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TITOLO TESI DI DOTTORATO:
Cardio-metabolic risk factors and quality of life in childhood-onset brain tumors with growth hormone deficiency.

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A Roberta, per avermi accompagnato in questi anni

e donato Lorenzo, speranza del mio futuro…

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Index of abbreviations

ACTH = adrenocorticotropic hormone

BMI = body mass index

CBCS = childhood brain cancer survivors

CCS = childhood cancer survivors

CRT = cranial radiotherapy

CVD = cardiovascular disease

DXA = dual-energy X-ray absorptiometry

GH = growth hormone

GHD = GH deficiency

HC = hip circumference

HDL = high-density lipoprotein cholesterol

HOMA IR index = homeostasis model assessment for insulin resistance

hrGH = human recombinant growth hormone

IGFBP3 = Insulin-like growth factor-binding protein 3

IGF1 = insulin-like growth factor 1

LDL = low-density lipoprotein cholesterol

MS = metabolic syndrome

OGTT = oral glucose tolerance test

PAT = peripheral artery tonometry

Quicki = quantitative insulin sensitivity check index

QoL = quality of life

RHI = reactive hyperemia index

ROI = region of interest

SDS = standard deviation score

TSH = thyroid stimulating hormone

VA = visceral adiposity

VWF = von Willebrand Factor

WC = waist circumference

WHO = World Health Organization

Abstract

Background: Several studies have reported an increased incidence of cardiovascular disease (CVD) in childhood brain cancer survivors (CBCS). Growth hormone (GH) replacement therapy may bring benefits to body composition, cardiovascular outcomes, and quality of life (QoL). However, in CBCS the optimal methodology for investigating the multifaceted aspects of visceral adiposity (VA) and the impact of GH on the cardiometabolic risk parameters, is still under investigation.

The aim of the study was to evaluate VA in CBCS with and without GH deficiency (GHD), and its relationship with anthropometric, biophysical and biochemical cardiometabolic parameters.

Methods: All CBCS, at least 2 years after the end of therapy, underwent a clinical evaluation including anthropometric measurements, biochemical metabolic profile, endothelial evaluation, body composition evaluation by Dual-energy X-ray absorptiometry (DXA), and QoL assessment.

Results: We recruited 48 CBCS with GHD (age 16.6 ± 4.9 years): 40 in treatment with human recombinant growth hormone (hrGH) and 8 not in treatment, and 12 controls (age 14 ± 4.7 years). In our study, insulin-like growth factor 1 (IGF1) standard deviation score (SDS) levels and hrGH daily dose showed a negative correlation with the WC and the main body composition parameters. Despite no significant differences in weight, body mass index (BMI) and waist circumference (WC), when compared to patients with GHD - not in treatment with hrGH, patients being treated with hrGH and patients with normal GH-pituitary function had a different body composition characterized by lower VA, higher HDL and adiponectin levels. The GHD negatively affects the QoL and fatigue perceived by parents compared to children in the "social functioning", "school functioning" and "general fatigue" domains.

Conclusion: Our results suggest that GHD is associated with different body composition and higher VA. BMI and WC alone might underestimate the metabolic risk in CBCS. The body composition evaluated by DXA may be a good marker of early cardiometabolic risk in CBCS and could be used to monitor the development of CVD. A tailored therapy with

hrGH aimed at achieving a proper body composition might be necessary to reduce cardiovascular risk in GHD children and adolescent cancer survivors.

Background

In recent years, the overall population of childhood cancer survivors (CCS) has improved. There are over 300 thousand CCS in Europe [1]. In long-term cancer survivors, the cumulative mortality 30 years after diagnosis is close to 20%, the CVDs are the most common causes of premature death [2]. In a large population of CCS, the cumulative incidence of coronary artery disease by the age of 45 years was 5.3% [3]. Furthermore, in CCS, the cumulative cardiovascular mortality does not seem to decrease over time [4] (Figure 1 and 2).

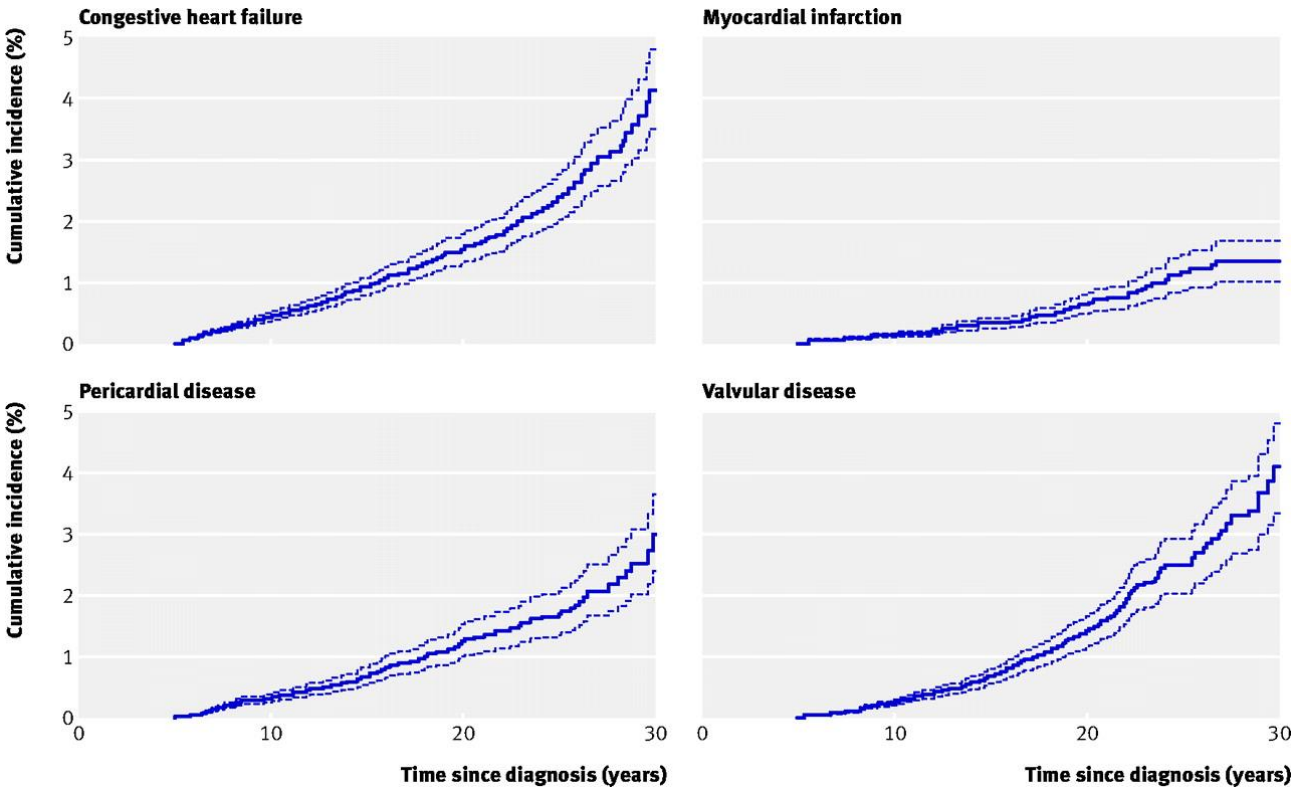

Figure 1. Cumulative incidence and 95% CI of cardiac disorders among childhood cancer survivors [5]

Endocrine late effects are the most common group of chronic conditions in brain CCS, occurring up to 50% of survivors. They include hypothalamic-pituitary, thyroid and gonadal dysfunction, bone disease, and cardiometabolic disorders. Sequelae may be associated both with the tumor type and location. In addition to the effects of surgery or direct endocrine gland involvement by the malignancy, treatments such as cranial radiotherapy (CRT) or chemotherapy may severely increase the risk of endocrine complications. The GHD is the most common anterior pituitary deficiency due to susceptibility of the hypothalamic pituitary axis to radiation damage: >24 Gy the deficiency

usually becomes manifest within 5 years, while for doses in the range of 18-24 Gy the deficiency may become manifest even after decades. Therefore, the risk should be

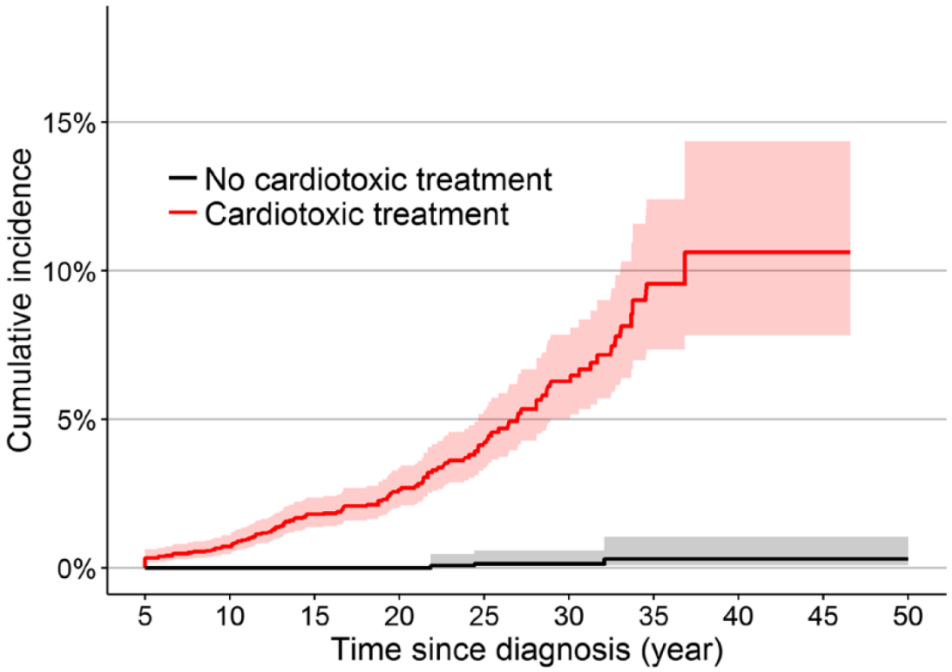

Figure 2. Cumulative incidence and 95% CI of heart failure for cardiotoxic treatment (anthracyclines, mitoxantrone, and radiotherapy involving the heart) with time since childhood cancer diagnosis as time scale. [6]

considered lifelong. There is evidence that survivors with endocrine deficiencies, exposed to CRT and abdominal RT, prolonged steroid treatment, and those with limitations in physical performance are at increased risk of gluco-metabolic disorders. Hypertension, obesity, dyslipidemia, and diabetes, clustered together as the metabolic syndrome (MS), are well-known independent risk factors for CVD [7]. The pathophysiologic process underlying CVD in CCS is multifactorial, in the last decades endothelial disfunction, adiposity and other indices of visceral fat are subjects of several studies due to their strong link with CVD [8].

A correlation between adiposity and CRT, often associated with GHD, has been confirmed in several studies [9, 10]. Long-term follow-up data demonstrate that endocrine late effects (Figure 3) continue to appear even after several years after the end of treatment, causing delays in the diagnosis and treatment of endocrine late effects [11], with potential repercussions on CVD and the QoL. The risk of obesity is higher in the female gender,

especially in children treated at a young age, and is radiation dose- and site-dependent [12, 13, 14].

