients had a tendency towards impaired IS-clamp (P = 0.06), which disappeared after correction for body composition (IS-clamp/kg FFM; P > 0.5). In the patients, time since CRT was positively correlated with percentage FM (r = 0.70, P = 0.02), and there was an independent negative association between serum FFA and IS-clamp (P = 0.05). Twelve months of GH treatment increased serum IGF-I (P = 0.003) and FFM (P = 0.02) and decreased percentage FM (P = 0.03), but no significant changes were seen in serum leptin/kg FM, serum FFA, FFA-clamp, serum insulin or IS-clamp (all, P ≥ 0.2).

CONCLUSIONS Young adult survivors of childhood ALL with GHD had increased fat mass, hyperleptinaemia and impaired insulin sensitivity, which could be a consequence of radiation-induced impairment of GH secretion or mediated by other hypothalamic dysfunctions, such as leptin resistance or other unknown factors, affected by CRT. Twelve months of individualized GH replacement therapy led to positive effects on body composition, but the hyperleptinaemia, hyperinsulinaemia and the impaired insulin sensitivity remained unchanged.

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Treatment of childhood acute lymphoblastic leukaemia (ALL), including multiagent chemotherapy and prophylactic cranial radiotherapy (CRT), has markedly improved the survival rate for children with ALL (Chessells et al., 1995). However, CRT in children frequently causes abnormal hypothalamic–pituitary function later in life (Constine et al., 1993) and GH deficiency (GHD) has been shown in childhood ALL even after low doses of CRT (Moëll et al., 1988; Brennan et al., 1998).

Adult hypopituitary patients, previously treated for pituitary tumours on conventional hormone replacement for multiple pituitary deficiencies, but without GH substitution, are insulin resistant (Johansson et al., 1995; Hew et al., 1996; Hwu et al., 1997). Whether survivors of childhood ALL, with a more limited reduction of pituitary function, have impaired insulin sensitivity has not yet been thoroughly investigated.

Leptin is produced by adipocytes and inhibits food intake via interaction with receptors at the hypothalamus (Zhang et al., 1994). Thus, leptin, like GH, plays an important role in the regulation of body composition and carbohydrate metabolism. It has been indicated that GHD patients, previously treated with prophylactic CRT for ALL, have elevated levels of leptin per unit fat mass (Brennan et al., 1999). It is, however, not known whether the hyperleptinaemia is caused by an altered regulation of leptin expression, due to GHD, or by leptin insensitivity at the hypothalamus caused by the irradiation. It would therefore be of interest to examine whether the hyperleptinaemia is reversible on GH replacement.
Despite beneficial effects on body composition by GH replacement (Jørgensen et al., 1989; Bengtsson et al., 1993), either deteriorated (Fowelin et al., 1993; Beshyah et al., 1995; Weaver et al., 1995; Christopher et al., 1998; Rosenfalck et al., 2000) or unaltered insulin sensitivity (McConnell et al., 2001; Khalifallah et al., 2001; Svensson et al., 2002) has been recorded. As it is considered that insulin resistance is an independent risk factor for cardiovascular disease (Reaven, 1988), we believe that it is of importance to investigate the effects on insulin sensitivity during GH replacement, especially in this group of young ALL survivors who are found to have additional cardiovascular risk factors (Oeffinger et al., 2001; Link et al., 2004).

The purpose of this study was therefore to assess whether young adult survivors of childhood ALL with irradiation-induced GHD have increased adiposity, changes in leptin, insulin and insulin sensitivity, compared to matched controls, and if so, to investigate whether these alterations are reversible on individualized GH treatment.

