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Blunted Response to a Growth Hormone Stimulation Test is Associated with Unfavorable Cardiovascular Risk Factor Profile in Childhood Cancer Survivors

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Abstract

Background—Childhood cancer survivors (CCS) are at risk for growth hormone (GH) deficiency. CCS are also at increased risk for early mortality from cardiovascular (CV) disease, but the association between GH levels and CV risk remains poorly understood. The goal of this study was to examine the cross-sectional association between stimulated GH levels and CV risk factors in CCS younger than 18 years.

Procedure—276 CCS (147 males, 14.4±2.6 years) ≥5 years after cancer diagnosis, and 208 sibling controls (112 males, 13.6±2.4 years) participated in this cross-sectional study, which included anthropometry, body composition, and metabolic studies. Blunted response (BR) was defined as peak GH level <7 μg/L after clonidine and arginine. Insulin sensitivity (M_{lbm}) was measured by euglycemic hyperinsulinemic clamp. Statistical analyses used linear and logistic regression accounting for sibling clustering, adjusted for age, sex, Tanner stage, and adiposity.

Results—34 (12%) CCS showed BR to GH stimulation. BR CCS were shorter and had a lower IGF-1 than controls; only 6 of 34 received cranial radiation therapy. CCS with normal stimulated GH response were similar to controls for CV risk factors. Conversely, BR CCS had greater adiposity, higher lipids, and lower M_{lbm} than controls. Differences in lipids and M_{lbm} between BR CCS and controls remained significant after adjustment for BMI or visceral fat.

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CONFLICT OF INTEREST STATEMENT
Nothing to declare

Conclusions—Blunted response to GH stimulation is prevalent in CCS youth and is associated with an unfavorable CV risk factor profile. Further studies are needed to establish the mechanisms of these associations.

Keywords

cardiovascular risk; growth hormone deficiency; cancer survivors; chemotherapy; insulin resistance; children

INTRODUCTION

Advances in cancer treatment, with high cure rates and improved survival, have resulted in a growing population of childhood cancer survivors (CCS), but have also led to an increase in premature morbidity and mortality due to late effects of treatment. Long-term follow-up studies have found cardiovascular (CV) disease among the leading causes of non-relapse mortality in CCS [1]. While overt CV disease usually does not appear until later in life, CV risk factors (obesity, hypertension, dyslipidemia, insulin resistance) can already be identified during childhood and young adulthood [2,3]. Since pathogenic processes leading to CV disease develop over time, early identification of CCS at risk, before establishment of overt disease, may provide an opportunity to prevent or halt CV disease progression.

Most investigations have studied CCS as adults, many of whom had already developed overt CV disease. A recent study showed that CCS during childhood have a higher burden of CV risk factors including greater adiposity, higher total cholesterol, low-density lipoprotein cholesterol (LDL-C), and triglycerides, and lower insulin sensitivity compared to healthy age and gender matched controls [3]. The cause of these metabolic alterations in CCS is not known, but they are likely related to cancer, secondary treatment effects, or both.

One of the most common endocrine complications of cancer treatment, affecting 30–40% of survivors, is growth hormone deficiency (GHD) [4]. GHD adults have increased adiposity (particularly in the visceral component), dyslipidemia (elevated concentrations of total cholesterol, LDL-C, triglycerides, and low high-density lipoprotein cholesterol [HDL-C]), and insulin resistance. Because CCS have increased prevalence of GHD, it has been speculated that GHD plays a role in development of CV risk in CCS [5–8]. However, the direct association of stimulated GH secretion with CV risk factors has not been examined in CCS during childhood. Importantly, this report compares CCS with a normal GH response to those with a blunted GH response and to controls, younger than age 18, by a thorough evaluation of CV risk factors including hyperinsulinemic euglycemic clamp studies as a measure of insulin sensitivity.

The present study's purpose was to examine: 1) the association of CV risk factors with GH secretion in response to GH stimulation test, and 2) whether this association is affected by adiposity in CCS under 18 years of age at examination. We hypothesized that a blunted response (BR) to GH stimulation test is associated with unfavorable levels of CV risk factors in CCS and that adiposity, albeit contributing to this association, does not entirely explain this relationship.