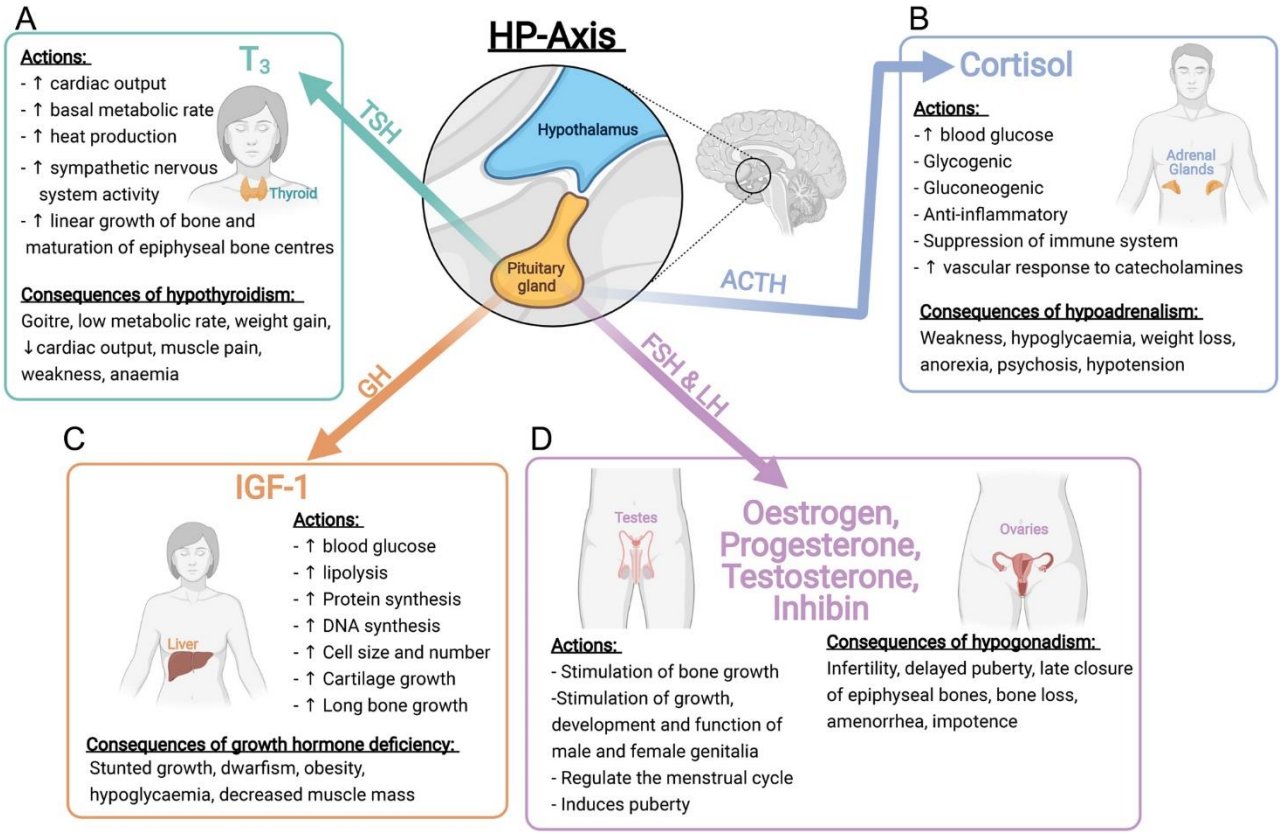

Figure 3. Summary of key hormones of the hypothalamic–pituitary axis, their functions, and the known consequences of their absence. [15]

When compared to BMI, adiposity is a broader term including more accurate anthropometric or densitometric measurements of adipose tissue accumulation, such as WC, waist/hip ratio and fat percentage or body composition assessed by DXA [16, 17, 18]. In CCS the VA, studied using DXA by identifying a non-standardized truncal region of interest (ROI), has been associated to body composition (fat mass) and adiposity (BMI, WC) and was more predictive of biochemical metabolic abnormalities. The multivariate predictive model showed that VA in the ROI was found to be positively related to WC and BMI, and was negatively related to IGF1 SDS levels. Patients with GHD showed an increased VA compared to patients without GHD [9, 19, 20]. This raises the question whether the low dosage of GH prescribed (for safety reasons) could result in an insufficient effect on the metabolism. Therefore, concern about safety might lead to sub-optimal treatment with GH in CCS. However, this hypothesis needs to be explored as studies

evaluating GH effects on cardiometabolic risk factors over time in neuro-oncological patients are still lacking.

QoL is an important measure of the effectiveness of cancer - and late effects - treatment. [21] Decreased QoL is a common feature in childhood and adolescent cancer survivors with GHD and adiposity. However, no studies assessing such outcomes have been conducted specifically in CBCS [22, 23].

The main aim of our study is to evaluate VA in CBCS with and without GHD at least 2 years after the end of therapy (T0). CBCS without GHD will be considered as controls.

The secondary aims of the study were to compare VA, several cardiometabolic parameters and biological markers of GH activity (body composition, lipid metabolism and gluco-metabolic parameters, biochemical and biophysical evaluation of endothelial function), QoL and fatigue in CBCS with GHD, and in the control group, at T0.

Patients and Methods

- **Subjects**

In this cross-sectional study, 60 patients (24 females, 36 males; mean age 16.1 ± 4.9 years; range 7.5-25.9) CBCS who were consecutively evaluated at the Endocrinological Department and Neuroncology Unit of Gaslini Institute of Genoa, between February 2020 and April 2022, were enrolled. To be eligible, subjects had to meet each of the following criteria: childhood brain tumor survivors, age greater than 4 and less than 26 years, at least 2 years cancer remission after completion of cancer treatments. The main exclusion criteria were syndromes associated with tumors (*i.e.* Turner syndrome, Klinefelter syndrome, Noonan syndrome) and congenital cardiac anomalies. All patients underwent a clinical and instrumental examination including blood testing, DXA scan, EndoPAT 2000 and QoL evaluations.

This study (MOMANADI002_Protocol Version _1.0_18/7/2019) was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Liguria region (protocol code 334/2019). Informed consent was obtained from all the patients and their families at the time of enrollment.

- **Statistical analyses**

The size of the study population was calculated by assuming (through personal and unpublished data) that the mean and standard deviation (SD) of the VA in the 2 groups would be 2,25 ± 1,0 kg in patients with GHD and 1,4 ± 0,7 kg in patients without GHD. We expected to enroll 75-80% of the patients with GHD, and 20-25% without GHD. Based on this perspective and by assuming an α error of 0.05, we determined that a sample of 60 subjects would provide >90% power calculation.

Descriptive statistics were generated for the whole cohort, data are described as mean and SD or median and range for continuous variables, and as absolute and relative frequencies for categorical variables. Non-parametric analysis (Mann-Whitney U-test) for continuous variables and the Chi square or Fisher's exact test for categorical variables were used to measure differences between groups. Spearman's correlation analysis was used to evaluate the relationships between body composition, anthropometric and MS parameters and IGF1 SDS o GH daily dose. The factors found to be significant upon uni- o bivariate analysis were

included in a multivariate analysis. P values ≤0.05 were considered statistically significant, and all P values were based on two-tailed tests. Statistical analysis was performed using SPSS for Windows (SPSS Inc, Chicago, Illinois USA).

- **Anthropometric evaluation, body composition and visceral adiposity measurement**

All patients underwent a clinical evaluation including measurement of height, weight, WC and hip circumference (HC). Height was measured by a Harpenden Stadiometer, with an accuracy of ± 1 mm. The weight was measured on a digital scale, with an accuracy of ± 0.1 kg. BMI was calculated as weight (kg) divided by height (m) squared and transformed into standard-deviation scores using the World Health Organization (WHO) reference values [24]. Obesity was defined by a BMI ≥ 95th percentile for age and sex in children and adolescents [25]. Central obesity was defined as a WC ≥ 90th percentile for age- and sex specific references in children and adolescence [26], and as an absolute WC ≥ 94 cm in adult males or ≥ 80 cm in adult females [27]. The MS was defined according to International Diabetes Federation Consensus Report [28]. (Figure 4)

Age group (years)	Obesity (WC)	Triglycerides	HDL-C	Blood pressure	Glucose
6–<10†	≥90th percentile				
10–<16	≥90th percentile or adult cut-off if lower	≥1.7 mmol/L (≥150 mg/dL)	<1.03 mmol/L (<40 mg/dL)	Systolic BP ≥130 or diastolic BP ≥85 mm Hg	FPG ≥5.6 mmol/L (100 mg/dL)** or known T2DM
16+(Adult criteria)	WC ≥ 94cm for Europid males and ≥ 80cm for Europid females, with ethnic-specific values for other groups*)	≥1.7 mmol/L (≥150 mg/dL) or specific treatment for high triglycerides	<1.03mmol/L (<40 mg/dL) in males and <1.29mmol/L (<50 mg/dL) in females, or specific treatment for low HDL	Systolic BP ≥130 or diastolic BP ≥85 mm Hg or treatment of previously diagnosed hypertension	FPG ≥5.6 mmol/L (100 mg/dL)** or known T2DM

BP: blood pressure; HDL-C, high-density lipoprotein cholesterol; FPG, fasting plasma glucose; IDF, International Diabetes Federation; T2DM, type 2 diabetes mellitus; WC, waist circumference.
*For those of South and South-East Asian, Japanese, and ethnic South and Central American origin, the cutoffs should be ≥90 cm for men, and ≥80 cm for women. The IDF Consensus group recognise that there are ethnic, gender and age differences but research is still needed on outcomes to establish risk.
†Metabolic syndrome cannot be diagnosed, but further measurements should be made if there is a family history of metabolic syndrome, T2DM, dyslipidemia, cardiovascular disease, hypertension and/or obesity.
**For clinical purposes, but not for diagnosing the MetS, if FPG 5.6-6.9 mmol/L (100-125 mg/dl) and not known to have diabetes, an oral glucose tolerance test should be performed.

Figure 4. The IDF definition of the at risk group and metabolic syndrome in children and adolescents. Diagnosing the metabolic syndrome requires the presence of central obesity plus any two of the other four factors. [28]

The pubertal developmental stage was assessed by physical examinations. The WC and HC were measured with a plastic tape measure with an accuracy of ± 0.1 cm. WC was measured midway between the lowest rib and the superior border of the iliac crest at the end of a normal expiration with a flexible nonelastic anthropometric tape, to the nearest 0.1 cm, as recommended by the WHO [29]. Therefore, the percentile for sex and age were assessed according to Xi et al. [26]. Blood pressure was measured with an automated electronic device

in the seated position after resting for at least 10 min. The elbow of the arm used for measurement was supported at heart level. The body composition was also measured by a DXA (DXA Lunar Prodigy, GE) to estimate total body lean and fat mass, trunk fat. A specific ROI for VA was examined, the ROI was obtained by manually selecting on the DXA scan a region delimited between the superior iliac crests and the thoracic rib cage. (Figure 5)

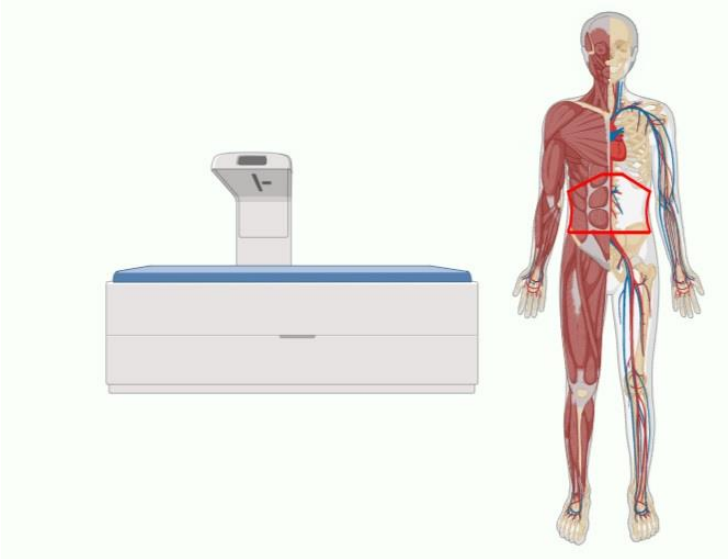

Figure 5. Truncal region of interest (ROI)

- **Biochemical evaluation of GH actions: gluco-metabolic parameters and lipid metabolism**

Venous blood samples were collected by venipuncture between 8 a.m. and 12 p.m., after an overnight fast. Samples were then transferred to the local laboratory and handled according to the local standards of practice. For the biochemical evaluation of lipid metabolism, the serum and plasma were immediately separated, and the lipid panel (triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL)) was quantified on the same day. An enzymatic colorimetric assay was used to determine total cholesterol, triglyceride, and direct HDL levels. Fasting plasma low-density lipoprotein cholesterol (LDL) was calculated using the Friedewald formula [30]. Adiponectin was quantified by immunoenzimatic test. The biochemical evaluation of gluco-metabolic parameters included the measurement of fasting glycemia and insulin, HbA1C, oral glucose tolerance test (OGTT), homeostasis model assessment for insulin resistance (HOMA IR index), HOMA-beta and quantitative insulin sensitivity check index (Quicki). OGTT was performed in 54 patients, 6 patients refused or vomited the sugar solution. After the administration of oral glucose at a dose of

1.75 g/kg (max: 75 g), glucose and insulin concentrations were measured at 0 and 120 min for all patients. Glycemia was determined using an enzymatic hexokinase assay, impaired fasting glucose and impaired glucose tolerance in OGTT were defined using the American Diabetes Association Standards of Medical Care in Diabetes [31] (Figure 6). Insulin concentrations were measured with the chemiluminescence immunoassay method. HOMA-IR was calculated as (fasting glucose mg/dl x 0,05551) x (fasting insulin uU/ml / 22,5). We evaluated IGF1 and IGFBP3 levels to estimate the bioavailable of IGF1 [32, 33].