Subjects and methods

Study group

The patients and controls gave their written informed consent and the study was approved by the Ethics Committee of the University of Lund. Characteristics of the 11 patients (10 females) are shown in Table 1. Median age at start of the investigation was 29 years (range 25–33 years). The patients were previously treated for ALL between the years 1972 and 1985, and the median age at diagnosis was 5 years (range 1–18 years). All patients were treated with prophylactic CRT, median dose 24 Gy (range 18–24 Gy) and the median time since CRT was 23 years (range 14–28 years). One patient (no. 3) had received spinal irradiation (23 Gy) and one patient (no. 7) was treated with GH from age 12 to 14 years. Chemotherapy was given according to the common protocols in the five Nordic countries (Gustafsson et al., 1981, 1987, 1998) to patients 7 and 11, and in the remaining patients according to the protocols of the Swedish Child Leukaemia Group (Gustafsson et al., 1981); for both protocols remission was induced with prednisolone and vincristine, and in some patients with doxorubicin and asparaginase. When consolidation therapy was given, it consisted of an intermediate dose of methotrexate, with citrovorum factor rescue, or of cyclophosphamide and cytostine arabinosides. The maintenance therapy included pentoxyl, methotrexate and sendoxan and CNS therapy included intrathecal methotrexate. All patients were in first remission. Median time between maintenance therapy and the present investigation was 20.5 years (range 13.5–22.5 years).

For each of the 11 patients, 10 potential control subjects, matched for sex and age, were selected randomly from a computerized register comprising the population in the catchment area for the patients. These control subjects were then contacted by telephone, complementary matching was made for body mass index (BMI) and the first eligible control who agreed to participate in the study was chosen. A difference in BMI in patients and controls of ±1.0 kg/m² or less was allowed.

At the time of the investigation four of the female patients had treatment with oral contraceptives [levonorgestrel + ethinylestradiol (n = 3), norethisterone + ethinylestradiol (n = 1)] compared to six of the female controls [levonorgestrel + ethinylestradiol (n = 4), norethisterone + ethinylestradiol (n = 1), desogestrel + ethinylestradiol (n = 1)]. Normal menstrual cyclic activity was present in all females prior to institution of oral contraceptives and in the females not receiving reproductive hormone treatment.

Table 1 Clinical characteristics and GH status in 11 patients treated for childhood acute lymphoblastic leukaemia (ALL)

<table>
<thead>
<tr>
<th>Patient no./Sex</th>
<th>Age at present investigation (years)</th>
<th>Age at diagnosis of ALL (years)</th>
<th>CRT dose (Gy)</th>
<th>Time since CRT (years)</th>
<th>GH peak after GHRH + arginine test (µg/l)</th>
<th>GH peak after insulin tolerance test (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F</td>
<td>31</td>
<td>7</td>
<td>24</td>
<td>23</td>
<td>6.2</td>
<td>2.2</td>
</tr>
<tr>
<td>2/F</td>
<td>25</td>
<td>4</td>
<td>24</td>
<td>21</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>3/F</td>
<td>33</td>
<td>4</td>
<td>23</td>
<td>28</td>
<td>4.2</td>
<td>1.0</td>
</tr>
<tr>
<td>4/F</td>
<td>30</td>
<td>6</td>
<td>24</td>
<td>23</td>
<td>6.2</td>
<td>1.3</td>
</tr>
<tr>
<td>5/F</td>
<td>32</td>
<td>7</td>
<td>24</td>
<td>25</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>6/F</td>
<td>26</td>
<td>6</td>
<td>24</td>
<td>20</td>
<td>9.6</td>
<td>2.0</td>
</tr>
<tr>
<td>7/F</td>
<td>25</td>
<td>1</td>
<td>20</td>
<td>23</td>
<td>9.2</td>
<td>0.2</td>
</tr>
<tr>
<td>8/F</td>
<td>27</td>
<td>3</td>
<td>24</td>
<td>24</td>
<td>2.6</td>
<td>0.4</td>
</tr>
<tr>
<td>9/F</td>
<td>29</td>
<td>5</td>
<td>24</td>
<td>24</td>
<td>10.4</td>
<td>0.5</td>
</tr>
<tr>
<td>10/F</td>
<td>26</td>
<td>2</td>
<td>20</td>
<td>23</td>
<td>12.7</td>
<td>0.8</td>
</tr>
<tr>
<td>11/M</td>
<td>33</td>
<td>18</td>
<td>18</td>
<td>14</td>
<td>8.5</td>
<td>3.0</td>
</tr>
</tbody>
</table>

CRT, cranial radiotherapy.

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Insulin sensitivity in survivors of childhood ALL

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Test (Ghigo et al., 2001) was performed in both patients and controls (Tables 1 and 2).