METHODS

Participants

This study was approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota Medical Center and Children's Hospitals and Clinics of Minnesota. Consent (and assent when appropriate) was obtained from children and their

parent/guardian(s). Participants were CCS, age 9–18 years at time of study, in remission, at least five years after cancer diagnosis, who had received treatment at the University of Minnesota Medical Center or the Children's Hospitals and Clinics of Minnesota. Recipients of hematopoietic cell transplant were excluded. A control group consisted of healthy siblings age 9–18 years who had never had cancer. Of 723 eligible CCS, 66 could not be located. The remaining 657 were contacted; consent was obtained from 319 (49%) CCS and 208 sibling controls. The 319 CCS participants and 338 CCS non-participants did not differ significantly in age, sex, race, diagnosis, age at diagnosis, or time from diagnosis to study evaluation. In the current report, 276 of the 319 CCS were included; 43 were excluded due to GH treatment previously or at the time of the study (n=36), or inability to perform GH stimulation test due to poor intravenous access, scheduling conflicts, or lab error (n=7).

Study procedures

All participants (CCS and controls) had height, weight, and waist circumference measured. Percent fat mass (PFM) and lean body mass (LBM) were estimated using dual-energy X-ray absorptiometry (DXA, Lunar Prodigy scanner, software version 9.3, General Electric Medical Systems, Madison, WI, USA). Visceral fat mass (VFM) was estimated with volumetrics from a single-slice abdominal computed tomography scan without contrast, with a Siemens Somaton Sensation 40 slice (Siemens Medical Solutions USA, Inc., Malvern, PA, USA). Systolic blood pressure was the average of two measurements from the right arm of rested, seated subjects. Euglycemic hyperinsulinemic clamps were performed after a 10–12 hour overnight fast to assess insulin sensitivity as previously described [3,9]. Insulin sensitivity was determined from the amount of glucose required to maintain euglycemia over the final 40 minutes of the clamp study, expressed as mg/kg/min of glucose per LBM (M_{lbm}). Plasma glucose was measured at bedside using a Beckman Glucose Analyzer II (Beckman Instruments Inc, Fullerton, CA). A correction factor (1.0278 × bedside glucose −15.029) was applied to adjust for differences in measurements between the bedside method and the central laboratory based on a randomly chosen subset of subjects. Serum insulin was determined by chemoluminescence immunoassay (Immulite Insulin DPC, Los Angeles, CA, USA). Serum lipids were analyzed from fasting blood samples, using a Vitros 5600 (Ortho-Clinical Diagnostics, Inc., Rochester, NY, USA). LDL-C was calculated by the Friedewald equation. Free thyroxine (free T4) was measured by competitive immunoassay. The following hormones were measured by chemiluminescent immunoassay: follicle stimulating hormone (FSH), insulin-like growth factor-1 (IGF-1), insulin-like growth factor binding protein-3 (IGFBP-3), and GH. Tanner staging of pubertal development was performed by trained pediatric providers and was based on breast and pubic hair development in girls and pubic hair development in boys. Exclusion of genital examination was found to improve patient compliance with the study protocol. Furthermore, testicular size may not be a reliable measure of pubertal development in boys who received chemotherapy due to seminiferous tubule dysfunction, which results in reduced testicular size [10]. Bone age X-ray was obtained as part of growth assessment.

GH stimulation test using clonidine and arginine [11] was performed in CCS only. Testing of healthy controls was not performed due to the potential for adverse reactions, namely hypotension. GH level was obtained at baseline, then +30, +60, +90 and +120 minutes after clonidine (5 mcg/kg up to 200 mcg by mouth), followed by infusion of arginine (0.5 grams/kg up to 30 grams) right after the +120 minutes blood draw, with GH levels at +140, +150, +160, +180, +210, and +240 minutes. Since the majority of subjects in all three groups were pubertal at study visit, no estrogen priming was deemed necessary.

A blunted GH response (BR) was defined as a stimulated GH level <7 μg/L, and a stimulated GH level ≥7 μg/L was defined as a normal GH response (NR) [12]. This conservative definition, compared to the usual cutoff of 10 μg/L used in clinical practice to

diagnose GHD [13], was chosen because of lack of growth velocity or parental heights that are usually used in combination with the response to a GH stimulation test to diagnose GH deficiency.

Hypothyroidism was defined by treatment with thyroid hormone replacement at the time of evaluation, or free T4 < 0.7 ng/dL (9 pmol/L). Hypogonadism was defined as delayed puberty or amenorrhea by report, or FSH > 40 IU/L in females, or LH > 10 IU/L and testosterone below normal for Tanner stage in males [14–16].