- **Biochemical and biophysical evaluation of GH actions on endothelial function and prothrombotic state**

FPG ≥126 mg/dL (7.0 mmol/L). Fasting is defined as no caloric intake for at least 8 h.*
OR
2-h PG ≥200 mg/dL (11.1 mmol/L) during OGTT. The test should be performed as described by WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*
OR
A1C ≥6.5% (48 mmol/mol). The test should be performed in a laboratory using a method that is NGSP certified and standardized to the DCCT assay.*
OR
In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥200 mg/dL (11.1 mmol/L).

DCCT, Diabetes Control and Complications Trial; FPG, fasting plasma glucose; OGTT, oral glucose tolerance test; WHO, World Health Organization; 2-h PG, 2-h plasma glucose. *In the absence of unequivocal hyperglycemia, diagnosis requires two abnormal test results from the same sample or in two separate test samples.

Figure 6. Criteria for the diagnosis of diabetes, American Diabetes Association (ADA) Standards of Medical Care in Diabetes

We estimated biochemical endothelial function and the prothrombotic state by evaluating circulating markers of endothelial dysfunction and the blood clotting profile (fibrinogen, protein S, protein C, factor VIII, von Willebrand Factor (VWF) antigen, homocysteine). Endothelial function was also evaluated by EndoPAT 2000 (Itamar Medical Ltd, Caesarea, Israel) which is a method used to assess biophysical endothelial function by measuring modifications in the digital pulse volume during reactive hyperemia. It is a non-invasive, reproducible, and operator-independent tool that can detect precocious microvascular endothelial dysfunction [34]. The technique provides values to calculate the reactive

hyperemia index (RHI) - peripheral artery tonometry (PAT), which is generated by brachial artery blood flow occlusion for 5 min, through rapidly inflating a blood pressure cuff to a supra-systolic pressure of 60 mmHg above the patient's systolic pressure or 200 mmHg. The PAT signal is measured by recording finger arterial pulsatile volume changes through plethysmographic biosensors that impart a uniform sub-diastolic pressure field to the fingers. The RHI is calculated automatically by the differences between post- and pre-occlusion PAT signal ratio in the occluded - and control - arm, corrected for baseline vascular tone [35] (Figure 7).

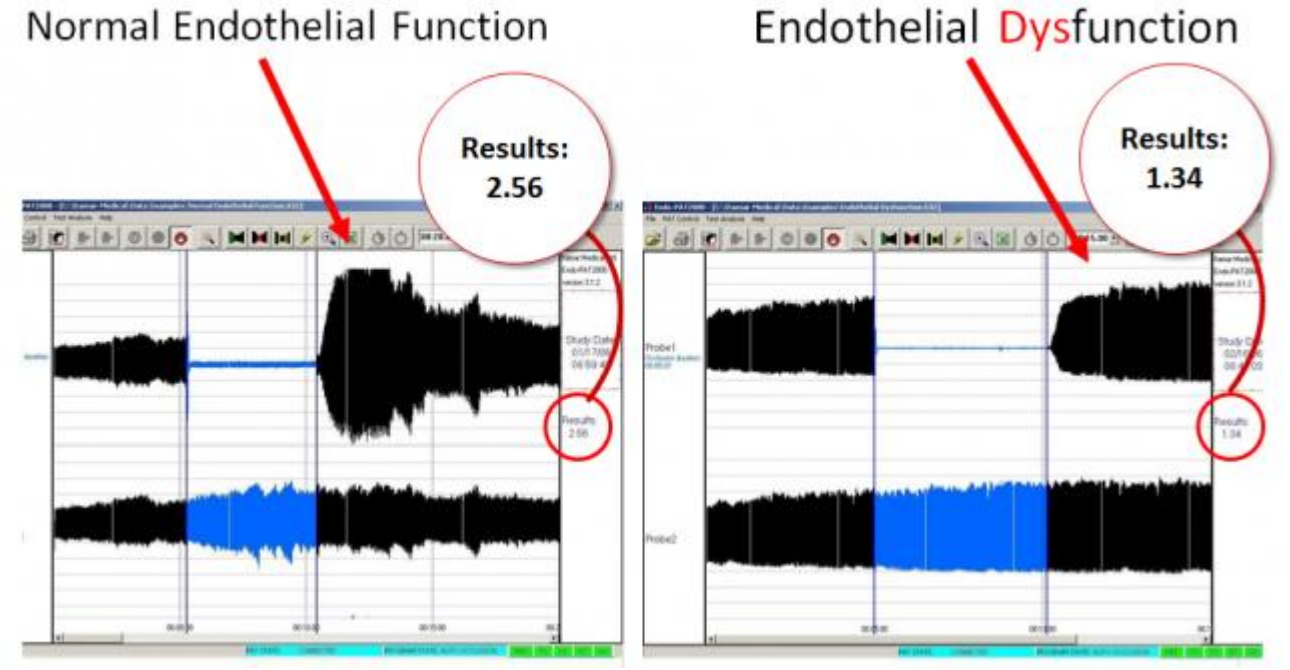

Figure 7. EndoPAT 2000 display screen at completion of test. [35]

- **Quality of life and fatigue**

We evaluated the QoL and chronicle fatigue level in the patients and their caregivers by using the following questionnaires: Pediatric Quality of Life Inventory (PedsQL) Generic Core Scales version 4.0 for QoL [36] and the PedsQL Multidimensional Fatigue Scale (PedsQL-MFS) version 3.0 for chronicle fatigue [37] (Figure 8). The PedsQL is a brief measure of multidimensional aspects of the QoL (Physical, Emotional, Social, School Functioning). The PedsQL-MFS is a questionnaire consisting of 18 items which evaluate the multidimensional aspects of fatigue (General Fatigue, Sleep/Rest Fatigue, Cognitive Fatigue). Each item is rated for how frequently symptoms of fatigue are a problem on a 5-point scale from 0 "almost never" to 4 "almost always". Items are transformed on a scale

from 0 to 100, as follows: 0=100, 1=75, 2=50, 3=25, 4=0. Both the PedsQL and the PedsQL-MFS questionnaires can be completed by parents (the Proxy Report) as well as children and young adults (the Self-Report) and are available in Italian.

*Considerando tutto quello che ti è successo nell'**ULTIMO mese**, quanto è un **problema** per te...*

LA MIA SALUTE E LE MIE ATTIVITÀ *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. Faccio fatica a camminare per più di 100 metri	0	1	2	3	4
2. Faccio fatica a correre	0	1	2	3	4
3. Faccio fatica a fare sport o attività fisica	0	1	2	3	4
4. Faccio fatica a sollevare cose pesanti	0	1	2	3	4
5. Faccio fatica a fare il bagno o la doccia da solo/a	0	1	2	3	4
6. Faccio fatica a fare lavoretti di casa	0	1	2	3	4
7. Ho male o dolore	0	1	2	3	4
8. Mi sento stanco/a	0	1	2	3	4

LE MIE EMOZIONI *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. Sono impaurito/a o spaventato/a	0	1	2	3	4
2. Mi sento triste	0	1	2	3	4
3. Sono arrabbiato/a	0	1	2	3	4
4. Ho difficoltà ad addormentarmi e/o a dormire	0	1	2	3	4
5. Mi preoccupo per quello che mi potrebbe succedere	0	1	2	3	4

COME STO CON GLI ALTRI *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. Ho difficoltà ad andare d'accordo con gli altri ragazzi/le altre ragazze	0	1	2	3	4
2. Gli altri ragazzi/Le altre ragazze non vogliono essermi amici/amiche	0	1	2	3	4
3. Gli altri ragazzi/Le altre ragazze mi prendono in giro	0	1	2	3	4
4. Non riesco a fare quello che fanno i ragazzi/le ragazze della mia età	0	1	2	3	4
5. È difficile per me fare quello che fanno i ragazzi/le ragazze della mia età	0	1	2	3	4

LA SCUOLA *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. È difficile stare attento/a in classe	0	1	2	3	4
2. Dimentico le cose	0	1	2	3	4
3. Ho difficoltà a seguire le lezioni in classe e a finire i compiti a casa	0	1	2	3	4
4. Sto a casa da scuola perché non mi sento bene	0	1	2	3	4
5. Sto a casa da scuola per andare dal dottore o in ospedale	0	1	2	3	4

*In questo **ULTIMO MESE**, quanto è stato **un problema** per te ...*

AFFATICAMENTO GENERALE *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. Mi sento stanco/a	0	1	2	3	4
2. Mi sento fisicamente debole (non forte)	0	1	2	3	4
3. Mi sento troppo stanco/a per fare le cose che mi piace fare	0	1	2	3	4
4. Mi sento troppo stanco/a per passare il tempo con i miei amici	0	1	2	3	4
5. Ho difficoltà a finire le cose	0	1	2	3	4
6. Ho difficoltà a iniziare le cose	0	1	2	3	4

AFFATICAMENTO LEGATO A SONNO/RIPOSO *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. Dormo più spesso del solito	0	1	2	3	4
2. È difficile per me dormire tutta la notte	0	1	2	3	4
3. Mi sento stanco/a quando mi sveglio al mattino	0	1	2	3	4
4. Riposo molto	0	1	2	3	4
5. Dormo spesso di giorno	0	1	2	3	4
6. Sto molto tempo a letto	0	1	2	3	4

AFFATICAMENTO COGNITIVO *(è un problema...)*	Mai	Quasi mai	Qualche volta	Spesso	Quasi sempre
1. È difficile per me concentrarmi su qualcosa	0	1	2	3	4
2. È difficile per me ricordare quello che gli altri mi dicono	0	1	2	3	4
3. È difficile per me ricordare quello che ho appena sentito	0	1	2	3	4
4. È difficile per me pensare in fretta	0	1	2	3	4
5. Ho difficoltà a ricordare quello a cui stavo pensando	0	1	2	3	4
6. Ho difficoltà a ricordare più di una cosa alla volta	0	1	2	3	4

Figure 8. Pediatric Quality of Life Inventory (PedsQL) Generic Core Scales version 4.0 for QoL [36] and the PedsQL Multidimensional Fatigue Scale (PedsQL-MFS) version 3.0

Results

- **Subject characteristics**

Of the 62 patients aged 5-25 years old who were invited to participate, 61 complied with the inclusion and the exclusion criteria, 1 adult patient refused to participate, therefore we enrolled 60 participants, 32 female (53.3%) and 28 males (46.7%).

The mean age at oncological diagnosis was 7 ± 4.6 years, at evaluation 16.1 ± 4.9 years and from the end of the oncological treatment to evaluation 7.2 ± 4.9 years. Forty-six (66.7%) patients received CRT (maximum mean dose on primary tumor 54.3 ± 8.3 Gy), of them 16 patients also received spinal irradiation. Forty-four (73.3%) underwent neurosurgery and 40 (66.7 %) were treated with chemotherapy. Initial oncological diagnoses were: 20 (33.3%) patients with tumors of the sellar region including the germ cell tumors, 15 (25%) low grade gliomas, 12 (20%) medulloblastomas, 8 (13.3%) other high grade tumors and 5 (8.3%) other low grade tumors. The primary lesions in the patients were located in the following regions: 30 (50%) in the sellar/parasellar region, 10 (16.7%) in the posterior fossa, 6 (10%) in the hemispheric, 4 (6.7%) in the pineal region and 9 (15%) were disseminated at diagnosis. The demographic and oncological characteristics of the 60 participants are shown in Table 1.

Table 1. Demographic and oncological features of the patients diagnosed with GHD (GHD group) and those with normal GH pituitary function (control group).