Study design

Investigations were performed once in the controls (baseline) and twice in the patients (baseline and after 12 months of GH treatment). Patients were treated with open-label biosynthetic human GH (Humatrope, Eli Lilly and Company, Indianapolis, USA) each evening by subcutaneous injection with a commencing dose of 0.1 mg/day. In the female patients the dose was increased over 5 weeks to 0.5 mg/day and in the male patient over 4 weeks to 0.4 mg/day. The dose was thereafter adjusted according to the response in serum IGF-I, which was measured every 3 months, and the aim was a serum IGF-I level in the middle of the normal reference range. At the end of the study the median GH dose was 0.5 mg/day (range 0.4–0.6 mg/day). GH dose reductions were necessary in patients 1 and 10 because of side-effects. The individual final GH doses are shown in Table 2.

BMI, waist–hip ratio (WHR) and body composition

BMI was calculated as kg/m² from body weight and standing height. In the standing position, waist circumference was measured with a soft tape at the level of the umbilicus and hip circumference was measured over the greater trochanters for calculation of the WHR. Body composition was measured in the supine position by bioelectric impedance analysis (BIA) using the BIA 101-S technique (RLI Systems, Detroit, MI, USA) with a 50-kHz, 800-µA instrument; fat mass (FM) and fat free mass (FFM) in kg were determined from equations supplied by the manufacturer and percentages were calculated from body weight.

Euglycaemic–hyperinsulinaemic clamp

Insulin sensitivity was determined by a euglycaemic–hyperinsulinaemic clamp (DeFronzo et al., 1979). After an overnight fast and insertion of catheters into antecubital veins in both arms, baseline samples of glucose and insulin were taken and a constant insulin (Actrapid 100 IU/ml, Novo Nordisk, Bagsvaerd, Denmark) infusion started (0.28 nmol/m² body surface area/min). After 4 min, a variable-rate 20% glucose infusion was added to maintain the blood glucose level at 5.0 mmol/l, as determined every 5 min. Samples for insulin assay were taken at 60 and 120 min. Insulin sensitivity (IS-clamp) was estimated as the glucose infusion rate during the second hour of the clamp divided by the mean 60 and 120 min insulin concentrations. Serum FFA was measured at 60 and 120 min and FFA-clamp was calculated as the mean of these two values.

Biochemical assays

Blood samples were drawn in the morning after an overnight fast. Serum samples were stored at −70 °C until analysis and samples drawn before and after GH treatment were analysed in the same assay, except for GH, IGF-I and blood glucose, which were analysed currently. Serum GH was analysed by an immuno-fluorometric method (Wallac Oy, Turku, Finland); the interassay coefficient of variance (CV) was 3% and the intra-assay CV ≤ 5%.

Serum IGF-I was measured by an immunoradiometric assay (Nichols Institute of Diagnostics, San Juan Capistrano, CA, USA) with intra- and interassay CVs of 13.3% or less. Venous blood glucose was measured with a HemoCue Blood Glucose Analyser (HemoCue, Angelholm, Sweden) (Ashworth et al., 1992). The instrument was calibrated daily using a standard microcuvette and weekly using a haemolysate (Eutrol, Wageningen, the Netherlands) with known glucose concentration. Serum

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Table 2 GH doses and changes in serum IGF-I and percentage fat mass after 12 months of GH treatment in 11 patients with GHD, previously treated for childhood acute lymphoblastic leukemia

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Final GH dose (mg/day)</th>
<th>s-IGF-I baseline/12 months GH (µg/l)</th>
<th>Change in percentage fat mass (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>144/166</td>
<td>−4.1</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
<td>207/311</td>
<td>−3.0</td>
</tr>
<tr>
<td>3</td>
<td>0.4</td>
<td>246/317</td>
<td>−9.0</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>108/254</td>
<td>−5.4</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>124/238</td>
<td>−8.5</td>
</tr>
<tr>
<td>6</td>
<td>0.4</td>
<td>142/183</td>
<td>−1.7</td>
</tr>
<tr>
<td>7</td>
<td>0.6</td>
<td>274/302</td>
<td>−0.6</td>
</tr>
<tr>
<td>8</td>
<td>0.5</td>
<td>111/199</td>
<td>+2.8</td>
</tr>
<tr>
<td>9</td>
<td>0.5</td>
<td>137/259</td>
<td>−1.2</td>
</tr>
<tr>
<td>10</td>
<td>0.4</td>
<td>156/197</td>
<td>−2.1</td>
</tr>
<tr>
<td>11</td>
<td>0.5</td>
<td>150/210</td>
<td>−5.4</td>
</tr>
</tbody>
</table>