Statistical analysis

All analyses used the SAS system (v. 9.2, SAS Institute, Cary, NC, USA). Height and weight SDs were calculated using 2000 CDC growth charts [17]. Analyses had the form of multivariate linear regression or logistic regression or multinomial regression (depending on the outcome) adjusting for age, sex, and Tanner stage, with some analyses also adjusting for adiposity using BMI and VFM. Generalized estimating equations (GEE) with robust standard errors, accounting for sibship, were used to test the association of GH status and CV risk factors. Adjusted averages for controls, CCS overall, and CCS subgroups are SAS's least-squares means. For simple, unadjusted comparisons among CCS groups but not controls, p-values are from a t-test or Fisher's exact test; for unadjusted comparisons involving CCS groups and controls, p-values are from GEE. For each outcome separately, a Bonferroni correction controlled type I error for pairwise comparisons between patient groups; for analyses of controls and 2 CCS subgroups, a two-sided p-value ≤ 0.0167 (0.05/3) was considered statistically significant. To investigate the association between BR CCS and exposure to chemotherapy, logistic regression including only CCS without CRT was used, adjusting for age, sex, and Tanner stage.

RESULTS

Characteristics of CCS with BR versus NR compared to controls are shown in Table I. Thirty four (12%) of CCS had BR to GH stimulation test. Age at cancer diagnosis did not differ between NR and BR CCS. Age at study was slightly higher in CCS than controls. The mean Tanner stage of pubertal development did not differ between CCS and controls, although NR CCS were slightly more advanced in puberty than controls. The vast majority of patients were pubertal. CCS, particularly those with blunted GH response, were shorter and had lower IGF-1 levels compared to controls. The major cancer diagnostic categories had similar representation in NR and BR groups and included solid tumors (sarcoma, kidney tumors, neuroblastoma, non-Hodgkin's lymphoma, and others), leukemia (acute lymphoblastic and acute myeloid), and CNS tumors (glial tumors, neuroectodermal tumors, retinoblastoma, and others). There was no significant difference in recurrence between NR group (7/242) and BR group (0/34; p=0.6). History of hypothyroidism was more common among CCS than controls, but all participants were euthyroid on thyroid hormone replacement at the study visit. Hypogonadism was also more common among CCS than controls. None of the participants were receiving sex hormones at the study visit (only 2 of 5 in the NR group and 1 of 2 in the BR group had elevated gonadotropin levels).

Table II shows comparisons of CV risk factors between controls and CCS according to GH status. CV risk factors in NR CCS did not differ from controls ("A vs. B" comparisons). In contrast, BR CCS had significantly greater adiposity compared to controls ("A vs. C" comparisons) by all measures and unfavorable lipid profile (higher total cholesterol, LDL-C, and triglycerides and lower HDL-C). BR CCS also were more insulin resistant (lower insulin sensitivity [M_{lbm}] and higher levels of fasting insulin) and had higher systolic blood pressure compared to NR CCS and controls. After adjustment for BMI or VFM, differences between BR CCS and controls remained significant for waist-to-height ratio, total

cholesterol, LDL-C and M_{lbm} (Table II), even after excluding patients with hypogonadism (Supplemental Table I), or after increasing GH cut-off to define BR to <10 μg/L (Supplemental Table II).

We further examined whether there is a linear relationship between stimulated GH levels and CV risk factors. Preliminary analyses using the loess smoother showed straight-line relationships between peak GH and each CV risk factor but with a change of the straight line's slope at peak GH about 12 μg/L, indicating stronger association when the maximum GH response was ≤12 μg/L. Thus, the association between peak GH value and each CV risk factor was analyzed using a linear regression with a change of slope at peak GH of 12 μg/L (Table III). A decrease in GH level was associated with an increase in all measures of body fatness, total cholesterol, LDL-C, triglycerides, fasting insulin, and systolic blood pressure and was associated with a decrease in HDL-C and M_{lbm} after adjustment for age, sex, and Tanner stage. For example, for each unit decrease in GH peak below 12 μg/L, on average BMI was higher by 0.8 kg/m^2, cholesterol was higher by 3.8 mg/dL, and insulin sensitivity (M_{lbm}) was lower by 0.6 mg/kg/min.

Since it has been previously reported that BMI and VFM may have a negative effect on GH secretion [18–21], additional analyses adjusting for BMI and VFM were performed and showed that peak GH levels were independently associated with a number of CV risk factors, including total cholesterol, LDL-C, triglycerides, and insulin sensitivity. Further analyses examined the association between IGF-1 SD and CV risk factors. Low IGF-1 SD (≤ −1.5) was associated with increased cholesterol level and increased VFM.