	All patients (*n* = 60)	**GHD group (*n* = 48)**	**Control group (*n* = 12)**
Sex number:			
male/female	28/32	24/24	4/8
Age at diagnosis:			
mean years ± SD	7 ± 4.6	7.5 ± 4.5	5 ± 4.9
Age at the evaluation:			
mean years ± SD	16.1 ± 4.9	16.6 ± 4.9	14 ± 4.7
Tumor grade WHO 2016:			
I – II	32/2	26/2	6/0
III - IV	2/24	1/19	1/5
Primary lesion involving hypothalamic-pituitary region:			
yes/no	30/30	27/21	3/9
Chemotherapy:			
n (%)	40 (66.7%)	32 (66.7%)	8 (66.7%)
Cranial radiotherapy:			
mean Gy ± SD	54.3 ± 8.3	54.1 ± 8.4	55.5 ± 9
n (%)	46 (76.7%)	**40 (83.3%)**	**6 (50.0%)***
Pituitary deficiencies:			
mean numbers ± SD	2.6 ± 1.5	**3.1 ± 1.2**	**0.5 ± 0.5*****

°p = 0.05, *p <0.05, **p <0.005, ***p <0.0005

- **Anthropometric evaluation, body composition and visceral adiposity measurement**

In the cohort, 48 patients (80%) had a GHD diagnosis during childhood or adolescence confirmed by the presence of typical clinical characteristics of GHD and by two different GH-stimulation tests. Forty of the patients are being treated with a standard dose of hrGH (hrGH group), while 8 refused or discontinued the treatment (no hrGH group) and 12 patients had no GHD (control group). The children started the rhGH treatment with a dose range of 0.018-0.032 ug/kg/day and adolescents 0.2-0.4 mg/day. At study enrollment, the children that are being treated with hrGH have a dose range of 0.018-0.031 ug/kg/day if non-obese or 3.2-6.3 mg/m2 of body surface area/week if obese, and adolescents have a dose range of 0.2 – 0.7 mg/day. A target of serum IGF1 levels within the age-adjusted reference range (<2 SDS), together with the growth velocity range for the children, was used to adjust the hrGH dose during the treatment. The mean time interval of GH treatment was 57.4 ± 49.2 months. Other hormone deficiencies were as follows: 38 (63.3%) thyroid stimulating hormone (TSH), 36 (60%) adrenocorticotropic hormone (ACTH), 21 (35%) gonadotropins and 16 (26.7%) antidiuretic hormone. The control group had lower numbers of pituitary deficiencies: 0.50 ± 0.52 vs 3.10 ± 1.21 in GHD group, $p < 0.0001$. MS was present in 5 (8.3 %) patients. In the cohort, 15 (25%) had a short stature (height SDS ≤ 2 SDS), 34 (56.7%) were obese and 29 (48.3%) were centrally obese. Due to the high prevalence of short stature in both groups, with the control group taller than GHD group (0.17 ± 1.40 vs -0.93 ± 1.64, $p < 0.05$) we also calculated the VA as waist-to-height ratio (WHtR): 40 (66.7%) patients had value ≥ 0.5, of them 25 patients were being treated with hrGH (62.5% of the group), 9 did not have GHD (75%), 6 had GHD but they were not being treated (75%). No significative differences were founded between GHD and control group for other anthropometric and endocrinological features, body composition and VA measurement as shown in Table 2, excluding the WC/HC ratio and the morning serum cortisol, both higher in the control group.

Table 2. Anthropometric and endocrinological features, cardiometabolic parameters of the GHD group and the control group.

Characteristic	All patients (*n* = 60)	GHD group (*n* = 48)	Control group (*n* = 12)
Height: mean cm ± SD	153.7 ± 16	154 ± 17	152.6 ± 11.9

mean SDS ± SD	-0.7 ± 1.7	**-0.9 ± 1.6**	**0.2 ± 1.4°**
genetic target SDS ± SD	-0.4 ± 0.9	-0.4 ± 0.9	0 ± 1
Weight:			
mean kg ± SD	61.2 ± 21.8	62.2 ± 22.4	57.2 ± 19.6
BMI:			
mean kg/m2 ± SD	25.1 ± 5.6	25.4 ± 5.7	23.8 ± 5
mean SDS ± SD	2 ± 1.5	2.1 ± 1.5	1.6 ± 1.7
Tanner stage:			
mean ± SD	3.5 ± 1.5	3.6 ± 1.5	3.1 ± 1.4
SBP:			
mean mmHg ± SD	111.2 ± 11.8	112.5 ± 12.3	105.8 ± 7.2
DBP:			
mean mmHg ± SD	65.7 ± 9.4	66.3 ± 9.8	63.2 ± 7
WC:			
mean cm ± SD	84 ± 13.7	84.4 ± 13.9	82.2 ± 13.1
HC:			
mean cm ± SD	94.2 ± 16.8	95.1 ± 17.7	90.6 ± 12.3
WC/HC Ratio:			
mean ± SD	0.9 ± 0.2	**0.9 ± 0.2**	**1 ± 0.1°**
WC/Height Ratio:			
mean ± SD	0.6 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
Pituitary deficiencies:			
TSHD n (%)	38 (63.3)	**36 (75.0)**	**2 (16.7)*****
ACTHD n (%)	36 (60.0)	**33 (68.8)**	**3 (25.0)***
Hypogonadism n (%)	21 (35.0)	**20 (41.7)**	**1 (8.3)***
DI n (%)	16 (26.7)	**16 (33.3)**	**0 (0)***
IGF1:			
mean ng/ml ± SD	211.7 ± 121.4	214 ± 132.4	202.4 ± 63
mean SDS ± SD	-1.5 ± 3.1	-1.6 ± 3.3	-0.9 ± 1.6
IGFBP3:			
mean ng/ml ± SD	3008.0 ± 1948.6	2987.4 ± 2017.2	3090.4 ± 1723.5
mean SDS ± SD	-1 ± 1.6	-1 ± 1.6	-1.1 ± 1.3
ALS:			
mean nmol/L ± SDS	128.3 ± 92.9	127.9 ± 94	129.8 ± 92.7
Other hormone levels:			
FT4 mean pg/mL ± SDS	9.3 ± 5.9	9.7 ± 5.8	
Cortisol h 8 ug/dL ± SDS	9 ± 6.4	**7.6 ± 5.8**	**14 ± 6****
DXA body composition:			
total body less head fat mass mean kg ± SD	24.8 ± 11.8	24.7 ± 12	25 ± 11.5
trunk fat mass mean kg ± SD	12.6 ± 7	12.6 ± 7.3	12.4 ± 6.1
ROI trunk fat mass mean % ± SD	44.3 ± 11.7	44.1 ± 11.9	45 ± 11
ROI trunk fat mass mean gr ± SD	1913 ± 1440.7	1914 ± 1442.3	1908.8 ± 1503.9
fat mass index kg/m2 ± SD	5.2 ± 2.4	5.1 ± 2.5	5.2 ± 2.1
total body less head lean mass mean kg ± SD	32.6 ± 12.1	33.3 ± 13	29.3 ± 7.1
trunk fat / total body fat mass ratio	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1
leg fat / total body fat mass ratio	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
four limbs fat / total body fat mass ratio	1.1 ± 0.3	1.1 ± 0.3	1.1 ± 0.3
Lipid metabolism:			
Triglycerides mean mg/dL ± SD	104.8 ± 52.1	109.2 ± 54.8	87.5 ± 36.9
cholesterol mean mg/dL ± SD	173.5 ± 33.9	175.9 ± 34.6	163.9 ± 30.5
HDL mean mg/dL ± SD	52.1 ± 13.3	51.9 ± 14.4	52.6 ± 8
LDL mean mg/dL ± SD	105.7 ± 28.6	108 ± 29.6	96.6 ± 23
adiponectin mg/L ± SD	8.8 ± 6.9	8.4 ± 6.9	10.5 ± 6.7
Gluco-metabolic parameters:			
fasting glucose mean mg/dL ± SD	87.2 ± 8.5	86.8 ± 6.8	88.6 ± 13.6
glucose OGTT 120′ mean mg/dL ± SD	118.8 ± 27.2	119.3 ± 29.1	116.7 ± 19.1
glucose OGTT max value mean mg/dL ± SD	142.2 ± 28.3	143.7 ± 29.2	136.7 ± 26
fasting insulin uU/mL	16.9 ± 9.8	16.4 ± 9.2	18.7 ± 12.1
insulin OGTT 120′ mean uU/mL ± SD	155 ± 172.8	159.4 ± 181.8	138.2 ± 139.2

insulin OGTT max value mean uU/mL ± SD	218 ± 132.9	227.9 ± 141.4	182.6 ± 97.1
Prothrombotic factors:			
protein S mean % ± SD	86.5 ± 19.1	86.9 ± 17	85.27±25.78
protein C mean % ± SD	110.5 ± 24	108.6 ± 25.5	116.91±17.43
FVIII mean % ± SD	138 ± 32.9	134.4 ± 31.7	150.4 ± 35.3
VWF Ag mean % ± SD	125 ± 50.1	118.4 ± 41.3	147.7 ± 70.6
fibrinogen mean mg/dL ± SD	283.6 ± 61.5	281.6 ± 59.1	290.8 ± 72.1
homocysteine mean umol/L± SD	14.9 ± 18.2	15.3 ± 18.2	13.3 ± 5.8
Endothelial function:			
EndoPAT RHI mean ± SD	1.6 ± 0.6	1.6 ± 0.6	1.4 ± 0.5
EndoPAT ln RHI mean ± SD	0.4 ± 0.4	0.4 ± 0.4	0.3 ± 0.5

°p = 0.05, *p <0.05, **p <0.005, ***p <0.0005

In the cohort, the IGF1 SDS levels showed a weak negative correlation with BMI (r = -0.26, p = 0.04), moderate with weight (r = -0.414, p = 0.001), WC (r = -0.428, p = 0.001) and to a higher extent with DXA body composition parameters. Interestingly, in bivariate analysis, both the IGF1 SDS levels and GH daily dose showed a negative correlation with VA (WC, ROI fat mass and trunk fat mass indices) (Figure 9a, 9b, 9c, 9d), and a positive correlation with other body composition indices including leg fat and four limbs fat (Table 3).

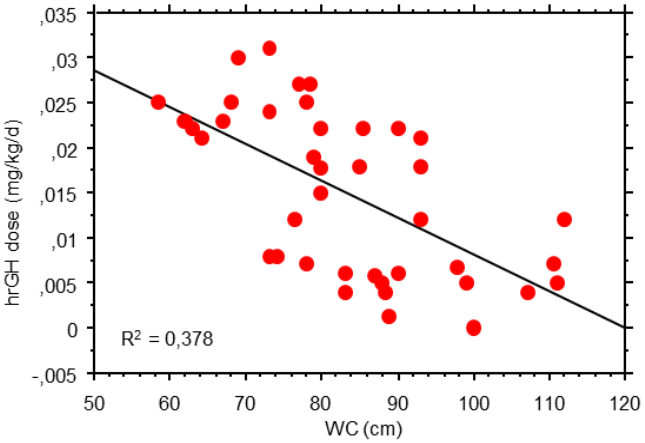

Figure 9a. Bivariate Scattergram for hrGH daily dose vs WC.

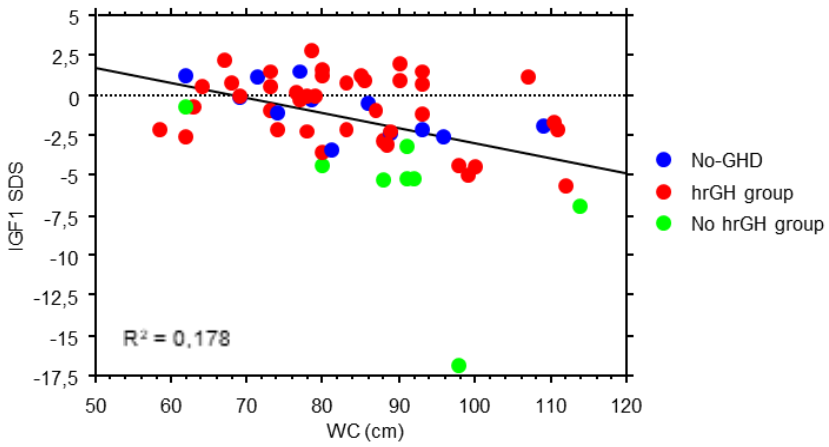

Figure 8b. Bivariate Scattergram for IGF1 SDS vs WC.

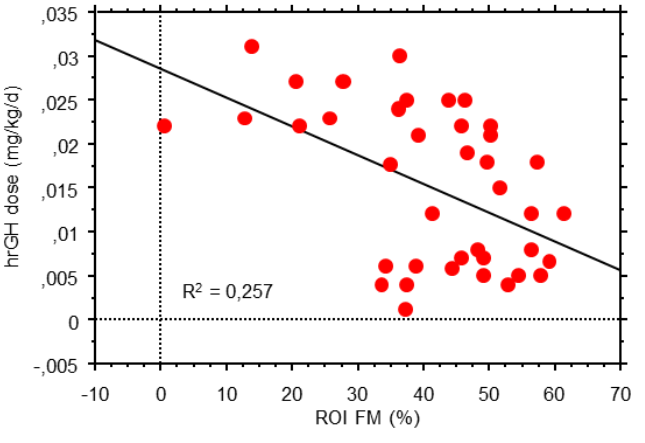

Figure 9c. Bivariate Scattergram for hrGH daily dose vs ROI FM.

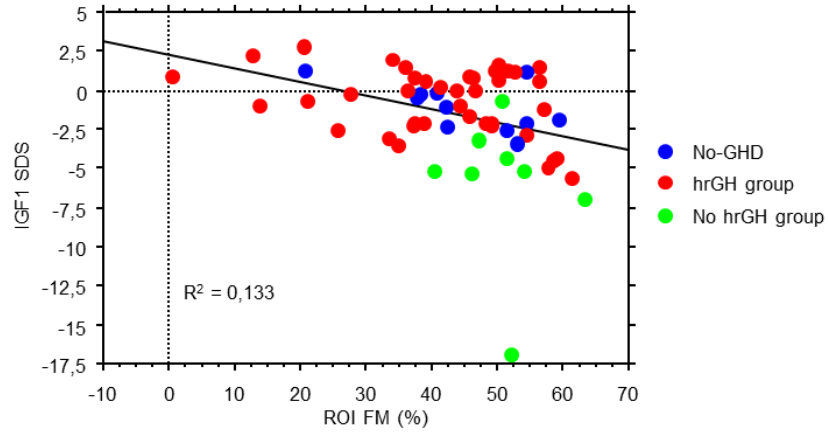

Figure 9d. Bivariate Scattergram for IGF1 SDS vs ROI FM.