Normal reference range for serum IGF-I: 122–400 µg/l.
insulin was measured with a competitive radioimmunoassay (RIA) with intra- and interassay CVs of <7% or less (Thorell & Larson, 1978). Free fatty acids (FFA) were determined spectrophotometrically (Wako Chemicals, Neuss, Germany). Serum leptin was analysed with a double-antibody RIA using rabbit anti-human leptin antibodies, 125I-labelled human leptin as tracer and human leptin as standard (Linco Res., St Charles, MO, USA) as described previously (Ma et al., 1996); the interassay CV was 7%.

**Statistical analysis**

Data are presented as median and range. Comparison of data before and after GH treatment and between patients and controls was made with the Wilcoxon matched-pair signed-rank test. Univariate correlations were assessed using Spearman’s rank order correlation test. A stepwise linear regression model was used to determine variables that predicted IS-clamp in the GHD patients. The level of significance was set at \( P \leq 0.05 \).

**Results**

**Hormone levels**

Baseline hormone levels in patients and controls are shown in Table 3. There was no significant difference in serum IGF-I levels in patients and controls (\( P > 0.5 \)). GH secretion assessed by a GHRH + arginine test showed a significantly lower peak GH response in the patients than in the controls (\( P = 0.005 \)). Serum levels of thyroid hormones, cortisol (both, \( P > 0.2 \)) and testosterone were similar in patients and controls, but serum prolactin was significantly lower in the patients (\( P = 0.02 \)). Peak serum cortisol was measured during the ITT and the response was normal in eight of the patients (≥572 nmol/l) and subnormal in three (range 364–464 nmol/l).

GH treatment for 12 months caused an increase in serum IGF-I (Table 2) in all patients (baseline; median 144 µg/l, range 100–274 µg/l vs. 12 months GH; median 230 µg/l, range 166–317 µg/l, \( P = 0.003 \)).

**Anthropometric data**

Anthropometric data in patients at baseline and after 12 months of GH treatment and in matched controls are shown in Table 4. At baseline, the patients were significantly shorter (\( P = 0.003 \)) and lighter (\( P = 0.007 \)) than the controls, but there was no significant difference in the WHR (\( P = 0.1 \)). The percentage FM was higher (\( P = 0.05 \)) and FFM lower (\( P = 0.003 \)) in the patients than in the controls, whereas no difference was seen in body FM (\( P = 0.5 \)).

Twelve months of GH treatment did not change BMI (\( P = 0.4 \)) or WHR (\( P > 0.5 \)), but significant changes were seen in body composition measured by BIA (Table 4), with a decrease in percentage FM (\( P = 0.03 \)) and an increase in FFM (\( P = 0.02 \)). Individual changes in percentage FM in the patients are shown in Table 2. After GH treatment, FFM was still significantly lower in the patients than in the controls (\( P = 0.003 \)), whereas percentage FM was similar in patients and controls (\( P > 0.5 \)).

**Biochemical parameters and IS-clamp**

Blood glucose was similar in patients and controls (\( P = 0.4 \)), but serum insulin was significantly (\( P = 0.02 \)) higher in the patient group (Table 5).

During the steady state of the clamp the mean blood glucose levels were similar in the patients at baseline (median 5.0 mmol/l; range 4.5–5.2 mmol/l), after 12 months of GH treatment (median 5.1 mmol/l; range 4.8–5.3 mmol/l) and in the controls (median 4.9 mmol/l; range 4.6–5.2 mmol/l). Serum insulin levels during the clamp in the patients were, before GH treatment, median 88 pmol/l; range 62.5–163.0 pmol/l, and after GH treatment, median 77.5 pmol/l; range 47.5–121.0 pmol/l, and in the controls, median 68.5 pmol/l; range 44.5–139.5 pmol/l. There