Regarding the impact of treatment exposures, as expected, the proportion of patients exposed to CRT was higher in BR CCS than in NR CCS (18% vs. 6% of patients, respectively; p=0.035), but the cumulative doses of radiation did not differ significantly between NR and BR CCS. When BR CCS who received CRT were removed from the analysis (N=6), the differences in CV risk factors between BR CCS (N=28) and controls remained significant (Table IV). Likewise, exclusion of patients with CNS tumors did not change the results. The cumulative dose of intrathecal chemotherapy and the proportion of patients who received it were similar between BR and NR groups. CCS exposed to 250–450 mg/m^2 of cisplatinum were overrepresented among BR CCS (4/34 BR CCS [12%] vs. 3/240 NR CCS [1%]; p= 0.008). In analyses adjusted for age at study, sex, Tanner stage, and BMI, in subjects not treated with CRT, there were no significant associations between BR and exposure to any individual chemotherapeutic agent.

DISCUSSION

In this cross-sectional study, the association between a hypothalamic-pituitary response to GH stimulation test and CV risk factors was examined in CCS during childhood. CCS with stimulated GH levels <7 μg/L (BR CCS) had adverse levels of CV risk factors compared to CCS with normal response to GH stimulation test and to controls. In contrast, CV risk factors in NR CCS were similar to controls. BR CCS had elevated total cholesterol, LDL-C, and triglycerides and reduced HDL-C levels, similar to those seen in adults with GHD with or without a history of cancer [5,22,23]. In addition, stimulated GH levels were linearly associated with adverse levels of CV risk factors, particularly for GH levels ≤12 μg/L, with the effect size/slope of the relationship being clinically relevant.

Low IGF-1 was inversely correlated with cholesterol levels and VFM. Studies in adult survivors of childhood cancer have both supported the inverse association between IGF-1 and visceral fat shown in the present study [24], and reported an opposite finding, showing no correlation [25]. It is not clear why the conflicting results were found, but they may be

related to differences in the methods used for visceral fat assessment or methods used for the IGF-1 assays.

Insulin resistance has been associated with adverse CV risk, independent of obesity [26,27]. In the present study, insulin resistance was greater in BR CCS compared to controls and NR CCS. Although insulin resistance is known to increase during puberty [9], it is unlikely to be related to the findings in this study, because Tanner stage of pubertal development was similar between these groups.

Studies in non-CCS children and adults have shown a strong inverse association between GH secretion and adiposity (BMI or VFM) [18–21]. In the current study, after controlling for adiposity, stimulated peak GH levels remained independently associated with adverse levels of CV risk factors, suggesting that adiposity does not entirely explain the association between blunted GH response and the unfavorable of CV risk profile. Because GH is a lipolytic hormone it is not surprising that body fat (particularly VFM) was higher in BR CCS [5,7,8,28–30].

While GHD may increase adiposity, a reverse mechanism of increased adiposity predisposing to blunted GH response is also plausible. The cross-sectional nature of the study design does not allow conclusions about causality or temporal occurrence of changes. However, in the general population obese children are usually taller and have higher IGF-1 levels compared to non-obese controls [31]. In contrast, in this study BR CCS were shorter and had lower IGF-I levels than controls. Nevertheless, in the present cohort of CCS, the results of the GH stimulation test should be interpreted with caution because of a potential for false-positives in children with increased adiposity.

Previous studies established a strong causal link between GHD and CRT [32]. Radiation-induced GHD is thought to be due to hypothalamic damage, because the hypothalamus is very sensitive to radiation, even more so than the pituitary gland [33]. However, in this study only a small minority of CCS with increased CV risk factors had either whole brain or focal brain radiation. Furthermore, when BR CCS who received CRT were removed from the analysis, the differences in CV risk factors between BR CCS and controls remained significant. The etiology of GHD in patients who did not receive CRT is currently unknown [34–36]. While exposure to cisplatinum was more prevalent among BR CCS in this study, in adjusted analyses we did not find a significant association between BR and exposure to any individual chemotherapeutic agent. Because all patients treated with chemotherapy received multi-agent regimens, overlapping toxicities may have precluded the detection of adverse effects by any individual chemotherapeutic agent. It is also conceivable that factors other than chemotherapy adversely affected the hypothalamic-pituitary-growth hormone axis in CCS by either acting directly on the anterior pituitary gland and/or by affecting hypothalamic regulation of GH secretion [37]. In this study, by using arginine, which inhibits somatostatin release, and clonidine, which stimulates α2-adrenergic receptors and increases GH-releasing hormone secretion from the hypothalamus, we were able to show that the hypothalamic-pituitary regulation of GH secretion was impaired. Hypothalamic damage *per se* can also be associated with increased CV mortality and unfavorable CV risk profile (obesity, hyperinsulinemia and insulin resistance) [38–41]. Thus, it remains to be determined whether the impaired GH secretion is causally related to the increased CV risk factor levels or is simply a marker of hypothalamic damage.