Table 3. Spearman's correlation coefficient (r) of the relation between body composition parameters and IGF1 SDS - GH daily dose.

Body composition parameters	IGF1 SDS		GH dose mg/kg/d	
	r	*p*	*r*	*p*
total body less head fat mass mean (kg)	-0.424	<0.001	-0.400	<0.005
trunk fat mass mean (kg)	-0.473	<0.0001	-0.442	<0.001
ROI trunk fat mass mean (%) ± SD	-0.333	<0.01	-0.376	<0.005
ROI trunk fat mass mean (gr)	-0.494	<0.0005	-0.427	<0.005
fat mass index (kg/m2)	-0.339	<0.01	-0.343	<0.01
trunk fat / total body fat mass ratio	-0.405	<0.001	-0.397	<0.005
leg fat / total body fat mass ratio	0.387	<0.005	0.403	<0.005
four limbs fat / total body fat mass ratio	0.398	<0.005	0.414	<0.005
total body less head lean mass mean (kg)	-0.317	<0.01	-0.312	<0.05

Multivariate analysis indicated that ROI trunk fat (gr) was independently, negatively, and significantly predicted (adjusted R^2 = 0.532) by GH daily dose (p = 0.03) and directly predicted by BMI SDS (p = <0.0001), after correction for metabolic syndrome parameters (Triglycerides, HDL, SBP, DBP, Glucose). Moreover, WC was independently and negatively predicted (adjusted R^2 = 0.600) by IGF1 SDS (p = 0.0004) and directly predicted by BMI SDS (p = <0.0001), after correction for other metabolic syndrome parameters triglycerides, HDL, SBP, DBP, Glucose). Similarly, multivariate analysis indicated that ROI trunk fat (%) was independently, negatively, and significantly predicted (adjusted R^2 = 0.446) by GH daily dose (p = 0.0058) and directly predicted by BMI SDS (p = <0.0001), after correction for metabolic syndrome parameters (triglycerides, HDL, SBP, DBP, Glucose). In addition, WC was independently and negatively predicted (adjusted R^2 = 0.422) by IGF1 SDS (p = 0.019) and directly predicted by BMI SDS (p = <0.0001), after correction for other metabolic syndrome parameters (triglycerides, HDL, SBP, DBP, glucose).

To understand the possible impact of hrGH on cardiometabolic risks, we analyzed the differences by dividing the GHD group in two subgroups based on their treatment with hrGH. As expected, patients with GHD not on hormone replacement treatment present significantly lower levels of IGF1 and IGF1 SDS, compared to the other two groups, and the mean level of IGFBP3 was pathologic. The control group had lower pituitary deficiencies

than both GHD subgroups. Interestingly, the patients with normal GH-pituitary function, and the patients being treated with hrGH had a lower VA as defined by the following ratios: trunk fat / total body fat mass ($p = 0.01$ and $p = 0.005$). Moreover, patients with GHD not in treatment with hrGH had a significantly different body composition, as showed by leg fat / total body fat mass ratio ($p = 0.03$, $p = 0.02$) and four limbs fat / total body fat mass ratio ($p = 0.007$, $p = 0.005$) (Figure 10). No significant differences were found between the three

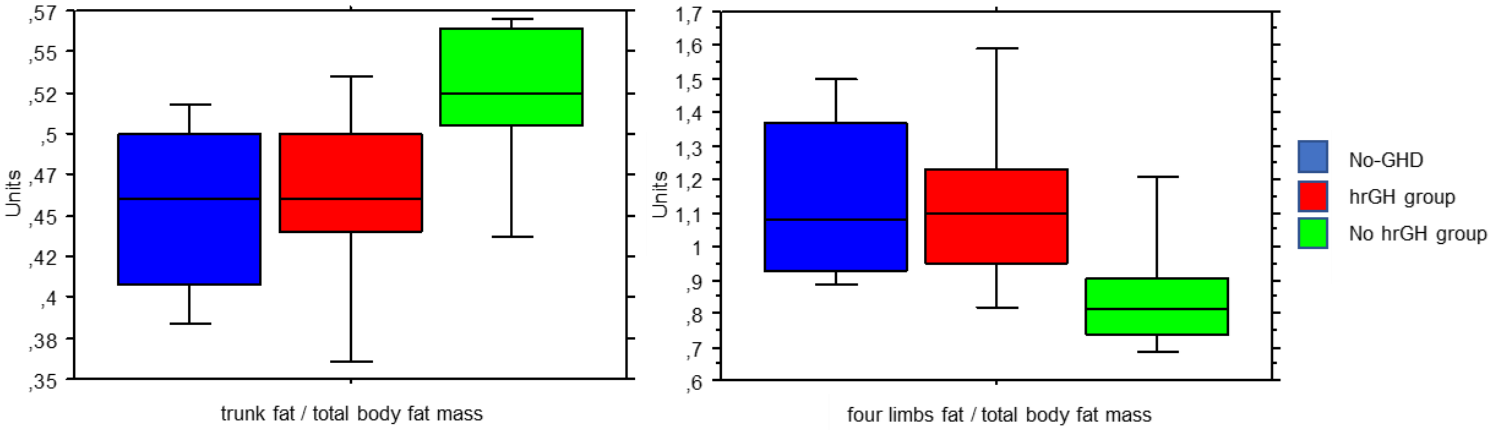

Figure 10. Body composition parameters in the control group (No-GHD) and GHD subgroups.

groups for other anthropometric and endocrinological features, VA (Figure 11) and body composition measurement as show in Table 4.

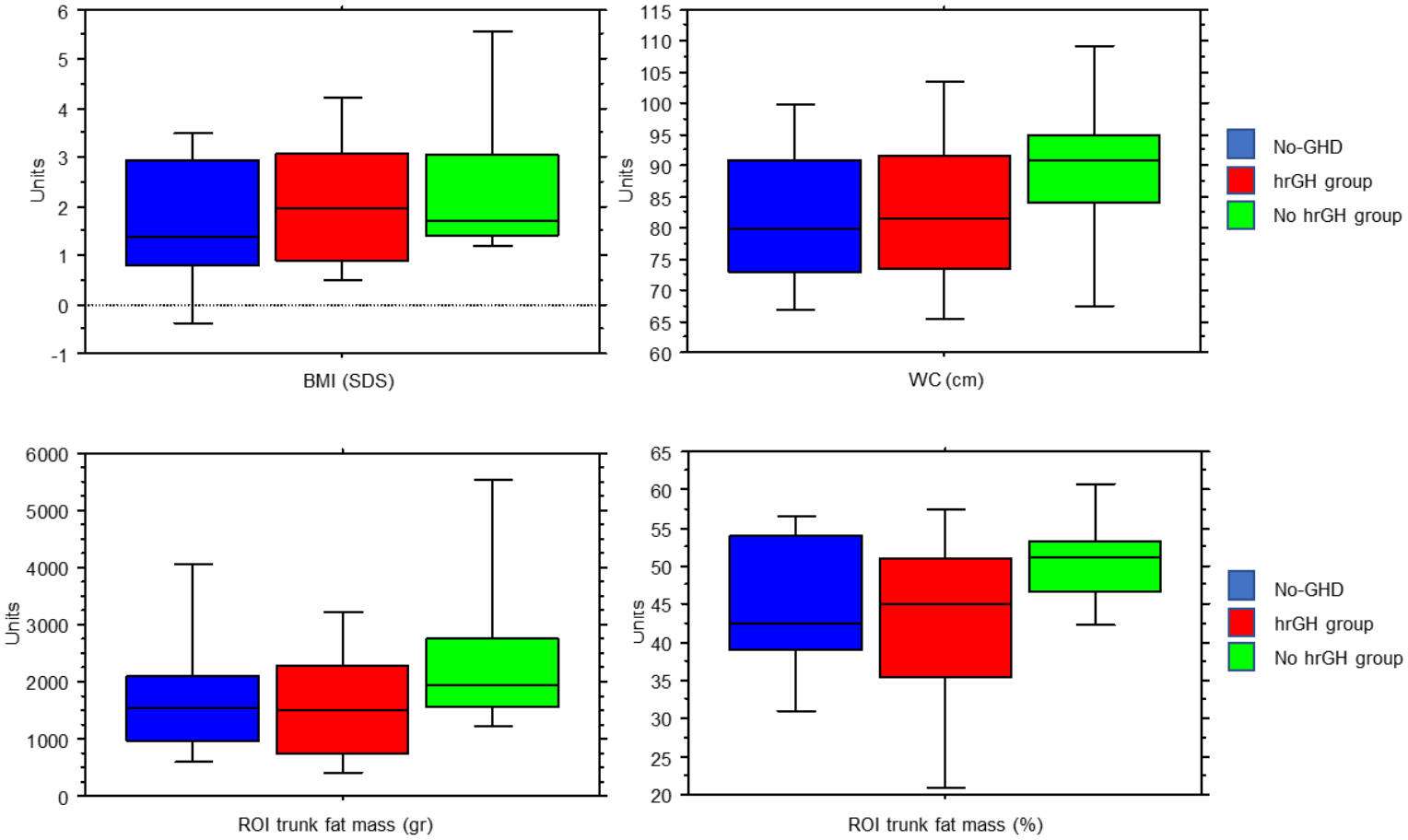

Figure 11. Visceral adiposity parameters in the control group (No-GHD) and GHD subgroups.

Table 4. Clinical features and cardiometabolic parameters of the patients with normal GH pituitary function (control group) and those diagnosed with GHD in treatment - (hrGH group) and not in treatment - with hrGH (no hrGH group).