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Serum hormone levels in 11 patients with GHD, previously treated for childhood acute lymphoblastic leukaemia, and 11 sex-, age- and BMI-matched controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHD patients</td>
<td>Controls</td>
</tr>
<tr>
<td>IGF-I (µg/l)</td>
<td>median (range)</td>
</tr>
<tr>
<td>144 (100–274)</td>
<td>189 (69–275)</td>
</tr>
<tr>
<td>GH peak after GHRH + arginine test (µg/l)*</td>
<td>2 (1.3–12.7)</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>1.7 (0.6–3.9)</td>
</tr>
<tr>
<td>Free T4 (pmol/l)</td>
<td>14.0 (12.0–17.0)</td>
</tr>
<tr>
<td>Free T3 (pmol/l)</td>
<td>5.6 (4.4–7.7)</td>
</tr>
<tr>
<td>Cortisol (nmol/l)</td>
<td>357 (258–1231)</td>
</tr>
<tr>
<td>Prolactin (µg/l)</td>
<td>9 (5–19)</td>
</tr>
<tr>
<td>Testosterone (nmol/l)**</td>
<td>15 0</td>
</tr>
</tbody>
</table>

*Data are missing in one of the control subjects.
**Serum testosterone was only measured in the male subjects.

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Insulin sensitivity in survivors of childhood ALL was a tendency towards a decreased IS-clamp in patients compared with controls (Table 5); however, this did not reach statistical significance \( (P = 0.06) \). This tendency disappeared after correction for body composition (IS-clamp/kg FFM) \( (P > 0.5) \).

Serum levels of fasting FFA, as well as FFA-clamp, were not significantly different (both, \( P \geq 0.2 \)) between the groups (Table 5). Serum leptin was higher in the patients \( (P = 0.02) \). A similar result was observed when leptin was expressed per kg FM \( (P = 0.01) \).

GH treatment for 12 months had no significant effect (all \( P \geq 0.1 \)) on blood glucose, serum insulin, IS-clamp, serum FFA, FFA-clamp, serum leptin or serum leptin/kg FM (Table 5).

Relationships between clinical, anthropometric, biochemical parameters and IS-clamp in the GHD patients before GH treatment

In the patients, at the baseline investigation, time since CRT was significantly positively correlated with percentage FM \( (r = 0.70, P = 0.02) \) and serum FFA \( (r = 0.67, P = 0.02) \) and negatively correlated with IS-clamp \( (r = -0.85, P = 0.001) \). Serum FFA was negatively associated with IS-clamp \( (r = -0.60, P = 0.05) \) (Fig. 2), but there was no significant correlation between percentage FM and IS-clamp \( (r = -0.45, P = 0.2) \). In a stepwise multiple regression model including serum FFA and time since CRT as independent variables and IS-clamp as the dependent variable, serum FFA remained significantly associated with IS-clamp \( (P = 0.05) \), whereas the relationship between time since CRT and IS-clamp was no longer statistically significant \( (P > 0.5) \).

There were no significant correlations between serum IGF-I, peak GH at the GHRH + arginine test or the ITT and IS-clamp \( (r < 0.50, P > 0.1) \).

Before GH treatment, serum leptin was correlated with BMI \( (r = 0.83, P = 0.001) \), FM \( (r = 0.76, P = 0.006) \) and percentage FM \( (r = 0.82, P = 0.002) \), but not significantly with serum insulin \( (r = 0.56, P = 0.07) \) or IS-clamp \( (r = -0.32, P = 0.3) \).
The present study revealed that young adult survivors of childhood ALL with GHD, due to primarily hypothalamic dysfunction, had similar fasting blood glucose levels as sex-, age- and BMI-matched controls, but significantly higher serum insulin levels. A rise in serum insulin in the presence of normoglycaemia is a sensitive marker of a deterioration in insulin sensitivity and points towards insulin resistance at baseline levels of insulin. To further examine insulin sensitivity in this patient group, we performed a euglycaemic–hyperinsulinaemic clamp. We then found that when the glucose infusion rate during the clamp was divided by the insulin levels, a tendency, although not significant ($P = 0.06$), towards insulin resistance was evident in the patients. The failure to reach significance is probably related to the low number of subjects, but may also be explained by the use of high insulin levels. Nevertheless, the clear tendency towards reduced insulin sensitivity in association with the increased basal insulinaemia suggests to us that reduced insulin sensitivity must be associated with these patients.