There is no consensus in the literature regarding a definitive GH stimulation test and no single GH stimulation test is uniformly used [42,43]. Some stimulation tests carry a risk of hypoglycemia (insulin tolerance test) [44], some can no longer be performed because the stimulant is no longer manufactured (GHRH) [45], and some require overnight

hospitalization (continuous overnight GH monitoring) with no clear advantage over stimulated GH response [46]. We chose a combined clonidine/arginine stimulation test because it is commonly used in children, has low risk of side effects, and has been validated in previous studies [11]. In clinical practice, the clinical diagnosis of GHD is based on a combination of GH stimulation test, auxological criteria, biochemical tests, and radiologic evaluation [13]. In this study, we have used the terminology "blunted GH response" to avoid labeling the patients as GHD based solely on the GH stimulation test. Importantly, our study's results are not intended as an endorsement of treating CCS with GH.

This study has a number of limitations. With a participation rate of 49%, selection bias cannot be excluded, although there were no significant differences between participating and non-participating CCS with respect to age, sex, race, diagnosis, and age at diagnosis. The study complexity, which required a two-day commitment by subjects and their parent/guardian, likely prohibited participation by some eligible subjects. The population was predominantly white non-Hispanic, thus the findings may not generalize to other racial/ethnic groups. Despite adjustment for age and puberty, CCS were slightly older than controls. Sex differences in outcomes were not addressed, due to lack of power for statistical analyses by sex within each GH status category. Lack of repeated measures of height and parental heights limit the ability to interpret the relation of BR to height. Since controls did not have a GH stimulation test, we cannot exclude the possibility that some controls may have had a blunted response. In addition, the sensitivity, specificity, and positive predictive value of the GH stimulation test is variable even at a GH cut-off <7 μg/L [47]. Finally, this was a cross-sectional study at a median of 10 years after cancer diagnosis, therefore the onset of BR in this cohort of CCS is not known.

In summary, the findings of the study highlight the association between blunted GH secretion and unfavorable CV risk factor profile among CCS during childhood. The widely documented tracking of CV risk factors from childhood into adulthood [48,49] and the high risk of premature CV disease in CCS provide a strong rationale for devising methods for early detection of CV risk in this population. The clinically relevant observations of this study are that: a) blunted response to GH stimulation test is common in CCS, even in the absence of CRT, and b) its association with an unfavorable CV risk profile suggests a potentially common mechanism. The current study lays the groundwork for longitudinal studies in CCS with more homogenous diagnostic and treatment profiles, which may help better understand the underlying mechanisms of these associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table I

Characteristics of study participants, controls vs. CCS divided by peak stimulated GH level.

Variable	Controls [A] N=208	NR CCS [B] N=242	BR CCS [C] N=34	p-value		
				A vs B	B vs C	A vs C
Male gender, N (%)	112 (54)	131 (54)	16 (47)	0.95	0.40	0.43
Ethnicity, N (%)						
Non-Hispanic	203 (98)	238 (98)	33 (97)	0.74	0.48	1.0
Hispanic	5 (2)	4 (2)	1 (3)			
Age at cancer diagnosis, yrs±SD	N/A	4.4±3.0	4.4±3.4	--	0.98	--
Age at study, yrs±SD	13.6±2.4	14.4±2.6	14.3±2.9	**0.0007**	0.82	0.21
Bone age at study, yrs±SD	13.8±2.7	14.6±2.6	14.9±2.7	**0.001**	0.50	**0.02**
Tanner stage, mean±SD	3.4±1.5	3.6±1.4	3.5±1.5	0.057	0.59	0.71
Stage, N (%)						
I	33 (16)	23 (10)	5 (15)			
II–III	63 (31)	70 (29)	9 (26)	**0.028**	0.63	0.57
IV–V	110 (53)	149 (61)	20 (59)			
Height SDs, mean±SD	0.4±1.0	0.2±1.0	0.0±1.1	**0.046**	0.25	**0.042**
<−2 SDs, N (%)	0 (0)	4 (2)	2 (6)	0.25	**0.025**	**0.004**
−2 to −1 SDs, N (%)	15 (7)	21 (9)	6 (18)			
IGF-1 SDs, mean±SD	−1.0±0.8	−1.1±0.8	−1.7±0.8	0.18	**0.0004**	**<0.0001**
< −2 SDs, N (%)	11 (5)	20 (8)	11 (32)	0.25	**0.0002**	**<0.0001**
IGFBP-3 SDs, mean±SD	−0.5±0.6	−0.6±0.6	−0.6±0.7	0.30	0.56	0.32
Hypothyroidism, N (%)	1 (0.5)	5 (2.1)	3 (8.8)	0.22	0.062	**0.009**
Hypogonadism, N (%)	0 (0.0)	5 (2.1)	2 (5.9)	0.06	0.21	**0.019**
Type of cancer, N (%)	N/A			--	0.73	--
Solid tumor		105 (43)	13 (38)			