Characteristic	hrGH group (n = 40)	No hrGH group (n = 8)	Control group (n = 12)	0 vs 1	0 vs 2	1 vs 2
Age at the evaluation:						
mean years ± SD	16.2 ± 4.8	18.2 ± 5.4	14 ± 4.7	0.33	0.15	0.11
Age at diagnosis:						
mean years ± SD	7.6 ± 4.5	6.9 ± 5	5 ± 4.9	0.69	0.20	0.79
Time diagnosis – evaluation:						
mean years ± SD	8.7 ± 4.6	11.4 ± 5.1	9 ± 4.9	0.16	0.95	0.38
Time between diagnosis – end of oncological treatments:						
mean years ± SD	7.2 ± 4.5	7.8 ± 6.4	6.9 ± 5.6	0.94	0.62	0.91
Cranial radiotherapy:						
mean Gy ± SD	54.1 ± 8.2	54.5 ± 9.8	55.5 ± 9	0.89	0.23	0.44
Pituitary deficiencies:						
mean numbers ± SD	3.2 ± 1.2	2.9 ± 1.5	0.5 ± 0.5	0.52	**0.0001**	**0.0001**
Tanner stage:						
mean ± SD	3.5 ± 1.5	4 ± 1.6	3.1 ± 1.4	0.29	0.34	0.13
Weight:						
mean kg ± SD	61.3 ± 22.1	66.8 ± 24.8	57.2 ± 19.6	0.56	0.66	0.34
Height:						
mean cm ± SD	153.5 ± 16.7	156.5 ± 19.8	152.6 ± 11.8	0.78	0.75	0.85
mean SDS ± SD	-0.9 ± 1.6	-1.1 ± 1.8	0.2 ± 1.4	0.80	0.06	0.16
genetic target SDS ± SD	-0.4 ± 1	-0.6 ± 0.7	-0.0 ± 1	0.65	0.29	0.18
BMI:						
mean kg/m2 ± SD	25.2 ± 5.6	26.5 ± 6.4	23.8 ± 5	0.60	0.38	0.21
mean SDS ± SD	2.1 ± 1.4	2.5 ± 1.8	1.6 ± 1.7	0.75	0.51	0.21
Body area surface:						
mean m2 ± SD	1.6 ± 0.4	1.7 ± 0.4	1.5 ± 0.3	0.56	0.47	0.27
WC:						
mean cm ± SD	83.4 ± 13.7	89.5 ± 14.8	82.2 ± 13.1	0.18	0.74	0.21
HC:						
mean cm ± SD	94.9 ± 17.7	96.2 ± 18.5	90.6 ± 12.4	0.99	0.38	0.52
WC/HC Ratio:						
mean ± SD	0.9 ± 0.2	0.9 ± 0.1	1 ± 0.1	0.92	**0.05**	0.21
WC/Height Ratio:						
mean ± SD	0.5 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.36	1	0.34
SBP:						
mean Hgmm ± SD	112.6 ± 12.1	113.6 ± 14.2	105.8 ± 7.2	0.84	0.07	0.47
DBP:						
mean Hgmm ± SD	66.6 ± 10.2	65 ± 8.5	63.17±7.00	0.67	0.23	0.85
Endocrinological assessment:						
IGF1 mean ng/mL ± SD	240.8 ± 128.1	80 ± 41.4	202.4 ± 63	**0.0001**	0.54	**0.0001**
IGF1 mean SDS ± SD	-0.8 ± 2.1	-6 ± 4.8	-0.9 ± 1.6	**0.001**	0.73	**0.001**
IGFBP3 mean ng/mL ± SD	4108.7 ± 1146.8	3030.5 ± 1680.7	3090.4 ± 1723.5	0.73	0.95	0.62
IGFBP3 mean SDS ± SD	-1 ± 1.2	-2.5 ± 2.8	-1.1 ± 1.3	0.07	0.39	0.16
ALS mean nmol/L ± SDS	134.3 ± 91.4	96.5 ± 106.5	129.8 ± 92.7	0.22	0.88	0.34
FT4 mean pg/ml ± SDS	9.6 ± 5.9	10.4 ± 5.6	7.8 ± 6.3	0.50	0.13	0.27
Cortisol mean ug/ml ± SDS	7.2 ± 5.8	9.1 ± 5.9	14 ± 6	0.25	**0.001**	0.30
DXA body composition:						
total body less head fat mass mean kg ± SD	24.1 ± 12.1	27.5 ± 12.1	25 ± 11.5	0.51	0.84	0.44
trunk fat mass mean kg ± SD	12.2 ± 7.3	14.8 ± 7.2	12.4 ± 6.1	0.21	0.73	0.49
ROI trunk fat mass mean % ± SD	42.7 ± 12.3	50.8 ± 6.7	45 ± 11	0.07	0.54	0.49
ROI trunk fat mass mean gr ± SD	1788.5 ± 1358.2	2541.5 ± 1774.9	1908.8 ± 1503.9	0.14	0.77	0.39
fat mass index kg/m2 ± SD	5 ± 2.6	5.9 ± 2.2	5.2 ± 2.1	0.26	0.82	0.44
total body less head lean mass mean kg ± SD	32.9 ± 13	35.2 ± 13.4	29.3 ± 7.1	0.60	0.71	0.27
trunk fat / total body fat mass ratio	0.457 ± 0.06	0.521 ± 0.05	0.455 ± 0.05	**0.005**	0.85	**0.01**

leg fat / total body fat mass ratio	0.381 ± 0.05	0.339 ± 0.04	0.394 ± 0.05	**0.02**	0.57	**0.03**
four limbs fat / total body fat mass ratio	1.129 ± 0.27	0.864 ± 0.21	1.144 ± 0.25	**0.005**	0.85	**0.007**
Lipid metabolism:						
Triglycerides mean mg/dL ± SD	109.7 ± 55	106.9 ± 57.4	87.5 ± 36.9	0.86	0.23	0.62
cholesterol mean mg/dL ± SD	178.1 ± 35.9	165.5 ± 26.8	163.9 ± 30.5	0.43	0.21	0.73
HDL mean mg/dL ± SD	53.6 ± 14.7	43.6 ± 9.4	52.6 ± 8	**0.02**	0.89	**0.02**
LDL mean mg/dL ± SD	108.9 ± 31.2	103.9 ± 21	96.6 ± 23.1	0.73	0.17	0.30
adiponectin mg/L ± SD	9.6 ± 7.2	3.4 ± 1.8	10.5 ± 6.7	**0.007**	0.72	**0.0001**
Gluco-metabolic parameters:						
fasting glucose mean mg/dL ± SD	86.8 ± 6.8	86.9 ± 7.1	88.6 ± 13.6	0.82	0.35	0.49
glucose OGTT 120′ mean mg/dL ± SD	115.7 ± 25.7	134.8 ± 39.1	116.7 ± 19.1	0.23	0.82	0.49
glucose OGTT max value mean mg/dL ± SD	142.4 ± 28.3	150.8 ± 37.8	136.7 ± 26	0.56	0.90	0.41
fasting insulin uU/mL	16.3 ± 8.8	16.9 ± 11.5	18.7 ± 12.1	0.94	0.71	0.78
insulin OGTT 120′ mean uU/mL ± SD	138.9 ± 126.1	246.5 ± 328.4	138.2 ± 139.2	0.65	0.85	0.66
insulin OGTT max value mean uU/mL ± SD	224.7 ± 142.9	244.6 ± 152.4	182.6 ± 97.1	0.59	0.60	0.41
HbA1C mean % ± SD	5.1 ± 0.3	5.2 ± 0.6	5.1 ± 0.5	0.96	0.94	0.77
HOMA IR mean mg/dL ± SD	3.8 ± 2.3	3.6 ± 2.2	3.7 ± 3.2	0.86	0.48	0.85
HOMA-beta mean ± SD	167 ± 62	169.4 ± 84.4	164.4 ± 100.7	0.65	0.71	1
Quicki mean ± SD	0.3 ± 0	0.3 ± 0	0.3 ± 0	0.90	0.87	0.97
Prothrombotic factors:						
protein S mean % ± SD	85.7 ± 16.6	91.8 ± 19	85.3 ± 25.8	0.35	0.96	0.48
protein C mean % ± SD	107.2 ± 27.4	114.7 ± 15.1	116.9 ± 17.4	0.51	0.33	0.79
FVIII mean % ± SD	134.2 ± 31.8	135.2 ± 33.8	150.4 ± 35.3	1	0.27	0.60
VWF Ag mean % ± SD	118 ± 39.6	120 ± 51.8	147.6 ± 70.5	0.88	0.31	0.54
fibrinogen mean mg/dL ± SD	277.5 ± 50	297.4 ± 88.1	290.8 ± 72.1	0.69	0.48	0.90
homocysteine mean umol/L± SD	14.3 ± 21.4	19.4 ± 14.7	13.3 ± 5.8	0.10	0.09	0.36
Endothelial function:						
EndoPAT RHI mean ± SD	1.6 ± 0.6	1.8 ± 0.7	1.4 ± 0.5	0.53	0.57	0.14
EndoPAT ln RHI mean ± SD	0.4 ± 0.4	0.5 ± 0.4	0.3 ± 0.5	0.51	0.39	0.54

- **Biochemical evaluation of GH actions: gluco-metabolic parameters and lipid metabolism**

The following gluco-metabolic parameters were analyzed without any differences in the three groups: fasting glucose and insulin, HbA1C, OGTT, HOMA IR index, HOMA-beta and insulin sensitivity check index (Quicki). Impaired fasting glucose was present in 2 patients being treated with hrGH and in 1 patient of the control group. Impaired glucose tolerance (n = 54) was present in 8 patients (2 with MS), 4 in the hrGH group, 3 not in treatment and 1 in the control group.

Of the 29 patients with central obesity, 5 had MS (2 criteria were present in 4 patients and 3 criteria in 1). Moreover, 3 patients were in treatment with metformin (1 without any criteria for MS) and 2 with oral cholesterol-lowering agents (1 without any criteria for MS). 2 patients with MS: 1 in treatment with hrGH metformin, 1 of no hrGH group had glycated hemoglobin >6%.

In bivariate analysis of MS parameters, both IGF1 SDS levels and GH daily dose showed a negative correlation with WC, and GH daily dose also a positive correlation with HDL (Table 5). Multivariate analysis indicated that WC was independently, negatively, and significantly predicted by IGF-1 SDS (adjusted R^2 = 0.309, p = 0.02) after correction for metabolic syndrome parameters (Triglycerides, HDL, SBP, DBP, Glucose). Moreover, WC was independently and negatively predicted (adjusted R^2 = 0.296) by GH daily dose (p = 0.03) and directly predicted by triglyceride levels (p = 0.04), after correction for other metabolic syndrome parameters (HDL, SBP, DBP, Glucose).

Table 5. Spearman's correlation coefficient (r) of the relation between metabolic syndrome parameters and IGF1 SDS - GH daily dose.

Metabolic syndrome parameters	IGF1 SDS		GH dose mg/kg/d	
	r	*p*	*r*	*p*
Waist circumference (cm)	-0.428	**<0.001**	-0.337	**<0.01**
Triglycerides (mg/dL)	-0.181	0.169	-0.056	0.672
HDL (mg/dL)	0.252	0.055	0.284	**<0.05**
SBP (Hgmm)	-0.212	0.104	-0.037	0.798
DBP (Hgmm)	-0.247	0.056	-0.007	0.955
Glucose (mg/dL)	0.159	0.222	0.117	0.367

Patients with GHD not in treatment with hrGH showed lower HDL levels (43.6 ± 9.4 mg/dl, n = 8), than those in treatment (53.6 ± 14.7 mg/dl, n = 40; p = 0.02) or those with normal GH-pituitary function (52.6 ± 8.0 mg/dl, n = 8; p = 0.02). There were no significant differences in triglyceride, total cholesterol and LDL levels between the control groups and both GHD groups (Figure 12).

However, according to the 2011 National Heart, Lung, and Blood Institute guideline [38], the hrGH group had borderline mean lipid levels of triglycerides and the total cholesterol, while LDL and HDL levels were normal/acceptable. In patients not treated with hrGH, the mean lipid levels of triglyceride and HDL were borderline, while total cholesterol and LDL levels were normal/acceptable. All lipid plasma levels were normal/acceptable in the control group. The prevalence of dyslipidemia in the three groups was as follows: triglycerides, in the control group 41.7% (2 high, 3 borderline), in the no hrGH group 50% (2 high, 2

borderline), in the hrGH group 52.5% (11 high, 10 borderline); total cholesterol, in the control group 25% (3 high), in the hrGH group 42.5 % (8 high, 9 borderline), in the no hrGH group 25% (1 high, 1 borderline); HDL, in the control group 16.7% (2 borderline), in the hrGH group 22.5% (4 borderline, 5 low), in the no hrGH group 62.5% (2 borderline, 3 low), p = 0.06; LDL, in the control group 16.7 % (2 high), in the hrGH group 40% (10 borderline, 6 high), in the no hrGH group 12.5% (1 high).

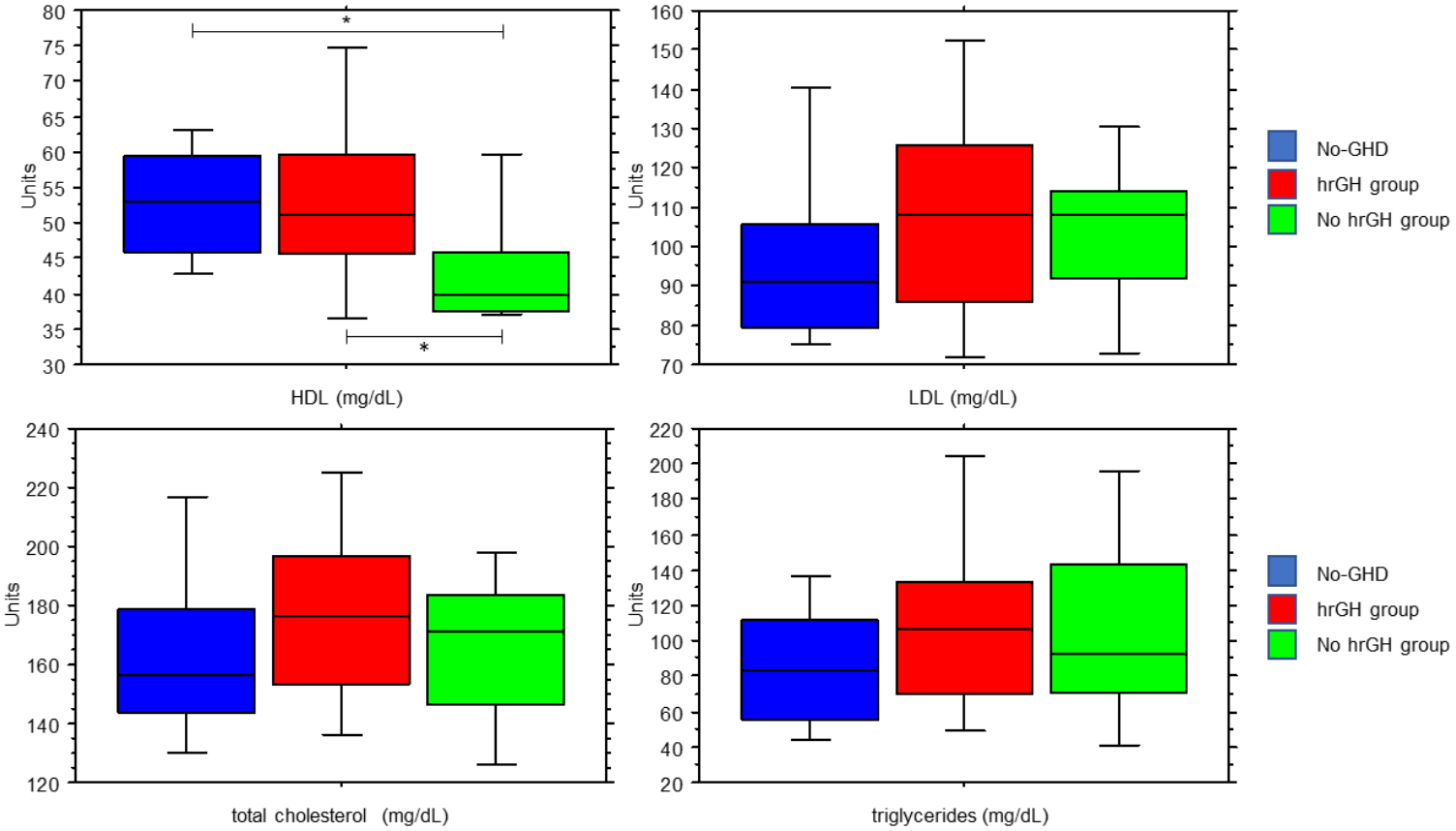

Figure 12. Lipid profile in the control group (No-GHD) and GHD subgroups.