All of the patients had severe GHD according to the results of the ITT. It has been shown that the hypothalamus is more sensitive to irradiation than the pituitary (Constine et al., 1993; Darzy et al., 2003). The observed lower peak GH response after ITT than after the GHRH + arginine test (Table 1) would support this, as hypoglycaemia causes GH release by actions at the hypothalamic level (Cordido et al., 1990), whereas the GHRH + arginine test also has a direct stimulatory effect on the pituitary (Barinaga et al., 1983).

In the present study the patients were not treated for any additional pituitary insufficiencies, allowing the exclusion of possible effects of glucocorticoids, thyroid hormone and testosterone substitution, which are all known to influence body composition and insulin sensitivity (Saunders et al., 1980; Andrews & Walker, 1999; Livingstone & Collison, 2002). Moreover, a similar number of female patients and controls were treated with oral contraceptives (four patients compared to six controls). The female patients were, however, not studied at the same time of their menstrual cycle, which may have a minor influence on the results, as there is a difference in insulin sensitivity between the follicular and the luteal phases (Diamond et al., 1989). The hypoglycaemia-induced hypocortisolism found in three of the patients could not explain the increase in insulin resistance, due to the insulin-antagonistic effects of cortisol (Cryer & Gerich, 1985). PRL has been shown to cause hyperinsulinaemia and insulin resistance (Pelkonen et al., 1982; Foss et al., 1995). In the present study, however, hyperprolactinaemia seemed not to be contributing to the alteration in insulin sensitivity, as the patients had significantly lower levels of serum PRL than the controls.

The finding in the present study that the patients had a higher percentage FM than their controls, despite similar BMI, explains the increase in insulin resistance, due to the insulin-antagonistic effects of cortisol (Cryer & Gerich, 1985). PRL has been shown to cause hyperinsulinaemia and insulin resistance (Pelkonen et al., 1982; Foss et al., 1995). In the present study, however, hyperprolactinaemia seemed not to be contributing to the alteration in insulin sensitivity, as the patients had significantly lower levels of serum PRL than the controls. The finding in the present study that the patients had a higher percentage FM than their controls, despite similar BMI, accords with the results from other studies (Brennan et al., 1999; Nysom et al., 1999). Nysom et al. (1999) found that in patients treated for ALL, the degree of adiposity was correlated with cranial irradiation or GHD but not with doses of chemotherapy or corticosteroids. This is in line with our results, showing that time since CRT was significantly correlated with percentage FM.

The present study revealed that young adult survivors of childhood ALL with GHD, due to primarily hypothalamic dysfunction, had similar fasting blood glucose levels as sex-, age- and BMI-matched controls, but significantly higher serum insulin levels. A rise in serum insulin in the presence of normoglycaemia is a sensitive marker of a deterioration in insulin sensitivity and points towards insulin resistance at baseline levels of insulin. To further examine insulin sensitivity in this patient group, we performed a euglycaemic–hyperinsulinaemic clamp. We then found that when the glucose infusion rate during the clamp was divided by the insulin levels, a tendency, although not significant ($P = 0.06$), towards insulin resistance was evident in the patients. The failure to reach significance is probably related to the low number of subjects, but may also be explained by the use of high insulin levels. Nevertheless, the clear tendency towards reduced insulin sensitivity in association with the increased basal insulinaemia suggests to us that reduced insulin sensitivity must be associated with these patients.

All of the patients had severe GHD according to the results of the ITT. It has been shown that the hypothalamus is more sensitive to irradiation than the pituitary (Constine et al., 1993; Darzy et al., 2003). The observed lower peak GH response after ITT than after the GHRH + arginine test (Table 1) would support this, as hypoglycaemia causes GH release by actions at the hypothalamic level (Cordido et al., 1990), whereas the GHRH + arginine test also has a direct stimulatory effect on the pituitary (Barinaga et al., 1983).