Variable	Controls [A] N=208	NR CCS [B] N=242	BR CCS [C] N=34	p-value		
				A vs B	B vs C	A vs C
Leukemia		87 (36)	12 (35)			
CNS tumor		50 (21)	9 (27)			
Cranial radiation, N (%)						
Whole brain	N/A	6 (3)	3 (9)	--	**0.035**	--
Focal brain		8 (3)	3 (9)			
None		228 (94)	28 (82)			
Cumulative dose, whole brain radiation, cGy						
Mean±SD	N/A	1890±221	2247±408	--	0.089	--
Median		1800	2340			

Unadjusted three-group comparisons: controls vs. CCS with peak GH ≥7 (NR) vs. CCS with peak GH <7 (BR). When all three groups had data, the p-value was computed using GEE except for hypothyroidism and hypogonadism, for which p-values are from Fisher's exact test for analyses including pairs of groups because counts were too low to use GEE. P-value is shown in bold font if it is less than 0.05. When only CCS had data, the p-value was computed using a t-test (age at diagnosis) or Fisher's exact test.

Table II

Comparison of CV risk factors between CCS, divided according to stimulated GH levels, and sibling controls.

Variable	Controls [A] N=208	NR CCS [B] N=242	BR CCS [C] N=34	p-value adjusted for age, sex, and Tanner stage			p-value also adjusted for BMI	
				A vs B	B vs C	A vs C	A vs C	B vs C
BMI (kg/m^2)	20.7±0.3	20.4±0.3	27.3±0.9	0.38	**<0.0001**	**<0.0001**	N/A	N/A
BMI percentile	57.7±2.3	56.4±2.2	85.5±2.8	0.52	**<0.0001**	**<0.0001**	N/A	N/A
Percent fat mass	24.9±0.8	25.2±0.7	39.0±1.4	0.73	**<0.0001**	**<0.0001**	N/A	N/A
Waist (cm)	68.2±0.8	68.1±0.7	82.9±2.1	0.93	**<0.0001**	**<0.0001**	N/A	N/A
Waist percentile	29.5±1.9	30.9±2.0	66.2±4.1	0.50	**<0.0001**	**<0.0001**	N/A	N/A
Waist (cm) to height (cm) ratio	0.43±0.005	0.43±0.004	0.53±0.01	0.31	**<0.0001**	**<0.0001**	**<0.0001** *	**0.0039** *
Abdominal visceral fat (cm^3)	19.7±0.9	18.2±0.7	38.6±3.4	0.12	**<0.0001**	**<0.0001**	0.02	**0.0103**
Total Cholesterol (mg/dL)	147.2±2.1	149.1±1.9	182.0±6.3	0.37	**<0.0001**	**<0.0001**	**0.0001** *	**0.0004** *
LDL-Cholesterol (mg/dL)	84.4±1.9	86.4±1.7	115.6±5.3	0.23	**<0.0001**	**<0.0001**	**0.0001** *	**0.0004** *
HDL-Cholesterol (mg/dL)	47.9±1.0	47.7±0.9	40.2±1.6	0.85	**<0.0001**	**<0.0001**	0.13	0.23
Triglycerides (mg/dL)	76.0±3.2	75.3±3.0	124.4±9.8	0.86	**<0.0001**	**<0.0001**	0.02	0.03
Glucose (mg/dL)	87.5±2.0	85.6±0.7	86.0±1.5	0.22	0.74	0.44	0.05	0.29
Insulin (mU/L)	8.8±0.5	7.5±0.5	18.8±3.1	0.05	**0.0004**	**0.0015**	0.22	0.11
Insulin sensitivity M_{lbm} (mg/kg/min)	14.6±0.4	13.8±0.4	10.8±0.7	0.04	**<0.0001**	**<0.0001**	**0.0029** *	0.06
Systolic BP (mmHg)	108.5±0.7	108.7±0.8	114.8±2.2	0.84	**0.0056**	**0.0044**	0.92	0.86