Biochemical and biophysical evaluation of GH actions on endothelial function and prothrombotic state

No significant differences were detected between the three groups for the mean of RHI-PAT and RHI-PAT logarithmic. When we used an RHI-PAT cut off of 1.35 to identify patients with endothelial dysfunction (sensitivity of 80% and a specificity of 85% in adults [39]), 20 patients had a lower RHI-PAT: control group n = 3 (25%); hrGH group n = 14 (35%); no hrGH group n = 3 (37.5%), p = 0.77. We used EndoPAT 2000 to evaluate endothelial function because it is a non-invasive, reproducible test and is less dependent on the operator than ultrasound. Figure 9 shows EndoPAT-RHI in endothelial dysfunction.

No significant differences were detected between the three groups for circulating markers of endothelial dysfunction and the blood clotting profile (fibrinogen, protein S, protein C, factor VIII, VWF Antigen, fibrinogen, homocysteine).

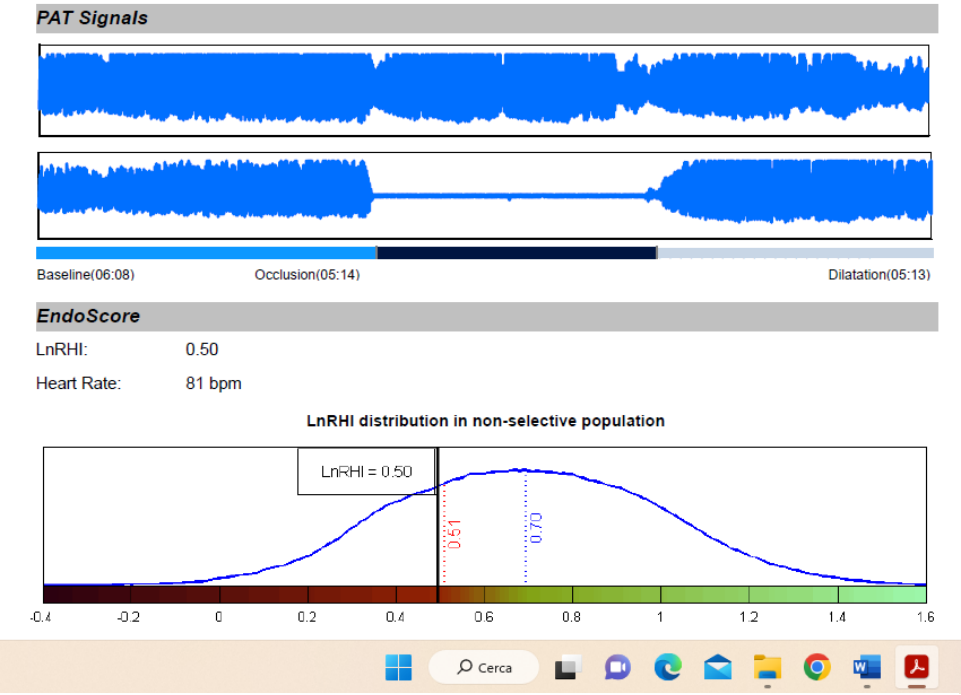

Figure 13. EndoPAT-RHI in endothelial dysfunction.

- **Quality of life and fatigue**

The PedsQL version 4.0 for QoL and the PedsQL-MFS version 3.0 for chronicle fatigue was proposed to all 60 families of them 52 parents (86.7%) accepted to fill both the questionnaires, but 1 parent completed only the PedsQL-MFS. Of the 60 children, 2 children were not able to fill the questionnaires due to severe visually impaired and 2 due to cognitive impairment, the PedsQL questionnaire was fully completed by 44 children (73.3%), while the PedsQL-MFS by 45 children (75%), respectively 12 (20%) and 11 (18.3) children did not complete the PedsQL and PedsQL-MFS questionnaire.

There were no significant differences in children and parents, between the control group and both the GHD groups in the overall perceived QoL (Figure 14a, 14b) and fatigue. However, the parents in the control group, showed a better psychosocial health in the dimension emotional functioning, than parents in GHD group (68.2 ± 17.5 vs. 82.5 ± 11.1; $p < 0.02$). This data is also confirmed when parents in the control group are compared to parents in the GHD group in treatment or not in treatment with hrGH (respectively, $p < 0.01$ and $p < 0.03$).

In children and in parents, the PedsQL and the PedsQL-MFS questionnaire showed significant correlations. To evaluate the agreement between children and parents concerning QoL and fatigue, the mean scores of both questionnaires were compared. In the control group, the mean scores did not significantly differ between children and their parents in all dimensions of the QoL and fatigue. The mean PedsQL total score was 81.8 ± 16.5 in the group of children and 77.7 ± 16.8 in the group of parents (p = 0.55), while mean PedsQL-MFS total score was 79.2 ± 22 in the group of children and 76.3 ± 16.9 in the group of parents (p = 0.75). Except for one family in which both child and parent had a score lower than 60 points, all other families' results were between 60 and 100, revealing a neutral or a good/very good evaluation of the QoL and fatigue. In the GHD group, mean PedsQL total score was higher in children than in parents (77.4 ± 16.1 vs 66.9 ± 16.1, p = 0.006), while mean PedsQL-MFS total score was 75.6 ± 16.5 for the children and 68 ± 20.3 for the parents (p = 0.08). The answers which showed significantly different mean values between children and parents were the domains "social functioning" and "school functioning" in PedsQL (p = 0.0009 and p = 0.048) and in PedsQL-MFS the domain "general fatigue" (p = 0.003). In the GHD group several children and parents reported a negative evaluation of the QoL and Fatigue with a score lower than 60 points.

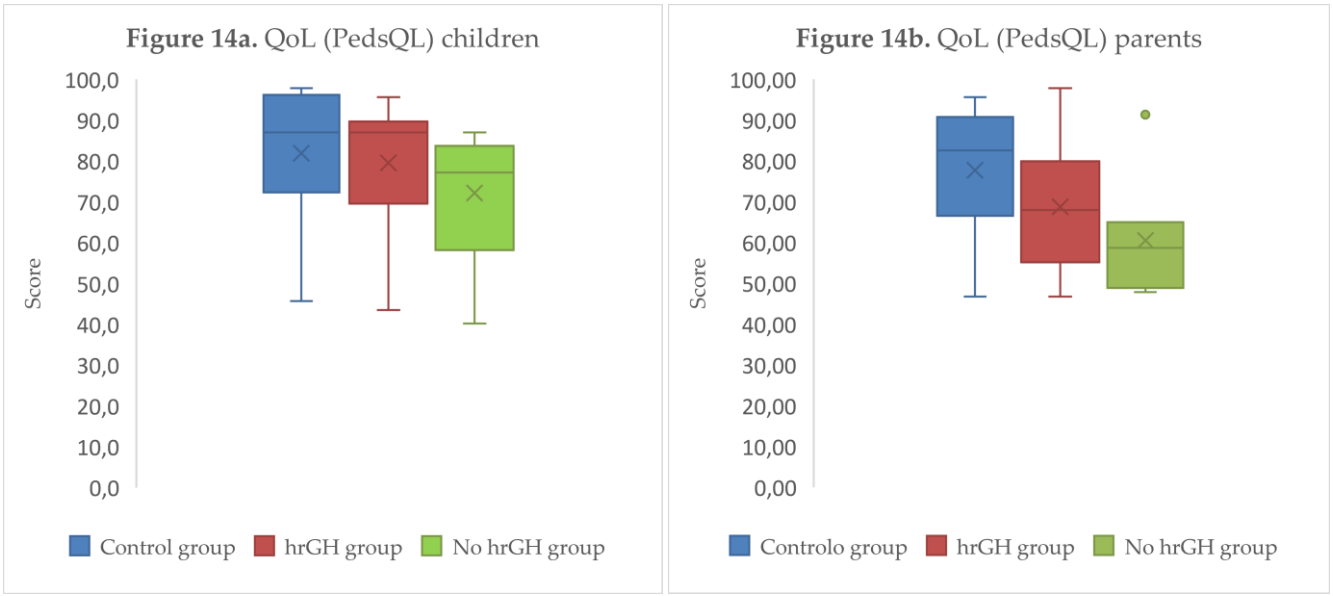

Figure 14 a,b. QoL in patients and their parents

Discussion

Obesity in children and adolescents has become a major global health challenge. The problem is even more serious in cancer survivors due to an increased risk of obesity and CVD [40]. In various studies the prevalence of obesity in CCS have been found to differ widely due to the heterogeneity of cancer populations. In our CBCS cohort, after a follow up of 9.2 years, the prevalence of obesity was 66.7% (58.3% in control group and 68.5% in GHD group). The prevalence was much higher than the Italian prevalence of obesity in healthy children and adolescents (9.3% and 6.2%) [41, 42]. The prevalence was also higher when compared to a Korean study in which obesity was found in 29.1% of 258 CCS (follow-up period, mean years of 9.2 ± 4.16) [43] or to the St. Jude Lifetime cohort consisting of 1996 CCS (follow up of 24.6 years) which had a prevalence of obesity of 36% [44]. In a large population of adults from the Childhood Cancer Survivor Study, after adjusting for age, sex and ethnicity, when obesity is determined using BMI (that is the most widely used indicator of CV risk in the general population) CBCS were more likely than siblings to be obese (Odds ratios and 95% confidence intervals were: Astrocytoma 1.4 1.2–1.7; Medulloblastoma 1.4 1.0–1.9; other central nervous system malignancy 1.1 0.7–1.6) [45]. In the St. Jude cohort, CRT (Odds ratios 1.66) was an independent risk factor for obesity and the risk of obesity were increased among survivors who were younger at diagnosis when compared to matched healthy controls [44].