In the present study the patients were not treated for any additional pituitary insufficiencies, allowing the exclusion of possible effects of glucocorticoids, thyroid hormone and testosterone substitution, which are all known to influence body composition and insulin sensitivity (Saunders et al., 1980; Andrews & Walker, 1999; Livingstone & Collison, 2002). Moreover, a similar number of female patients and controls were treated with oral contraceptives (four patients compared to six controls). The female patients were, however, not studied at the same time of their menstrual cycle, which may have a minor influence on the results, as there is a difference in insulin sensitivity between the follicular and the luteal phases (Diamond et al., 1989). The hypoglycaemia-induced hypocortisolism found in three of the patients could not explain the increase in insulin resistance, due to the insulin-antagonistic effects of cortisol (Cryer & Gerich, 1985). PRL has been shown to cause hyperinsulinaemia and insulin resistance (Pelkonen et al., 1982; Foss et al., 1995). In the present study, however, hyperprolactinaemia seemed not to be contributing to the alteration in insulin sensitivity, as the patients had significantly lower levels of serum PRL than the controls.

The finding in the present study that the patients had a higher percentage FM than their controls, despite similar BMI, accords with the results from other studies (Brennan et al., 1999; Nysom et al., 1999). Nysom et al. (1999) found that in patients treated for ALL, the degree of adiposity was correlated with cranial irradiation or GHD but not with doses of chemotherapy or corticosteroids. This is in line with our results, showing that time since CRT was significantly correlated with percentage FM.

It is well known that adult patients with GHD have changes in body composition, with an increase in fat mass and a reduction in lean body mass, reversible on GH treatment (Jørgensen et al., 1989; Bengtsson et al., 1993). Similar changes were also seen after GH treatment in the present study, indicating the importance of GHD for the observed adiposity.

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Leptin insensitivity due to radiation-induced damage of the hypothalamus is another potential cause for the increase in fat mass in the patients investigated. The finding that patients previously treated with CRT for ALL have hyperleptinaemia (Birkebaek et al., 1998; Brennan et al., 1999), which was also seen in the present study, would support this hypothesis. Another possibility for the increase in leptin is the GHD. It has been indicated that GH/IGF-I could have direct regulatory effects on leptin production (Boeni-Schnetzler et al., 1996; Bianda et al., 1997; Elimam et al., 1999), which would suggest that the increase in leptin instead could be a consequence of GHD. In the present study serum leptin per kg FM was not affected by GH treatment, which speaks against the assumption that the elevated leptin production in this group of patients is entirely an effect of GHD.

Recent research has revealed that several neuropeptides besides leptin regulate appetite through actions at the hypothalamus (Hillerbrand et al., 2001), and future studies will show whether these neurotransmitters are affected in this group of irradiated patients.

The patients investigated with isolated GHD had alteration in insulin sensitivity comparable with that seen in GHD patients with replacement therapy for additional pituitary deficiencies (Johansson et al., 1998; Hew et al., 1996; Hwu et al., 1997). In the present study, there were no significant correlations between serum IGF-I, peak GH at the provocative tests (GHRH + arginine test and ITT) and IS-clamp, speaking against a direct effect of GHD on insulin sensitivity. These findings may, however, be difficult to interpret, as the results of these tests are not the whole reflection of GH sufficiency.

Table 6 Effect of GH treatment on glucose homeostasis in some studies of patients with GHD

<table>
<thead>
<tr>
<th>Study</th>
<th>Age (years)</th>
<th>GH dose (mg/day)</th>
<th>Duration of GH treatment</th>
<th>Glucose</th>
<th>Insulin</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fowelin et al. (1993)</td>
<td>9</td>
<td>49 (38–63)</td>
<td>2/2</td>
<td>6 weeks</td>
<td>↑</td>
<td>↑</td>
<td>Clamp</td>
</tr>
<tr>
<td>Weaver et al. (1993)</td>
<td>22</td>
<td>45 (20–60)</td>
<td>0.7</td>
<td>6 months</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
<tr>
<td>Hwu et al. (1997)</td>
<td>9</td>
<td>28 (20–33)</td>
<td>0.6</td>
<td>12 months</td>
<td>⇑</td>
<td>⇑</td>
<td>HOMA</td>
</tr>
<tr>
<td>Christopher et al. (1998)</td>
<td>11</td>
<td>41.7 (41)</td>
<td>0.8</td>
<td>2 years</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
<tr>
<td>Al-Shoumerie et al. (1998)</td>
<td>13</td>
<td>47 (24–65)</td>
<td>0.8</td>
<td>1 year</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
<tr>
<td>Bilow and Erfurth (1999)</td>
<td>10</td>
<td>27 (21–28)</td>
<td>0.5</td>
<td>9 months</td>
<td>⇑</td>
<td>⇑</td>
<td>OGTT</td>
</tr>
<tr>
<td>Rosenfeld et al. (2000)</td>
<td>11</td>
<td>37.6 (26–57)</td>
<td>0.5</td>
<td>2.5 years</td>
<td>⇑</td>
<td>⇑</td>
<td>OGTT</td>
</tr>
<tr>
<td>Chrousildos et al. (2000)</td>
<td>12</td>
<td>52 ± 10</td>
<td>0.7</td>
<td>7 years</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
<tr>
<td>McConnell et al. (2001)</td>
<td>13</td>
<td>47 (34–64)</td>
<td>0.5</td>
<td>6 months</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
<tr>
<td>Khalfallah et al. (2001)</td>
<td>6</td>
<td>39 (27–50)</td>
<td>0.4</td>
<td>1 year</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
<tr>
<td>Svensson et al. (2002)</td>
<td>11</td>
<td>48 (20–62)</td>
<td>0.6</td>
<td>7 years</td>
<td>⇑</td>
<td>⇑</td>
<td>Clamp</td>
</tr>
</tbody>
</table>