All values are expressed as mean±SE. Three-group comparisons: controls vs. CCS with peak GH ≥7 (NR) vs. CCS with peak GH <7 (BR) are adjusted for age at study (continuous), gender (male vs. female), and Tanner stage with additional adjustments for body mass index (BMI) and visceral fat mass (VFM). The p-value was calculated from a GEE analysis, which accounts for intra-family correlation. A Bonferroni-adjusted significance threshold for p-value of 0.0167 (shown in bold font) was used to account for 3 comparisons between pairs of groups. Conversion factor to SI units: 0.0259 for total cholesterol, LDL-C and HDL-C (mmol/l), 0.0113 for triglycerides (mmol/l), 0.0555 for glucose (mmol/l), 6.945 for insulin (pmol/l).

*Also significant when adjusted for VFM.

Table III

Linear association between cardiovascular risk factors and peak GH in CCS, allowing different slope (strength of association) for peak GH ≤12 and peak GH >12.

Variable	Peak GH	Adjusting for age, sex, Tanner stage		p-value for slope, also adjusting for BMI or VFM	
		Slope (SE)	p-value	BMI	VFM
BMI (kg/m^2)	≤12	−0.80 (0.094)	**<0.0001**	N/A	**0.0341**
	>12	−0.12 (0.032)	**0.0003**	N/A	0.10
BMI percentile	≤12	−3.06 (0.576)	**<0.0001**	N/A	0.57
	>12	−0.93 (0.196)	**<0.0001**	N/A	**0.001**
Percent fat mass (DXA)	≤12	−1.65 (0.198)	**<0.0001**	**0.0028**	**0.005**
	>12	−0.41 (0.068)	**<0.0001**	**<0.0001**	**<0.0001**
Waist (cm)	≤12	−1.82 (0.220)	**<0.0001**	0.08	N/A
	>12	−0.25 (0.075)	**0.001**	0.63	N/A
Waist percentile	≤12	−4.04 (0.580)	**<0.0001**	0.30	N/A
	>12	−0.83 (0.198)	**<0.0001**	**0.0294**	N/A
Waist-to-height ratio	≤12	−0.01 (0.001)	**<0.0001**	**0.0002**	**0.0031**
	>12	−0.00 (0.000)	**0.0003**	0.27	0.12
Abdominal visceral fat (cm^3)	≤12	−2.65 (0.266)	**<0.0001**	**<0.0001**	N/A
	>12	−0.32 (0.090)	**0.0005**	0.14	N/A
Total Cholesterol (mg/dL)	≤12	−3.76 (0.654)	**<0.0001**	**<0.0001**	**<0.0001**
	>12	−0.18 (0.222)	0.43	0.54	0.62
LDL-C (mg/dL)	≤12	−3.19 (0.572)	**<0.0001**	**<0.0001**	**0.0001**
	>12	−0.16 (0.195)	0.42	0.56	0.57
HDL-C (mg/dL)	≤12	0.92 (0.239)	**0.0002**	0.1242	0.15
	>12	0.07 (0.081)	0.37	0.98	0.87
Triglycerides (mg/dL)	≤12	−7.00 (1.052)	**<0.0001**	**<0.0001**	**0.0014**
	>12	−0.14 (0.358)	0.70	0.52	0.45
Fasting glucose (mg/dL)	≤12	−0.17 (0.184)	0.36	0.89	0.76
	>12	0.03 (0.063)	0.60	0.33	0.34
Fasting insulin (mU/L)	≤12	−1.37 (0.210)	**<0.0001**	**0.0092**	0.39

Variable	Peak GH	Adjusting for age, sex, Tanner stage		p-value for slope, also adjusting for BMI or VFM	
		Slope (SE)	p-value	BMI	VFM
	>12	−0.09 (0.071)	0.20	0.67	0.38
Insulin sensitivity M/lbm (mg/kg/min)	≤12	0.56 (0.101)	**<0.0001**	**0.0005**	**0.0046**
	>12	−0.09 (0.033)	**0.0046**	**0.0005**	**0.0003**
Systolic BP (mmHg)	≤12	−0.93 (0.259)	**0.0004**	0.88	0.28
	>12	0.14 (0.088)	0.11	**0.0012**	**0.0142**