In CBCS adiposity is hypothesized to be associated with direct cancer damage and late effects of treatment especially surgery and irradiation of the hypothalamic-pituitary axis due to alterations in the production of GH and neuronal damage of leptin sensitivity [44, 46,]. In this study, a high level of adiposity (WC > 90° percentile) was present in 44% of GHD group and 66.7% of control group. In addition, a high level of adiposity was present in 39.1% of the patient group that received CRT and in 78.6% of patient group that did not received this treatment. The high prevalence of adiposity also in the non-irradiated group (in a special population such as CBCS) may reflect a high rate of neurological late effects, fatigue and low QoL that reduces physical activity [47] and results in alterations of body composition with high levels of fat mass and low levels of muscle mass (also known as

sarcopenic obesity), which then causes a reduction in energy expenditure and worsens the metabolic damage [48, 49]. (Figure 15)

In our study, the cardiometabolic profiles of CBCS with normal GH pituitary function were compared with GHD cohort in - and not in – treatment with hrGH. The results of this study found a different body fat distribution characterized by higher fat mass in the four limbs and VA in the patients not in treatment with hrGH, whereas the BMI, WC and other classic anthropometric parameters associated to cardiovascular risks were not significantly different between the three groups. In addition, patients with GHD not in treatment with hrGH showed lower adiponectine levels (3.37±1.79 mg/l, n = 8) than those in treatment (9.56 ± 7.15 mg/l; p = 0.007) or those with normal GH-pituitary function (10.50 ± 6.72 mg/l; p = 0.0001). Today, weight and BMI are frequently the only parameters used to classify obesity in epidemiological studies and also in clinical practice. However, neither measurement may accurately distinguish between fat and fat-free mass [50]. The idea behind the BMI was conceived in 1832 by Belgian anthropologist and mathematician Quetelet. He made a simple observation that weight increases with the square of height, but he did not consider it as a

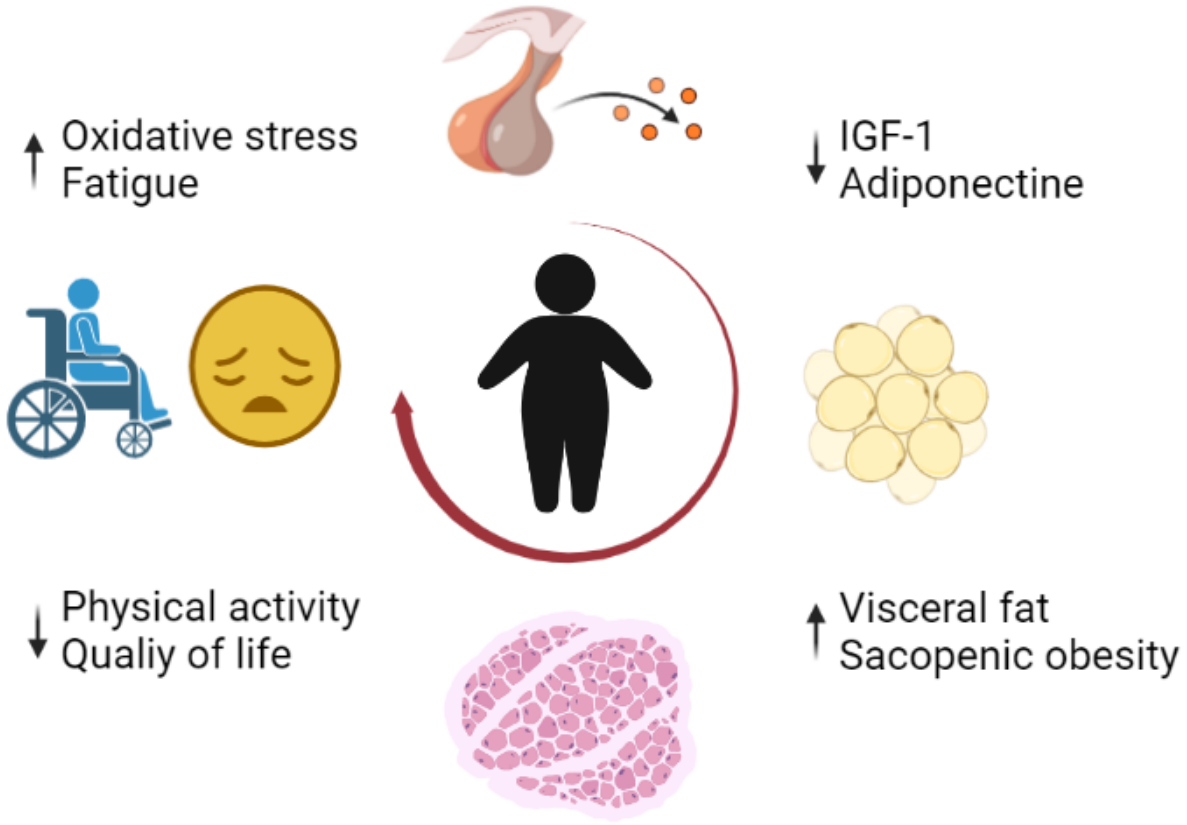

Figure 11. Schematic illustration of sarcopenic obesity in CBCS

health measure according to the current concept of obesity, for which it has been used from the second half of the following century. Our study supports the increasing evidence that BMI values might underestimate adiposity, while the body composition and accumulation of visceral fat, estimated by DXA, are more reliable measures to predict the development of cardiovascular risk in children and young adults [9] as it may allow prediction of changes in fat distribution that will evolve over time into VA. However, since DXA is time consuming and financially costly, BMI still remains the most used measurement to study obesity even in high risk populations. Visceral adiposity can also be precisely measured by magnetic resonance imaging or computed tomography scan. The MRI involves no ionizing radiation but is expensive, time-consuming and impractical for routine use [51], therefore WC measurement is still the most common alternative parameter used to assess VA. WC is easier and cheaper to evaluate, and therefore in the recent decades it has been under investigation in many studies as a tool to better predict the risk of CVD and mortality in adults [50, 52]. Furthermore, WC remains the essential component of MS as defined by the joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society and International Association for the Study of Obesity [53]. Cutoffs of WC to define central obesity have been proposed by several authors, including for different populations of adults by the IDF [54, 55], and in children and adolescents by Xi et al. in an international study [26]. We preferred to use International Waist Circumference Percentile Cutoffs for children and adolescents and WC values for Europeans, recommended by the IDF, for young adults (94 cm for men and 80 cm for women), due to better sensitivity and specificity to identify CV risk [56]. In addition, the 90th WC percentile cutoff linked better, at the age of 18 years with the criteria for adult central obesity [26]. Compared to BMI, WC and WHtR seem to be more strongly associated with cardiometabolic risk factors, CVD, and premature death [57, 58]. However, in a special population such as adolescent and young adult survivors of childhood brain cancer also WC might underestimate the peculiarity of pathological fat mass distribution which is the sarcopenic adiposity where the the pathological body composition involves the whole body more extensively than general population.

In CBCS GHD is the most common pituitary disorder, and CVD is the leading non-cancer cause of premature mortality in this population [59]. The prevalence of GHD and CVD increases over time, and both may also develop decades after the end of cancer treatments. The risk of CVD in CBCS is approximately 10 times higher than their siblings. Therefore, lifelong surveillance is of critical importance in CBCS [60].

In our study IGF1-SDS levels showed significant moderate negative correlations with the main anthropometric and body composition parameters. However, in the literature, the data on the relationship between circulating IGF-1 and all causes of mortality, including CVD, are still controversial. The meta-analysis carried out by Burgers et al. in 2011 suggested a U-shaped dose–response association between IGF-1 levels and risk of all-cause mortality [61]. This data was confirmed in 2021 by Xie et al., in a large prospective study with 380 997 participants [62], and in 2021 by Rahmani et al. in a meta-analysis on 30876 patients [63]. In both studies, the dose–response analyses showed a U-shaped relation between IGF-1 and the risk of mortality with both high and low values of IGF-1 associated with an increased risk of mortality.

Several studies have found a correlation between GHD, low levels of IGF-1 and chronic conditions such as CVDs, diabetes mellitus, VA and sarcopenia. Only a few studies focused on the cardiovascular and metabolic effects of hrGH in CBCS. The cardio-metabolic risk is linked to an altered body composition with high adiposity, which is associated to a low chronic pro-inflammatory state and promotes a pro-atherogenic metabolic state characterized by insulin resistance, hypertension, dyslipidemia, dysregulated adipokine secretion and a pro-thrombotic state. These modifiable risk factors appear to be synergistic with endothelial, vascular and cardio-toxic late effects of cancer treatments [8, 64]. (Figure 16)

The GH-IGF1 plays a role in the regulation of body composition in childhood and adults through its anabolic and lipolytic actions [65]. However, data from difference studies about the lipid profile is discordant. Murray et al. [66] showed that CCS patients with GHD had

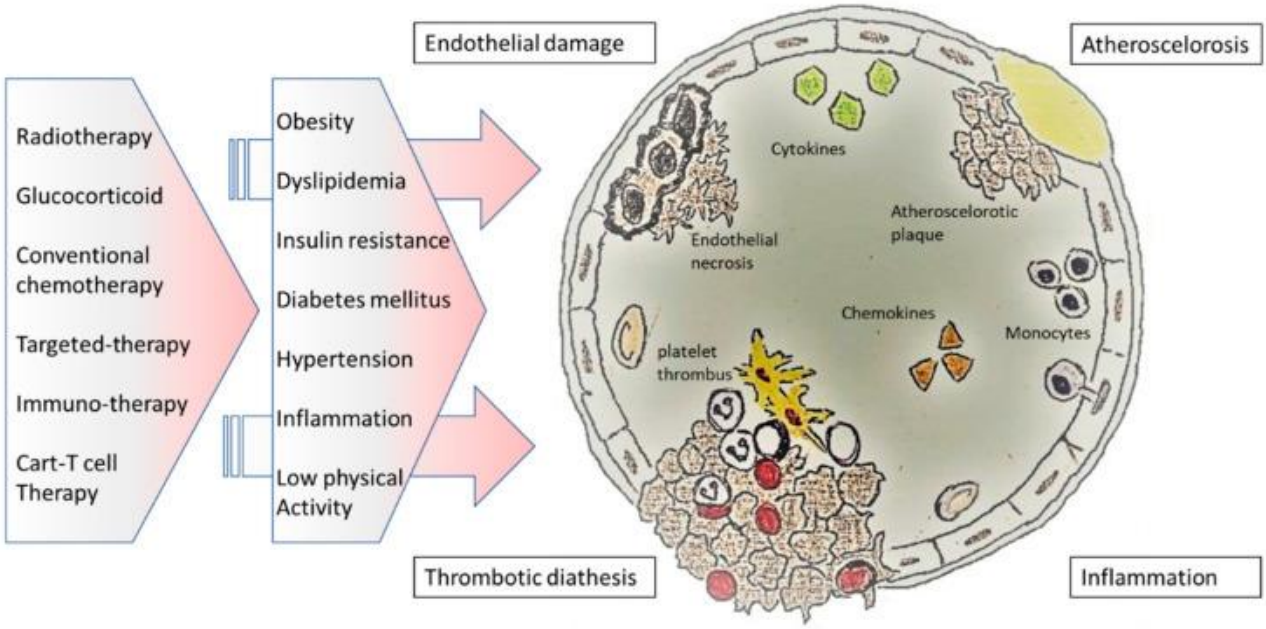

Figure 12. Etiopathogenesis of endothelial dysfunction in cancer survivors

higher total cholesterol, low-density lipoprotein cholesterol, and triglyceride levels. However, in the same study after 12 months of hrGH, there was no significant difference in lipid profiles of CCS versus controls, but there were improvements in the body composition in the male subgroup and in total cholesterol and triglyceride levels in the female subgroup. In another study treatment with hrGH improved the high-density lipoprotein cholesterol levels and the prevalence of MS in survivors of acute lymphoblastic leukemia [67], compared with an untreated control group.

A GHD may potentiate survivors' risk of adiposity and metabolic disease and thus also CVD. In order to further highlight the need to better understand the impact of GH on cardiometabolic risk factors, we evaluated the GH actions on several anthropometric, biophysical and biochemical cardiometabolic parameters. Our results suggest that in CBCS with a GHD hrGH may mitigate the pathological modification of VA, the lipid profile and adipokine production. Therefore, in the long-term optimizing the hrGH therapy may prevent adiposity-linked metabolic disorders and CVD.

Moreover, the GHD negatively affects the QoL and fatigue perceived by parents compared to children in the "social functioning", "school functioning" and "general fatigue" domains. To the best of our knowledge this is one of the first studies that analyzes in detail

cardiometabolic risk factors, QoL and fatigue in a population of children and young adults who survived a tumor of the central nervous system.

One of the strengths of our study is the high enrolment rate, together with the analysis of the QoL and the fatigue perceived by patients and their parents. Most families were very collaborative, and only 1 family did not accept the invitation to participate in the study. As regards to the sample selection bias, no preconditions were present that may have affected the answers (such as when using patient associations for the enrollment). On the other hand, our cases came from the Gaslini Hospital, which is a tertiary referral centre hub for severe clinical cases (also from other Italian regions), therefore the mix of histology tumors and prevalence of endo-metabolic late effects is not a true reflection of the prevalence in the Italian population of brain cancer survivors. Although our findings cannot be generalized to the whole population of Italian CBCS, there was no evidence of selection biases that may have affected our results. However, the Covid-19 pandemic situation may have influenced the enrollment bias due to the fact that patients with less late effects preferred to postpone their clinical checks. Another possible limitation is the absence of a healthy control group, and the lack of data on the neuromotor limitations of the children and the socio-economic status of the families.

Conclusion:

CBCS have a high risk of adiposity and cardiometabolic disease. Emphasizing the need to reduce potentially modifiable risk factors, a tailored therapy with hrGH may have therapeutic potential in the management of the cardiovascular risk. For that purpose, the therapy should also be modulated to consider modifications in the body composition particularly the adipose tissue and the altered level of adipokines and lipoprotein, taking into consideration the molecular mechanisms underlying the U-shaped relationship between serum IGF1 levels and the increased risk of mortality due to CVD.

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