Clamp, euglycaemic–hyperinsulinaemic clamp; HOMA, homeostatic model assessment; MIST, modified insulin suppression test; OGTT, oral glucose tolerance test; IS, insulin sensitivity; AUC-g, area under the curve for glucose after an oral glucose tolerance test; AUC-i, area under the curve for insulin after an oral glucose tolerance test.

After correction for body composition (IS-clamp/kg FFM), the clear tendency of a difference in IS-clamp between patients and controls disappeared. This indicates that the impaired insulin sensitivity is primarily caused by the observed alterations in body composition. FFA, which is elevated in obesity (Kelley et al., 2001), may mediate the impaired insulin sensitivity observed in our study; perhaps via an altered Randle cycle (Randle et al., 1963). This would be consistent with the independent negative association between serum FFA and IS-clamp, which was observed at baseline.

In several previous studies, some degree of worsening of insulin sensitivity has been reported after GH treatment in GHD adults (Table 6). In the present study, and in other more recent studies, the GH dose was titrated against IGF-I instead of being based on body weight, and as a consequence there have been less negative effects on the glucose metabolism (Bilow & Erfurth, 1999; Khalfallah et al., 2001; McConnell et al., 2001; Svensson et al., 2002). Thus, despite the powerful anti-insulin effect of GH, insulin sensitivity has been unchanged after GH therapy, the explanation probably being the favourable changes in body composition.

Except for one study (Hwu et al., 1997), an improvement in insulin sensitivity has not been demonstrated after GH treatment in GHD adults (Table 6). In the present study there was a decrease in percentage FM and an increase in FFM, but FFM was still significantly lower in the patients than in the controls after GH treatment. Furthermore, there was no reduction in serum FFA after GH treatment for 12 months, which might also have
contributed to the lack of improvement in insulin sensitivity. There is a possibility that GHD is not the sole factor leading to insulin resistance in this group of patients or that the benefits of GH therapy are outweighed by other negative effects on the carbohydrate metabolism. The control subjects in the present study were only investigated at baseline, which might influence the comparison between the patients post-GH treatment and the control group. However, the relatively short time period of 12 months means that these alterations would probably not change the results significantly.

In summary, young adult survivors of childhood ALL with GHD had impaired insulin sensitivity compared to controls matched for sex, age and BMI; this could be caused by the alterations seen in body composition, including increased FM and decreased FFMI. This might in turn be a consequence of radiation-induced impairment of GH secretion or other hypothalamic dysfunction, such as leptin resistance, or other as yet unknown factors affected by CRT. Twelve months of individualized GH replacement therapy led to positive effects on body composition, but the hyperleptinaemia, hyperinsulinaemia and impaired insulin sensitivity remained unchanged. The reason for the lack of improvement in insulin sensitivity after GH replacement is unknown and needs further investigation.

Acknowledgements
We thank Karin Odh and Lilian Bengtsson for expert technical assistance. The study was supported by Eli Lilly and Company, Swedish Research Council grants (14257 and 6834), and the LLO-foundation, Swedish Diabetes Association, Region Skåne and the Faculty of Medicine, Lund University.

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