Slopes were obtained from linear regression with adjustment for age, sex, and Tanner stage. For each CV risk factor, the row labeled “Peak GH ≤12” is the rate of change (slope) of the risk factor as a function of peak GH for peak GH ≤12; the row labeled “Peak GH >12” is the rate of change (slope) of the risk factor as a function of peak GH for peak GH >12. P-value is shown in bold font if it is less than 0.05. Some of the associations between stimulated GH levels and CV risk factors remained significant after additional adjustment for body mass index (BMI) or visceral fat mass (VFM).

Table IV

Comparison of CV risk factors between CCS who did not receive cranial radiation, divided according to stimulated GH levels, and sibling controls.

Variable	Controls [A] N=208	NR CCS [B] N=228	BR CCS [C] N=28	p-value adjusted for age, sex, and Tanner stage			p-value also adjusted for BMI	
				A vs B	B vs C	A vs C	A vs C	B vs C
BMI (kg/m^2)	20.7±0.3	20.4±0.3	28.1±0.9	0.50	**<0.0001**	**<0.0001**	N/A	N/A
BMI percentile	57.7±2.3	56.9±2.2	87.6±2.9	0.68	**<0.0001**	**<0.0001**	N/A	N/A
Percent fat mass	24.9±0.8	25.0±0.7	39.6±1.5	0.76	**<0.0001**	**<0.0001**	N/A	N/A
Waist (cm)	68.2±0.8	68.1±0.7	84.7±2.2	1.0	**<0.0001**	**<0.0001**	N/A	N/A
Waist percentile	29.5±1.9	31.3±2.0	71.2±3.9	0.46	**<0.0001**	**<0.0001**	N/A	N/A
Waist (cm) to height (cm) ratio	0.43±0.005	0.43±0.004	0.54±0.01	0.28	**<0.0001**	**<0.0001**	**0.0011**	0.03
Abdominal visceral fat (cm^3)	19.7±0.9	18.0±0.8	40.0±4.0	0.12	**<0.0001**	**<0.0001**	0.08	0.04
Total Cholesterol (mg/dL)	147.2±2.1	149.6±1.9	176.0±5.4	0.24	**<0.0001**	**<0.0001**	**0.0004**	**0.0019**
LDL-Cholesterol (mg/dL)	84.4±1.9	86.7±1.7	111.9±4.7	0.19	**<0.0001**	**<0.0001**	**0.0001**	**0.0009**
HDL-Cholesterol (mg/dL)	47.9±1.0	48.0±0.9	38.4±1.6	0.94	**<0.0001**	**<0.0001**	0.04	0.06
Triglycerides (mg/dL)	76.0±3.2	75.3±3.0	127.8±11.3	0.96	**<0.0001**	**<0.0001**	0.04	0.06
Glucose (mg/dL)	87.5±2.0	85.7±0.7	86.6±1.7	0.29	0.61	0.67	0.13	0.43
Insulin (mU/L)	8.8±0.5	7.6±0.5	20.8±3.7	0.07	**0.0005**	**0.0012**	0.19	0.11
Insulin sensitivity M_{lbm} (mg/kg/min)	14.6±0.4	13.8±0.4	10.4±0.7	0.04	**<0.0001**	**<0.0001**	**0.0023**	0.04
Systolic BP (mmHg)	108.5±0.7	109.0±0.8	116.7±2.3	0.66	**0.001**	**0.0005**	0.49	0.72

All values are expressed as mean±SE. Three-group comparisons: controls vs. CCS with peak GH ≥7 (NR) vs. CCS with peak GH <7 (BR), excluding CCS who received whole or focal brain radiation, adjusted for age at study (continuous), gender (male vs. female), and Tanner stage with an additional adjustment for body mass index (BMI). The p-value was calculated from a GEE analysis, which accounts for intra-family correlation. A Bonferroni-adjusted significance threshold for p-value of 0.0167 (shown in bold font) was used to account for 3 comparisons between pairs of groups. Conversion factor to SI units: 0.0259 for total cholesterol, LDL-C and HDL-C (mmol/l), 0.0113 for triglycerides (mmol/l), 0.0555 for glucose (mmol/l), 6.945 for insulin (pmol/l).